# Fertilizing Crops to Improve Human Health: A Scientific Review





International Fertilizer Industry Association

## Fertilizing Crops to Improve Human Health: **A Scientific Review**

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#### Foreword by IFA and IPNI

Traditionally, fertilizers have been used to maintain or restore soil fertility, increase crop yield and to a lesser extent improve crop quality. Their management has been progressively improved to optimize their economic return, while minimizing negative impacts on the environment. More recently, there is increasing attention to another dimension: managing fertilizers such that they also contribute to healthy and productive lives for all.

Good human health not only requires enough calories but also sufficient intake of all essential nutrients. There are a number of success stories, such as the use of zinc fertilization in Central Anatolia in Turkey and the supplementation of fertilizers with selenium in Finland. These initiatives have offered effective solutions to major human health problems. They should be implemented in a larger number of countries where similar deficiencies in soils, crops and humans are widespread. Fertilization practices can also impact the composition of food products. Enhancing the levels of health-beneficial compounds could be considered a fertilization objective as well. For instance, potassium fertilization can enhance lycopene concentration in tomato and the isoflavone content of soybean seed.

In order to better understand how fertilizer use can enhance human health, the International Plant Nutrition Institute (IPNI) and the International Fertilizer Industry Association (IFA) decided in 2008 to launch an extensive scientific literature review of the state of knowledge in this complex domain. Leading scientists in the identified fields were invited to draft chapters. All the chapters have been peer-reviewed by academic scientists in order to ensure the publication presents a thorough and balanced analysis.

This book is intended for all stakeholders with an interest in nutritional aspects, from the agricultural sector to the human health community. We trust that this reference document will provide the necessary scientific basis for developing and promoting new fertilizer recommendations aimed at alleviating the burden of nutrition insecurity and will stimulate further research in this area. It is an essential contribution to the fertilizer industry's efforts to improve fertilizer management practices through implementation of 4R Nutrient Stewardship for delivering economic, social and environmental benefits.

Luc M. Maene	Terry L. Roberts
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#### **Foreword by IFPRI**

Fertilizer has been instrumental in global agricultural development over the past century, most notably in the Green Revolution—the concerted effort that spread across Asia beginning in the mid-1960s to increase food production through the introduction of a package of modern inputs consisting of improved seeds, fertilizer, and crop protection products along with improved policies and incentives. These advances contributed to a doubling of world cereal production in the past four decades, enabling billions more people to be fed. While fertilizer has been highly influential in increasing the quantity of food produced, it also holds enormous potential for improving human welfare by improving the quality of food.

Micronutrient deficiencies, or "hidden hunger," affect the lives of approximately 2 billion people around the world. In developing countries, more than 10 million children under the age of 5 die each year; 60 percent of these deaths are related to malnutrition. About 1.6 billion people are anemic due to iron deficiency, vitamin A deficiency results in the death of around 1 million children each year, iodine deficiency during pregnancy contributes to the mental impairment of nearly 20 million babies annually, and deficiency in zinc would be responsible for about 800,000 deaths annually from diarrhea, pneumonia and malaria in children under 5. Agriculture can play an important role in combating hidden hunger and its consequences, as discussed extensively at the International Food Policy Research Institute 2020 Vision Conference on "Leveraging Agriculture for Improving Nutrition and Health" (http://2020conference.ifpri.info), through the application of science and technology that advances both the quantity and quality of food. Many parts of the world that suffer from hidden hunger also endure low agricultural productivity. Enabling farmers to access fertilizers at affordable prices is vital. Utilizing fertilizer to address deficiencies of key nutrients, including zinc and selenium, will require an integrated approach and involve numerous actors. While challenges include efficiently incorporating fertilizer throughout the agricultural value chain, opportunities abound for the private sector to engage more effectively with farmers and farm organizations, national governments, and international agencies.

The fertilizer community sits atop a significant resource for advancing nutritional outcomes. *Fertilizing Crops to Improve Human Health: A Scientific Review* comes at a timely and critical juncture in the intersection between agriculture, nutrition, and health. The information and analysis presented in this volume offers a distinct opportunity to leverage fertilizer for improving the nutrition and health of many vulnerable people around the world.

Rajul Pandya-Lorch International Food Policy Research Institute (IFPRI) viii | Fertilizing Crops to Improve Human Health: A Scientific Review

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# Symbols commonly used throughout this publication

A1	Aluminum
В	Boron
С	Carbon
Ca	Calcium
CaCO <sub>3</sub>	Calcium carbonate
Cd	Cadmium
Cl-	Chloride
Cu	Copper
$CuSO_4$	Copper sulphate
F	Fluorine
Fe	Iron
Fe <sup>2+</sup>	Ferrous iron
Fe <sup>3+</sup>	Ferric iron
H⁺	Hydrogen ion
HCO <sub>3</sub> -	Bicarbonate
Ι	Iodine
К	Potassium
KC1	Potassium chloride (also muriate of potash or MOP)
K <sub>2</sub> O	Oxide form of K, used in trade to express K content of fertilizer
$K_2SO_4$	Potassium sulphate (also sulphate of potash or SOP)
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Ν	Nitrogen
Na	Sodium
NaCl	Sodium chloride
$N_2$	Dinitrogen
NH <sub>3</sub>	Ammonia
$\mathrm{NH}_4^+$	Ammonium
Ni	Nickel
$NO_2^-$	Nitrite
$NO_3^-$	Nitrate

Р	Phosphorus
Pb	Lead
PO <sub>4</sub> <sup>3-</sup>	Phosphate
$P_2O_5$	Oxide form of P, used in trade to express P content of fertilizer
Pu	Plutonium
S	Sulphur
Se	Selenium
Si	Silicon
SO <sub>4</sub> <sup>2-</sup>	Sulphate
Zn	Zinc

Introduction/Executive Summary

### Fertilizing Crops to Improve Human Health: a Scientific Review

Tom W. Bruulsema, Patrick Heffer, Ross M. Welch, Ismail Cakmak and Kevin Moran<sup>1</sup>

A large proportion of humanity depends for its sustenance on the food production increases brought about through the application of fertilizers to crops. Fertilizer contributes to both the quantity and quality of the food produced. Used in the right way applying the right source at the right rate, time and place and on the right crops, it contributes immensely to the health and well being of humanity.

Since 1948, the World Health Organization has defined human health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." Reflection on this definition leads one to realize that responsibility for human health extends well beyond the critically important domain of medical science to include many other disciplines. The awarding of the 1970 Nobel Peace Prize to Dr. Norman Borlaug indicates a high level of recognition of the linkage of agricultural sciences to this definition of human health.

The increasing use of fertilizer in agricultural crops has boosted production per unit area, increasing the total supply of food as well as contributing to the quality of food and its content of essential trace elements. Increased production of the crops most responsive to fertilizer has also changed the mix of crops produced and their match to the nutritional needs of the human family.

For symbols used commonly throughout this book see page xi.

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There is no human health without food. The mission of agriculture is more than producing food commodities; it is to supply foods that nourish human health. Fertilizer use supports that mission. Sustainable agricultural development and sustainable fertilizer use must increasingly focus on nourishing human health, towards a goal of healthy and productive lives for all in the context of a burgeoning world population. While the current role of fertilizers in supporting human health is large, the opportunities to expand it even further are also substantial.

Sustainable development requires a vision that extends beyond the immediate and important concerns of productivity and profitability at the farm level to encompass design of agricultural systems to provide better human nutrition. This review aims to provide accurate knowledge of the multiple linkages to crop qualities that influence human health. The industry's 4R Nutrient Stewardship approach—application of the right source at the right rate, right time and right place—will need to include these linkages as part of the definition of "right."

#### **Food and Nutrition Security**

**Food security** exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food. Nutrition security means access to the adequate utilization and absorption of nutrients in food, in order to be able to live a healthy and active life (FAO, 2009).

Between 1961 and 2008, the world's population grew from 3.1 to 6.8 billion. In the same period, global cereal production grew from 900 to 2,500 Mt (Figure 1), with much of the growth due to the increase in world fertilizer use from 30 to over 150 Mt. Without fertilizer use world cereal production would be halved (Erisman et al., 2008).



Figure 1. Global cereal production and total fertilizer consumption 1961-2011 (FAO 2012; IFA 2012).

By doubling the quantities of new N and P entering the terrestrial biosphere, fertilizer use has played a decisive role in making possible the access of human-

kind to food. However, not all have access. Chronic hunger still haunted the existence of one-sixth of the world's people in 2009. By 2050, according to the FAO, the human population would require a 70% increase in global agriculture output compared to that between 2005 and 2007 (FAO, 2012). Future yield increases expected through genetic improvement will still depend on replenishment of nutrients removed by using all possible sources, organic and mineral, as efficiently as possible.

**Nutrition Security.** In addition to yield, plant nutrition affects other important components of human nutritional needs, including the amounts and types of carbohydrates, proteins, oils, vitamins and minerals. Many of the healthful components of food are boosted by the application of mineral nutrients. Since most farmers already fertilize for optimum yields, these benefits are easily overlooked. Trace elements important to human nutrition can be optimized in the diet by applying them to food crops.

Opportunity exists to improve yields and nutritional quality of food crops such as pulses, whose yields and production levels have not kept pace with population growth. Ensuring that such crops maintain economic competitiveness with cereals requires policies that reward farmers for producing the nutritional components of greatest importance to human health.

**Micronutrient malnutrition** has been increasing, partially as a consequence of increased production of staple cereal crops. Other micronutrient-rich crops, particularly pulses, have not benefited as much from the Green Revolution. Having become relatively more expensive, they now comprise a smaller proportion of the diets of the world's malnourished poor.

Biofortification of crops can be an effective strategy for moving large numbers of people from deficient to adequate levels of Fe, vitamin A and Zn. The choice of genetic and/or agronomic approaches to biofortification depends on the micro-nutrient. The two approaches can also be synergistic and complementary.

In staple crops, genetic approaches are most effective for Fe and vitamin A, while agronomic approaches including fertilizers can boost the Zn, I and Se levels in foods. While deficiencies of I and Se do not limit the growth of plants, correction of Zn deficiency can benefit both crops and consumers of crops. Fertilizing cereals with Zn and Se improves both concentration and bioavailability of these trace elements. Timing of foliar application of micronutrients seems to be a critical agronomic practice in maximizing grain accumulation of micronutrients, such as Zn. According to the results obtained from field experiments, foliar spray of Zn late in growing season results in much greater increase in grain Zn concentration when compared to the earlier foliar applications, particularly in the endosperm part that is the most commonly eaten part of wheat grain. A large proportion of soils worldwide are deficient in Zn (**Table 1**), and the proportion of people at risk of Zn malnourishment, while varying regionally, is also substantial (**Table 2**).

Table 1. Proportion of agricultural soils deficient in mineral elements (based on a survey of 190 soils worldwide – Sillanpaa, 1990).

	,
Element	%
Ν	85
Р	73
K	55
В	31
Cu	14
Mn	10
Mo	15
Zn	49

**Table 2.** Global and regional estimates of the<br/>proportion of the population at risk<br/>of inadequate Zn intake (Hotz and<br/>Brown, 2004).

Region	Population at Risk, %
N. Africa and E. Mediterranean	9
Sub-Saharan Africa	28
Latin America & Caribbean	25
USA and Canada	10
Eastern Europe	16
Western Europe	11
Southeast Asia	33
South Asia	27
China (+ Hong Kong)	14
Western Pacific	22
Global	21

#### **Functional Foods**

**Calcium, Mg and K** are essential macro mineral nutrients for humans. The essential functions of these mineral elements in humans are similar to those in plants, with the striking exception of Ca's major role in bones and teeth. Their content in plants is influenced by their supply in the soil. Thus, in addition to assuring optimal crop production, fertilization practices may contribute to meeting the requirements for these minerals in human nutrition. Calcium deficiencies occur in countries where diets depend heavily on refined grains or rice (e.g. Bangladesh and Nigeria). Adequate Mg intake is not easily defined, but studies suggest a significant number of adults, even in the United States, do not consume adequate amounts. Similarly, a recommended daily allowance for K intake has not been defined, but only 10% of the men and less than 1% of women in the United States take in as much as or more than the adequate intake of 4.7 g/day.

**Carbohydrates, proteins and oils.** Applying N to cereals adds to the protein they produce, as well as their yields. In rice, while N has its largest effects on yield, it can slightly increase protein and protein quality, since the glutelin it promotes has higher concentrations of the limiting amino acid, lysine, than do the other proteins it contains. In maize and wheat, protein may increase with N rates higher than needed for optimum yield, but the improvement in nutritional value may be limited by low concentrations of the essential amino acid lysine. An exception is the Quality Protein Maize developed by plant breeding: its lysine

concentration remains high when more N is applied. In potatoes, N increases starch and protein concentration while P, K and S enhance protein biological value. Oil composition of crops changes little with fertilization, though oil production is increased wherever yield-limiting nutrient deficiencies are alleviated.

Management tools that more precisely identify optimum source, rate, timing and placement of N will help improve the contribution of fertilizer to production of healthful proteins, oils and carbohydrates. Genetic improvements to N use efficiency may require careful attention to impact on protein quantity and quality in cereals. However, nutrient management practices such as late foliar applications or controlled-release technologies can boost N availability for protein production while keeping losses of surplus N to a minimum.



Figure 2. Yield and protein of wheat respond to applied N fertilizer.

**Health-functional quality of fruits and vegetables.** Scientific evidence from numerous sources has demonstrated that judicious fertilizer management can increase productivity and market value as well as the health-promoting properties of fruits and vegetables. Concentrations of carotenoids (Vitamin A precursors) tend to increase with N fertilization, whereas the concentration of vitamin C decreases. Foliar K with S enhanced sweetness, texture, color, vitamin C, beta-carotene and folic acid contents of muskmelons. In pink grapefruit, supplemental foliar K resulted in increased beta-carotene, and vitamin C concentrations. Several studies on bananas have reported positive correlations between K nutrition and fruit quality parameters such as sugars and ascorbic acid, and negative correlations with fruit acidity.

In addition to effects on vitamins, fertilizers can influence levels of nutraceutical (health-promoting) compounds in crops. Soybeans growing on K-deficient soils in Ontario, Canada had isoflavone concentrations about 13% higher when fertilized with K. Potassium has also been reported to promote concentrations of lycopene in grapefruit and in tomatoes. Broccoli and soybeans are examples of plants that can contribute Ca and Mg to the human diet. When crops like these are grown in acid soils of limited fertility, applying lime can boost the levels of these important minerals.

The potent antioxidant pigments lutein and beta-carotene generally increase in concentration in response to N fertilization. Together with vitamins A, C and E, they can help lower the risk of developing age-related macular degeneration, which is one of the leading causes of blindness.



#### **Risk Reduction**

**Plant disease.** In cereals deficient in Cu, ergot (Claviceps sp.) is an example of a food safety risk caused by a plant disease that can be controlled by application of Cu fertilizer. By immobilizing and competing for mineral nutrients, plant pathogens reduce mineral content, nutritional quality and safety of food products from plants. While many other specific diseases have known plant nutritional controls, there is a knowledge gap on the optimum nutrition for controlling the plant diseases most relevant to food safety.





Application of Cu fertilizer (CuSO<sub>4</sub> crystal on right) has been an effective treatment in ergot-prone soils.

Managing nutrition influences diseases and their control. Strategies to reduce plant disease through plant nutrition include:

- the development of cultivars that are more effective in taking up Mn
- · balanced nutrition with optimum levels of each nutrient
- attention to forms and sources suited to the crop (e.g. nitrate versus ammonium, chloride versus sulphate)
- timing, applying N during conditions favoring plant uptake and growth response
- integration with tillage, crop rotation, and soil microbes

**Farming systems.** Organic farmers apply strategies for plant nutrition that differ from those of other producers. Do these differences influence the healthfulness of the food they produce? Owing to the restricted sources for nutrient supply, organic farming cannot provide sufficient food for the current and growing population in the world. Also, because organic production systems rely heavily on ruminant animals and forage crops for the cycling of nutrients, the proportions of food types produced do not match the requirements of healthy diets. An imbalanced dietary composition can cause health problems as a result of insufficient supply of essential nutrients or excessive supply of other food constituents.

The composition of foods produced does show small changes explained by plant physiological responses to differences in N supply. Vitamin C is increased, but A and B vitamins, protein and nitrate are reduced under organic farming. Higher levels of nitrate in conventionally grown foods do not threaten and may be beneficial to human health. Despite the great interest in food quality among supporters of organic agriculture, focussing on food supply and dietary composition is most important for human health.

**Remediating radionuclides.** When soils become contaminated with radionuclides, as for example after accidents with nuclear reactors in Chernobyl or Fukushima, limiting plant uptake becomes an important goal for protecting human health. Studies on soils from the Gomel region of Belarus showed that levels of radiocaesium (<sup>137</sup>Cs) and radiostrontium (<sup>90</sup>Sr) in crops declined in response to increasing soil exchangeable K, with K applied as either fertilizer or manure. These radionuclide levels also declined with addition of dolomitic limestone, and N and P fertilizers. The involvement of rural inhabitants in processes of self-rehabilitation and self-development is a way to improve people's life quality on radioactive contaminated territories.

#### Summary

The foregoing demonstrates the very large role fertilizer plays in improving crop attributes relevant to the health of humankind.

Given the important role of fertilizers in promoting food and nutritional security, it becomes all the more important to invest in research aimed at optimizing the

benefits associated with their use. Research needs to support the adoption of 4R Nutrient Stewardship to ensure that the right source is applied at the right rate, at the right time, and in the right place. This concept—embraced by the fertilizer industry—defines "right" as that most appropriate for addressing the economic, social and environmental aspects of sustainability, all three of which are critical to sustain human health. Coupled with appropriate strategic changes to farming systems toward production of a better balance of foods to address the true



Applying the right source of plant nutrients at the right rate, time and place enables improvement of crop quality.

nutritional needs of the human family, an emphasis on 4R Nutrient Stewardship in agronomic research and extension will enhance the benefits and minimize the potential negative impacts associated with fertilizer use. **FCHH** 

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Chapter 1

# The Role of Plant Nutrition in Supporting Food Security

Terry L. Roberts and Armando S. Tasistro<sup>1</sup>

#### Abstract

One-sixth of the world's people were chronically hungry in 2009. Competing and increasing requirements for food, feed, and biofuels necessitate future cereal production increase of 70% by 2050. Expansion of harvested area and increasing crop productivity are the only options available for increasing food production, with the latter being the most important. Advances in biotechnology, new genetics, improvements in agronomic management, and increased efficient management of fertilizers will be necessary to significantly increase crop yields. Commercial fertilizer accounts for 40 to 60% of the world's cereal production and will continue to play a vital role in the future in closing the gap between actual and attainable crop yields. Other sources of nutrients such as animal manures, green manures, or biological fixation should be used when available or combined with non-organic nutrient sources. Fertilizer best management practices and nutrient stewardship, based on 4Rs—applying the right source, at the right rate, in the right time, and the right place—based on scientific principles, provide guidelines and a global framework to ensure fertilizers are used efficiently and effectively in helping the world achieve food security.

#### Introduction

Food security is a multi-dimensional phenomenon that exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food which meets their dietary needs and food preferences for an active and healthy life (FAO, 2003). In this chapter we will analyze mainly the role of plant nutrition with regards to the amounts of food produced globally, recognizing that the production of enough food is a necessary—but not sufficient—condition for attaining food security. Between 1961 and 2008, the world population grew from 3.1 to 6.8 billion, and although global gross production of cereals increased from 0.9 billion metric tons (t) to a record high of 2.5 billion t in the same period (**Figure 1**), one-sixth of the world's population (1.02 billion people) were still

For symbols used commonly throughout this book see page xi.

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Figure 1. Change in global cereal production and population, 1961-2008.

chronically hungry in 2009, the highest level of undernourishment in 40 years (FAO, 2009a). Although the number of hungry people fell off in the 1970s and 1980s, it began to increase since the mid-1990s as the per capita cereal production started to decline, despite a slower population growth (**Figure 2**). Growth in population has slowed in recent years, but is still expected to reach 9.2 billion by 2050 (United Nations, 2008).

Almost all of the hungry are in the developing world: 63% in Asia and the Pacific and 26% in Sub-Saharan Africa. FAO estimates that 33 countries are currently facing a food crisis (FAO, 2010a). In 2008, wheat and maize prices tripled and the price of rice increased even more, compared to their early 2005 levels (Beddington, 2010) sparking food riots in poor nations. Increased food demand in the developing world, high oil prices, biofuels, high fertilizer prices, low global cereal stocks, and market speculation were blamed for the crisis (Glenn et al., 2008).

The livestock sector is a major contributor to creating demand for grains and oilseeds. Greater amounts of grain are being demanded by the livestock sector due to the change in diets that is accompanying the increasing urbanization and affluence of the population, as shown by the growth in global meat production, which doubled in three decades (1970 to 2000) from 11 to 27 kg per person (**Figure 2**), and is forecast to reach 44 kg per person by 2050 (Alexandratos et al., 2006). In China, for instance, meat consumption grew from 9 kg per person to more than 50 kg over that same time period. Feed use of cereals grew at about the same rate as livestock growth in the 1970s (2.4% per annum). But growth fell to about 1% in the subsequent two decades, even though livestock kept growing at over 2% per annum, suggesting feed conversion efficiency was improving. In 1999/01, feed use of cereals was estimated at 666 million metric tons (M t) or 35% of total world cereal use.

Biofuels are also intensifying the demand for cereals as the production and use of biodiesel and ethanol have increased dramatically in recent years. Global ethanol production tripled between 2000 and 2007, largely due to growth in the USA



Figure 2. Change in cereal and meat production per capita from 1961-2008 (FAO, 2010b).

and Brazil, and biodiesel expanded from less than 1 billion liters to almost 11 billion liters (OECD-FAO, 2008). In 2007/08, 110 M t of coarse grains were used to produce ethanol, which was equivalent to around 10% of the total global utilization of coarse grains (FAO, 2009a). FAO (2009a) cited OECD-FAO projections that put global biofuel production at 192 billion liters in 2018, which would increase the demand for agricultural feedstocks (sugar, maize, oilseeds) possibly resulting in higher food prices. Beyond 2018, Alexandratos et al. (2006) suggested that 200 M t of cereals might be going to biofuels by 2050.

The competing and increasing requirements for food, feed, and biofuels make it more difficult to attain the Millennium Development Goal of halving world hunger by 2015 from the 1990-92 World Food Summit baseline of 842 M (FAO, 2008). Although future cereal production must increase, predictions differ as how much the increase should be. Glenn et al. (2008), in the most recent *State of the Future* report, suggested that food production has to increase 50% by 2013 and double in 30 years. The 2009 World Summit on Food Security projected global cereal production would have to increase 70% and output double in developing countries if we are to feed an extra 2.3 billion by 2050 (FAO, 2009a).

How can the world increase food production by 50 to 70%, or double it in the next 30 to 40 years? There are only two ways to increase crop production: expansion of harvested area (i.e. expand cropped land and/or cropping intensity) or increase crop productivity.

#### **Increasing Cereal Production**

Strategically, global food security will continue to depend on rice, wheat, and maize, as these three crops still occupy 58% of the annual crop area and provide about 50% of food calories. Rice and wheat have been essential suppliers of energy for the population of developing countries since 1960, whereas maize has provided over 60% of energy in commercial animal feeds (Fischer et al., 2009).

About 1.6 billion ha of the world's 13.4 billion ha of land area is under cultivation. After considering non-agricultural uses (forest cover, protected areas, urbanization, etc.), ecological fragility, low fertility, toxicity, disease incidence, lack of infrastructure, and other constraints an estimated additional arable area of some 70 M ha may come into crop production by 2050 (an expansion of 120 M ha in developing countries offset by a 50 M ha decline in developed countries) (FAO, 2009b). Increases in cropping intensity (i.e. multiple cropping or short fallow periods) over the projection period could add another 40 M ha, giving a total increase in harvested area of 110 M ha.

Sub-Saharan Africa and Latin America have the most potential for area expansion. Farmers in Sub-Saharan Africa are projected to bring another 20 M ha of cereal production under the plow between 1997 and 2020 and farmers in Latin America, 8 M ha, but the rest of the developing world will account for only another 13 M ha (Rosegrant et al., 2001). However, lack of infrastructure and technology, environmental concerns (some land has to come from forested areas), political will, and other opposition will make land expansion difficult. Therefore, a more favorable scenario for meeting future food needs is one in which increased crop production comes from greater yields on existing farm land.

Even without new genetic advances there are opportunities right now to increase yields. Average farm yields in many regions are normally unsurprisingly below potential yields. Lobell et al. (2009) surveyed the literature on wheat, rice, and maize cropping systems and found that average yields range between 20% and 80% of potential yields in probably all of the major cropping systems of the world. Potential yield was defined as the yield of an adapted crop cultivar when grown under favorable conditions without limitations from water, nutrients, pests or diseases. Lobell et al. (2009) also concluded that several major rice and wheat systems of the world had yields that approached 70% to 80% of yield potential, but none had passed beyond that point, which suggested that it marked a limit to yield gap reduction.

Neumann et al. (2010) analyzed current vs. attainable yields—the latter calculated by means of stochastic frontier production functions—frontier yields for these authors represent what can be currently produced, without taking into account genetic improvements that may result in higher potential yields—and concluded that on average the present actual global yields of wheat, maize, and rice are 64%, 50%, and 64% of their frontier yields, respectively.

The successful application of intensification (i.e. closing the yield gap between actual and attainable yields) depends on a thorough understanding of the nature and strength of region-specific constraints. Grain yields in developing countries lag behind those in developed countries and yields differ greatly among developing countries. Some of the yield gap described above may result from biophysical limitations, such as inadequate climate (e.g. temperatures and rainfall distribution), lack of irrigation, topography, and low soil fertility. In addition, socio-economic circumstances such as access to markets and credit, governmental support



Figure 3. Anticipated impact of improvements in agronomics, breeding, and biotechnology on average maize yields in the USA (Edgerton, 2009).

policies, and access to educational programs by producers, also play a critical role. The inadequate and improper use of inputs and other cultural practices is often a consequence of ignorance or lack of means to access better options.

Many believe that biotechnology holds the key to producing more food. The genetics and biotech industries have assured us they can deliver increased crop yields, promising leaps in yield potential of 3 to 4% per year (Fixen, 2007). Monsanto, the world's largest seed company, has pledged to develop new varieties of maize, soybeans, and cotton by 2030 that will yield twice as much grain and fiber per acre while using two-thirds the water and less N (Monsanto, 2008; Edgerton 2009). These kinds of technological advances will be required if we hope to feed the world's hungry, however history shows that genetic advances alone may not be able to solve the world's food shortage. Cassman and Liska (2007) point out the 40-year trend for USA maize yields have been linear with an annual increase of 112 kg/ha or a 1.2% relative gain compared to the current 9.2 t/ha yields. This 1.2% annual yield increase has been made possible, among other factors, by the positive interactions between technological advances such as the introduction of better genotypes (including hybrids and transgenic Bt insect resistant maize), soil testing and balanced fertilization, expansion in irrigation, and conservation tillage.

Undoubtedly, a blend of improved crop management and biotechnological advances will be needed to significantly increase productivity. Edgerton (2009) explained Monsanto's pledge to double maize yields would require a combination of conventional breeding, marker-assisted breeding, biotechnology traits, and continued advances in agronomic practices (**Figure 3**), assuming that agronomic management (better planting density, increased fertilizer use efficiency, and improvements in soil management) will proceed at current historical rates, based on estimates of Duvick (2005). Current thinking about genetic manipulation of crops, both in the private and public research sectors, includes the use and improvement of conventional and molecular breeding, as well as molecular genetic modification, to adapt our existing food crops to increasing temperatures, decreased water availability in some places and flooding in others, rising salinity, changing pathogen and insect threats, and increasing crops' nutrient uptake and use efficiency (Fedoroff et al., 2010). However, there are limits as to how much N use could be reduced, given the role of N in plant protein and recognizing that a 10 t/ha maize crop contains about 100 kg N/ha as protein in the grain (Edgerton, 2009).

#### **Role of Fertilizers in Cereal Productivity**

Globally, commercial fertilizer has been the major pathway of nutrient addition, and by more than doubling the quantities of new N and P entering the terrestrial biosphere, has played a decisive role in making possible the access of humankind to food (Vitousek et al., 2009). While inherent soil fertility, climatic conditions, crop rotation, and management make it difficult to quantify exactly how much crop yield is due to the use of fertilizer, global cereal production and fertilizer consumption are closely correlated (**Figure 4**). One-third of the increase in cereal production worldwide and half of the increase in India's grain production during the 1970s and 1980s have been attributed to increased fertilizer consumption (Bruinsma, 2003). Since the mid-1960s, 50 to 75% of the crop yield increases in developing countries of Asia have been attributed to fertilizers (Viyas, 1983, cited by Heisey and Mwangi, 1996).

More recent data on the essentiality of adequate plant nutrition are provided by Fischer et al. (2009) who mentioned the unpublished results of an assessment of the constraints and possibilities for rice in South Asia carried out by the International Rice Research Institute (IRRI) in 2008 using expert knowledge. According to the estimates, current rice yield (5.1 t/ha) was, on average, constrained by 1.9 t/ha (37%); 10% by inadequate plant nutrition, 7% by diseases, 7% by weeds, 5% by water shortage, and 4% by rats. A similar assessment carried out for rainfed lowland and upland rice in South Asia, with a current yield of 1.8 t/ha, showed that the gap with potential yield (68%) was due to poor nutrient availability (23%), disease (15%), and weeds (12%).

Better plant nutrition has also been an important agronomical tool in raising the potential yield of crops. The positive response of solar radiation use efficiency by



Figure 4. Global cereal production and total fertilizer consumption 1961-2011 (FAO 2012; IFA 2012).

crops to leaf N content has been documented in a wide range of crops: wheat, maize, sorghum, peanut, cowpea, soybean, and mungbean (Muchow and Sinclair, 1994; Bange et al., 1997).

Assuming an average fertilizer application on cereals in developing countries of at least 100 kg/ha of nutrients, the current growth rate in fertilizer use of 3.6% per year, and a grain to nutrient response of 5:1, Fischer et al. (2009) calculated that an amount of 18 kg/ha additional yield would be added annually, which is equivalent to a 0.6% increment. This is a major contribution if we compare it to an estimated growth rate of the average cereal yield of the developing countries of 1% per year (Bruinsma, 2003).

The contribution of commercial fertilizer to crop yield has also been estimated through the use of omission trials and long-term studies comparing yields of unfertilized controls to yields with fertilizer. Long-term trials are particularly useful because they integrate the effects of year, climate, pest and disease stress, etc. Stewart et al. (2005) reviewed data representing 362 seasons of crop production and reported 40 to 60% of crop yield can be attributed to commercial fertilizer inputs. A few examples will be cited here.

	Estimated crop	% reduction	
Crop	Baseline yield	Without N	from no N
Maize	7.65	4.52	41
Rice	6.16	4.48	27
Barley	2.53	2.04	19
Wheat	2.15	1.81	16

## **Table 1.** Estimated effect of omitting N fertilizer on cereal yields in the USA(Stewart et al., 2005).

**Table 1** shows that by omitting N fertilizer in the USA, average cereal yields declined 16 to 41%. Eliminating N from soybeans and peanuts (both leguminous crops) had no effect on yield (data not shown). Had the studies measured the effect of eliminating P and K, the reductions were expected to be significant.

The Magruder Plots, established in 1892 in Oklahoma, are the oldest continuous soil fertility research plots in the USA Great Plains. Nutrient treatments have changed since the plots were established, with annual N (37 to 67 kg/ha) and P (15 kg/ha) applications starting in 1930. Averaged over 71 years, N and P fertilization in these plots was responsible for 40% of wheat yield (**Figure 5A**). The Sanborn Field at the University of Missouri was started in 1888 to study crop rotation and manure additions on wheat. Commercial fertilizer was introduced in 1914. Although application rates have varied over the years, comparing the plots receiving N, P, and K fertilizer to the unfertilized control showed that



Figure 5. Yield attributed to fertilizer: (A) N and P from 1930 to 2000, in the Oklahoma State University Magruder plots; (B) N, P, and K from 1889 to 1998 in the University of Missouri Sanborn Field plots; (C) N, P, K, and lime from 1955 to 2000 in the University of Illinois Morrow plots; and (D) N with adequate P and K vs. P and K alone from 1852 to 1995 (years between 1921 and 1969 excluded because part of the experiment was fallowed each year for weed control) in the Broadbalk Experiment at Rothamsted, England (Stewart et al., 2005).

fertilizer contributed to an average of 62% of the yield over the 100-year period (**Figure 5B**). The Morrow Plots at the University of Illinois were established in 1876. Early fertility treatments on the maize included manure, rock phosphate, and limestone, but commercial fertilizers (N, P, and K) and lime were not started until 1955. The NPK + lime treatments averaged 57% more maize yield than the control treatments (**Figure 5C**). The Broadbalk Experiment at Rothamsted, England has the oldest continuous field experiments in the world. Winter wheat has been grown continuously since 1843. Application of N fertilizer with P and K over many decades has been responsible for 62 to 82% of wheat yield compared to P and K applied alone (**Figure 5D**). From 1970 to 1995, growing high-yield-ing winter wheat continuously receiving 96 kg N/ha, omitting P decreased yield an average of 44% and omitting K reduced yields by 36%.

These long-term studies from temperate climates clearly show how essential fertilizer is in cereal productivity, accounting for at least half of the crop yield.

Fertilizer is even more critical to crops in the tropics where slash and burn agriculture devastates inherent soil fertility. Stewart et al. (2005) refer to examples of continuous grain production in the Amazon Basin in Brazil and in Peru, where fertilizer applied the second year after slash-and-burn clearing was responsible for over 80% of crop yield.

Although the above examples demonstrate the crucial role of N, P, and K fertilizers in increasing crop production, secondary nutrients and micronutrients have comparable importance. The attainment of higher yields through the application of N, P, and K might lead to lower concentrations of other nutrients because of what has been labeled a "dilution effect" (Davis, 2009). Plants need an adequate and balanced supply of all nutrients, including secondary and micronutrients. Therefore, fertilizing with only NPK, without ensuring proper supplies of other limiting nutrients, is counterproductive as it reduces the efficiency of utilization of all nutrients.

Additionally, the need for micronutrients is especially critical for elements like Zn and B, which are suspected as being deficient in almost every country (Sillanpaa, 1982). Deficiencies of other micronutrients like Cu, Cl, Fe, Mn, Mo, and Ni are more soil and crop specific. For example, in India deficiency of Zn is reported to be the most widely occurring nutritional disorder in plants, next only to N and P in lowland rice, and after N, P, K, and S in oilseed and pulse crops (Rattan and Datta, 2010).

Plant nutrients can also be effectively supplied by organic sources. Optimal nutrient management begins with the utilization of on-farm sources of nutrients, then supplementing them with commercial fertilizers. Inorganic and organic nutrients should be used in a balanced fashion and within the context of other best management practices for cultivar selection, crop protection, water management, planting dates and densities, and for other aspects of good agronomic management. All nutrient sources should be managed in a complementary way in an integrated plant nutrient management (IPNM) approach (Roy et al., 2006) that includes assessing residual soil nutrient supplies, soil productivity potential for crops, site-specific crop nutrient requirements, quantifying nutrient value of onfarm nutrient sources (e.g. manure and crop residues), determining supplement nutrients to be met with off-farm sources, and developing appropriate nutrient management plans considering source, time of application, and placement. By concentrating on the nutrient supply aspects of crop production IPNM focuses in nourishing the crop as efficiently as possible while minimizing adverse environmental impacts.

There are abundant results that show that often, best yields are achieved when organic and inorganic nutrients are applied together. **Table 2** shows results from a 9-year field trial with dryland finger millet in Bangalore, India. Highest yields were obtained when recommended rates of fertilizer were applied in combination with 10 t/ha of farmyard manure. Integrating the organic and inorganic nutrients allowed grain yields of at least 3 t/ha in 8 of the 9 years of the study.

Annual treatment	Mean grain yield, t/ha	Number of years in which grain yield (t/ha) was:			
		<2	2-3	3-4	4-5
Control	1.51	9	0	0	0
FYM	2.55	1	6	2	0
NPK <sup>1</sup>	2.94	0	5	4	0
FYM (10 t/ha) + NPK <sup>1</sup>	3.57	0	1	5	3

**Table 2.** Effect of fertilizer and farmyard manure (FYM) on millet yield and yieldstability over 9 years in Bangalore, India (Roy et al., 2006).

<sup>1</sup>Fertilizer 50-50-25 (kg/ha N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O)

Integrated soil fertility management (ISFM) is a component of IPNM that incorporates all aspects of plant nutrient uptake, including nutrient demand, through the integration of improved genetics and the biological and physical dimensions of soil fertility that can improve nutrient uptake (Alley and Vanlauwe, 2009). It is defined as "...the application of soil fertility management practices, and the knowledge to adapt these to local conditions, which optimize fertilizer and organic resource use efficiency and crop productivity. These practices necessarily include appropriate fertilizer and organic input management in combination with the utilization of improved germplasm." ISFM strives to maximize the interactions that result from the combination of fertilizer, organic inputs, improved germplasm, and farmer knowledge. IPNM and ISFM adhere to the same principles as 4R nutrient management discussed later in this chapter. The concept of ISFM has best been adopted in sub-Saharan Africa. Alley and Vanlauwe (2009) provide a thorough discussion of the concept and improvements in crop productivity that result from mixtures of commercial fertilizers and organic inputs.

Agricultural production cannot be increased substantially without commercial fertilizers, but fertilizers also play an important role in improving crop quality and the nutritional component of crops. The positive impact of NPKS-containing fertilizers and certain micronutrients (e.g. Zn, Ni, and Mo) on the accumulation of nutrients (e.g. vitamins, minerals, and proteins) and nutraceuticals in many crops is well documented (Grunes and Allaway, 1985; Allaway, 1986; Bruulsema, 2002; Wang et al., 2008). Micronutrient fertilization, especially with Zn, is proving to be a cost effective strategy to address micronutrient malnutrition in human diets (Bouis and Welch, 2010; Shetty, 2009).

#### **Evolution of Global Fertilizer Consumption**

During the period 1961-2008, global fertilizer consumption increased steadily through the 1980s, and then declined through the mid-1990s. From the mid-1990s, consumption started to rise again until 2008 when it dropped 6.8% from 2007 levels (**Figure 6**) mainly due to large decreases in  $P_2O_5$  (10.5%) and  $K_2O$  (19.8%).



**Figure 6.** World fertilizer (N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O) consumption from 1961 to 2008 (IFA 2010).

Fertilizer consumption in developing countries has been growing since the Green Revolution, and currently accounts for 68% of global fertilizer use. Fertilizer use is also currently higher in developing countries than in industrial countries (**Figure 7A**), where it reached a plateau, and fell markedly in countries that were part of the Former Soviet Union after they adopted a market economy.

Asia has had the highest and fastest growth in fertilizer use, whereas current application rates in Latin America exceeds those in North America (**Figure 7B**). However, commercial fertilizer use in sub-Saharan Africa is dreadfully low (i.e. less than 8 kg/ha) due among other reasons to high prices and poor markets (Morris et al., 2007). Low fertilizer use explains a large part of the lagging productivity growth in that region.

Fertilizer is a world market commodity subject to global supply, demand, and market fluctuations. Recent years have seen unprecedented demand for fertilizer and record prices (**Figure 8**). World price (USD per metric ton) for fertilizer remained relatively constant from 2000 through 2005/06: urea (FOB Middle East) ranged from USD 115 to 215, diammonium phosphate (DAP; FOB US Gulf Export) from about USD 150 to 230, and potash (FOB Vancouver) from USD 123 to 160 (Pike and Fischer, 2010). But, in 2007 international prices started to escalate, due to rising global demand (strong crop commodity prices and increasing ethanol production), a falling US dollar, higher transportation costs and a shortage of supply (TFI, 2008; IFA, 2008), peaking in September and October of 2008, with urea reaching about USD 350, DAP USD 1,014, and potash USD 580. Prices declined in 2009, but to a higher baseline than pre-2008 prices.

## Fertilizer Best Management Practices (BMPs) and Nutrient Stewardship

Assuming an average nutrient content of harvested grain of 1.83% N, 0.33% P, and 0.44% K (IPNI unpublished data), the 2.52 billion t of grain harvested in



Figure 7. Regional trends in fertilizer use, 1961-2007 (FAO, 2010b).



Figure 8. Average monthly price of urea, diammonium phosphate (DAP), and muriate of potash from January, 2000 to March, 2010 (Pike & Fischer, 2010).

2008 would remove an estimated 46.2 M t of N, 19.2 M t of  $P_2O_5$ , and 13.3 M t of  $K_2O$ . Total nutrient uptake could be much higher varying with residue management, crop yield and variety, soil fertility, and climatic conditions.

However, a doubling of production in the next 3 to 4 decades does not necessarily imply a doubling in nutrient removal. As Dobermann (2006) has noted, except for Oceania and Eastern Europe/Central Asia, cereal yields in many industrialized regions have continued to increase in the past 20 years without significant increases in N fertilizer use. Substantial increases in fertilizer use efficiency can also have similar results (Tilman et al., 2002).

Improving nutrient use efficiency (NUE) is a challenge whose importance will increase in the coming years due to the dependence of fertilizer production on non-renewable raw materials and the need to minimize adverse environmental impacts such as atmospheric, soil, and water pollution.

Nutrient use efficiency is a dynamic indicator of nutrient management that can be applied at different levels of evaluation (e.g. country, region, and farm). The methodologies employed in measuring NUE are often confusing because of the variety of definitions and terms used to describe it. Snyder and Bruulsema (2007) reviewed common definitions and applications relative to fertilizer BMPs.

Evidence of improved NUE for N is available from the USA, where the partial factor productivity of N (kg of grain per kg of N applied) increased from 42 kg grain per kg N in 1980 to 57 kg grain/kg in 2000, during a time when maize yields grew 40%. In addition to stagnating fertilizer-N use, N fertilizer efficiency was boosted by the use of modern hybrids with greater stress tolerance, better crop management practices—such as conservation tillage, higher seed quality and higher plant densities—and improved N management (Dobermann and Cassman, 2002; Dobermann and Cassman, 2004). Furthermore, there were major institutional factors that contributed to such progress in fertilizer N management such as effective research and development, grower and grower adviser education, and adequate infrastructure (Fixen and West, 2002). Similar developments in improved NUE for N have been observed in other developed countries.

Nutrient use efficiency for P fertilizers has been considered by many to be inherently low because first year recovery of applied P is relatively low (i.e. less than 20%) compared with other nutrients. However P fertilizer use efficiency is often high (i.e. up to 90%) when evaluated over time and using the balance method, which calculates P recovery as the percentage P removal by crop of the P applied (Syers et al. 2008). The efficient use of P sources is essential because, if managed inappropriately, P supplied by manures or commercial fertilizers can contribute to eutrophication of surface waters. In addition, phosphate rock is a finite, nonrenewable resource which must be used not wastefully.

Fertilizer BMPs play a vital role in increasing NUE by matching nutrient supply with crop requirements and minimizing nutrient losses from fields. The approach is simple: apply the correct nutrient in the amount needed, timed and placed to meet crop demand. Applying the 4Rs—right source (or product) at the right rate, right time, and right place—is the foundation of fertilizer BMPs (Roberts, 2007).

A global framework describing how the 4Rs are applicable in managing fertilizer around the world has been developed by the International Plant Nutrition Institute (Bruulsema et al., 2008) and the International Fertilizer Industry Association (IFA, 2009). Although fertilizer management is broadly described by the four "rights", determining which practice is right for a given farm is dependent on the local soil and climatic environment, crop, management conditions, and other site-specific factors. The purpose of the framework is to guide the application of scientific principles to development and adaptation of global BMPs to local conditions, while meeting the economic, social, and environmental goals of sustainability.

It is clear that increasing NUE will be more knowledge intensive. As mentioned earlier, achieving greater productivities and efficiencies requires considerable
emphasis in education of growers and their network of advisers. It will not be possible to attain such gains in productivities and efficiencies in developing countries without a reduction in the poverty levels. Moreover, the improvement in the income levels of small farmers will reflect also in their capacity to purchase fertilizers, other needed tools, and food itself. As FAO and OECD point out (FAO, 2009a; Dewbre, 2010), ensuring an adequate supply of food at the aggregate level, globally or nationally, does not guarantee that all people have enough to eat unless they have the means to buy food.

## Summary

Global food security continues to be one of the greatest challenges of the 21<sup>st</sup> century. The population has doubled in the last 50 years to 6.8 billion and global cereal production has more than doubled reaching 2.5 billion t, yet one-sixth of the world's population (1.02 billion) were undernourished in 2009. To meet the expected population growth global cereal production will need to increase 70% by 2050. Competition for food, feed, and biofuels are putting greater pressure on alleviating global hunger as more grain is needed for direct consumption and for producing the animal-based protein diets increasingly demanded in the developing world, and the growing demand for biofuels in developed countries.

Biotechnology and genetic advances will be critical to increasing crop yields, but meeting the world's escalating food needs cannot be achieved by biotechnology alone. Without adequate plant nutrition, the world would produce only about half as much staple foods and more forested lands would have to be put into production. Plant nutrients from organic and inorganic sources are needed for higher crop production. Inorganic fertilizer plays a critical role in the world's food security, but highest yields are often the result of using organic and inorganic sources together. Integrated soil fertility management (i.e. optimizing fertilizer and organic resources with improved germplasm) is critical to optimizing food production and efficient use of plant nutrients. The 4Rs—right source at the right rate, right time, and right place—are the underpinning principles of nutrient management and can be adapted to all cropping systems to ensure productivity is optimized. **FCHH** 

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Chapter 2

## Micronutrient Malnutrition: Causes, Prevalence, Consequences and Interventions

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## Introduction

Billions of people in developing countries suffer from an insidious form of hunger known as micronutrient malnutrition. Even mild levels of micronutrient malnutrition may damage cognitive development, lower disease resistance in children, and reduce the likelihood that mothers survive childbirth. The costs of these deficiencies in terms of lives lost and poor quality of life are staggering.

The primary underlying cause of micronutrient malnutrition is poor quality diets, characterized by high intakes of food staples, but low consumption of animal and fish products, fruits, lentils, and vegetables, which are rich sources of bioavailable minerals and vitamins. As such, most of the malnourished are those who cannot afford to purchase high-quality, micronutrient-rich foods or who cannot grow these foods themselves.

Agricultural research and agricultural policy needs to be brought to bear to improve nutrition. In the past, the nutrition community for the most part has ignored food-based interventions as a means to reduce malnutrition. The agricultural community has regarded farming primarily as a means to provide employment and improve the incomes, and has similarly given low priority to the essential role of agriculture as the primary supplier of vitamins, minerals, and other life-sustaining compounds.

The first section of this chapter discusses how agriculture, food prices, and household incomes set the context for the types of diets that the poor can afford to eat, the prevalence of micronutrient malnutrition, and the conditions which will drive the effectiveness of various types of interventions that can be

For symbols used commonly throughout this book see page xi.

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implemented to reduce micronutrient malnutrition. The second section discusses the numbers of people affected globally by mineral and vitamin deficiencies and what are functional consequences of these deficiencies. The third section describes agricultural and non-agricultural inventions, and their relative cost-effectiveness, that are currently being used to address the problem of micronutrient deficiencies.

# Agriculture Sets the Context for Improvements (or Not) in Micronutrient Malnutrition

This paper provides some perspectives on the underlying economic factors that drive this outcome, with the objective of providing a better understanding of the importance of the potential of agricultural inventions (often neglected) to improve the current situation. Interventions to improve the minerals and vitamins supplied by crop and marketing systems should be understood in the context of: a) agricultural and economic development over time, and b) household resource allocation decisions at any given point in time. In this context, per capita food intakes at the household level generally are primarily a function of: i) household income, and ii) food prices in the context of falling real cereal prices over the past several decades, and more recently, their subsequent increase.<sup>2</sup>

### **Dietary Quality and Household Income**

**Table 1** shows per capita energy intake and share of food expenditures by broad food groups by income group for three countries. At low incomes the poor give priority to purchasing food staples, the most inexpensive source of energy, to keep from going hungry. Then at the margin as income increases, they buy non-staple plant foods (e.g. lentils, fruits, vegetables) and animal products (including fish) because of a strong underlying preference for the tastes of these non-staple foods.

In **Table 1**, diets are expressed in terms of energy (and not minerals and vitamins), because non-staple plant foods and animal products are denser than food staples in bioavailable minerals and vitamins. Percentage increases in mineral and vitamin intake rise much more sharply with income than do energy intake. Animal products are the most expensive source of energy, but the richest sources of bioavailable minerals and vitamins.

There is a natural underlying tendency, then, for dietary quality to improve as economic development proceeds. As household income rises and demand for non-staple plant foods and animal products rises, prices for these better quality foods will tend to rise, all things being equal. These price signals, in turn, will give rise to supply responses from agricultural producers. The essence of economic (in this case agricultural) development is that technological improvements will be stimulated (e.g. development of higher yielding varieties either through

<sup>2</sup> Food prices in any given locality are a function of market access (supply) and local food culture (demand). Certainly there are individual differences in preferences for particular foods across households and individuals (sometimes driven by education/knowledge) which creates variance around average consumption levels for particular income, or other socioeconomic groups.

	Bangladesh				Kenya				Philippines					
Food Group	Income Tercile			All	In	Income Quartile			All	Income Quartile			All	
	1	2	3	House- holds	1	2	3	4	House- holds	1	2	3	4	House- holds
					Per Cap	ita En	ergy In	take						
Staples	1805	1903	1924	1879	1283	1371	1388	1394	1360	1361	1431	1454	1381	1406
Non-Staple Plant	281	347	394	340	256	348	363	464	357	197	229	304	395	281
All Animal	44	61	89	64	112	120	161	187	145	67	102	118	207	124
Total	2130	2311	2407	2283	1651	1839	1912	2045	1862	1625	1762	1876	1983	1811
Food Budget Share (Percent)														
Staples	46	41	36	40		Data	Not A	vailab	le	43	36	28	24	33
Non-Staple Plant	32	35	36	34						30	36	39	37	35
All Animal	22	24	28	26						27	28	33	39	32
Total	100	100	100	100						100	100	100	100	100

**Table 1.** Per capita energy intakes (calories/day) and food budget shares by broadfood group by income group for three countries (Graham et al., 2007).

public or private investments in agricultural research), which in turn will lead to more efficient production, faster supply growth rates, and eventually lower nonstaple food prices.

It is the role of public food policies to influence this long-run process so that aggregate growth is rapid and so that all socio-economic groups (importantly the malnourished poor) share in the benefits of this growth. With this as background, we now briefly examine the role of the Green Revolution in influencing food prices.

### Dietary Quality, Food Prices, and the Green Revolution

**Figure 1** shows the percentage increases in developing country population, in cereal production, and in pulse production between 1965 and 1999. Developing country population doubled during this period. It is the great achievement of the Green Revolution that cereal production more than doubled due to rapid technological change. After adjusting for inflation, real cereal prices have fallen over time despite the doubling of developing country population. As suggested in **Table 1**, the poor spend a high percentage of their income on food staples, and lower cereal prices free up income that eases their burden and can be spent on a range of necessities, including better quality food.

Pulse production in **Figure 1** is representative of increases in production for any number of non-staple plant and animal foods. Production increased significantly, but did not keep pace with growth in demand – due both to population growth and income increases as developing country economies have grown. There was no commensurate technological change in the non-staple food sector. Consequently,



**Figure 1**. Percent changes in cereal and pulse production and in population, 1965-1999 (Graham et al., 2007).



Figure 2. Indices of inflation-adjusted prices for Bangladesh 1973-75 = 100 (Bouis et al., 1998).

inflation-adjusted prices of many non-staple foods have increased over time, as shown in **Figure 2**.

Given these relative price changes over time, energy (rice in the case of Bangladesh) becomes more affordable, but dietary quality (non-staples) more expensive. As shown in **Figure 3**, expenditures for non-staple plant foods and fish and meat exceed those for rice (Bouis et al., 1998).

The data cited in **Figure 3** were collected in the mid-1990s in rural Bangladesh after rice prices (adjusted for inflation) had fallen considerably from the early 1970s, and non-staple food prices had risen significantly.



Figure 3. Share of energy source and food budget in rural Bangladesh (Bouis et al., 1998).

This change in relative prices – lower food staple prices and higher non-staple food prices – has made it even more difficult for the poor to achieve mineral and vitamin adequacy in their diets. Certainly, for those poor whose incomes have remained constant, price incentives have shifted the diet more and more toward reliance on food staples – in the absence of knowledge about the importance for health of a nutritious diet and what relatively inexpensive non-staple foods can provide in terms of minerals and vitamins. This has led to a worsening of mineral and vitamin intakes for many segments of developing country populations, micronutrient malnutrition, poor health, and much misery.

To reiterate, the long-run task of public food policy is to stimulate growth in the non-staple food sector (sometimes referred to as "high-value" agriculture) through any number of instruments – agricultural research, education, building infrastructure, improving markets for agricultural inputs and outputs, to name a few. However, this is a several-decades-long process. In the meantime, there are specific, cost-effective steps (such as biofortification, adding Zn and Se to fertilizers) that can be taken to utilize agriculture to improve mineral and vitamin intakes in the shorter run.

## Dietary Quality and the Recent Rises of Staple Food Prices in the Post Green Revolution Period

Rapid increases in yields of rice and wheat, and maize led to the declining prices for food staples, as exemplified for Bangladesh in **Figure 2**. However, in part due to declining public investments in agricultural research over the past two decades, high growth rates in cereal yields in developing countries could not be sustained. Population, of course, continued to grow. As incomes increased in China, Share of expenditures before price rise



## Figure 4. Share of food groups and non-food in total expenditures before and after rises in food staple prices (Bouis et al., 2011a).

Note: Diagram for "before price rise" is based on data collected in 1995/96; diagram for "after price rise" is based on simulations shown in Table 3.

India, and other developing countries, greater demand for animal products led to increased use of cereals for animal feed. Use of cereals as bio-fuels also increased demand. These longer-run supply and demand factors put underlying pressures on food staple prices to begin to rise. Finally, short-term draw downs in global cereal food stocks and weather shocks caused by drought in major producing countries, led to very rapid and substantial increases in food staple prices in the first half of 2008. Speculation also contributed to the 2008 price increase (Piesse and Thirtle, 2009) and as the speculator bubble burst, prices fell somewhat; however, the underlying longer-run pressures continue, so that 2011 has seen prices rise to a new high. What are the consequences of such prices for dietary quality of the poor?

The poor must, at all costs, protect their consumption of food staples to keep from going hungry. Bangladeshis, for example, must now spend more for rice. This leaves less money to spend on non-staple foods and non-foods as illustrated in **Figure 4**.

Economists simulate/predict the changes in diet caused by rising food prices through use of price and income "elasticities" which provide estimates of percentage changes in quantities in foods consumed for given percentage changes in prices and incomes. An example for rural Bangladesh of a "demand elasticity matrix" is shown in **Table 2**.

Examining particular values in the demand elasticity matrix above, if income

Share of expenditures after price rise

doubles (a 100% increase), then the quantity of staple food consumption is predicted to increase by 5% (see final column); that is, the staple income elasticity of 0.05 = +5% / +100%. In contrast in terms of magnitude, if income doubles, then non-staple food consumption (plants and animal/fish aggregated) is predicted to increase by 110% (1.10 = +110% / +100%). These are referred to as "income" elasticities.

	Budget Shares	Staples	Non- Staples	Non-Foods	Income
Staples	0.35	-0.20	0.10	0.05	0.05
Non-Staples	0.35	-0.27	-0.95	0.12	1.10
Non-Foods	0.30	-0.62	-0.18	-1.20	1.99

#### Table 2. Demand elasticity matrix for rural Bangladesh (Bouis et al., 2011a).

## Table 3. Simulation results for rural Bangladesh, assuming a 50% increase in staple and non-staple food prices and no change in income (Bouis et al., 2011a).

Non-Staple Food Income Elasticity	1.0	1.1	1.2	1.3	1.4
% Change in Iron Intakes	-27	-29	-30	-32	-34
% Change in Energy Intakes	-14	-14	-15	-16	-16
% Change in Expenditures on Food Staples	43	43	43	43	43
% Change in Expenditures on Non-Staples	-23	-29	-34	-39	-44
% Change in Expenditures on Non-Foods	-23	-17	-10	-5	1
Absolute Change in Food Staple Calories	-74	-74	-73	-73	-72
Absolute Change in Non-Staple Food Calories	-196	-210	-224	-238	-251

Notes: The results outlined within lines correspond to the food demand matrix shown in **Table 2**; the initial daily total calorie intake was assumed to be 2,000, divided between staples (1,600) and non-staples (400). Staples were assumed to provide 50% of total Fe intake, and non-staples the other 50%.

If the price of staples (rice in the case of Bangladesh) increases by 50%, then the quantity of staples consumed decreases by 10% (-0.20 = -10% / +50%; see column labeled "Staples"). This is referred to as an "own-price" elasticity. If the price of non-staples increases by 50%, then the quantity of food staples increases by 5% (0.10 = +5% / +50%; see column labeled "Non-Staples"). This is referred to as a "cross-price" elasticity.

Using the elasticities in the matrix above, changes in quantities consumed can be predicted for varying levels of price rises. These changes in quantities, in turn, can be converted into changes in nutrient intakes. **Table 3** shows simulation results for an assumed 50% increase in both staple and non-staple foods, but no changes in non-food prices and incomes.

The following observations may be made from **Table 3**:

• As an order of magnitude, Fe intakes decline by 30%. Energy intakes decline by 15%; however, note that *the decline in energy intakes is primarily due to the decline in consumption of non-staple foods*.

• Expenditures on food staples increase markedly due to inelastic demand; expenditures for non-staple foods and non-foods decline.

• To the extent that non-staple foods are considered a "luxury" (non-staple income elasticities at the high end near 1.4), the poor adjust by reducing non-staple food expenditures and non-food expenditures are little affected; to the extent that non-staple foods are considered more of a necessity (non-staple income elasticities at the lower end near 1.0), the poor adjust by reducing expenditure on both non-staple foods and non-foods.



**Estimated percentile of requirement** 



The distribution of Fe requirements is modeled from a factorial accounting for body size, age, menstrual blood loss, and contraceptive use (Food and Nutrition Board and Institute of Medicine, 2001). A Monte Carlo simulation with n >1,000 was used. The estimated average iron intake among Filipino women is 7 mg/day, and the estimated average iron intake if only high-iron (12 mg/kg milled) biofortified rice is consumed is 11 mg/day. Source: John Beard, Pennsylvania State University.

How significant is a 30% decline in Fe intakes? To obtain some sense of this, **Figure 5** shows the cumulative distribution of women meeting their Fe intake requirements at various levels of average Fe intake. Because individual-specific requirements for Fe (and other nutrients) vary, some women meet their requirements at an average intake of 7 mg Fe/day (30% in the diagram) and others do not (70% in the diagram).

Given a food price increase of 50%, Fe intakes would decline by an estimated 30% from 7 mg Fe/day to about 5 mg Fe/day (indicated by the food price simulation in the diagram). This would mean that only 5% of women would be meeting their daily requirements – an increase of 25 percentage points in women who are no longer consuming their required Fe intakes.

The results presented in **Tables 2** and **3** are derived from consumption and nutrition data collected in rural Bangladesh. How generalizable are these findings to other regions in developing countries?<sup>3</sup>

Budget shares allocated by the poor in Africa, Asia, and Latin America to staple foods, non-staple foods, and non-foods are of similar magnitude as those in **Table 2**. This is simply because of limited incomes, the need to avoid hunger by purchasing large amounts of food staples (roughly one-third of total expenditures before the food price rise), and having to allocate remaining income between: i) the desire for some variety in the diet in addition to food staples (roughly one-third of total expenditures), and ii) a range of necessities such as housing, clothing, sanitation, and so forth (roughly the last third of total expenditures). Thus, demand elasticities for the poor should not vary markedly from those shown in **Table 2**.

Declines in Fe intakes due the food price increases; however, may be particularly large in the case of Bangladesh for two reasons:

(i) milled rice (the primary staple in Bangladesh) has a relatively low Fe density; still, rice provides 40-45% of the Fe in the total diet of the poor in Bangladesh (Arsenault, 2010). In other countries, say where whole wheat is consumed as the primary staple (which has a much higher Fe density than milled rice) staples will provide a higher share of total Fe ( > 50%); consequently, sharp declines in non-staple food consumption will not result in as large percentage declines in total Fe intakes (although bioavailability of Fe in the total diet may decline due to loss of animal and fish foods).

(ii) in some countries, especially in Africa, poor populations may eat significant amounts of three or four food staples which are available concurrently

<sup>3</sup> D'Souza and Jolliffe (2010) looked at the increase in wheat prices in Afghanistan. They found it to be associated with lower dietary diversity. They did not estimate a full demand system, so it is not possible to determine the underlying differences in elasticities between staple foods and non-staples.

Brinkman et al. (2010) combined different methods (simulation and regression) to look at the impact of higher food prices on dietary diversity. They focused on Nepal, Haiti and Niger, and they found that consumers reduced dietary diversity when faced with higher food prices.

Jensen and Miller (2008) looked at the impact of higher food prices on calorie intakes in China around 2006. They did not find any significant effect and concluded that Chinese consumers have preserved their energy intakes by substituting cheaper calories for more expensive ones.

Skoufias et al. (2010) looked at how the ratio of staple calories (over total calories) changed after the 1998 economic crisis (negative income shock). They found that the starchy staple ratio did not change during the crisis, while specific micronutrients (Fe, Ca, vitamin B1) were very sensitive to the income shock during the crisis.

during any given season. In such cases if there are sharp increases in the prices of certain staple foods, consumers can substitute the more inexpensive staples for the ones whose prices have risen. To the extent that staples are rich in energy/calories, this will protect total energy intake, while saving income for purchase of more non-staples than would otherwise have been the case.

#### **Effects on Farm Income of Rising Food Prices**

While rising food prices hurt poor consumers, agricultural producers will be helped on the income side by high market prices for their products. To what extent will this compensate for a loss in food and nutrient intakes on the consumption side? To answer this question, we take the result for Bangladesh shown in **Table 3** and assume that total income (on a nominal basis) has risen by 35%.<sup>4</sup>

A 35% increase would be in the maximum range for a landowning household that depended primarily on their farm output for income. It is an interesting threshold also for the reason that the household has the option to choose to spend this extra income to just compensate for the increased cost of food (initially 70% of income goes for food expenditures, with a 50% increase in food prices then imposed).

The results for simulating a 50% increase in food prices and a 35% increase in nominal income are shown in **Figure 4**. Note that energy and Fe intakes still decline (although by lower amounts). Because of the increase in the price of food, expenditures for non-foods become relatively more attractive. The household does not choose to maintain the same food intake choices as before.

# Consequences, Prevalence, and Trends of Micronutrient Malnutrition

As all living organisms, humans have evolved to depend on food as sources of minerals and vitamins. Without these compounds, vital functions and complex interactions with the environment that allow them to respond and adapt to stimuli cannot take place optimally or at all. These compounds are known as essential nutrients or micronutrients. In contrast to the macronutrients (i.e. protein, fat, and carbohydrate), the average daily dietary intake requirement for micronutrients are measured in milligrams or smaller quantities. Micronutrients are a diverse group of dietary components necessary to sustain health. Nine trace elements (Fe, Zn, Cu, Cr, Se, I, F, Mn, and Mo) and 13 vitamins (vitamin A, vitamin B1, B2, B6 and B12, Niacin, Folate, Pantothenic acid, vitamin C, vitamin D, Biotin, vitamin E, and vitamin K) have been identified as essential to humans (Bogden and Klevay, 2000).

Some micronutrients are known to have very specific metabolic roles and bio-

<sup>4</sup> For example, a 35% increase in nominal income could be achieved for a 50% rise in farm output prices, no increase in input costs, with farm income accounting for 70% for total household income.

markers associated with their different physiologic compartments, while others do not. For instance, vitamin A is stored almost entirely in the liver stellate (Ito) cells (Blomhoff et al., 1990) and is vital for the retinal night vision cycle (Rando, 1990) as well as for preserving the integrity of the physical barrier against infections at the mucosal lining of the gastrointestinal and respiratory tracts (West et al., 1991); folate-mediated one-carbon reactions are important for biosynthetic pathways of DNA, RNA, cell membrane lipids, and some neurotransmitters. Folate (folacin, folic acid) reduces blood homocysteine, plays a role in red blood cell (RBC) formation, protein metabolism, cell growth and division, and prevents neural tube defects (i.e. spina bifida) and anencephaly; Fe is necessary for RBCs to carry oxygen within hemoglobin molecules, for normal neurotransmitter chemistry, the organization and morphology of neuronal networks, and the neurobiology of myelination (Lozoff and Georgieff, 2006); and iodine-containing hormones modulate growth in every living cell with particular impact on the nervous system during fetal life and infancy (Zimmerman et al., 2008). On the other hand, other nutrients (such as Zn) are involved in multiple metabolic pathways, some of which are still incompletely defined (Golden, 1994). Zinc, for instance, is ubiquitous in humans, playing a vital role in protein synthesis, cellular growth, and cellular differentiation (Hotz and Brown, 2004). Some micronutrients serve as prohormones (e.g. vitamin D) (DeLuca and Zierold, 1998), while other vitamins (Vit. C, Vit. E, Vit. A) and some minerals (Cu, Zn, and Se) display or enable antioxidant activities in more complex biochemical systems (Heyland et al., 2005).

Given the primary (dietary) origin of most micronutrient deficiencies and the intricate association between undernutrition and infection, it is logical to suppose that single deficiencies are the exception and not the rule in public health (Black, 2001). Undoubtedly, however, some deficiencies are more common and have more dire health consequences for the individuals and groups affected. Hence, in a world with limited resources to tackle the maladies that affect the poverty-ridden and food-insecure masses, it has become practice to prioritize these deficiencies in terms of millions affected, lives threatened, attributable deaths, disability-adjusted life years caused, and the existence of cost-effective control interventions among other parameters to assign priority to vitamin A, Fe, I, and Zn. Even though the little attention paid to the prevention of neural tube closure defects associated with periconceptional folate deficiency has been rightly called a public health travesty comparable to withholding measles immunizations from populations at risk (Pitkin, 2007), investment in food fortification and supplementation in developing countries with folate remains conspicuously low (Botto et al., 2005).

Roughly more than one-third of the world's population is at risk of one or more micronutrient deficiencies. The most common trace element deficiencies in order of prevalence are Fe (~1.6 million; de Benoist et al., 2008a), I (~2.0 billion; de Benoist et al., 2008b), and Zn (~ 1.5 billion; Hotz and Brown, 2004), most likely followed by Se (Brown and Authur, 2002), and Cu (Madsen and

Jonathan, 2007). The most widely prevalent vitamin deficiencies of public health significance are vitamin A with 190 million pre-school children and 19 million pregnant women at risk (WHO, 2009) and folate with roughly 300,000+ newborn infants affected by neural tube defects (Botto et al., 2005). Vitamins B12 and D trail behind, yet without solidly proven functional effects to merit the consensus of scientists regarding their global prevalence or their ascent to a first tier in the world of public health malnutrition.

The estimated regional prevalence of the four principal micronutrient deficiencies is described in **Tables 4** and **5**. It should be noted, however, that such figures do not portray the daily human drama experienced by the affected one-half of the world's population which agglomerates in Asia and Africa. In these populations, the poorest bear the brunt of preventable mental disability and diminished physical performance of children and adults, maternal and fetal-child deaths, and other long-term negative effects that constrain socioeconomic development. The lack of each nutrient deteriorates human health independently but their combination undermines the potential of human capital at the individual and collective levels in additive or synergistic fashion which is very difficult to measure accurately.

Anemia, due primarily to Fe deficiency, but also to varying degrees due to chronic infection and other nutritional deficiencies depending on the socio-ecological context of each population, affects 1.6 billion people worldwide. Iron deficiency leads to mental impairment in children (Lozoff and Georgieff, 2006), maternal mortality when severe (Allen, 1997), and lower capacity for physical work in children and adults (Haas and Brownlie, 2001). Vitamin A deficiency causes blindness, impairs immune response and increases mortality from infections such as measles in children (West, 2002). Twenty-percent of the world population is at risk of Zn deficiency<sup>5</sup> (Table 5) resulting from inadequate dietary Zn intake and causing stunting (Brown et al., 2009a) and mortality in children (Walker et al., 2009), often from diarrhea and upper respiratory infections. In 2008, the Maternal and Child Undernutrition Study Group (Lancet, 2008) published estimates of the burden of disease associated with micronutrient malnutrition. According to these estimates while stunting, severe wasting and intrauterine growth retardation (IUGR) together account for 2.2 million deaths and 21% of disability-adjusted life-years (DALYs).6 The deficiencies of two micronutrients associated with the immunologic system (Zn and vitamin A) are responsible for an additional 1.0 million deaths and 9% of the DALYs lost (Black et al., 2008). The World Health Organization has estimated that approximately 800,000

<sup>5</sup> Indicators of Zn deficiency prevalence are currently under review by the World Health Organization. Prevalence may be as high as 28.5% globally if starting rates are selected as the chosen proxy indicator (Shrimpton 2010).

<sup>6</sup> One DALY can be thought of as one lost year of "healthy" life. It attempts to measure the number of days spent in ill health due to a preventable disease or condition (in the case of morbidity) and the number of days lost to premature death in the case of mortality. This annual measure allows the addition not only of morbidity and mortality outcomes, but also short-duration conditions such as diarrhoea with longer term ones such as night blindness. It also takes into account the severity of functional outcomes.

**Table 4.** Global and regional prevalence (%) of the principal vitamin and mineraldeficiencies.

WHO region	Vitam Defici		(proxy f	Iodine <sup>3</sup>		
	Preschool-age children	Pregnant women	Preschool-age Children	Pregnant Women	Non-pregnant women	School-age children
Africa	44.4	13.5	67.6	57.1	47.5	40.8
Americas	15.6	2	29.3	24.1	17.8	10.6
Europe	19.7	11.6	21.7	25.1	19	52.4
Eastern Mediterranean	20.4	16.1	46.7	44.2	32.4	48.8
South-East Asia	49.9	17.3	65.5	48.2	45.7	30.3
Western Pacific	12.9	21.5	23.1	30.7	21.5	22.7
Global	33.3	15.3	47.4	42	30.2	31.5

1 Global Prevalence of Vitamin A deficiency in populations at risk 1995–2005,: WHO Global Database on Vitamin A deficiency

2 Worldwide Prevalence of Anaemia 1993-2005, World Health Organization, 2008

3 Iodine deficiency in 2007: Global progress since 2003; World Health Organization, 2008

## **Table 5.** Global and regional estimates of the proportion (%+S.D.) of the popula-tion at risk of inadequate Zn intake.

Region	Population at risk, % ± S.D.
N. Africa and E. Medit.	9.3 ± 3.6
Sub-Saharan Africa	$28.2 \pm 15.0$
Latin America & Caribbean	$24.8 \pm 12.0$
USA and Canada	$9.5 \pm 1.3$
Eastern Europe	$16.2 \pm 10.5$
Western Europe	$10.9 \pm 5.2$
South-East Asia	33.1 ± 5.9
South Asia	$26.7 \pm 9.4$
China (+ Hong Kong)	14.1
Western Pacific	22.1 ± 8.2
Global	$20.5 \pm 11.4$

Source: IZiNCG, Estimated risk of zinc deficiency by country, FNB 2004; vol. 25(supplement 2).

maternal and perinatal deaths (1.5% of all deaths in these age groups) and ~130,000 young children deaths are attributable to Fe deficiency, an attributable

loss of 35 million life years (2.4% of global DALYs) with roughly one-third occurring in South East Asia and another one-third in Sub-Saharan Africa (Stoltzfus et al., 2004). Iron deficiency in women of reproductive age alone may account for 0.4% of DALYs. Allowing for co-exposure to these four micronutrient deficiencies, severe wasting, growth stunting, IUGR, and suboptimum breastfeeding, globally these nutrition-related factors account for 35% of child deaths and 11% of the total disease burden.

## Consequences of Individual Micronutrient Deficiencies Iron deficiency

Iron is required in all tissues of the body for basic cellular functions such as oxygen transport (hemoglobin), oxygen storage (myoglobin), energy transfer within cells (cytochromes), and is critically important in muscle, brain and RBCs (Yehuda and Mostofsky, 2009). A body deprived chronically of sufficient dietary Fe to meet its daily requirements will progress from depletion of Fe stores to insufficient Fe at the level of tissues with high Fe demand (i.e. bone marrow, striated muscles, and brain) and finally to anemia, defined as a reduction in the normal circulating quantity of oxygen-carrying protein hemoglobin, which results in inadequate delivery of oxygen to vital organs. Because all human cells depend on oxygen for survival, varying degrees of anemia can have a wide range of clinical consequences. Therefore, because anemia is simple and inexpensive to measure it has been used as the hallmark of Fe deficiency severe enough to affect RBC formation but it is not a reliable indicator of Fe deficiency. However, Fe deficiency is not the sole cause of anemia in most populations and may have multiple contributing factors in the same individual (parasites that cause blood loss, vitamin A deficiency, chronic infection or blood loss, etc.) (Hershko and Skikne, 2009). The often quoted 50% proportion of anemia (WHO, 2008) as being caused by Fe deficiency has never been properly validated across ecological regions and populations.

On the one hand, because of the high Fe demands of infant growth and pregnancy, these two life cycle stages are the most vulnerable to Fe deficiency disorders (Preziosi et al., 1997). On the other hand, chronic Fe deficiency anemia is seldom a direct cause of death; however, moderate or severe Fe deficiency anemia can produce sufficient hypoxia to aggravate underlying pulmonary and cardiovascular disorders, which may lead to death (Horwich et al., 2002). Nonetheless, Fe deficiency may affect individuals throughout their lives when their diets are based on staple food crops with little meat intake (Zimmerman et al., 2005) and/ or frequent exposure to infections that cause blood loss.

The one-fifth of perinatal mortality and one-tenth of maternal mortality in developing countries often cited as attributable to Fe deficiency may be overestimates related to inadequate hemoglobin cut-off points and the true prevalence of severe anemia, the varying proportion of Fe deficiency anemia across populations, and the incidence of underlying factors that are aggravated by severe anemia, the paucity of properly conducted research on this topic, among other reasons (Rush, 2000). There is a growing body of evidence in support of a causal association between Fe deficiency anemia in early childhood and reduced intelligence in mid-childhood (Lozoff, 2008). Available evidence presents a robust case for the causal role of Fe deficiency on decreased physical fitness and aerobic work capacity through mechanisms that include oxygen transport and respiratory efficiency within the muscle (Haas, 2001), which ultimately decreases work productivity and income, particularly in economies based on physically demanding labour.

## Zinc deficiency

Inadequate intake of bioavailable Zn, and to some extent increased losses, lead to Zn deficiency since only animal flesh is a good source of bioavailable Zn, and phytates inhibit absorption (Hotz and Brown, 2004). Therefore, populations relying primarily on a plant-based diet are susceptible. Significant loss of Zn during diarrheal illness also contributes to an unfavourable balance in Zn nutriture (Castillo-Duran et al., 1988).

Severe Zn deficiency is rare. It was defined in humans in the early 1900s as a condition characterized by short stature, underdeveloped secondary sexual characteristics and a body with long legs and a short trunk in prepubertal males, impaired immune function, skin disorders, and low appetite (Prasad, 1991). In the past 40 years, Zn deficiency has evolved from a rarity in human nutrition to an important global public health nutritional problem (Mathers et al., 2006) with the understanding of the adverse effects of subclinical deficiency.

Worldwide, Zn deficiency results in increased risk of lower respiratory tract infections, diarrhea, and malaria (Black, 2003a). It is thought to be responsible for approximately 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrhoeal disease (Caulfield and Black, 2004).

Zinc deficiency is estimated to be responsible for about 800,000 deaths annually from diarrhea, pneumonia, and malaria in children under five (Caulfield and Black, 2004). The highest attributable burden of pneumonia and diarrhea occurs in Sub-Saharan African countries with high child and very high adult mortality rates, and in South Asian, Eastern Mediterranean, and American countries with high child, high adult mortality rates. Sub-Saharan Africa accounts for practically the entire attributable malaria burden (Mathers et al.,2006).

## Vitamin A deficiency

Vitamin A is essential for maintaining eye health and vision, growth, and immune function. Typically the concurrence of several conditions (i.e. low dietary intake, malabsorption, and increased excretion of vitamin A associated with measles and other common illnesses) precipitate the overt signs of vitamin A deficiency (VAD). VAD results from low intake of animal tissues, inadequate intake of plant sources of pro-vitamin A carotenoids and inadequate intake of dietary fat along with the latter (Sommer, 2008). Severe and prolonged VAD can be identified by the classic ocular signs of xerophthalmia ("eye dryness"), such as corneal lesions and eventually blindness, and remains the leading cause of preventable blindness in children (WHO, 2009). Some signs of Xerophthalmia (i.e. conjunctival xerosis and corneal ulcers) may also be found in systemic autoimmune diseases such as lupus erythematosus and rheumatoid arthritis (Roy, 2002). However, less florid manifestations of VAD are more common, and while the biochemical assessment of vitamin A status is not without limitations, recent advances in field friendly technology (portable computerized pupillary dark adaptometry goggles) to assess night vision in women and children are promising for situation assessment and program impact evaluation (Labrique et al., 2009).

It has been estimated that in total about 0.8 million (1.4%) of deaths worldwide can be attributed to vitamin A deficiency among women and children (1.1% in males and 1.7% in females). Attributable DALYs account for ~1.8% of global disease burden (Black, 2003b). Again, children under five years of age and women of reproductive age are at highest risk of this nutritional deficiency and its adverse health consequences, with the largest prevalence and numbers of affected in parts of South East Asia (30-48%) and in Africa (28-35%) (Rice et al., 2004).

More recently, a meta-analysis of nine randomized placebo-controlled trials in children 6–59 months showing risk reduction with Vitamin A supplementation was reported in the Lancet Maternal and Child Nutrition Series (Black et al., 2008). Calculation of relative risks of cause-specific mortality produced a relative risk of 1.47 (95% CI 1.25–1.75) for diarrhea mortality and 1.35 (0.96–1.89) for measles mortality as a result of vitamin A deficiency as a whole. Additionally, the findings from three trials of vitamin A supplementation of newborn infants in Asia show reductions in mortality in the first six months of life. The results from these trials are applied in the first six months of life to indicate a relative risk of 1.25 for all deaths due to infection and two-thirds of deaths due to prematurity.

### **Iodine deficiency**

Iodine deficiency is the most common preventable cause of impaired mental development and brain damage (Zimmerman et al., 2008). "Endemic cretinism" a form of severe mental retardation closely identified with fetal and neonatal I deficiency represents the extreme of a broad spectrum of reproductive, neurological and endocrinological abnormalities collectively known as I deficiency disorders (IDD). IDD include lower birth weight, increased infant mortality, impaired motor skills, hearing impairment, hypothyroidism, increased susceptibility to nuclear radiation, iodine-induced hyperthyroidism, and neurological dysfunction of various degrees depending on the timing and duration of the insult (WHO et al., 2007). IDD have been estimated to result in the loss of 2.5 million lost years of life (0.2% of total) with approximately 25% of this burden concentrating in the poorest African countries, 17% in South East Asia, and 16% in Eastern Mediterranean region (WHO, 2002).

## Interventions to Control and Prevent Micronutrient Malnutrition

Effective interventions to control vitamin and mineral deficiencies have been available typically in the forms of centrally processed fortified foods and

condiments/sauces, dietary diversification/modifications, or medicinal supplements (i.e. large doses of retinol in standard 50,000, 100,000, and 200,000 IU capsules or syrup; iodized oil capsules; and iron-only or multiple micronutrient tablets, syrups and dispersible powders containing Fe, etc.). Biofortification, the purposeful increase of key micronutrients through plant breeding and genetic modification of staple food crops grown and consumed by rural communities in developing countries – as well as agronomic bifortification, putting trace minerals in fertilizers - is both a fortification and dietary modification alternative. Iron-biofortified rice (Haas et al., 2005) and carotenoid-rich sweet potato (Low et al., 2007) have proven efficacious for enhancing micronutrient status in Filipino women and Mozambican children, respectively. Efforts are underway to assess the efficacy of other biofortified staple crops (high Zn rice and wheat, high Fe beans and pearl millet, high pro-vitamin A carotenoid-rich cassava and maize). The nutrient-specific interventions have been extensively reviewed elsewhere (Bhutta et al., 2008) and will not be addressed individually in depth here but rather used to draw meaningful lessons that can be applied across interventions in national program settings.

The Lancet Series on Maternal and Child Undernutrition showcased the contributions of micronutrient interventions to achieving Millennium Development Goals 1 (target 1C, undernutrition), 4 (child mortality reduction), and 5 (target 5A, maternal mortality reduction). Partly in response to the call for international coordination in this publication, The Micronutrient Forum partners created the 2008 Innocenti Process, which critically reviewed the evidence from real-world micronutrient deficiency control programs implemented at scale; actively engaged country-level program managers and implementers; and built consensus among key stakeholder groups on what makes successful programs succeed or fail (Klemm et al., 2009). The process identified overarching intervention-specific issues affecting micronutrient program implementation (**Table 6**). In summary, stakeholders need leadership, coordination and more resources, while country implementation teams require guidance, empowerment and stronger monitoring, evaluation and performance and impact documentation for national programs and international assistance in this area to improve.

Based on the strength of evidence on their performance and impact, The Innoceti Process classified large-scale micronutrient interventions into:

1) Interventions with strong evidence of effective implementation and impact at large-scale (i.e. pre-school vitamin A supplementation, mass fortification of salt with I, sugar with vitamin A and folic acid-fortified wheat flour);

2) Micronutrient interventions needing further confirmation of implementation effectiveness and impact (maternal iron and folic acid supplementation, and mass iron fortification programs; and

3) Emerging micronutrient interventions that hold promise but lack implementation experience at large scale (i.e. home-based fortification

**Table 6.** Cross-cutting issues affecting the ability to accelerate scaling-up and to<br/>document evidence-based, effective large-scale micronutrient (MN)<br/>programs (Klemm et al., 2009).

- Key stakeholders share common MN goals but lack the leadership to coordinate priority-setting, advocacy, and action;
- Stakeholder groups within the MN community do not communicate effectively with one another;
- Stakeholders have misaligned and often competing priorities and approaches at both global and country levels. This has impeded coordinated actions and slowed progress in achieving common goals;
- The MN community has not adequately engaged with broader nutrition, health, or development initiatives;
- The MN community has not harnessed the full potential of private sector resources, expertise, and delivery mechanisms to improve MN products, services, and delivery platforms;
- Country teams lack guidance and are not empowered to assess needs systematically and facilitate evidence-based decision-making;
- Weak program monitoring, evaluation, and documentation of performance and impact of MN interventions hinders efforts to strengthen programs, advocacy, accountability, and guidance to country-level managers;
- Achieving MN goals is impeded by the overall paucity of nutrition funds; and,
- Limited funding for implementation research restricts our understanding of how best to strengthen the design, management, implementation, evaluation, and financing of MN programs at scale.

with micronutrient powders (Dewey and Adu-Afarwuah, 2008); incorporating Zn supplementation<sup>7</sup> as an adjunct treatment to low osmolarity oral rehydration salts and continuing child feeding for managing acute diarrhea (Thapar and Sanderson, 2004); poverty reduction strategies, such as conditional cash transfers, microcredit, and agricultural interventions that include nutrition components). The cost per DALY saved associated with some of these interventions is summarized in **Table 7**. It is our opinion that biofortification of staple food crops has quickly inserted itself into the third category, although the recent success with provitamin A (orange flesh) sweet potato would place sweet potato biofortification in the second category.

<sup>7 20</sup> mg/day for 10-14 days for children 6 months and older, 10 mg/day for children under 6 months of age.

Intervention	Africa	Asia
Interventions with evidence of effective implementation at large-scale	:	
Vitamin A Supplementation, 50% coverage	26-52	55
Vitamin A Fortification, 50% coverage	21-41	22
Interventions needing further confirmation of impact at large-scale		
Iron supplementation, 50% coverage <sup>2</sup>	30	70
Iron fortification, 50% coverage <sup>2</sup>	27	43
Promising and emerging interventions		
Vitamin A biofortified sweet potato, 40% coverage <sup>3</sup>	9	
Vitamin A biofortified maize, 40% coverage <sup>3</sup>	11-18	
Zinc biofortifed rice, 60% coverage <sup>3</sup>		2-7
Zinc supplementation, 50% coverage <sup>1</sup>	476-823	7

#### Table 7. Range in average cost (USD) per DALY saved.

1 WHO-CHOICE, http://www.who.int/choice/results/en/

2 Baltussen et al. (2004). "Iron Fortification and Supplementation are Cost-Effective Interventions to Reduce Iron Deficiency in Four Subregions of the World" The Journal of Nutrition.

3 Meenakshi et al. (2010). "How Cost-Effective is Biofortification in Combating Micronutrient Malnutrition? An Ex Ante Assessment" World Development.

## Supplementation

A very basic difference between successful I and vitamin A deficiency prevention and the less fortunate battle against Fe and Zn-deficient diets is the body's ability to absorb and store significantly greater amounts of the former two from a single mega dose dispersed in oil, whereas the absorption of Fe and Zn is affected by other dietary components and by homeostatic regulatory mechanisms which result in minute amounts being absorbed and stored daily. Notwithstanding the recommendation to switch to lower, more physiological doses, a typical 20,000 IU retinol dose suffices a lactating woman's requirements for over 100 days; a single low dose of 0.4 ml of iodized poppy seed oil with 200 mg of elemental I fills a school age child's requirements for one year (Zimmerman et al., 2000). Preventive Fe supplementation typically means daily intake of 15-30 times the daily requirement during 30 to 90+ days, depending on the target group. Iron (or multiple micronutrient) supplements can be consumed weekly in particular settings where high compliance is fostered (e.g. school settings) for longer periods of time. However this modality of supplementation is not efficacious during pregnancy given the short time available to build up Fe stores.

Moreover, regarding compliance with long drawn daily preventive supplementation regimes (as for Zn and Fe deficiencies), they are difficult to adhere to because of consumer fatigue, undesirable gastrointestinal side effects (heartburn, metallic aftertaste, diarrhea, etc). In addition, these deficiencies do not have overt, alarming signs like enucleated eye balls and corneal scars of VAD and monstrous goiters and cretins of IDD. For governments and agencies supporting supplementation programs, it is much more cost-effective to procure and distribute one capsule per at-risk individual every 6-12 months than 30 or 90 tablets every 1-3 months. Hence, to be effective and sustainable, programs for Fe and Zn deficiency control and prevention require year round logistic support and effective monitoring systems integrated with and not parallel to other health care delivery strategies.

Supplementation with large doses of preformed vitamin A (retinol) at least twice a year has drastically decreased blindness and other ocular signs caused by VAD among children under five years of age in the developing world. The rationale of vitamin A supplementation (VAS) is that it reduces general child mortality by 20-30%, particularly in settings with low measles immunization coverage (around 350,000 deaths avertable per year) (Sommer, 2008). International guidelines recommend regular dosing with vitamin A capsules for a target of all children between the ages of six months and five years, in all countries with child mortality greater than 70 in 1,000 live births (UNICEF, 2007). Although initially conceived as a short-term emergency intervention VAS is an essential component of the public health nutrition armamentarium as long as and wherever food insecurity remains at current levels.

While the efficacy of Zn supplementation to prevent and treat diarrheal disease has been confirmed by several studies (Santosham et al., 2010), there is very little program experience with preventive Zn supplementation. On the other hand, in the past five years, over 45 countries have successfully changed national childhealth policies to include Zn in their diarrhea treatment guidelines (Fischer-Walker et al., 2009). Preventive vitamin A supplementation and preventive and therapeutic Zn supplementation were classified as the most cost-effective public health interventions available to decrease child mortality by the 2008 Copenhagen Consensus Experts (Lomborg, 2007) and are considered among the most effective interventions for improving maternal and child health and nutrition (Bhutta et al., 2008).

#### **Food Fortification**

Food fortification has been extensively reviewed by the World Health Organization (Allen et al., 2006). Food fortification is, in general, efficacious and cost-effective in the medium and long-term to improve micronutrient status. The clearest example of effectiveness is the iodization of common salt to prevent I deficiency disorders, an intervention that has prevented millions of still births, abortions, newborns with severe mental and neurological damage (cretins and less severe results of in utero and neonatal iodine deficiency), hypothyroid persons, and millions of public dollars in health care services associated with the consequences of this deficiency (Zimmerman et al., 2008). Iodine fortification reduces the incidence of IDD by 73% (Mahomed and Gülmenzoglu, 1997). The addition of synthetic folic acid to cereal flours (mainly wheat) is another successful example of the effectiveness of centrally processed staple foods to prevent congenital defects (spina bifida and anencephaly, among others) and folate deficiency anemia. Since closure of the neural tube occurs before the 20th week of gestation and defects in its closure are associated with folate deficiency around the time of conception, the timing of adequate folate is critical and can best be achieved by adding the folic acid to a food consumed daily in regular amounts by all women of child bearing age, as is the case with this intervention. Since flour production already includes the use of additives to enhance bread-making properties and shelf life, adding essential nutrients to flour is simple and highly affordable, given a certain level of industrial sophistication. However the ideal food-nutrient combination is country specific and depends on the dietary deficit of the nutrient(s) under consideration, the usual intake of the potential food vehicles by the target population group (amount and frequency), etc.

Flour fortification at small-scale mills in Africa has been tried but no successful experiences with these types of milling networks have been published. Small community roller mills in Nepal have been successfully adapted to add micronutrients with a locally invented premix dosifier.

Dary (2007) recently reviewed Fe fortification programs and concluded that with respect to the Fe fortification of foods and condiments (salt, soy/fish sauce, sugar, etc) the key factor limiting Fe content is technological, that is, the incompatibility between the Fe compounds and the food matrix. In fact, Fe must be added in relatively low amounts to prevent undesirable changes in the sensory properties of flours. The maximum feasible amount varies with the Fe compound used (e.g. around ~30 mg Fe/kg flour from ferrous sulphate, ~55 mg Fe/kg flour from ferrous fumarate, and 60-80 mg Fe/kg flour from electrolytic Fe for low-extraction, highly refined wheat flours, and lower levels of Fe from NaFe(II)EDTA for high-extraction unrefined flours) (Hurrell et al., 2010). The magnitude of the biological impact of a fortified food would be related to the proportion of the estimated average requirement (EAR) or the recommended nutrient intake (RNI) supplied and absorbed. In countries with significant Fe deficiency, it would require an additional Fe intake of at least 60% EAR to improve Fe stores and at least 90% EAR to decrease nutritional anemia. Other examples of efficacious Fe fortification have been documented for table salt (Zimmerman et al., 2003), rice (Diego et al., 2006), sugar (Viteri et al., 1978), soy sauce (Chen et al., 2005) among others, but their large-scale effectiveness under free market conditions or public program conditions are still under study.

The addition of retinol palmitate to sugar (Ribaya-Mercado et al., 2004) has been used effectively in Guatemala, El Salvador, Honduras, and Nicaragua since the 1980s, but not in Zambia, as a strategy to reduce vitamin A deficiency (Arroyave, 1981). The addition of retinol to cooking oil and oil-based foods (margarine) has been increasing in Africa but there are no publications attesting to its efficacy.

An interesting supplementation-fortification hybrid technology has been making great progress in the fight against childhood and infancy nutritional anemia. Stanley Zlotkin conceived the use of multiple micronutrient powders (Sprinkles<sup>TM</sup>) to fortify complementary foods at the household level as a solution to the worldwide rejection of, and lack of compliance with, traditional Fe

syrups and drops by children and mothers. Daily use of one sachet of 0.5-1.0g of a multiple micronutrient containing powder for 1-3 months has efficaciously reduced anemia in several different settings (Ghana, Nepal, India, China, Bolivia, Mexico, etc.; Zlotkin et al., 2004). Among its key elements for success are high consumer acceptance because of its ease of use, a specific marketing audience, the attractive packaging, and the lack of metallic taste in the food, achieved by microencapsulation of the Fe compound.

Inconsistent results have been generated regarding the impact of Zn fortification of food. Whereas zinc-fortified foods result in significantly increased Zn intake and positive net absorption of additional Zn, only a few studies have found positive impacts of Zn fortification on serum Zn concentrations or functional indicators of Zn status (Brown et al., 2009). Additional research is needed to elucidate the reasons for the inconsistent findings to date. A recent review of this topic (Hess, 2009) suggests that the choice of food vehicles, the age group and Zn status of the study populations, or particular aspects of the study design will have to be properly addressed in future research. And because of the "benefits of increasing intake in populations at high risk for Zn deficiency, the documented increase in total Zn absorption that occurs following Zn fortification, the absence of any adverse effects, and the relatively low cost of adding Zn" current research gaps on the efficacy of Zn fortification should be pursued as a high priority.

#### **Dietary Diversity and Modification of Feeding Habits**

Diets in developing countries generally are monotonous and insufficient to provide energy and several nutrients, so intervention strategies need to also emphasize an increase in total food intake, in addition to a constant and greater variety of foods. Undeniably, the degree and distribution of micronutrient deficiencies depends on the political and economic situation, the level of education and sanitation, the season and climate conditions, food production, cultural and religious food customs, breast-feeding habits, prevalence of infectious diseases, the existence and effectiveness of nutrition programs, and the availability and quality of health services. Single nutrient or single food approaches cannot adequately tackle malnutrition in developing countries. In a given rural, food-insecure population where the reach of supplements and fortified foods is insufficient, sustainable improvement of dietary adequacy may be accomplished through food-based approaches that rely on the availability of agricultural and animal husbandry resources and behavior modification interventions. Small-scale efforts have demonstrated that dietary diversification can be effectively achieved through consumption of a broad variety of foods (i.e. home gardens and small livestock production) (Tontisirin et al., 2002). Morally irrefutable, the statement that in food-insecure settings households should be educated and supported to increase production of dark-green leafy vegetables, yellow and orange fruits, eggs, milk, fish and small animal stock is notably based on scant scientific evidence, most of which comes from small-scale development and research projects. The only notable large-scale application of this approach is Brasil's Fome Cero (Zero Hunger) National Program (FAO, 2007), which has linked community development policies to national programs for the alleviation of hunger and malnutrition, with an emphasis on increasing the variety of foods consumed and eliminating constraints to access to a diverse diet using locally produced foods – probably the best strategy for decreasing micronutrient malnutrition (and hunger) sustainably among the urban and rural poor (Gómez-Calera et al., 2010).

## Agricultural Approaches Biofortification

## Rationale for Biofortification

Modern agriculture has been largely successful in meeting the energy needs of poor populations in developing countries. In the past 40 years, agricultural research in developing countries has met Malthus's challenge by placing increased cereal production at its center. However, agriculture must now focus on a new paradigm that will not only produce more food, but deliver better quality food as well.<sup>8</sup>

Through plant breeding, biofortification can improve the nutritional content of the staple foods poor people already eat, providing a comparatively inexpensive, cost-effective, sustainable, long-term means of delivering more micronutrients to the poor. This approach will not only lower the number of severely malnourished people who require treatment by complementary interventions, but will also help them maintain improved nutritional status. Moreover, biofortification provides a feasible means of reaching malnourished rural populations who may have limited access to commercially marketed fortified foods and supplements.

Unlike the continual financial outlays required for traditional supplementation and fortification programs, a one-time investment in plant breeding can yield micronutrient-rich plants for farmers to grow around the world for years to come. It is this multiplier aspect of biofortification across time and distance that makes it so cost-effective.<sup>9</sup>

## **Comparative Advantages of Biofortification** Reaching the Malnourished in Rural Areas

Poor farmers grow modern varieties of crops developed by agricultural research centers supported by the Consultative Group on International Agricultural

<sup>8</sup> An important part of the overall solution is to improve the productivity of a long list of nonstaple food crops. Because of the large number of foods involved, achieving this goal requires a very large investment, the dimensions of which are not addressed here.

<sup>9</sup> A review of progress in biofortification under the HarvestPlus program for seven food staple crops is provided in Bouis et al. (2011). In general, nutrient targets in breeding aim to achieve 30-50% of the Estimated Average Requirement, taking into account retention of nutrients in storage, processing, and cooking and bioavailability. HarvestPlus research has shown that there is no inherent tradeoff between Fe, Zn, and provitamin A content and yield. More resources need to be invested in a breeding program to achieve high nutrient content combined with the same pace of advancement in yield. However, the potential public health benefit is far higher than this extra cost/investment in breeding.



Figure 6. Biofortification improves status for those less deficient and maintains status for all at low cost.

Research (CGIAR) and by national public and private agricultural research systems (NARS), and disseminated by non-governmental organizations (NGOs) and government extension agencies. The biofortification strategy seeks to put the micronutrient-dense trait in the most profitable, highest-yielding varieties targeted to farmers and to place these traits in as many released varieties as is feasible. Moreover, marketed surpluses of these crops make their way into retail outlets, reaching consumers in both rural and urban areas. The direction of the flow, as it were, is from rural to urban in contrast to complementary interventions that begin in urban centers.

## Cost-Effectiveness and Low Cost

Biofortified staple foods cannot deliver as high a level of minerals and vitamins per day as supplements or industrially fortified foods, but they can help to bring millions over the threshold from malnourishment to micronutrient sufficiency. **Figure 6** shows this potential schematically when a high percentage of the iron-deficient population is mildly deficient. For those who are severely deficient, supplements (the highest cost intervention) are required.

In an analysis of commercial fortification in 2003, Horton and Ross (2003) estimated that the present value of each annual case of Fe deficiency averted in South Asia was approximately USD 20.<sup>10</sup>

Consider the value of 1 billion cases of Fe deficiency averted in years 16-25

<sup>10</sup> A World Bank study in 1994 assigns a present value benefit of USD 45 to each annual case of Fe deficiency averted through fortification (a mix of age-gender groups). The same study gives a present value of USD 96 for each annual case of vitamin A deficiency averted for pre-schoolers.

after biofortification research and development project was initiated (100 million cases averted per year in South Asia). The nominal value of USD 20 billion (1 billion cases times a value of USD 20 per case) must be discounted because of the lags involved between the time that investments are made in biofortification and when benefits are realized. At a three percent discount rate, the present value would be approximately USD 10 billion, and at a 12 percent discount rate, the present value would be approximately USD 2 billion. This benefit is far higher than cost of breeding, testing, and disseminating high Fe and high Zn varieties of rice and wheat for South Asia (< USD 100 million in nominal costs).

## Sustainability of Biofortification

Once in place, the system described in the previous section is highly sustainable. The major fixed costs of developing the varieties and convincing the nutrition and plant science communities of their importance and effectiveness are being covered by programs such as HarvestPlus (www.harvestplus.org). However, the nutritionally improved varieties will continue to be grown and consumed year after year. To be sure, recurrent expenditures are required for monitoring and maintaining these traits in crops, but these recurrent costs are low compared with the cost of the initial development of the nutritionally improved crops and the establishment, institutionally speaking, of nutrient content as a legitimate breeding objective.

#### **Fortification of Fertilizers**

Use of micronutrient-fortified fertilizers represents another important agricultural approach to enrichment of food crops with micronutrients. There are examples, involving Zn, Se, and I, demonstrating that a fertilizer approach is a quick and effective approach in biofortifying food crops with targeted micronutrients. This approach might be, however, not affordable in some countries where application of fertilizers is very restricted due to lack of resources and/or limited availability of fertilizers.

Particular attention should be paid to use of micronutrient-fortified fertilizers in soils where chemical availability of micronutrients and thus their root uptake are very significantly reduced due to extreme soil conditions such as very high pH and very low levels of organic matter. Almost 50 % of the cereal-cultivated soils globally have a Zn deficiency problem.

Both macronutrient fertilizers containing N, P, K, and S, and certain micronutrient fertilizers (e.g. Zn, I, Co, Mo, and Se) can have significant effects on the accumulation of nutrients in edible plant products (Allaway, 1986; Grunes and Allaway, 1985). Depending on the severity of soil deficiency, use of micronutrient fertilizers can also contribute to increase in grain yield as demonstrated in India, Australia and Turkey in zinc-deficient wheat and rice soils (Graham et al., 1992; Cakmak, 2008). In India, field experiments on rice and wheat showed that application of zinc-enriched urea (up to 3% Zn) significantly enhanced both grain Zn concentration and grain yield in rice and wheat (Shivay et al., 2008). Other micronutrient fertilizers such as Fe have very little effect on the amount of the micronutrient accumulated in edible seeds and grains when they are applied to soils or when used as foliar sprays (Welch, 1986). This is because of their very limited phloem sap mobility (Welch, 1999).

For Zn, I, and Se, increasing the soil-available supply to food crops can result in significant increases in their concentrations in the edible portions of plant products (Graham et al., 2007; Welch, 1995). Increasing the supply of Zn and Se to wheat (*Triticum aestivum* L.) significantly improved both the total amount and bioavailability of Zn and Se in wheat grain (Cakmak, 2008; Haug et al., 2008; House and Welch, 1989). Foliar application of Zn fertilizers (especially late season applications) is also highly effective in increasing Zn concentration in the endosperm part that is the most commonly eaten part of cereal grains (Cakmak et al., 2010).

For Fe, providing more to plants than required for maximum yield does little to further increase the Fe in edible seeds and grains.

Recently published data indicate importance of N fertilizers in improving root uptake and grain deposition of Zn and Fe in wheat (Kutman et al., 2010). Generally, grains with high protein concentration also contain high amounts of Zn and Fe, suggesting that grain protein is a sink for Zn and Fe.

Interestingly, the micronutrient, I, supplied in irrigation water, can greatly increase the levels of I in edible portions of food crops alleviating the debilitating disease, cretinism, as well as other I deficiency disorders in populations dependent on irrigated food crops grown on low I soils (Cao et al., 1994; Ren et al., 2008). In Finland, Se added to fertilizers and applied to soils increased the Se status of the entire Finnish population (Mäkelä et al., 1993).

These results highlight the importance of fertilizers as an effective agricultural tool to improve the nutritional health of people in the developing world. For more detailed information concerning the effects of fertilization practices on micronutrient accumulation in plant foods readers are referred to Welch (2001), Cakmak (2008) and Chapter 4 by Lyons and Cakmak (2011) in this book.

### Homestead Food Production Programs (HFPPs)

This intervention has been developed by Helen Keller International (HKI) and implemented in Bangladesh, Cambodia, Nepal, and the Philippines, primarily in response to high prevalence of vitamin A deficiency in rural areas. Working through local NGOs, the approach consists of promoting home gardening and small livestock production, importantly in conjunction with provision of nutrition education. NGOs provide households with materials needed to get started, such as seeds and seedlings. At first these programs emphasized only vegetable and fruit production. But because new research by nutritionists indicated low bioavailability of provitamin A carotenoids from vegetable sources, small animal production (which provides preformed vitamin A, or retinol) was added to these programs.

Gardens are classified into three types: i) *traditional gardens* are seasonal, found in scattered non-permanent plots, producing just a few types of vegetables; ii) *improved gardens* are also seasonal, but fixed plots which produce a wide range of vegetables; iii) *developed gardens* produce a wide range of vegetables all year round. Programs have been successful in having a large majority of participating households create developed gardens.

Impact studies of HFPPs have shown that greater food availability in conjunction with nutrition education, has led to higher household income, increased consumption of higher quality foods, increased vitamin and mineral intake, lower prevalence of micronutrient deficiencies, and empowerment of women. In two decades of operation in Bangladesh, HFPPs have improved the food security for nearly five million vulnerable people (nearly 4% of the population) in diverse agroecological zones across much of the country (Spielman and Pandya-Lorch, 2009).

#### Introduction of Nutrient-Dense, Novel Foods into Food Systems

To illustrate this strategy, consider the case of the introduction of orange-flesh sweet potato (for which there are specific lines very dense in provitamin A carotenoids) into a food system that is severely deficient in vitamin A. Where white sweet potato varieties are already being consumed, as in many parts of Sub-Saharan Africa, this is the biofortification strategy discussed above.

In other areas such as parts of South Asia, however, sweet potato may be a completely novel crop. This will be a much more difficult "sell" to consumers, depending on the acceptability of the texture, taste, smell, and other any other organoleptic properties of this novel food in the local culture.

In either case, a communication strategy needs to be developed, directed not only at users but at policymakers and diffusers of this technology (diffusers ultimately report to policymakers who provide, or do not provide, an enabling environment to implement the dissemination strategy). High yielding, high profit varieties and effective communication creates farmer demand for vines, thereby ensuring suppliers and market linkages for supplies. Consumer demand would need to be motivated by a message of improved nutrition through effective communication. Finally, after the initial public investment introducing the new crop into the food system, at some point public activities would need to be withdrawn, leaving in place a supply-demand marketing chain operating within the market economy.

### **Cost-Benefit Analysis of Alternative Interventions**

Virtually all the interventions discussed above are highly cost effective. Since the consequences of micronutrient malnutrition are several and varied, and include both morbidity and mortality outcomes, an assessment of the health benefits that

result from any intervention necessitates the use of a metric that can be added across these outcomes in a meaningful manner. The reduction in the DALY burden in a community that results from the implementation of an intervention, or DALYs saved or averted is a common measure of its health impact. Cost-effectiveness figures are therefore commonly expressed in terms of costs per DALY saved (Disease Control Priorities Project 2008).

**Table 7** presents a range of cost-effectiveness figures for three categories of interventions: pre-school vitamin A supplementation fortification, which, as noted above, have demonstrated evidence of effective implementation at a large-scale; Fe supplementation and fortification, interventions which need to further demonstrate effectiveness at large-scale; Zn supplementation and biofortification, emerging interventions with promise.

Cost effectiveness figures can be interpreted at two levels: first, to examine whether the benefits exceed the cost, and second, to compare across interventions. Virtually all the numbers cited in **Table 7** fall under the 'highly cost-effective' category; in other words, even when viewed as stand-alone interventions, these all merit investment, for the benefits far outweigh the costs.

And although differences in methodology used in computing the figures cited in **Table 7** preclude a direct comparison across interventions, it is clear that biofortification compares favorably with vitamin A and Zn fortification and supplementation. The reason for this is not hard to find. Because biofortification is an agriculture-based strategy, the costs are incurred upfront in developing and deploying biofortified planting materials. Once these become part of the cropping pattern of farmers, the micronutrient embodied in the seed yields benefits year-after-year. Recurring costs, primarily incurred on maintenance breeding to ensure that the trait is retained in all successive varietal releases are a low percentage of total costs. Further, despite rapidly changing diets, model-based simulations suggest that staple foods will continue to be the mainstay of diets of the poor in the years to come, especially in rural areas (Msangi et al., 2010). In contrast, both fortification and supplementation require annual investments to reach target populations.

Given the magnitude of the problem, it is likely that a multiplicity of interventions is likely to be necessary to achieve impact, and the actual mix will depend on costs. For example, in the case of biofortification, costs are generally higher for vitamin A crops than for Zn crops, since the presence of beta-carotene renders the crop a distinct orange colour, in contrast to the white varieties that are commonly consumed. The unfamiliar colour may necessitate greater investments in behavior change communication than may be necessary for an invisible trait. However, research thus far suggests that the orange colour is unlikely to be an impediment to adoption (Chowdhury et al., 2009; Meenakshi et al., 2010; Stevens and Winter-Nelson, 2008). Also, because infrastructure for the dissemination of new technologies in agriculture is generally better in Asia than in Africa, the biofortification intervention is likely to be cheaper in Asia. Therefore, which combination of interventions will work best in a particular country will need to be worked out on a case-by-case basis.

## Conclusion

Ultimately, good nutrition depends on adequate intakes of a range of nutrients and other compounds, in combinations and levels that are not yet completely understood. Thus, the best and final solution to eliminating undernutrition as a public health problem in developing countries is to provide increased consumption of a range of non-staple foods. However, this will require several decades to be realized, informed government policies, and a relatively large investment in agricultural research and other public and on-farm infrastructure (Graham et al., 2007).

In conceptualizing solutions for a range of nutritional deficiencies, interdisciplinary communication between plant scientists and human nutrition scientists holds great potential. Human nutritionists need to be informed, for example, about the extent to which the vitamin and mineral density of specific foods, as well as compounds that promote and inhibit their bioavailability, can be modified through plant breeding. Plant breeders need to be aware of both the major influence that agricultural research may have had on nutrient utilization in the past (e.g. the bioavailability of trace minerals in modern varieties versus bioavailability in traditional varieties), and the potential of plant breeding for future improvements in nutrition and health.

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## Perspectives on Enhancing the Nutritional Quality of Food Crops with Trace Elements

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## Abstract

Humans require at least 10 essential trace elements (B, Cu, F, I, Fe, Mn, Mo, Ni, Se, and Zn). The foods produced from farmer fields are the primary suppliers of these nutrients. Vast numbers of people, primarily women, infants, and children, are afflicted with trace element deficiencies (notably Fe, I, Se, and Zn) mostly in the resource-poor countries of the developing world. Micronutrient malnutrition (which includes both trace element and vitamin deficiencies) is the result of dysfunctional food systems based in agricultural systems that do not meet all human nutritional needs. Agricultural tools can be used to address micronutrient malnutrition. These tools include the biofortification strategies of plant breeding and use of trace element fertilizers. Zinc may be the key nutrient in reducing micronutrient malnutrition in many nations because nutrient interactions with Zn are important issues. Breeding staple plant foods for higher levels of prebiotics in edible portions is suggested as the most effective means of improving the bioavailability to humans of essential trace elements in plant foods. This review advocates that it is imperative that agriculture be closely linked to human nutrition and health and that fertilizer technology be used to improve the nutritional quality of staple food crops that feed the world's malnourished poor.

## Introduction

Living organisms contain most of the 90 naturally occurring elements on earth. Some elements normally occur in living tissues in high amounts while others are generally present in very small amounts at "trace" levels. Thus, the term "trace element" was coined to distinguish those elements that normally occur at low levels in biological tissues from those elements that usually occur at higher

Abbreviations specific to this chapter: DcytB = Duodenal Cytochrome B; DMT1 = Divalent Metal Transporter 1; CIMMYT = Centro Internacional de Mejoramiento de Maiz y Trigo; WHO = World Health Organization. For symbols used commonly throughout this book see page xi.

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concentrations in living tissues (Mertz, 1987; Pais and Jones, Jr., 2009). Table 1 identifies the 73 elements classified as trace elements because they are normally present at low levels in biological systems compared to the nine major essential elements for plants, animals and humans (H, C, N, O, K, Mg, Ca, P, and S). Sodium and Cl<sup>-</sup> are also major essential elements for animals and humans. Of the 73 trace elements, nine are commonly accepted as essential for animals and humans (Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, and I). For plants, the trace elements generally accepted as essential include Fe, Zn, Mn, Cu, Ni, Cl-, B, and Mo. Cobalt is essential for some plants that utilize symbiotic N fixation as a major source of N; Si and Na have been reported to be essential for some plant species, but have not been proven to be essential for all higher plants (Epstein and Bloom, 2005; Welch, 1995). Others are considered by some to be essential for animals and humans depending on what criteria are used to define essentiality; these include B, F, Li, Si, V, Ni, As, Cd, Sn, and Pb (Nielsen, 1993; Nielsen, 1997). Still others may be proven to be essential in the future (Welch, 1995). This chapter primarily focuses on the trace elements (i.e. micronutrient elements) proven to be essential for humans that have been shown to be limiting in the diets of numerous resource-poor people in the world causing widespread micronutrient malnutrition and related poor health, reduced worker productivity, stagnating development efforts and death to many. These micronutrient elements are Fe, Zn, Se, I, and Co.

Table 1. The 73 elements classified as trace elements in biological systems (shown
with green shading) among the 90 naturally occurring elements on earth.
Macronutrient elements and rare gases are shown without green shading.

Н																	Не
Li	Be											В	С	Ν	0	F	Ne
Na	Mg											Al	Si	Р	S	CI	Ar
К	Ca	Sc	Ti	V	Cr	Mn	Fe	Со	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Мо		Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Те	I	Xe
Cs	Ва	La	Hf	Та	W	Re	Os	lr	Pt	Au	Hg	TI	Pb	Bi	Ро	At	Rn
Се	Pr	Nd		Sm	Eu	Gd	Tb	Dy	Но	Er	Tm	Yb	Lu				
Fr	Ra	Ac	Th	Pa	U												

In this chapter, we attempt to demonstrate the close linkages that exist between agriculture and trace element deficiencies in the world and why it is imperative that these linkages be used to find sustainable solutions to trace element deficiencies in humans especially for resource-poor populations globally.

## **Requirements of Plants, Animals, and Humans**

### Plants

Numerous environmental and genetic factors interact to determine the required levels of essential trace elements in different organs and tissues of plants. These interacting factors are complex and include the plant's genetic makeup and

Element	Plant species, part	Deficient	Adequate	Toxic
Fe	Soybean ( <i>Glycine max</i> L. Merr.), shoot	28-38	44-60	
	Pea (Pisum sativum L.), leaf	14-76	100	>500
	Corn (Zea mays, L.) leaf	24-56	56-178	_
	Tomato (Lycopersicon esculentum Mill.), leaf	93-115	107-250	_
Mn	Soybean (Glycine max L. Merr.), leaf	2-5	14-102	>300
	Potato (Solanum tuberosum L.), leaf	7	40	—
	Tomato ( <i>Lycopersicon esculentum</i> Mill.), leaf	5-6	70-400	—
	Wheat (Triticum aestivum L.), shoot	4-10	75	>750
	Sugar beet ( <i>Beta vulgaris</i> L.), leaf	5-30	7-1200	>1200
Zn	Potato (Solanum tuberosum L.), leaf	<30	30-87	
	Tomato (Lycopersicon esculentum Mill.), leaf	9-15	65-200	>500
	Corn (Zea mays, L.). leaf	9-15	>15	—
	Oat (Avena sativa L.), leaf	<20	>20	—
	Wheat (Triticum aestivum L.), shoot	<14	>20	>120
Cu	Cucumber (Cucumis sativa L.), leaf	<2	7-10	>10
	Potato (Solanum tuberosum L.), shoot	<8	11-20	>20
	Tomato (Lycopersicon esculentum Mill.), leaf	<5	8-15	>15
	Corn (Zea mays, L.) leaf	<2	6-20	>50
	Wheat (Triticum aestivum L.), shoot	<2	5-10	>10
Ni	Soybean (Glycine max L. Merr.), leaf	<0.004	0.05-0.1	>50
	Cowpea (Vigna unguiculata L. Walp), leaf	<0.1	>0.1	—
	Barley (Hordeum vulgare L.), whole grain	<0.1	>0.1-0.25	—
	Oat (Avena sativa L.), leaf	<0.2	>0.2	
В	Broccoli (Brassica olearaces L.), leaf	2-9	10-71	
	Potato (Solanum tuberosum L.), leaf	<15	21-50	>50
	Tomato (Lycopersicon esculentum Mill.), leaf	14-32	34-96	91-415
	Corn (Zea mays, L.), shoot	<9	15-90	>100
	Wheat (Triticum aestivum L.), straw	4.6-6.0	17	>34
Mo	Tomato (Lycopersicon esculentum Mill.), leaf	0.13	0.68	>1000
	Barley (Hordeum vulgare L.), shoot	_	0.03-0.07	
	Broccoli (Brassica olearaces L.), shoot	0.04		
C1	Potato (Solanum tuberosum L.), leaf	210	2580	>5000
	Sugar beet ( <i>Beta vulgaris</i> L.), leaf	40-100	>200	

## **Table 2.** Concentration ranges of essential trace elements in common food crops<br/>( $\mu$ g/g, dry weight) (Modified from Welch, 1995).

Data from: Jones, Jr., 1991; Chapman, 1966; Asher, 1991.

environmental factors (biotic and abiotic stresses) including various soil factors, pathogen pressures, and weather conditions during growth (Welch, 1995). Thus, both dynamic physiological and environmental features interact to determine the micronutrient concentrations at which deficiencies or toxicities occur. **Table 2** presents examples of the range of micronutrient concentrations (from deficient to toxic) found in some organs of important crop species grown under typical field conditions. **Table 3** shows the critical concentrations (i.e. the lowest concentration in a selected tissue that is associated with maximal growth rates) of essential trace elements in some organs reported for three important crop species. More information on the ranges of essential trace element concentrations in plants can be found in other reviews (Reuter and Robinson, 1997; Jones, Jr., 1991; Bennett, 1993; Chapman, 1966).

**Table 3**. Critical concentrations of essential trace elements in maize (Zea mays L.), soybean (Glycine max L.), and wheat (Triticum aestivum L.) plants (in μg/g, dry weight) (Modified from Welch, 1995).

Micronutrient	Corn <sup>†</sup>	Soybean <sup>‡</sup>	Wheat <sup>§</sup>
Fe	25	30	25
Mn	15	20	30
Zn	15	15	15
Cu	5	5	5
Ni	—	<0.004¶	<0.1#
В	10	25	15
Mo	0.2	0.5	0.3
C1-**			

<sup>†</sup> Leaf below ear at tasseling.

<sup>\*</sup> Youngest mature leaves and petioles after pod formation.

- <sup>§</sup> Entire shoot at boot stage of development.
- ¶ Entire shoot
- \* Mature grain

<sup>++</sup> The critical concentration of Cl<sup>-</sup> for these plant species has not been established. For many species it may be as low as 35 μg/g, dry weight, or as high as several 1000 μg/g, dry weight (Römheld and Marschner, 1991; Jones, Jr., 1991).

### **Animals and Humans**

As with plants, the range of essential trace elements in animal and human tissues can vary widely depending on genetic, physiological, nutrition and health status and environmental variables. **Table 4** lists the essential trace elements for animals and humans and examples of some deficiency implications, functions, estimated dietary needs for adult males and rich food sources. More information on the levels of these nutrients in animal and human tissues and their requirements can be found in other reviews (Mertz, 1986; Mertz, 1987; World Health Organization, 1996; Pais and Jones, Jr., 2009).

# **Table 4.** Essential trace elements for animals and humans: examples of somedeficiency implications, functions, estimated dietary needs and rich foodsources<sup>†</sup> (Table from Welch, 2001).

Element	Deficiency and Function(s)	Human Dietary Need <sup>†</sup> ; Rich Food Sources
As	impaired fertility and increased perinatal mortality; depressed growth; conversion of methionine to its metabolites; methylation of biomolecules	12 μg/d (est.); fish, grain and cereal products
В	impaired Ca utilization in bone; more severe signs of vitamin D related rickets; decreased apparent ab- sorption of Ca, Mg, and P; impaired metal functions in older women and men (>45 years old); <i>cis</i> -hydroxyl reactions with biomolecules; cell membrane integrity	0.5 - 1.0 mg/d (est.); non- citrus fruits, leafy veg- etables, nuts, and pulses
Cr	impaired glucose tolerance; impaired growth; el- evated serum cholesterol and triglycerides; increased incidence of aortic plaques; corneal lesions; decreased fertility and sperm count; potentiates insulin action	33 μg/d (est.); processed meats, whole grain products, pulses, and some spices
Cu	hypochromic anemia; neutropenia; hypopigmenta- tion of hair and skin; impaired bone formation with skeletal fragility and osteoporosis; vascular abnor- malities; steely hair; metal cofactor in numerous metalloenzymes (e.g. cytochrome oxidase, caerulo- plasmin, superoxide dismutase, etc.)	1.5 - 3.0 mg/d; organ meats, seafood, nuts and seeds
F	status as an essential trace element debated; benefi- cial element because of its effects on dental health	1.5 - 4.0 mg/d; tea, marine fish consumed with bones
Ι	wide spectrum of diseases including severe cretin- ism with mental retardation; enlarged thyroid (goi- ter); essential constituent of the thyroid hormones	150 μg/d; seafood, iodized table salt; milk; I concen- trations in plant foods vary greatly depending on vari- ous environmental factors including the geochemical environment, fertilizer, food processing and feed- ing practices
Fe	Fe deficiency erythropoiesis with low Fe stores and with work capacity performance impaired; Fe deficiency anemia with reduced hemoglobin levels and small red blood cells; impaired immune func- tion; apathy; short attention span; reduced learning ability; constituent of hemoglobin, myoglobin and a number of enzymes	15 mg/d; meats, eggs, vegetables and iron- fortified cereals
Mn	poor reproductive performance; growth retardation; congenital malformations; abnormal bone and car- tilage formation; impaired glucose tolerance; metal activator of many enzymes (e.g. decarboxylases, hydrolases, kinases, and transferases); constituent of pyruvate carboxylase and superoxide dismutase in mitochondria	2.0 - 5.0 mg/d; whole grain and cereal products, fruits and vegetables, tea

#### Table 4. (Continued)

Element	Deficiency and Function(s)	Human Dietary Need <sup>‡</sup> ; Rich Food Sources
Mo	retarded weight gain; decreased food consumption; impaired reproduction; shortened life expectancy; neurological dysfunction; dislocated ocular lenses, mental retardation; cofactor (molybdopterin) in sulfite oxidase and xanthine dehydrogenase	75 - 250 μg/d; milk, beans, breads and cereals
Ni	depressed growth and reproductive performance; impaired functioning and body distribution of several nutrients (e.g. Ca, Fe, Zn, vitamin $B_{12}$ ); cofactor for an enzyme that affects amino acids and odd-chained fatty acids derived from the propionate metabolic pathways	<100 µg/d; chocolate, nuts, dried beans, peas and grains
Se	endemic cardiomyopathy (Keshan disease); white muscle disease; endemic osteoarthoropathy (Kash- in-Beck disease) with enlargement and deformity of the joints; liver necrosis; exudative diathesis; pancreatic atrophy; growth depression; depressed activity of 5'-deiodinase enzymes that produce tri- iodothyronine ( $T_3$ ) from thyroxine ( $T_4$ ); impaired immune response to viral infections; anticarceno- genic activity; essential component of glutathione peroxidase and "selenoprotein-P"	55 - 70 μg/d; seafood, organ meats; meats; cereal grains grown on Se-rich soils; Brazil nuts; Se con- centrations in plant foods can vary greatly depend- ing on the available Se content of the soil where grown and the plant spe- cies grown
Si	depressed collagen content in bone with skull struc- ture abnormalities; long bone abnormalities; de- creased articular cartilage, water, hexosamine, and collagen; decreased levels of Ca, Mg, and P in tibias and skulls under Ca deficiency conditions	5 - 20 μg/d (est.); unre- fined grains, cereal prod- ucts; root and tuber crops
V	death proceeded by convulsions; skeletal deformi- ties; increased thyroid weight; participates in oxida- tion of halide ions and/or the phosphorylation of receptor proteins	<10 μg/d (est.); shellfish, mushrooms, black pepper, dill seed
Zn	loss of appetite; growth retardation; skin changes; immunological abnormalities; difficulty in par- turition; teratogenesis, hypogonadism; dwarfism; impaired wound healing; suboptimal growth, poor appetite, and impaired taste acuity in infants and children; diarrhea; impaired immune function; constituent of numerous enzymes; cellular mem- brane stability function	15 mg/d; animal prod- ucts especially red meats, cheese, legume seeds and pulses

<sup>†</sup> Sources of information: World Health Organization, 1996; National Research Council, 1989.

\* Reported daily allowances are for adult men. For elements not generally recognized as essential, the "est." indicates values that are only estimates from the literature.

## **Global Perspectives on Trace Element Deficiencies**

All biological systems depend on essential nutrients in balance to thrive. Lack of any one nutrient will lead to loss in productivity, disease states, and ultimately death. Thus, it is paramount that all biological systems receive their required nutrients in appropriate amounts during all seasons. Nearly all human food systems on earth are dependent on agriculture as their primary supplier of nutrients. If agriculture cannot provide adequate amounts of all nutrients, these food systems become dysfunctional and malnutrition ensues. The question of how agriculture can best feed a burgeoning human population when faced with unprecedented challenges occupies the minds of world leaders today. The human population is already as much as three times the population defined by global ecologists as sustainable (Evans, 1998). Moreover, the land available for productive agriculture is nearing its maximum; other resources, such as energy and fertilizer, are also reaching resource limits. In such a context, this chapter focuses on the use of nutrient fertilizers that themselves may be reaching limits but a consideration of trace element needs of these future crops offers some scope for optimism because essential trace elements can greatly increase the efficiency of use of the macronutrients (e.g. N, P, and K) in food systems.

Trace element deficiencies in soils worldwide were studied by Sillanpaa (Sillanpaa, 1990; Sillanpaa, 1982). He investigated 190 representative soils from around the world. While 190 is a small sample of the total number of soil types, this survey was easily the most detailed nutritional study of soils ever completed. In particular, Sillanpaa utilized field experiments with fertilizers and several crops, and conducted plant analysis on their tissues, a strategy that is more sensitive for assessing trace element requirements than the more common soil analysis tests. Therefore, Sillanpaa's work gives us a better overall perspective on the incidence of trace element and macronutrient deficiencies worldwide. In particular, his use of growth responses to a target nutrient when all other nutrient requirements have been met is particularly meaningful and so rarely used in soil surveys. By this means, Sillanpaa was able to assess what he called the latent deficiency of each element (**Table 5**), which is the severity of deficiency of other nutrient elements is relieved.

Deficiency	Ν	Р	K	В	Cu	Fe	Mn	Мо	Zn
Acute	71	55	36	10	4	0	1	3	25
Latent	14	18	19	21	10	3	9	12	24
Total	85	73	55	31	14	3	10	15	49

**Table 5.** Percentage of 190 worldwide soils deficient in N, P, K, B, Cu, Fe, Mn, Mo,and Zn (Data from Sillanpaa, 1990).

Sillanpaa's prodigious work therefore gives us the most detailed and valid picture of life in the soil of planet Earth from the viewpoint of the mineral nutrients.

Firstly, deficiencies of macronutrients N, P, and K are the most common, these being deficient for crops in 55 to 85% of all soils. Deficiencies of essential trace elements are also widespread if slightly less frequent than for macronutrients. For example, nearly half of all soils are deficient in Zn, but only 25% of soils express Zn deficiency in the natural state while another 24% of all soils were Zn deficient for crop growth when greater limitations, generally N, P, and K, were first treated with the appropriate fertilizer. Thus, the overall extent of Zn deficiency in world soils matches the extent of Zn deficiency in the human population and published maps for each factor are remarkably similar (Hotz and Brown, 2004; Alloway, 2008; Graham, 2008). The similarity in both pattern and extent suggests the possibility of direct causation; however, there is no such similarity of pattern and extent for B. Boron is the second most common micronutrient deficiency in crops, being deficient in 31% of all soils in Sillanpaa's study, yet B is not yet fully established as an essential trace element for humans and symptoms of B deficiency in humans are rare if any (Hunt, 2003). Contrary to expectations, Fe deficiency in plants is not common (only 3% in Sillanpaa's study) whereas Fedeficiency anemia is the most common mineral deficiency in humans with estimates varying from 35 to 80% of the world's population (Kennedy et al., 2003; Mason and Garcia, 1993). While Fe-deficiency anemia can be induced by other nutrient deficiencies in humans, genetic diseases and infections, it is generally regarded as dominantly due to Fe deficiency itself. Furthermore, Se and I are each deficient in soils occupied by nearly 1 billion humans, but are not known to be deficient for any land plants because they have not been shown to be essential for higher plants (Graham, 2008). While Se and I fertilizers are effective in eliminating deficiencies of these elements in animals and humans (Lyons et al., 2004; Cao et al., 1993) the farmer will not see a benefit in yield, nor will consumers see any visible difference in the harvest to denote that the food is more nutritious (see Graham et al., 2001 and 2007 for more information on issues concerning farmer and consumer acceptance of biofortified crops).

Because some trace element deficiencies are more common in acidic soils (B, Mo), others more common in alkaline soils (Mn, Fe), and Cu in highly organic and in sandy soils, it is highly likely that most soils are deficient in at least one trace element as well as several macronutrients. It follows that trace element deficiencies are just as widespread as those of macronutrients, but diagnosis is more difficult because high-quality analytical technique is necessary, together with an understanding of the potential for complex interactions between nutrients. Although essential trace elements are not expensive because of the small amounts required, it is costly to ignore them as they can severely limit the benefits of the costly macronutrient fertilizers used.

## Major Factors Affecting Available Levels of Essential Trace Elements in Food Systems

The levels of nutrients in soils are inherently higher in soils derived from mineral-rich 'basic' rocks, such as basalt and diorite, and lower in acidic rocks such as granite and rhyolite. Also important are the age of the parent materials, the extent of weathering and leaching by rain and the time over which these processes of soil development have occurred (Donald and Prescott, 1975).

The major factor affecting the availability of accessible trace elements in presentday soils is soil pH. Low pH decreases availability of B through leaching and it must be replaced by fertilizing with borates; whereas Mo is bound in acid soils and is commonly corrected by adding agricultural lime. On the other hand, high pH, especially in subsoil, decreases availability of the transition metals, Mn, Cu, Co, Fe, Zn, and Ni. High pH is not so easily corrected as low pH and the best agronomic approach is generally to add more of the limiting trace element (Cakmak, 2008). Often, a better strategy, especially where the alkalinity occurs primarily in the subsoil, is to sow varieties of crops more tolerant of these trace element-deficient subsoils. Tolerant varieties are usually more efficient at absorbing the limiting nutrient than standard varieties and may also store more in the seeds or other edible plant parts. As edible portions are often the seeds for next year's crop the agronomic advantage may also be in having a better crop establishment in the next generation (Graham et al., 2001).

Topsoil drying also decreases the plant's ability to absorb micronutrients from otherwise available forms (Holloway et al., 2010) and plants must obtain micronutrients from subsoils where availability is often low because of high pH and low density of roots. Under these conditions micronutrient-efficient genotypes express their superiority. Where the whole soil profile has been dried, water itself becomes the limiting factor.

The interaction of soil organic matter status and availability of micronutrients is an interesting one of mutual dependence. In our experience, organic matter does not accumulate in micronutrient-deficient soils and its build-up depends on relief of all nutrient deficiencies, because most organic matter comes from plant production in the soil itself. By binding nutrients in plant-available forms, organic matter contributes to sustainable productivity in soils that are are already moderately productive; in other words, nutrient deficiencies are more fundamental to increasing productivity than low soil organic matter.

## Impacts of the First 'Green Revolution' on Micronutrient Malnutrition in Resource-Poor Populations in Developing Countries

The population explosion following World War II resulted in a threat of mass starvation by 1960, a threat that led to the effort now known as the 'green revolution'. High yielding new varieties of maize, wheat, and rice combined with N, P, and K fertilizers and disease control in the crops dramatically increased food production in populous areas of Asia, and gradually spread to other areas of need, so that by 1980 the world was again in surplus for basic staples.

A decade later, the WHO began to recognize a widespread increase in Fe deficiency in humans, especially in resource-poor countries where vitamin A deficiency was also increasingly severe, followed later by increasing recognition of Zn deficiency in humans (WHO, 1996). Concurrently, deficiency of Se in large



**Figure 1.** Percent change in cereal and pulse production and in human populations from 1965 to 1999 for select countries, developing nations and world population (Graham et al., 2007).

areas of China and Africa became of increasing concern while I deficiency had been an increasing concern globally since the 1970s (Hetzel, 1989). In short, the importance of trace element deficiencies appeared to rise as the threat of energy and protein deficiency declined.

Pulse production in South Asia did not increase as dramatically as production of wheat and rice (Graham et al., 2007; Figure 1). As a result, per-capita pulse production actually declined. In some areas of the Bangladesh panhandle, pulse production had given way entirely to production of the 'green revolution' varieties of rice, the cheapest energy source available. Pulses are generally much denser in micronutrients (i.e. vitamins and essential trace elements) than rice (or wheat) and we have argued that the rise in micronutrient deficiencies in human populations was due to this replacement of a traditional pulse-rice-based or pulsewheat-based diet with rice or wheat alone (Welch and Graham, 1999; Welch et al., 1997). While this replacement went against tradition and culture, population pressure on the land and the much greater and more reliable yield of rice and its consequent lower price were the drivers of radical change. Coupled with this is the greater susceptibility of pulses to disease and environmental stresses such as flooding (to which rice is especially tolerant), drought, and heat. Thus, we have hypothesized that micronutrient deficiencies in human populations at epidemic proportions were the direct result of the first 'green revolution'. The unique, global nature of this event puts the above assertion beyond any prospect of rigorous scientific proof ('one treatment in one replication'), but as a highly rational working hypothesis, it directs how a second 'green revolution' must be focused if we are to avoid the colossal human cost of a further rise in the global

burden of micronutrient deficiencies and their impact on overall health of the human population.

Graham (2008) has argued the case for ranking Zn deficiency the most significant adverse micronutrient effect on humans of the first 'green revolution' and therefore, Zn fertilizer requirements (additional to N, P, K, and S) warrants special attention in the second 'green revolution' program. This is partly because Zn is the most widespread deficiency of a trace element for crops and also because of the role of Zn in Fe homeostasis in the human body (Yamaji et al., 2001; Iyengar et al., 2009; Balesaria et al., 2010). Correction of Se and I deficiencies may also make food-derived Fe more bioavailable (Lyons et al., 2004) and the synergy between Fe, Zn, and vitamin A has been well established since the 1970s (Thurlow et al., 2005). We argue that fertilizer enhancement of the trace elements Zn, I, Fe, Co, and Se and the deployment of provitamin A carotenoid-rich target crops together must rank of equal status with yield enhancement and environmental sustainability in this new effort for food security and healthier lives for all.

#### **Time-dependent Dilution of Grain-micronutrients**

Many observations of grain nutrient concentrations declining over historical time have been reported (Fan et al., 2008 and references therein) and this clearly has impact on nutrition of humans at the population level. However, these trends are the result of multiple factors and it is not so easy to deduce what possible causes should be addressed to reverse them. On the other hand, Ortiz-Monasterio (Monasterio and Graham, 2000) studied time trends in the impact of wheat breeding on nutrient concentrations in grain of wheat in a way that minimized time co-variants. All major CIMMYT wheat varieties released over the previous 40 years were grown together at Obregon and El Batan, Mexico. The excellent progress in breeding for yield was demonstrated in the trial, but there was only a small decrease in grain Fe and Zn concentrations over breeding time, despite the major yield increase.

#### Lessons Learned

The 'comparability' of nutrition and yield can be demonstrated in the reports published by Li and Haas (Li et al., 1994; Zhu and Haas, 1997) which showed that mildly Fe-deficient women needed 5 to 10% more calories to do the same physical work as an Fe-replete control-group. A 10% increase in wheat yield takes about 20 years to achieve in current Australian wheat breeding programs (Australian Agronomy Conference, 2008), in which time, additional micronutrient traits could be incorporated instead and achieve the same work capacity in a target population, and better health! Thus, in satiating a target population, breeding for micronutrient density may achieve greater health and at least equal work capacity for the same quantum of breeding activity. Additionally, there is need for higher yielding and stress-tolerant pulse crops that can compete with cereals for a part of the productive land. The ultimate goal of the second 'green revolution' must be adequate nutrition for all, not just adequate calories, and if achieved it will deliver far greater health (i.e. physical and mental capacity) and sustainability than did the first 'green revolution'. Nothing less than this complex

of goals will be an acceptable target for the second 'green revolution'. Higher yields of cereals, the first 'green revolution target', will not serve well an increasingly over-populated and under-nourished human race the second time around.

## **Bioavailability of Trace Elements in Foods to Humans**

The amount of a trace element that is absorbable and utilized by the body from a meal (i.e. the bioavailable amount) is an important parameter to consider when developing micronutrient-enhanced food crops that will have measurable impact on reducing micronutrient malnutrition in target populations (Welch, 2008; Hotz et al., 2007). Some trace elements are lost during processing and cooking; some are made unavailable for absorption from the gastrointestinal tract by binding to substances (antinutrients) in the meal that prevent their absorption from the gut or interfere with their utilization in the body once absorbed, making them metabolic inactive (Hotz et al., 2007; Fairweather-Tait and Hurrell, 1996; Welch, 2002). Furthermore, some may be absorbed into microbiota in the intestine being potentially lost from the body when microorganisms are excreted.

Determining the bioavailable amount of a trace element in a diet is extremely complex and difficult to assess in human populations because of the myriad of interacting factors involved (Welch and House, 1984; Welch and Graham, 2004; Matzke, 1998; World Health Organization, 1996). Thus, clinical efficacy trials under highly controlled conditions employing trace element isotopes (either radioactive or stable isotopes) are usually performed to measure trace element bioavailability in vivo from plant foods to humans (Turnlund, 2006). These types of studies are relatively expensive and of limited value with respect to free living populations eating mixed diets in developing nations although currently, in vitro isotope studies are the only method available to determine trace element bioavailability in humans. Further, many of the studies are carried out using isotopes added to food matrixes (i.e. "extrinsic tags") because labeling foods with isotopes intrinsically (i.e. using an isotope to label a plant during growth by adding it to the growth media) greatly increases the cost of isotope studies. Unfortunately, "extrinsic tags" are always equivocal because the added isotope "tag" may not equilibrate completely with the trace element bound to intrinsic factors in the food or in the diet as a whole (Jin et al., 2008). Generalizations gleaned from such clinical studies may not always reflect the true bioavailability of trace elements from plant foods to resource-poor people living in developing nations (Graham et al., 2001; Welch, 2002; Welch and Graham, 1999). The true impact of biofortified plant foods to the health of these target populations can only be ascertained by doing human effectiveness trials [i.e. performing studies in target populations in the local area before and after introduction (along with a control group) of essential trace element enhanced (biofortified) crops to a region and measuring the effectiveness of the intervention on improving the nutrition and health outcomes of the communities]. However, well designed effectiveness studies are difficult to carry out, very expensive and time consuming. For these reasons, model systems have been developed to aid plant breeders, in consultation with human nutritionists, in screening crops for nutritional quality traits

using *in vitro* human intestinal cell models (e.g. the Caco-2 cell model), animal models (e.g. rats, pigs and poultry) and algorithms to predict bioavailable levels of trace elements in crop breeding lines. All of these models have limitations which should be understood before using them in biofortification programs.

### The Human In Vitro Caco-2 Cell Model

Caco-2 cells are human epithelial colorectal adenocarcinoma cells cultured in vitro. They mimic small intestinal mucosal enterocytes in absorbing nutrients and can be used to rapidly screen plant foods for bioavailable Fe from in vitro digestions of plant foods, meals, and other experimental preparations (Sharp, 2005; Glahn et al., 1998; Glahn, 2009). Limitations of the in vitro Caco-2 cell model are that it is a tissue culture model based on cells that mimic human small intestinal cells and on an *in vitro* digestion methodology that may not reflect the effects of whole-organism intrinsic factors that can interact with the digestion of foods and absorption of nutrients from the gastrointestinal tract. It excludes the role of microbiota in the intestine, especially the large intestine, and their potential effects on trace element absorption. Further, as normally employed, it does not include dietary interactions with plant food constituents that can influence the bioavailability of trace elements. However, it is rapid and inexpensive allowing the ability to screen large numbers of plant genotypes in breeding programs. It is imperative that such Caco-2 cell screenings be followed up by animal models and human efficacy clinical trials before selecting nutrient enhanced genotypes for further advancement in wide scale breeding activities because of these limitations.

#### **Animal Models**

Various animal models have been used to determine trace element bioavailability from foods. These include small rodents (e.g. mice and rats), poultry, pigs, and primates. However, there are differences between these species and humans that should be addressed before selecting an animal model (Baker, 2008). Mice, rats, and poultry models have been used extensively because they are easily used, inexpensive and require little food to maintain and small doses of experimental material to perform experiments. Poultry models are inexpensive but they are not mammals having shorter intestines compared to mammals which could result in less efficient absorption of trace elements compared to humans. Pigs are thought to be the best model for mineral bioavailability studies although they are relatively expensive to use compared to small rodent or poultry models and require more experimental material to feed (Miller and Ullrey, 1987). They have a much longer intestinal system compared to humans which could result in higher absorption efficiencies compared to humans. While primates are the closest animal model to humans, their use is extremely expensive and they are difficult to maintain and use in bioavailability experiments.

#### Algorithms

Various algorithms (i.e. predictive equations) have been developed to try to predict the bioavailability of Fe and Zn from plant foods, meals, and diets (Beard et al., 2007; Hotz, 2005; Reddy, 2005; Lynch, 2005; Hunt, 1996). They rely on determining the concentration of a nutrient in a food/meal/diet and then allowing for the inclusion of factors that estimate the effects of inhibitors or enhancers of nutrient absorption from the food/meal/diet. For Fe, their use has been questioned because they do not predict the change in Fe status of people in efficacy trials held over long periods of time (Beard et al., 2007). Thus, it is highly recommended that current algorithms to estimate trace element bioavailability not be used as screening tools in plant breeding programs because none has proven to be accurate in predicting trace element bioavailability to free living populations at high risk of developing deficiencies of these nutrients.

## **Reactions with Food Components in the Human Gut**

Diet-related factors that can interact to influence the bioavailability of trace elements negatively or positively are numerous and include multiple food components such as: the physicochemical mineral forms (e.g. non-specific adsorption, solubility, trace element complex formations and ligand binding), trace element oxidation state [e.g. Fe<sup>2+</sup> and Fe<sup>3+</sup>], antinutrients (see below), promoter substances (see below), and competitive and non-competitive inhibition of trace element transport protein binding sites in intestinal enterocyte plasma membranes by elements with similar binding and chemical properties. Thus, all food components as eaten have to be considered when determining the bioavailability of trace elements in plant foods from a meal (Matzke, 1998). Some of the most studied factors are discussed below.

## Effects of Processing, Cooking, and Meal Components

Food processing, preparation, and cooking methods all have effects on the amount of a trace element retained in a meal and its ultimate bioavailability from plant foods as consumed (Matzke, 1998; Duchateau and Klaffke, 2009). There are numerous processing techniques that can have impact on the losses or gains of trace elements and their bioavailability from foods. These include: soaking, milling, polishing, heat treatments (e.g. boiling/cooking, blanching, steaming, pasteurization, parboiling, sterilization, canning, baking, and frying), drying, freezing, fermentation, germination, extrusion, packaging, storage, and home preparation methods. It is beyond the scope of this review to cover all of these potential processing and cooking techniques on trace element bioavailability. Refer to the following reviews for in-depth discussions of this topic (Matzke, 1998; Hotz and Gibson, 2007; Gibson et al., 2007; McClements and Decker, 2010; Hemery et al., 2007).

## Antinutrients (Inhibitors)

Staple legume seeds and cereal grains can contain high levels of antinutrients which can inhibit the absorption of polyvalent trace element cations (e.g.  $Fe^{3+}$  and/ or  $Zn^{2+}$ ) from the gut, reducing their bioavailability to humans. **Table 6** lists examples of some known antinutrients found in edible seeds and grains. There are other

unidentified antinutrients in plant foods because known antinutrients cannot account for all the negative effects of certain plant foods on trace element bioavailability; further research is needed to identify them. By far the most studied antinutrient in food crops is phytic acid (*myo*-inositolhexaphosphoric acid) that is known to inhibit Fe, Zn, and other polyvalent cation bioavailability to humans (Kumar, 2010). Certain phenolic and polyphenolic compounds have also been studied extensively as they relate to Fe bioavailability (Bravo, 1998).

Antinutrient	Major Staple Plant Food Sources
Phytic acid	Whole legume seeds and cereal grains
Phenols & polyphenols	Beans, sorghum, other whole cereal grains
Certain fibers	Whole legume seeds and cereal grains
Hemagglutinins (i.e. lectins)	Most legume seeds and wheat grain
Heavy metals (e.g. Cd, Hg, Pb)	Seeds and grains from crops grown in heavy metal polluted soils (e.g. Cd in rice grain)

Table 6. E	Examples of antinutrients in staple plant foods affecting trace element
n	metal bioavailability.

It is possible to greatly reduce the levels of antinutrients in staple seeds and grains through plant breeding by traditional breeding approaches or by including transgenic molecular biological approaches. However, this should be done with caution because many antinutrients play important beneficial roles in plant metabolism as well as in promoting human health.

### Phytic Acid

Phytate levels in staple plant foods can be reduced through plant breeding using low-phytate mutant genotypes or via genetic engineering. Doing so is not without risk. Phytate plays important roles in plant metabolism. Phytate is the major storage site for P in seeds. Phosphorus is hydrolyzed from phytate during germination for use in early embryo and radical growth by activation of seed phytases. Low-phytate mutants store much more P in the seed as inorganic P which rapidly diffuses away from the embryo and radical during imbibition. If the soil is low in available P, lowering phytate in the seed could have negative impacts on seedling growth. Significantly reducing phytate in seeds of staple food crops to levels that are needed to increase Fe and Zn bioavailability may decrease crop productivity especially when these crops are planted in P-deficient soils. For example, Meis et al. (2003) reported that low-phytate soybean seeds had significantly lower field emergence rates, lower viability, lower germination rates and lower cold vigor compared to normal-phytate seeds. Oltmans et al. (2005) reported that soybean seedling emergence was significantly reduced in low-phytate seeds compared to normal phytate seeds despite an identical total P in the seeds of compared lines.

Phytic acid has also been reported to have health benefits for humans. Some of the beneficial effects of phytate include:

- Decreases the risk of cancer (human cells tested include colon adenocarcinoma, erythroleukemia, mammary adenocarcinoma and prostrate adenocarcinoma
  - Up-regulates tumor suppressor genes (p53 and p21) in HT-29 human colon carcinoma cells
  - Involved in signal transduction pathways, cell cycle regulatory genes, differentiation genes, oncogenes and tumor suppressor genes
- Inositolpentaphosphoric acid (IP5) is shown to be a powerful anticarcinogen
- May play a role in preventing heart disease
  - Lowers serum cholesterol and triglycerides
  - Natural antioxidant lowering lipid peroxidation
  - Hydrolysate products may function in second messenger transduction systems
- Functions in neurotransmission, in exocytosis and in efficient transport of messenger RNA
- May lower renal calculi formation
- Decreases heavy metal bioavailability (e.g. Cd, Hg, Pb)
- Phytate, as a Zn-phytate complex, is required for iRNA editing enzymes and as such is required for all life

(From Zhou and Erdman, Jr., 1995; Liao et al., 2007; Grases et al., 2002; Shamsuddin, 1999; Saied and Shamsuddin, 1998; Shamsuddin et al., 1997; Jariwalla, 1992; Macbeth et al., 2005; Hanson et al., 2006; Lee et al., 2006).

Therefore, significantly reducing phytate in staple food crops may have a negative effect on chronic disease rates in populations dependent on these staples for sustenance. What should be done to reduce the negative effects of phytate on essential trace metal cation bioavailability to humans? Foods can contain promoter or "enhancer" substances that promote the bioavailability of essential trace metals even in the presence of antinutrients such as phytate in the diet. Increasing the levels of these substances in staple food crops is a highly desirable strategy to use and will be discussed subsequently.

### **Phenols and Polyphenols**

Phenols are found in numerous plant tissues as secondary plant metabolites. As a group, polyphenols are compounds that contain more than one phenol group per molecule. Polyphenols are usually divided into hydrolyzable tannins, condensed tannins and phenylpropanoids. The consumption of phenolic- and polyphenolic-rich plant foods has been shown to be beneficial to human health, lowering the risks of chronic diseases such as heart disease and cancers (Bravo, 1998; El Gharras, 2009). However, many of these phenolic and polyphenolic compounds are also antinutrients that can bind numerous trace elements in diets making them unavailable for absorption from the gut (Slabbert, 1992; Bravo, 1998). Others may act as antioxidants reducing the oxidation state of certain trace elements, such as  $Fe^{3+}$  to  $Fe^{2+}$  promoting their bioavailability (Duthie et al., 2000; Andjelkovic et al., 2006; Boyer et al., 1990). Most research on their effects on trace-element bioavailability has focused on Fe and Zn in food crops (Lopez and Martos, 2004). It is imperative that the chemical structure and functions of phenols in edible portions of staple food crops be known before trying to reduce their levels in these crops and so to enhance Fe bioavailability, assuring no adverse consequences to crop productivity and human health.

## Promoters (Enhancer Substances)

Some of the known trace element promoter substances found in foods, along with major dietary sources that can negate the effects of antinutrients in plant foods, are listed in **Table 7**. Unfortunately, only a few promoter substances have thus far been identified in plant foods [see (Graham et al., 2001; Welch, 2002; Graham et al., 2007; House, 1999)]. More research should focus on identifying promoter substances because knowing their identity would allow for

Substance	Trace Element	Major Dietary Sources
Certain organic acids (e.g. ascorbic acid, fumarate, malate, citrate)	Fe and/or Zn	fresh fruits and vegetables
Heme-Fe (e.g. hemoglobin)	Fe	animal meats
Certain amino acids (e.g. methionine, cysteine, histidine)	Fe and/or Zn	animal meats
Long-chain fatty acids (e.g. palmitate)	Zn	human breast milk
Meat factor (sulphated glycos- amineglycans, polypeptides rich in cysteine residues)	Fe and/or Zn	animal meats
$\beta$ -carotene and provitamin A carotenoids	Fe, Zn	dark green and orange vegetables
Inulin and other non-digestible carbohydrates (i.e. prebiotics)	Fe, Zn	chicory, garlic, onion, whole wheat grain, Jerusalem artichoke
Certain polyphenols (e.g. tannic acid, quercitin)	Zn	colored bean seeds, red wine, green tea, sorghum grain

Table 7. Examples of substances in diets that promote the bioavailability of Fe
and Zn from staple plant foods to humans (Welch, 2001).

breeding strategies to significantly increase their levels in staple food crops. Many of these compounds are normal plant metabolites and only small changes in their concentration may have significant effects on the bioavailability of essential trace elements. Further, the molecular mechanisms controlling their levels in plants may require fewer genes to regulate their biosynthesis. If so, it may be much easier for plant breeders to breed for such traits because of the fewer genes involved compared to the numerous genes required to manipulate the uptake, translocation, re-translocation and deposition of essential trace elements in edible portions of crop plants (Grotz and Guerinot, 2006; Welch, 1995). Therefore, it is highly recommended that plant breeders closely scrutinize this strategy when attempting to biofortify food crops as sources of essential trace elements for people. Further *in vivo* human efficacy studies may need to be carried out to confirm the enhancing effects of some of these substances that have been shown to be promoters in animal models. Following is a discussion of some promoter substances.

#### **Organic Acids**

Ascorbic acid (vitamin C) promotes the bioavailability of non-heme-Fe in plant foods to humans, primarily because it is capable of reducing  $Fe^{3+}$  to  $Fe^{2+}$  [as well as other transition metals (e.g. Cu<sup>2+</sup> to Cu<sup>1+</sup>)]. It has been the most studied Fe promoter substance identified in plants (Lopez and Martos, 2004; Fairweather-Tait, 1992). The Fe<sup>2+</sup> ion is the primary form of inorganic Fe transported by mucosal cells in the intestine via DMT1 in the apical enterocyte plasma membrane. Reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> causes destabilization of Fe<sup>3+</sup> ligand bonds with various organic and inorganic ligands (carboxyl-amines, phosphate esters, etc.) releasing Fe from these bonds making Fe more available for absorption from the diet. The reduction of ionic  $Fe^{3+}$  to  $Fe^{2+}$  can also occur by the action of the apical mucosal plasma membrane ferric reductase, DcytB (Donovan et al., 2006). Unfortunately, ascorbic acid is prone to oxidation to dehydroascorbate during cooking and storage, losing its ability to reduce Fe<sup>3+</sup> and its enhancing effects. Various other organic acids (e.g. citrate, fumarate, malate, oxalate, etc.) can form stable and soluble complexes with various trace element metal ions such as Fe<sup>3+</sup> and Zn<sup>2+</sup> helping keep these metal ions soluble during digestion thereby potentially promoting their absorption via intestinal mucosal cells depending on other dietary constituents and the physiological status of the individual (House, 1999).

#### **Amino Acids**

Some amino acids have been shown to promote the absorption of Fe, Zn, and other trace elements (Mertz, 1987; Mertz, 1986). For example, cysteine can promote both Fe and Zn bioavailability. Cysteine contains a reduced sulfhydryl group that can reduce some trace metals and also form soluble complexes with Zn<sup>2+</sup> and Fe<sup>2+</sup> ions making them more soluble and improving their absorption by mucosal cells (Li and Manning, 1955). Peptides rich in cysteine residues can promote Zn and Fe bioavailability. Cysteine can also form soluble complexes with other trace element cations enhancing their bioavailability. Histidine can form stable complexes with trace element cations such as Zn<sup>2+</sup> and Fe<sup>2+</sup> enhancing their absorption by mucosal cells (Freeman, 1973). Methionine can promote Zn absorption but does not form very stable complexes with  $Zn^{2+}$  ions. Apparently, methionine is required for the efficient absorption of Zn playing some biological role in its transport by mucosal cells. Thus, methionine deficiency will result in reduced Zn absorption rates (House et al., 1997).

#### Meat Factors

Animal meats are known to promote the absorption of non-heme-Fe and Zn from staple plant foods high in antinutrients such as phytate. Many attempts have been made to identify this factor in meats without complete success (Hurrell et al., 2006; Huh et al., 2004; Welch and House, 1995). Most research suggests that part of the positive effects of meat on Fe and Zn bioavailability is the result of peptides derived from meat that are rich in cysteine and histidine residues. Sulphated glycosamineglycans released from meat during digestion have also been suggested to play a role in the meat factor effect (Huh et al., 2004). More research is still needed to delineate the actual identity of the meat factor.

#### Prebiotics

Prebiotics are food substances that promote the growth of beneficial microorganisms in the intestine. The most studied are the non-digestible carbohydrates such as inulin (a fructan). These compounds have been shown to have positive effects on promoting the bioavailability of mineral nutrients (e.g. Fe, Zn, Ca, and Mg) (Manning and Gibson, 2004). The effects of human gut microbiota and their effects on human nutrition and health are just beginning to be recognized. Clearly, the effect of intestinal microbiota on our ability to utilize food, nutrients and phytochemicals is immense (Dethlefsen et al., 2007; Food and Agriculture Organization and WHO, 2006; Manning and Gibson, 2004). For trace element nutriture, probiotics (beneficial intestinal bacteria that promote health) may play important roles in their bioavailability from the diet which is discussed below.

The human intestine contains more bacteria than the eukaryotic cells of the body (i.e. at least 10 trillion microbial cells compared to about one trillion body cells). Microbiotic metabolic activity in the gut is equal to that of the body's vital organs and microbial tissue can account for 60% of the dry weight of feces (Steer et al., 2000). Studies have shown that host-microbe interactions are essential to normal mammalian physiology including metabolic activity and immune homeostasis (Dethlefsen et al., 2007). This microbial activity provides energy from undigested food substrates, trains the immune system, prevents growth of pathogens, transforms certain nutrients and beneficial phytochemicals into utilizable substrates, synthesizes certain vitamins, defends against certain diseases, stimulates cell growth, prevents some allergies, improves mineral absorption, produces anti-inflammatory effects, and so improves gut health in general.

Shifting the gut microbiota populations to more probiotic bacteria through dietary means may also have enhancing effects on Zn and other trace element absorption (Bouis and Welch, 2010). Providing prebiotics may overcome the negative effects of antinutrients on essential trace metal bioavailability because many bacteria in the gut can degrade antinutrients such as phytate and polyphenols releasing their bound metals and allowing absorption by enterocytes lining the intestine. Probiotic systemic effects on inducing the genes controlling the absorption of Fe and other metals from the intestine may enhance the bioavailability of these essential trace elements. Of equal and possibly more importance is the role of prebiotics in improving gut health and the intestine's ability to absorb and utilize numerous nutrients, regulate the immune system, and protect against invasion by pathogenic organisms. Thus, increasing the levels of prebiotics in staple food crops is an extremely important strategy to enhance the nutrition and health of malnourished people worldwide, especially resource-poor families with poor gut-health living in less sanitary environments. Current knowledge suggests that this strategy will be genetically more feasible (fewer genes in play) than the current HarvestPlus strategy of increasing the density of only a few nutrients in staple crops by plant breeding (www.harvestplus.org).

## **Interactions among Nutrients**

### **In Plant Nutrition**

An interaction is said to exist when the magnitude of response of an organism to a given level of one factor depends on the level of another factor. As an example, yield of a crop in a soil deficient in both N and P increases much more when both are added together than the sum of the responses of each added alone (synergy). Most interactions between two added essential nutrients that are deficient in the growth medium of plants are of this positive, synergistic type (unless other nutrients are more deficient still). In cases of unrecognized deficiencies, the addition of a fertilizer nutrient that is not the most deficient in the system can cause a yield decrease (antagonism) or at the very least no response. Such negative outcomes underline the need for advice from an experienced agronomist supported by the appropriate high quality plant analyses. An antagonistic interaction may occur between two micronutrients such as Cu and Zn (Gartrell, 1981). Typically in these cases, adding the more deficient nutrient results in a yield increase at relatively small cost.

Nutrients interact with other factors in the environment that also vary in severity so creating the possibility of interactions between fertilizers and environmental stresses. Any nutrient deficiency is likely to aggravate the effect of an environmental stress such as heat, cold, drought, water logging, fungal pathogens, salinity, direct drilling, topsoil drying, herbicide damage and seasonal differences such as the timing of the break of the season. For example, deficiencies of many trace elements and/or too much N commonly aggravate fungal pathologies (Graham, 1983; Graham and Webb, 1991; Wilhelm et al., 1985; Sparrow and Graham, 1988; Thongbai et al., 1993). Another vital interaction with nutrients is crop genotype. Some varieties are more tolerant of particular nutrient deficiencies than others; other genes control greater loading of trace elements into grain. Breeding for such tolerance is rewarding, and is often achieved empirically by plant breeders. These traits are both major-gene (Graham, 1984) and quantitative in nature (Loneragan et al., 2009; Cakmak et al., 2010), and are most valuable

when they provide tolerance to particular nutrient deficiencies in sub soils where the simple fertilizer option is not practicable. For trace element deficiencies in soils, breeding has been successful for some nutrients, including Fe, Mn, Cu, Zn, and B, but commonly these traits are quantitative, involving up to 20 loci [for Fe, (Fehr, 1982); for Zn, (Lonergan et al., 2009)].

#### In Human Nutrition

Micronutrient deficiencies in humans are well-researched and cover a large number of essential trace elements and vitamins. However, interactions between nutrients are not as well researched as in plants because of the great cost and difficulty of such clinical studies in humans. The synergy among Fe, Zn, and vitamin A is a system that was researched several decades ago (Thurlow et al., 2005; Kennedy et al., 2003; Garcia-Casal et al., 1998, and references therein). Each micronutrient can enhance the absorption, transport, or utilization of the others so that where two or all three of them are deficient, treatment of both or all three deficiencies at once with quite modest doses will achieve a marked recovery in health. Selenium, I, and Fe appear to have synergistic interactions in the deficiency range in the same manner (Lyons et al., 2004; Hotz et al., 1997; Contempre et al. 1991). Such synergies and antagonisms are characteristic of the micronutrients and emphasize the importance of addressing all deficiencies together in order to improve health.

#### The Link of Plant Nutrition to Human Nutrition

Deficiencies of essential trace elements in human populations, especially vegetarian populations, are obviously linked to the concentrations in their food plants and ultimately to the concentrations in the soils supporting their crops. However, these nutritional links are both weak and indirect for several reasons. Firstly, of the more than 40 essential nutrients for humans, only 17 elements are essential for plants. All the organic nutrients, mostly vitamins, can be synthesized *de novo* in plants and so are not plant nutrients that are by definition supplied externally. Secondly, it is the concentrations of nutrients in young leaves that determine a plant's sensitivity to micronutrient deficiencies whereas, in humans, it is the concentrations in the edible portions that are consumed and pass to the human gut where highly selective absorptive systems of the human body largely determine what is absorbed. Despite these complexities at the level of the individual, a general link appears to exist at the population level and as has been mentioned, a pattern of similarity at a global level exists between the distribution of Zn deficiency in the world soils and the prevalence of Zn-deficient diets in human populations (Alloway, 2008).

## Strategies to Address Micronutrient Malnutrition in Humans Using Agricultural Tools

*Biofortification strategies.* Biofortification is a name given to agricultural efforts to improve nutritional value of food crops (staples, mainly). The primary effort is through plant breeding, both conventional and biotechnological, but nutritional value can also be improved by the use of trace element fertilizers. Like plant

breeding, fertilizers can be used both to increase the yield and the concentration of specific nutrients in plant parts.

Plant breeding and biotechnology. The HarvestPlus Program (www.harvestplus. cgiar.org) utilizes mainly plant breeding to improve the nutritional quality of cereals, pulses and root crops for Fe, Zn, and vitamin A. Conventional breeding uses quantitative traits for breeding for Fe-dense and Zn-dense cereals, beans, and potato and major genes for raising the  $\beta$ -carotene concentrations in sweet potato, cassava, and potato. Improved lines have been developed and the traits have been moved into adapted cultivars of several crops for use in seven target countries in Africa and South Asia. The program is now in the second phase where these first-wave biofortified varieties are being used for proof-of-concept feeding trials in target areas. Ongoing breeding efforts produce more nutrient-dense, higher-yielding varieties for future release in new regions of the develop-ing world (Pfeiffer and McClafferty, 2007).

Workers in many institutions around the world are using biotechnology strategies to develop superior, nutrient-dense lines (e.g. Fe, Zn, and provitamin A carotenoids), but to date there have been problems of stability, density, yield penalty, public acceptance, and regulation to overcome and no high-density transgenic crops have been released for farmer use.

In our opinion, the greatest prospects for high impact on micronutrient malnutrition in subsistence populations in developing countries may be through breeding staple crops with high levels of prebiotics, as discussed previously, although human efficacy trials of prebiotic enriched biofortified crops need to be carried out. We have already demonstrated that the major modern cereals, wheat, rice, maize, and sorghum host genetic variation for prebiotic content of edible parts and the genetics is relatively simple and less affected by environment (Huynh et al., 2009; Stoop et al., 2007; Weyens et al., 2004). Finally, the absorption of Fe and Zn, as well as Ca and Mg, may be improved together (Manning and Gibson, 2004; Yasuda et al., 2006). Preliminary animal model trials have begun with staples supplemented with exogenous prebiotics and will extend to new varieties with sufficient natural prebiotic content (see for example Yasuda et al., 2006). Thus, in our view, clinical feeding trials with relevant populations are an urgent need.

*Fertilizer biofortification.* Fertilizer technologies for biofortifying staple food crops (frequently referred to as agronomic biofortification) with essential trace elements have been 'on the shelf' for decades, although a more recent technology, fluid fertilizers, may be an important advance that will benefit both yield and micronutrient value compared to current solid fertilizers (Holloway et al., 2008). The fertilizer biofortification strategy is likely to be a relatively poor means of biofortifying Fe because of its rapid oxidation and binding to soil colloids as well as the plants' tight homeostatic control of Fe uptake and translocation in plants, compared to biofortifying for Zn, I, and Se (Lyons et al., 2004). Because plants can synthesize vitamin A *de novo*, it is not a plant nutrient. The fertilizer strategy is likely to be most successful for Zn because of its widespread deficiency in soils,

crops and humans, as demonstrated in Turkey and Australia (Cakmak, 2009; Holloway et al., 2008) and its effectiveness and relatively high Zn fertilizer availability worldwide.

Graham (2008) has argued that one of the reasons for the rise of micronutrient deficiencies in humans during and after the first 'green revolution' was the extensive use of N and P fertilizers on the new high-yielding varieties where N and P had not been used before. He argued that when soil Zn status is low, additional N and P aggravated the low Zn status of the soil/crops and induced more extensive and overt deficiencies of Zn in these new varieties (Loneragan and Webb, 1993).

It is essential that the proposed 'new green revolution' use all these available agricultural tools to enhance the nutritional quality of plant food products if we are to find sustainable solutions to micronutrient malnutrition in the world.

## **Concluding Remarks**

Over 30 million people die of malnutrition each year making it by far the leading cause of death globally (Bouis and Welch, 2010). Many of these deaths are the result of deficiencies of essential trace elements, especially Fe, Zn, and I. Malnutrition, including trace element deficiencies, is the result of dysfunctional food systems based in agricultural systems that provide the nutrients to feed the world. Thus, farmers should be thought of as nutrient providers. Unfortunately, agriculture has never had an unequivocal goal of improving human health and the nutrition and health communities have never used agricultural tools as a primary strategy to address malnutrition. This must change! The first 'green revolution' staved off famine for millions by producing bumper crops of rice, wheat and maize but had the unforeseen consequence of reducing diet diversity and contributing to the rapid growth in micronutrient malnutrition in the developing world. The future requires that we closely link agriculture to human health to find sustainable ways to reduce micronutrient deficiencies. Biofortification of staple food crops through plant breeding is one such strategy that can contribute to reducing micronutrient malnutrition. Another is the use of fertilizer technologies applied to increase certain essential trace elements in the crops that feed the world's poor. The inclusion of animal/fish meats in the diets of the poor is another strategy. There is nothing more important than supplying all the nutrients required for good health, felicity, and longevity of the human race. The sustainable means to this end must come from agriculture. **FCHH** 

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Chapter 4

# Agronomic Biofortification of Food Crops with Micronutrients

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# Abstract

Agronomic biofortification of food crops might be an effective component of a food system strategy to reduce micronutrient malnutrition in human populations. The suitability of different mineral micronutrients for this approach is reviewed. In general, Fe is an unsuitable candidate for agronomic biofortification, while I and Co can be effective, especially for increasing concentration of these micronutrients in leaves. Agronomic biofortification can be highly effective for Zn and Se. For Zn, a combination of soil and foliar application (or two strategic foliar applications around late booting and early milk stages) appears most effective, and Zn sulphate is a suitable, inexpensive form to use. For crops growing on low Zn soils, there can be additional benefit of likely yield increase in the following crop grown with Zn-biofortified seeds. The traits of tolerance to low Zn soil and high accumulation of Zn in grain are controlled by separate genetic systems. For Se, depending on soil type, either soil or foliar application can be highly effective. As with Zn, timing of foliar application is important: a single application around mid booting stage or early milk stage is often effective. Sodium selenate is generally much more effective than selenite for soil application. Both Zn and Se are valuable, essentially non-renewable, resources; hence further research to maximize the efficiency of their application to food crops or foods is very important. This includes combining foliar application of Zn and Se with urea, application of organic materials, and intercropping. Application of micronutrients together with fungicides or insecticides to contribute to biofortification of food crops appears to be a further important research area. Farmers would need a yield

Abbreviations specific to this chapter: ATP = adenosine tri-phosphate; CIAT = Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture); DTPA = di-ethylene tri-amine penta-acetic acid; EDTA = ethylene diamine tetra-acetic acid; IDD = iodine deficiency disorders; KBD = Kashin-Beck disease; NWAFU = Northwest Agricultural and Forestry University, Yangling, China; UK = United Kingdom; UVB = ultraviolet-B; WHO = World Health Organisation.

For symbols used commonly throughout this book see page xi.

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incentive to apply micronutrients to their crops, which would usually be obtained only on low Zn soils, or a subsidy.

# Introduction

Malnutrition is the most important cause of global mortality, with over 50% of deaths due to diet-related diseases. Micronutrient deficiencies, notably Fe, Zn, Se, I, and various vitamins are widespread globally, affecting well over half of the world's population, and several deficiencies often occur together (WHO, 2003). Dysfunctional food systems fail to deliver optimum nutrition to populations. It is no longer sufficient to consider agriculture solely in terms of total production; rather it needs to be viewed as the core of a productive, sustainable, nutritious food system (Graham et al., 2001).

Biofortification of staple crops with micronutrients by breeding/genetic engineering (*genetic* biofortification) or by fertilization (*agronomic* biofortification) to achieve higher micronutrient concentration in edible parts is an important part of a strategy to address dietary deficiencies (Storsdieck gennant Bonsmann and Hurrell, 2008), with the potential to reach the neediest of the population, usually the rural poor. Other methods include increasing dietary diversity, process fortification, direct supplementation and supplementation of livestock (Lyons et al., 2003; Haug et al., 2007).

It is important that any biofortification strategy does not compromise agronomic and end-use characteristics in order to attract/retain the interest of producers and consumers. A farmer will not be interested in a high-Fe wheat which yields lower than his usual variety (Bouis and Welch, 2010; Cakmak et al., 2010a). High grain mineral levels are not detectable by consumers, thus raising issues such as product identification and branding (Pfeiffer and McClafferty, 2007). Bioavailability of micronutrients in food products is another important factor (Welch and Graham, 2004).

Previous research suggests that genetic biofortification may be more suitable for increasing pro-vitamin A carotenoids and Fe, whereas an agronomic strategy may be more effective for Zn, Se, and I (Cakmak, 2008; Lyons et al., 2008). For pro-vitamin A carotenoids there exists substantial genotypic variation in sweet potato, banana, and cassava to support a conventional breeding approach (Chavez et al., 2000, 2005; Bouis and Welch, 2010; Genc et al., 2010). Genetic engineering also has an important role in micronutrient biofortification as shown by the high-carotenoid *Golden Rice* (Potrykus, 2003). Biofortification of food crops with micronutrients by using the classical or modern breeding tools or by applying transgenic approaches is a long-term process. In addition, the success of genetic biofortification may also depend on the readily available amounts of micronutrients (i.e. Zn, Se, and Fe) in the soil solution. Agronomic biofortification is a short-term solution to the problem and represents a complementary approach to genetic biofortification. In the following, the possibilities for agronomic biofortification with individual micronutrients will be discussed.

# Iron, Iodine, and Cobalt

#### Iron

Grains of most modern wheat cultivars with high yield potential are poor sources of micronutrients, especially Fe and Zn (Cakmak et al., 2010a). Iron concentration in grain is generally in the range of 20 to 35 mg/kg (Rengel et al., 1999; Zhang et al., 2008) and is occasionally over 100 mg/kg (Rengel et al., 1999). However, grains of ancient wheats such as *Triticum dicoccoides* usually have higher concentrations of micronutrients than modern bread wheats, with Fe commonly in the 40 to 100 mg/kg range (Cakmak et al., 2004; White and Broadley, 2005, 2009; Cakmak et al., 2010a).

Genetic variability is being intensively exploited under the HarvestPlus Biofortification Challenge Program (www.harvestplus.org) to improve modern wheat cultivars and other staple food crops for both high concentrations and high bioavailability of Fe and Zn in grains (Cakmak et al., 2010a; Genc et al., 2010). Moreover, close relationships between concentrations of protein, Fe, and Zn have been found in the grain of wheat, triticale, maize, and sorghum. This suggests that the genes controlling their concentration are co-segregating (Cakmak et al., 2010a). Thus, selection for higher protein in wheat could be expected to increase grain Fe and Zn concentration as well.

On a cautionary note, a plausible target level of 40 mg/kg Fe in white wheat flour may be difficult to attain as most of the Fe is removed during milling, and bioavailability of non-heme Fe (which constitutes all plant-derived Fe and over 50% of animal-derived Fe) is low, usually in the range 2 to 20%, compared with 15 to 35% for heme Fe (Storsdieck gennant Bonsmann and Hurrell, 2008).

Iron has proved to be difficult to biofortify, especially by agronomic means (Rengel 1999; Welch 2001). Inorganic Fe fertilizers applied to soil are usually ineffective due to rapid conversion of Fe<sup>2+</sup> into plant-unavailable Fe<sup>3+</sup> forms (Rengel et al., 1999; Frossard et al., 2000; Zhang et al., 2008). Iron provided in chelate form is usually more available, but is expensive and may be effective at overcoming Fe deficiency but be only marginally better than inorganic Fe for increasing grain Fe concentration. Foliar application of FeSO<sub>4</sub> has been a little more effective than soil application at increasing grain Fe concentration in cereals, and can increase yield of crops growing on soils with low Fe availability (Rengel et al., 1999).

Process fortification with Fe has a long history, and foods which have been used successfully for Fe fortification include rice, fish, soy sauce, wheat flour and maize flour, milk, and infant formulas. Large-scale fortification of flour or salt can be an effective way to supply Fe to the urban poor, but reaching remote rural poor populations is difficult (Storsdieck gennant Bonsmann and Hurrell, 2008).

These issues suggest that genetic engineering may prove to be the best way to increase bioavailable Fe in food crops. For example, Fe concentration in rice can be increased up to three-fold by incorporating the ferritin gene from soybean (Goto et al., 1999). The current challenge for Fe biofortification is to show that Fe can be increased to nutritionally useful levels and be bioavailable (Storsdieck gennant Bonsmann and Hurrell, 2008). In the meantime, greater dietary diversity (e.g. increasing consumption of legumes, leafy vegetables and nuts, especially if meat, eggs or fish are either unavailable or too expensive) should not be overlooked.

#### Iodine

Supplementation using iodised salt has proved effective in alleviating iodine deficiency disorders (IDD) in many countries (Rengel et al., 1999); thus I biofortification is perhaps less of a priority than biofortification with Zn, Se, or Fe, given the cost-effectiveness of salt iodisation (Storsdieck gennant Bonsmann and Hurrell, 2008). However, in some places these programs have failed due to infrastructure or cultural problems. In such cases a *food system* approach based on agronomic biofortification may be necessary, and in one area this was a spectacular success. In Xinjiang province in north-west China, potassium iodate (5%) was dripped into irrigation canals and resulted in a three-fold increase in soil I levels, a two-fold increase in wheat straw I, a 50% reduction in infant mortality, and IDD were largely eliminated. Benefits were evident up to seven years later (Cao et al., 1994; Jiang et al., 1997). This program provides an example of effective agronomic biofortification by *fertigation*.

Plants generally accumulate more I when it is supplied as iodate rather than iodide (Mackowiak and Grossl, 1999; Dai et al., 2006), despite the likelihood that iodate needs to be reduced to iodide for plant uptake (Mackowiak and Grossl, 1999). Moreover, iodate is more stable, especially in tropical climates (Diosady et al., 2002).

In field trials conducted by CIAT and the University of Adelaide in Colombia with cassava, there was no increase in I in storage roots from targeted application of 115 g I/ha (as iodide) to soil four weeks after planting (Lyons, G., F. Calle, Y. Genc, and H. Ceballos, unpublished, 2008). In China, in field trials on the Loess Plateau conducted by the Northwest Agriculture and Forestry University (NWAFU) and the University of Adelaide using a similar I application (but in the form of iodate, and comparing soil and foliar application), there was no I increase in maize, wheat or soybean grain or potato tubers. Cabbage was the only crop where I increased significantly (Wang, Z., H. Mao, G. Lyons, unpublished, 2010).

Iodine in plants is transported almost exclusively in xylem (Mackowiak and Grossl, 1999), hence it is relatively easy to biofortify leaves (and thus leafy vege-tables such as cabbage, lettuce, spinach) using soil-applied iodate, but difficult to increase I levels in grain or storage roots/tubers (Mackowiak and Grossl, 1999). Nevertheless, the Xinjiang program demonstrates that human I status can be significantly improved when I-enriched leaves and rice/wheat husks are eaten by animals and chickens, whose products, or who themselves, are subsequently eaten by humans.

#### Cobalt

Cobalt is required for  $N_2$  fixation by *Rhizobium* species in legumes and in root nodules of certain non-legumes (e.g. alder, *Alnus glutinosa*). In legumes grown in Co-deficient soils, root nodule activity generally increases when Co is supplied (Yoshida, 1998; Marschner, 2002). However, there is a lack of evidence for a direct role of Co in the metabolism of higher plants. Cobalt is essential for ruminants, as rumen microflora are able to synthesise enough vitamin B12 (in which Co is a co-factor) to meet the animal's needs (Marschner, 2002). Humans and other non-ruminants require pre-formed vitamin B12, which has an important role in red blood cell formation and is sometimes referred to as "the antipernicious anaemia factor" (Krautler, 2005). Vitamin B12 is supplied in animal and certain microbial products, but generally not in plants. Thus biofortification of plants with Co can benefit humans if provided through plants consumed by ruminants, which incorporate it in vitamin B12.

# Zinc and Selenium

Evidence to date suggests that Zn and Se are the most suitable mineral micronutrients for biofortification, in particular using the agronomic approach.

#### Zinc

#### Breeding for higher grain Zn

As discussed above for Fe, plant breeding represents a promising and cost effective strategy for biofortification of food crops with Zn. However, achievement of a desirable increase in grain Zn concentration by breeding depends largely on existence of sufficient genetic variation for seed/grain Zn concentration and maintenance of an adequate pool of available Zn in soils. Moreover, genetic variation for grain Zn concentration within or among the high-yielding cereal species is, however, very narrow and not promising to contribute to a successful breeding program. In a recent review paper, Cakmak et al. (2010a) reported genetic variation for grain Zn for a range of wheat germplasm. On average, in modern wheat germplasm from different origins grain Zn concentrations ranged from 24 to 44 mg/kg, while in low-yielding germplasm of different wild wheats the range of grain Zn concentration was between 36 to 132 mg/kg. These results suggested that wild wheats represent a promising genetic source to be exploited in breeding programs aiming at improving grain Zn concentration.

Among wild wheats screened for grain Zn, *Triticum dicoccoides* showed the largest genetic variation and the highest grain Zn concentration in grain (Cakmak et al., 2004). Highly promising *Triticum dicoccoides* genotypes have been identified containing up to 190 mg/kg (Cakmak et al., 2004; Peleg et al., 2008). Since wild wheats have generally very low grain yields, higher concentrations of Zn in wild wheats should be carefully evaluated due to "concentration effects" resulting from low grain yield capacity. In the studies using transgenic approaches, large increases in seed concentrations of Zn and also Fe have been reported following expression of the targeted proteins in seeds (e.g. ferritin) (Goto et al., 1999; Lucca et al., 2006; Drakakaki et al., 2005). However, most of these studies did not report seed yield per plant. It is important to highlight that biofortification of seeds with Zn and Fe at desirable levels for human nutrition should be realized without loss in grain yield. Otherwise, acceptability and release of the newly developed biofortified genotypes may be seriously restricted.

The plant breeding approach might be also adversely affected by low levels of plant available Zn concentrations in soils. Nearly half of the cereal-cultivated soils are affected by low levels of plant available Zn concentrations due to adverse soil conditions such as low levels of soil moisture and organic matter and high levels of soil pH and CaCO<sub>2</sub> (Cakmak, 2008). Soil moisture is a key factor in occurrence of Zn deficiency in plants. The transport of Zn to root surfaces takes place via diffusion that is largely influenced by soil moisture (Marschner, 1993). Any decline in soil moisture significantly depresses transport of Zn to the root surface and thus its uptake by roots. Cereals, especially wheat, are mainly cultivated in semi arid regions where topsoil is often dry and root uptake of Zn reduced. It is therefore not surprising that Zn deficiency in wheat often occurs when water supply to soil is impaired due to limited precipitation and irregular distribution of rainfall as reported for Australia (Graham et al., 1992) and Turkey (Ekiz et al., 1998; Bagci et al., 2007). Maintaining a high amount of plant available Zn in soil in semi-arid regions is a particular issue to contribute to grain Zn concentration and also better grain yield.

In Turkey, where soil Zn deficiency is a well-known problem, grain Zn concentrations of various wheat cultivars range between 15 to 25 mg/kg and 8 to 12 mg/kg on soils with adequate and low concentrations of plant available Zn, respectively (Cakmak et al., 2010a). High soil pH and low soil organic matter have been shown to be the main reasons for low Zn availability to plant roots in Turkish soils. Similar soil problems and widespread occurrence of soil Zn deficiency have been also reported for India, Pakistan, China and several other developing countries. There are nearly 50 M ha of low Zn soils in China which are found mostly in northern, calcareous soils (Zou et al., 2008). It is therefore not surprising that there is a close geographical overlap between the reported soil Zn deficiency and incidence of human Zn deficiency in different countries (Cakmak, 2008).

In soils with adverse soil chemical conditions and thus low plant available Zn concentrations, the genetic capacity of the new biofortified genotypes to accumulate Zn at desirable levels for human nutrition could be seriously hampered. This may affect the success of breeding programs for enrichment of food crops with Zn. Therefore, maintenance of an adequate level of plant available Zn in soils is a critical issue for biofortification of food crops with Zn. Recently it has been reported that continual root uptake and transport into seeds during the grain filling period is of great importance for accumulation of Zn into grain (Waters and Grusak, 2008; Kutman et al., 2010). These results emphasize that plant breeding and agronomic biofortification approaches should not be considered as separate

approaches to the problem; by contrast, they are complementary approaches and act synergistically.

#### Agronomic biofortification with Zn

Application of Zn fertilizers is a rapid solution to the problems of both Zn deficiency and low Zn in grain. Zinc fertilizer trials have been conducted for different food crops; but these experiments focused more on correction of Zn deficiency and increasing grain yield. Little attention has been paid to nutritional quality of grains and measurement of grain Zn concentrations. With the start of the HarvestPlus Biofortification Challenge Program, there is a growing interest in biofortification of food crops with Zn by using plant breeding and agronomic approaches.

## Types and rates of Zn fertilizers

Zinc sulphate (ZnSO<sub>4</sub>) is the most commonly used Zn fertilizer applied either as Zn sulphate heptahydrate (with 7 mol water) or as Zn sulphate monohydrate (1 mol water) in agriculture. Other compounds including Zn oxide (ZnO) and Zn-oxy-sulphate are also being used increasingly. Use of ZnO as a source of Zn is popular due to its cheaper price and higher content of Zn per molecule. Recent advances in particle size management of micronutrient fertilizers (Moran, 2004) indicate that ZnO may represent a good source of Zn for coating seeds and addition to granular fertilizers and foliar applications because of modifications in its chemical availability in soils and on plant leaves. As discussed below, in terms of correcting Zn deficiency in crop plants, ZnO and ZnSO, are similarly effective but in terms of their role in biofortification of food crops with Zn, ZnSO is more effective than ZnO (Mordvedt and Gilkes, 1993; Cakmak, 2008; Shivay et al., 2008). Zinc-containing compound fertilizers are used extensively, especially in Turkey, India, Australia, and South Africa. A well-known chelated form of Zn is ZnEDTA, but due to its high cost, its use in agriculture is limited. In addition, ZnEDTA is not superior to ZnSO, in correction of the Zn deficiency problem. Martens and Westermann (1991) reported 0.5 to 1.0 kg Zn/ha as the most commonly used rates of Zn in foliar applications. Foliar application of Zn fertilizers can be performed by using either ZnSO<sub>4</sub> or chelated forms of Zn (e.g. Zn-EDTA). Timing of foliar Zn application is probably the most critical factor determining the effectiveness of foliar applied Zn fertilizers in accumulation of Zn in grains. It is expected that large increases in loading of Zn into seed can be achieved when foliar Zn fertilizers are applied to plants at a late growth stage (Yilmaz et al., 2007; Cakmak, 2008). In a recent paper it has been shown that foliar spray of Zn late in the growing season in wheat (e.g. at heading and early milk stage) grown under field conditions resulted in much greater increases in grain Zn concentration when compared to the applications of Zn at earlier growth stages such as at the stem elongation and booting stages (Table 1; Cakmak et al., 2010b). Increases in concentration of whole grain Zn through soil and/or foliar Zn applications were also well reflected (proportionally) in all grain fractions analyzed (e.g. embryo, aleurone, and endosperm fractions), especially in the

endosperm, the part predominantly consumed in food products in target countries (Table 1).

#### **Table 1.** Zinc concentrations of whole grain and the grain fractions bran, embryo, and endosperm of durum wheat cultivar Selcuklu grown under field conditions with (50 kg ZnSO<sub>4</sub>·7H<sub>2</sub>O/ha) and without soil Zn application and foliar spray of 0.5 % ZnSO<sub>4</sub>·7H<sub>2</sub>O (approx. 4 kg ZnSO<sub>4</sub>·7H<sub>2</sub>O/ha) at different growth stages in the Konya location (Cakmak et al., 2010b).

Soil Zn		Zn concentration, mg/kg				
appl., kg/ha	Foliar Zn application stages	Whole grain	Bran	Embryo	Endosperm	
	Control (no Zn)	11.7	20	38	8	
0	Stem + Booting	18.8	28	47	10	
0	Booting + Milk	26.9	35	62	15	
	Milk + Dough	25.4	41	63	15	
	Control (no Zn)	21.7	33	52	11	
50	Stem + Booting	25.5	34	58	13	
50	Booting + Milk	29.3	45	69	16	
	Milk + Dough	25.4	41	63	15	
	LSD <sub>0.05</sub> for soil Zn application	1.8	3.0	3.4	1.0	
	$\mathrm{LSD}_{_{0.05}}$ for foliar Zn application	2.6	4.8	4.2	4.8	

On soils with very low plant-available Zn, foliar application of Zn was also very effective in reducing the phytate concentration in grain (Erdal et al., 2002; Cakmak et al., 2010a). Previously, it has been shown that Zn-deficient plants have higher root uptake and root-to-shoot translocation capacity for P (Loneragan et al., 1982; Cakmak and Marschner, 1986). Phosphorus is the main storage compound of phytate in grain. Consequently, a reduction in root uptake and shoot transport of P by Zn fertilization caused reduction in phytate concentration in grain and thus in phytate/Zn molar ratio (Cakmak et al., 2010a). The phytate/Zn molar ratio is believed to be a good indicator for bioavailability of Zn in diets. By complexing Zn, phytate has a significant role in reducing bioavailability of Zn in diet and utilization of Zn in the human body.

There are various examples showing that the Zn fertilization strategy is a quick and effective way in biofortifying food crops with Zn. Field tests on Zn deficient soils in Central Anatolia showed that soil Zn application of  $ZnSO_4$  improves not only grain yield but also grain Zn concentrations. In the case of the combined application of Zn through soil and foliar, increases in grain Zn concentrations are particularly high, resulting in increases of up to three-fold. Effectiveness of soil Zn application in improving grain Zn concentration was also showed in India and Australia. Rates of 25 to 50 kg ZnSO<sub>4</sub> per ha are generally used in fertilization of soils with Zn (Cakmak, 2008).

Zinc-enriched fertilizers like Zn-coated urea or Zn-enriched NPK fertilizers have been used for many years in Turkey, Australia, and South Africa. Such fertilizers seem to be highly promising for adoption by farmers since their use does not require additional field operations. Field studies using Zn-coated urea fertilizers in India showed impressive results in terms of both improving grain yield and increasing grain Zn concentration in rice and wheat (Shivay et al., 2008). For example, in aromatic rice growing in rice-wheat cropping systems, application of prilled urea enriched with Zn (in form of ZnSO) up to 3% of the prilled urea enhanced grain yield from 3.87 to 4.76 and improved grain Zn concentration from 27 mg/kg to 42 mg/kg. In terms of benefit-cost ratio, 1.0% Zn-enriched urea was the most economic rate (Shivay et al., 2008). The suitability of ZnO as a source of Zn fertilizer has been discussed in the literature. Most papers indicate that ZnO and ZnSO, are equally effective in correction of Zn deficiency (Mordvedt and Gilkes, 1993). However, field trials with Zn-enriched urea in India demonstrated that although the differences were not large, urea fertilizers coated with ZnSO, always produced better results than urea coated with ZnO in terms of increasing grain yield and Zn concentrations in rice and wheat (Shivay et al., 2002) (Table 2).

		Rice	Wheat		
Treatments	Grain yield, t/ha	Grain Zn concentration, mg/kg DW	Grain yield, t/ha	Grain Zn concentration, mg/kg DW	
Prilled Urea	3.99	30	3.72	40	
Zn-Enriched ureas					
1% Zn as ZnO	4.46	36	4.14	46	
1% Zn as ZnSO <sub>4</sub>	4.67	39	4.25	49	
2% Zn as ZnO	4.95	43	4.39	49	
2% Zn as ZnSO <sub>4</sub>	5.15	48	4.53	51	

**Table 2.** Grain yield and grain Zn concentration of rice and wheat as affected by Zn-enriched urea applications at the research farm of IARI, New Delhi. Data show average values of 2-year field trials. Statistical details provided in the cited article (Shivay et al., 2008).

#### Influence of agronomic factors on grain Zn

Agronomy offers further practices to improve grain Zn concentration such as application of organic amendments into soil and changes in cropping systems. Increasing evidence is available in the literature showing that addition of different organic materials into soils as compost or farmyard manures can greatly improve solubility and spatial availability of Zn, and the total amount of

plant-available Zn (e.g. DTPA-extractable Zn) in soils (Srivastava and Sethi, 1981; Arnesen and Singh, 1998; Asada et al., 2010).

Existence of a strong positive relationship between soil organic matter and soluble Zn concentrations in rhizosphere soil was reported in a study of 18 different soils collected in Colorado (Catlett et al., 2002), indicating the importance of organic matter in improving spatial availability of Zn to plant roots, especially in soils with very low organic matter content (Marschner, 1993). Cropping systems and inclusion of legumes in rotation systems also have important effects on soil fertility and solubility of mineral nutrients, including micronutrients (Cakmak, 2002). In the case of biofortification of dicots with micronutrients, intercropping dicots together with cereal species is useful. Compared to monocropping, intercropping peanut with barley or maize increases biological activity and chemical availability of various nutrients in the rhizosphere, especially micronutrients, leading to increases in shoot and seed concentrations of Zn and Fe (Inal et al., 2007; Zuo and Zhang, 2009). Cereal crops belong to the stategy-II plants and release Feand Zn-mobilizing compounds (so-called phytosiderophores) from their roots when suffering from Zn or Fe deficiency. One possible reason for the enhanced uptake and accumulation of Zn and Fe in dicots under intercroping with cereals might be related to the root release of phytosiderophores (Zuo and Zhang, 2009).

Recent studies indicate that N nutritional status of plants greatly affects grain accumulation of Zn and also Fe. Greenhouse trials showed that enrichment of wheat grains with Zn by applying soil and/or foliar Zn fertilizers is maximized when the N nutrition regime of plants was improved either by soil or foliar application of N fertilizers (e.g. urea) (Kutman et al., 2010). According to these authors, N and Zn act synergistically in increasing grain Zn concentration in wheat when Zn and N are sufficiently high in growth media or plant tissues. Interestingly, in the case of low Zn supply or low tissue Zn concentrations, increasing N application has no effect on grain accumulation of Zn (Kutman et al., 2010). More attention should be paid to N management in cultivation of food crops and in establishing breeding programs for effective biofortification of grains with Zn and also Fe.

#### Tolerance to low Zn soils and accumulation of Zn in grain: two genetic systems.

Another aspect that should be mentioned here is the relationship between low Zn tolerance and grain Zn accumulation. The genetic systems affecting (i) tolerance to Zn deficiency in soils and (ii) accumulation of Zn in grain appear to have a different basis. Genotypes having high tolerance to low Zn soils do not necessarily accumulate high Zn in grain, and even opposite results are reported. For example, rye shows exceptionally high tolerance to low Zn in severely low Zn calcareous soils (Cakmak et al., 1998), while durum and bread wheats are particularly affected by low Zn, yielding poorly. The high tolerance of rye to low Zn is attributed to different mechanisms, including release of Zn-mobilizing phytosiderophores from roots, formation of Zn (Cakmak et al., 1999). Neverthe-

less, grain Zn concentration of rye grown under very low Zn soil without any sign of Zn deficiency symptoms and little reduction in grain yield, ranged from 8 to 12 mg/kg (Cakmak et al., 1998). When compared with wheat having similar grain yield and grown under the same field conditions, grain Zn concentrations in rye are still lower than in wheat. Thus low concentrations of Zn in rye grain cannot be ascribed to a dilution effect. Similarly, several low-Zn tolerant wheat cultivars from Turkey (Cakmak et al., 1999) and Australia (Graham et al., 1992) have lower grain Zn concentration than many low-Zn sensitive wheat cultivars, even under Zn-adequate conditions. These results suggest that under Zn deficiency, Zn-deficiency tolerant genotypes extract Zn from soils at amounts that are sufficient only for maintenance of healthy growth and appropriate yield. Apparently, these low-Zn tolerant genotypes do not accumulate Zn in grain exceeding the need for seed development and formation. Based on these results it can be concluded that tolerance of plant genotypes to low soil Zn and high accumulation of Zn in grain are controlled by separate, unrelated genetic systems.

#### Benefits from enrichment of seeds with Zn.

Enrichment of seeds or grains provides additional benefits in terms of agronomic performance of seedlings and final yield. During seed germination and early development of seedlings, high levels of Zn in seeds are required to ensure better germination, seedling establishment and protection against different environmental stress factors including soil-borne pathogens (Welch, 1991; Cakmak, 2008). The benefits of high seed-Zn on plant growth and yield become pronounced, especially on Zn deficient soils. Grain yield of plants derived from seeds containing 0.4  $\mu$ g Zn per seed (i.e. around 10 mg Zn/kg) was only half that of plants which were derived from seeds containing almost three-fold more Zn in seed (Yilmaz et al., 1998). Priming seeds with ZnSO<sub>4</sub> is another tool for enrichment of seeds with Zn. Harris et al. (2008) with chickpea and wheat and Slaton et al (2001) with rice showed impressive improvements in growth and yield when seeds were primed with Zn. In priming of wheat and chickpea seeds, 0.3% Zn for 10 h and 0.05% Zn for 6 h were used (Harris et al., 2008).

## Selenium

The importance of Se to human health (in terms of antioxidant, anti-inflammatory, anti-cancer, anti-viral, and anti-ageing activity, along with key roles in the thyroid, brain, heart, and gonads) is highlighted by its status as the only micronutrient to be specified in the human genome, as selenocysteine, the twentyfirst amino acid (Rayman, 2002). Selenium's anti-cancer effects are discussed in Combs and Lu (2006).

Selenium in a food system depends mainly on the levels of plant-available Se in soils used for agriculture. The element is ubiquitous but unevenly distributed, hence the high variability in population and sub-group Se status that can be seen globally (**Table 3**; Lyons et al., 2008). As presented, soil pH plays an important role in grain accumulation of Se. Selenium's availability in soils depends on pH, redox potential, cation exchange capacity, and levels of S, Fe, Al, and C (Ylaranta,

1983a; Banuelos and Schrale, 1989; Combs, 2001; Broadley et al., 2006; Li et al., 2008; Lin, 2008).

**Table 3.** Total soil Se level compared to Se level in cereal grain grown on the same soil (as an indicator of plant-available Se) at four locations (Lyons et al., 2004, 2010).

Location	Soil type	pH (H <sub>2</sub> O)	Total soil Se, µg/kg	Se in cereal grain, µg/kg
Yongshou, China	Ishumisol	8.3	700	20
Minnipa, S Australia	Calcareous Xerochrepts	8.6	80	720
Charlick, S Australia	Typic Natrixeralf	6.6	85	70
East Zimbabwe	Typic Kandiustalf (ex granitic parent material)	5.0	30,000	7

Strategies to increase Se intake include eating foods which are high Se accumulators (e.g. Brazil nuts), sprouting seeds in Se-rich media, producing foods on high-Se soils, supplementation of livestock, food fortification, individual supplementation, breeding food crops for enhanced Se accumulation, and use of Se fertilizers (Lyons et al., 2003; Haug et al., 2007). In the following the plant breeding approach and the agronomic biofortification strategy will be discussed in more detail.

# Genetic biofortification

Genotypic variation in Se accumulation has been reported for several food crops. For example, a 15-fold variation in Se concentration in Brassica vegetables (Combs, 2001), a four-fold variation in tomatoes (Pezzarossa et al., 1999), and some variation in Se concentration in rice grain (Lyons et al., 2005a). However, studies with wheat suggest that although genotypic differences may exist in modern wheat cutivars, they are likely to be insignificant in comparison with background soil variation, which for Se can exist at a microspatial (metre-to-metre) level. For example, at a trial site in South Australia a six-fold variation in grain Se concentration was observed among four replications of one wheat cultivar grown together in the same field (Lyons et al., 2005b).

Transgenic approaches have been studied, and mainly focus on increasing shoot Se concentration through knowledge of S and Se uptake and assimilation (Broadley et al., 2006; Sors et al., 2009). For example, an Indian mustard (*Brassica juncea*) that over-expresses ATP sulphorylase accumulates more Se for phytoremediation of a Se-contaminated soil in California (Banuelos et al., 2005). However, enhanced uptake efficiency for selenate (the most soluble, mobile Se

form) may be of limited value for crops grown in soils of very low available Se, where most of the Se is present as selenite, selenide, and elemental Se (Cary and Allaway, 1969; Lyons et al., 2008). Selenium is immobile under reducing conditions; elemental Se or metal selenides are likely to form under low pH/low redox conditions. Selenate is the major Se species in soil solution at high redox; selenite at medium redox, and selenide at low redox (Broadley et al., 2006). It is notable that in most soils, plant-available Se comprises only around 2 to 3% of total Se (Tan et al., 2002).

# Agronomic biofortification

# The suitability of selenate

Selenium appears to be particularly suited to agronomic biofortification of food crops. In the form of selenate, Se is readily taken up by plants growing on most soils of pH 5.5 to 9.0; it is transported easily throughout the plant; it accumulates in edible parts, and it is converted to organic forms, mainly selenomethionine, which is relatively evenly distributed throughout the cereal grain, and thus can be abundant in milled products like white flour and polished rice. Selenium in the forms usually found in food is generally highly bioavailable and suitable for humans and animals (Lyons et al., 2003).

Studies in Europe and North America since the 1970s demonstrate the effectiveness of agronomic biofortification using sodium selenate, and these have been reviewed by Lyons et al. (2003) and Broadley et al. (2006). Most studies have shown selenate (where Se exists in its highest oxidation state, +6), whether applied to the soil or as a foliar fertilizer, to be much more effective than selenite (Se +4). In many soils, selenite is readily adsorbed on clay colloids and becomes poorly available to plants. Dry climate, low organic matter, high temperature, high soil pH, and aeration are likely to increase the selenate: selenite ratio in the soil and hence the availability of Se to plants (Combs, 2001). In China, applications of Se-enriched manure have been found to be more effective than selenite in biofortifying various crops, including tea (Hu et al., 2002).

## Soil versus foliar application

The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, method of basal application, and time of foliar application. Ylaranta (1983b) found basal and foliar selenate to be equally effective at the low (10 g/ha) rate, foliar better at 50 g/ha, and both equal at the high rate of 500 g/ ha. Ten g/ha of foliar selenate, using a wetting agent, raised wheat grain Se level from 16 to 168  $\mu$ g/kg on the clay soil, while 9 g basally applied raised it to just 77  $\mu$ g/kg. Overall, foliar application was the more effective method, except where growth was poor due to low rainfall (Ylaranta, 1984).

In field trials in South Australia, where drought stress is a common factor in cereal crops, it was found that Se applied as sodium selenate to the soil at seeding was more effective than post-anthesis foliar application, even on soils of variable pH, Fe, S, and organic carbon content. Soil application of selenate (at rates from

4 to 120 g Se/ha), increased grain Se concentration progressively from 0.062 to 8.33 mg/kg, and this 133-fold increase occurred at the site with less favourable soil traits for Se availability: lower baseline Se level, lower pH and higher Fe, S and carbon, while the foliar application of selenate at the highest application rate increased grain Se concentration from 0.062 to 1.24 mg/kg, a 20-fold increase at the same site. Recent field trials in China (on loess soil) and Colombia (on a range of soils) showed that application of selenate to soil and (in China for winter wheat) foliar application of selenite were effective at biofortifying food crops (Lyons, G., F. Calle, Y. Genc, and H. Ceballos, unpublished, 2007; Wang, Z., H. Mao, and G. Lyons, unpublished, 2010).

# Se biofortification of pasture and forage crops

Selenium-responsive conditions in livestock include white muscle disease (cattle, sheep, pigs, poultry), exudative diathesis (poultry), pancreatic degeneration (poultry), liver necrosis (pigs), "ill-thrift" (cattle, sheep, poultry), as well as impaired reproduction and immunity in all of these species (Reilly, 1996). Pastures and forage crops have a long history of agronomic biofortification with Se, especially in New Zealand, which is renowned for its low-Se soils. *Selcote Ultra*°, a prilled 1% w/w Se product made of sodium and barium selenate, has been popular with graziers in New Zealand and Canada since the 1980s. It can be applied either directly or mixed with NPK fertilizer and is normally top-dressed annually in early spring, and usually applied at 10 g/ha (Broadley et al., 2006; Beaton and Foster, 2009).

The residual effect of Se treatments (other than slow-release forms like barium selenate) has been found to be low, even when applied at high rates (Ylaranta, 1983a,b; Gupta, 1993). No Se build-up has been observed in New Zealand, where Se fertilization has been practised since the 1970s, and positive responses continue to be obtained from Se application (Oldfield, 1999).

## Efficiency and Se target level

Recent field trials in the UK compared the fate of Se applied in either granular or liquid form to wheat. It was found that all selenate applications were effective, but spring application was more effective than winter application. A sizeable amount of Se remained in straw (and thus could be beneficial if used in animal feed), and percent Se recovery in grain increased with application rate, with 14% recovered at an application of 10g Se/ha. The authors calculated that this application at a national level would increase the grain Se concentration of UK wheat from around 30 to 300  $\mu$ g/kg. This would be an impressive increase, particularly when considering the high yields of UK wheat (Broadley et al., 2010). A desirable target for Se in biofortified crops can be postulated to be in the range of 250 to 300  $\mu$ g/kg on a dry weight basis, when international surveys of Se status of soils, crops, animals and humans, along with estimated optimum intake (at least in terms of maximising selenoenzyme activity) are considered (Combs, 2001; Rayman, 2002; Lyons et al., 2003).

The national Se fertilizer program in Finland (discussed below) shows that an increase in grain Se level as described by Broadley et al. (2010) would have a large effect on population Se status. However, at just 14% Se recovery in grain it can be argued that large-scale agronomic biofortification of cereals with Se would be somewhat wasteful of a relatively scarce trace element. If Se agronomic biofortification is to occur, whether locally or nationally, it is desirable to do it as efficiently as possible, especially as Se can be considered as a valuable resource which is difficult to recycle (Haug et al., 2007).

## Finland: nationwide agronomic biofortification with selenium

As a response to low dietary Se intakes and the understanding that this may be a risk factor for cardiovascular disease, which occurred at high rates in Finland in the 1960s and 1970s, Finland's government mandated the addition of Se (as selenate) to all multi-nutrient fertilizers from 1984 (see **Box 1**).

Box 1:	Finland: Se biofortification at a national level.
1970:	East Karelia has the highest heart disease rates in the world Low available Se in soils Se supplementation of livestock feeds commences
1984.	Heart disease (especially in men) begins to decline National Se biofortification program commences
	Se in spring wheat grain increases from 10 (pre-1984) to 250 $\mu$ g/kg Human Se intake trebles
	Human plasma Se level doubles (55 to 107 $\mu$ g/l) Heart disease continues to decline (at the same rate as pre-1984)
2010:	Heart disease relatively low (due to less smoking, improved diet and exercise, and possibly higher Se status) No detrimental effecs of Se observed Se still added to fertilizers at 10 mg/kg
Reference	res: Aro et al., 1995: Broadlev et al., 2006: Eurola et al., 1990: Hartikainen, 2005: Makela et al.,

References: Aro et al., 1995; Broadley et al., 2006; Eurola et al., 1990; Hartikainen, 2005; Makela et al., 2005; Varo et al., 1994.

Initially, rates of Se were 16 mg/kg of fertilizer used for grain production and horticulture and 6 mg/kg for fertilizer used for pasture and hay production. The program was so successful in raising plant Se concentration and human Se status that the higher application was removed in 1990, leaving the 6 mg/kg rate for all fertilizers (Broadley et al., 2006). For example, the Se level in all domestic cereal grains in Finland pre-1984 was 0.01 mg/kg or less, while in the late 1980s, spring wheats typically contained around 0.25 mg/kg, and for the less-fertilized winter wheat, around 0.05 mg/kg (Eurola et al., 1990). Then, in 1998 Se supplementation was increased to 10 mg/kg of fertilizer for all crops (Broadley et al., 2006). The program, which represents a genuine food system approach for improving

human nutrition, has been an effective method to increase the Se status of the entire population. Indeed, dietary Se intakes trebled and plasma Se concentrations nearly doubled within three years of the program's commencement (Aro et al., 1995; Hartikainen, 2005). Environmental parameters have been closely monitored since the Se program began and effects on the water ecosystem from Se supplementation of fertilizers has not been observed (Makela et al., 2005).

The Finnish experiment demonstrates the safety, effectiveness, ease, and costefficiency of this approach to raise Se levels in a human population. However, it is difficult to isolate the effects of a single factor, such as dietary change, from other factors that can be involved in the aetiology of such conditions as cancer and cardiovascular disease. There have been significant decreases in the rates of cardiovascular disease and certain cancers in Finland since 1985. But with no controls for comparison, this cannot be ascribed to Se alone (Varo et al., 1994; Hartikainen, 2005).

#### Additional agronomic considerations of Se biofortification

#### Phytotoxicity

Toxic plant tissue levels of Se are generally above 5 mg/kg (Reilly, 1996), and there is wide variation in susceptibility of plant species to Se toxicity. For example, tobacco and soybeans are relatively sensitive to Se in culture media (Martin and Trelease, 1938), while wheat is relatively tolerant of high levels of available Se in soil. One study found a critical tissue concentration (in whole tops harvested at 30 days) for Se toxicity as high as 325 mg/kg, which suggests that toxicity would not occur in the range of selenate application rates between 10 and 200 g Se/ha, that would be recommended for biofortification of wheat (Lyons et al., 2005c).

#### Selenium benefits to plants

Unlike Zn, Se is generally not considered to be essential for higher plants (it is for some algae), and low-Se soils appear neither to inhibit plant growth nor to reduce crop yield (Shrift, 1969; Reilly, 1996). However, a number of studies have found beneficial effects from low doses of applied Se, including increased growth in ryegrass (Lolium perenne) and lettuce (Lactuca sativa) exposed to UVB radiation (Hartikainen and Xue, 1999). These responses were associated with inhibition of lipid peroxidation through increased glutathione peroxidase activity (Xue and Hartikainen, 2000). A study using fast-cycling *Brassica rapa* reported an increase in seed production from addition of low doses of selenite to the culture solution, which was associated with an increase in respiration (Lyons et al., 2009). Other researchers have found increased tuber yield and increased starch concentration in young leaves in potato (Solanum tuberosum) (Turakainen et al., 2004) and upregulation of starch hydrolyzing enzymes associated with increased shoot biomass and increased respiration in mungbean (*Phaseolus aureus*) (Malik et al., 2010) with Se fertilization. It is clear that Se, when administered in certain forms and at low doses can be beneficial to higher plants, especially when they are exposed to oxidative stress, but the element has not been demonstrated to be essential at this stage.

In recent trials in China, repellent effects against a range of pests and pathogens (including spider mites, (*Tetranychus cinnabarinus*) and potato blight (*Phytophthora infestans*) were observed in maize, soybean and potato which had been biofortified with Se, Zn, and I applied to the soil at planting in a glasshouse pot trial. The biofortified plants yielded higher than controls. These anti-pest effects were not observed in later field trials (Z. Wang, H. Mao, G. Lyons, et al., unpublished, 2010). Interestingly, the leaf Se concentrations were not especially high in maize and soybean (4 to 15 mg/kg) in this glasshouse study, while other studies on Se's pest-repellent effects have found much higher leaf Se levels (500 to 800 mg/kg) are required to be effective (Hanson et al., 2003; Freeman et al., 2007). This suggests that the combined high levels of Se, I, and Zn in the leaves may have enhanced the repellent effect, and warrants further investigation.

## Sulphur effects

Sulphur (as sulphate) has been found to inhibit Se uptake in plants in numerous studies due to competition effects as Se is taken up largely by the main S transporter (Lauchli, 1993; Lyons et al., 2004b; White et al., 2004). Moreover, Adams et al. (2002) found a negative correlation between grain Se and S, and between grain Se and soil S application rate. Gypsum (calcium sulphate, which is applied at rates of up to 10 t/ha to treat sodic soils) and high-S fertilizers like single superphosphate, ammonium sulphate, and potassium sulphate, are likely to reduce Se concentration in crops.

Recent UK trials found differing effects of S on accumulation of Se in wheat grain, depending on soil pH. Applied S decreased grain Se concentration in controls at both sites, in accordance with previous studies. However, when S and Se were applied together, grain Se was increased on the low pH, S-sufficient soil but decreased on the high pH, low S soil (Stroud et al., 2010). However, most of the Se in these soils was in the form of selenite, the plant availability of which is more likely to be affected by influences on phosphate transporters, rather than sulphate transporters (Li et al., 2008).

#### Commercialisation of Se-biofortified wheat

It is clear that agronomic biofortification of cereals with Se is effective, inexpensive and provides desirable, bioavailable forms of Se. Novel wheat (or other cereal) products that contain enhanced levels of organic Se due to agronomic biofortification could be considered as *functional foods*, which are likely to provide human health benefits. In South Australia, Se-biofortified flour is marketed, and several bakeries sell high-Se bread and biscuits made from this flour.

## Potential health benefits of selenium-biofortified foods

It has become evident that Se-biofortified cereals are very effective at increasing body Se status, with selenomethionine well retained in muscle. Moreover, Se-biofortified broccoli, which contains Se mostly in the Se-methylselenocysteine form, along with other anti-cancer agents including sulphoraphanes, is one of the most promising anti-cancer functional foods (Finley, 2003; Liu et al., 2009).

On a cautionary note, there is a fairly narrow gap between deficient and toxic Se intakes for humans, and some researchers consider that the upper safe limit of Se intake in humans may be even lower than previously thought (Vinceti et al., 2009). Equivocal and conflicting findings for the roles of Se in human health, including risk of cancer and cardiovascular disease, are common. Selenium's actions and effects on humans are complex (Fairweather-Tait et al., 2010; Lyons, 2010). The rates of most cancers and their trends over the past 30 years in Finland (where crops have been biofortified with Se) are comparable with those in other Scandinavian countries with lower population Se status. On the other hand, studies in France and Italy (where Se status is relatively low) found that low blood Se in people over 65 years is a strong predictor of mortality over the next 6 to 9 years (Akbaraly et al., 2005; Lauretani et al., 2008), and it is hypothesised that low Se status is a risk factor for HIV/AIDS in Africa (Foster 2003).

# Conclusion

In general, Fe is not suitable for agronomic biofortification. Iodine concentration in leaves can be increased by this method, but it is difficult to increase I levels in grain or tubers/storage roots. Cobalt can be agronomically biofortified, but needs to reach humans via the ruminant route in order to be useful in terms of vitamin B12. For Zn and Se it can be highly effective for a range of crops, and is a promising strategy for increasing the status of these micronutrients in human populations, with probable consequent health benefits. For crops growing on low Zn soils, there is the added benefit of likely yield increase in the next crop, grown with higher-Zn seed.

For Zn, a combination of soil and foliar application (or two strategic foliar applications around late booting and early milk stages) appears to be the most effective agronomic biofortification method, and  $ZnSO_4$  is generally an effective, relatively cheap form of Zn to use for this purpose. Zinc-enriched fertilizers such as Zn-coated urea are a practical way to fertilize/biofortify with Zn. It is notable that tolerance of plant genotypes to low Zn soil and high accumulation of Zn in grain are controlled by separate genetic systems. Maintenance of adequate N nutritional status of plants appears to be an important agronomic practice in maximising biofortification of food crops with Zn and Fe.

For Se, depending on soil type, soil or foliar application can be highly effective, and as with Zn, timing of foliar application is important: one application around mid booting stage should be sufficient. Selenate is generally much more effective than selenite for soil application, and is also usually more effective for foliar application. Selenium in food crops, especially cereals, is usually highly bioavailable.

Both Zn and Se are valuable micronutrients, and are generally non-renewable resources which should be conserved. Hence, it is important to research ways to

maximize the efficiency of their application to food crops or foods. This includes further work on combining foliar application of Zn and Se with urea; application of different organic materials; and intercropping. In addition, further research on the bioavailability of Zn in various crops is required, and in particular the effect of different agronomic practices on phytate/Zn ratios. Food products biofortified with these micronutrients are potential health-promoting *functional foods*. Importantly, farmers would generally need a yield incentive to apply micronutrients to their crops. **FCHH** 

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Chapter 5

# Calcium, Magnesium and Potassium in Food

Forrest Nielsen<sup>1</sup>

# Abstract

The biochemical and physiological functions and consequences of deficient intakes, which show the nutritional importance of Ca, Mg, and K for humans, are reviewed. The dietary recommendations and food sources for these essential mineral elements for humans are presented. Factors that can influence the dietary intake and availability of these minerals for humans are discussed, including plant nutrition, and thus fertilization, impacts. Calcium, Mg, and K are essential for plants, in which they are widely distributed and have biochemical roles similar to those in animals and humans. Thus, foods of plant origin always contain measurable amounts of these minerals because of their need for growth and development. Increasing the amount of Ca to the root increases the amount of Ca in plants. Magnesium preferentially accumulates in grain when soil availability is low, but when Mg supplies approach adequacy, vegetative structures become storage sinks for Mg. As a result, Mg in foods can vary depending upon the environment in which they were grown. Increasing K to roots increases the K content of all organs of plants except seeds and grain. Thus, increasing soil K through fertilization may increase the K content of fruits and vegetables but not cereal grains. The preceding indicates that plant nutrition, which is impacted by fertilization, influences the amount of Ca, Mg, and K provided by foods of plant origin towards their requirements by humans.

# Introduction

Calcium, Mg, and K are essential macro mineral nutrients for animals and humans. The essential functions of these mineral elements in animals and humans

Abbreviations specific to this chapter: AI = Adequate Intake; ATPase = Adenosine Triphosphatase; CRP = C-reactive protein; DRI = Dietary Reference Intake; EAR = Estimated Average Requirement; FAO/WHO = Food and Agriculture Organization/World Health Organization;  $\mu M$  = micromolar; mmol = millimoles; NHANES = National Health and Nutrition Examination Survey; RDA = Recommended Dietary Allowance; RNI = Recommended Nutrient Intake; UL = Tolerable Upper Limit. For symbols used commonly throughout this book see page xi.

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are similar to those in plants. Animals and humans require much larger amounts of Ca than do plants, owing to its role in skeletal growth and maintenance. Because of this difference, Ca may be considered a micro mineral nutrient for plants, although it is most often classified a secondary macro nutrient. Calcium, Mg, and K have major metabolic functions throughout animals and plants, and thus are consistently found in food. Thus, in addition to assuring optimal crop production, fertilization practices may influence the meeting of Ca, Mg, and K requirements for humans.

# Calcium

#### **Nutritional Importance for Humans**

**Biochemical and Physiological Functions.** Calcium has three major metabolic functions. Calcium is a second messenger that couples intracellular responses to extracellular signals, an activator of some functional proteins, and indispensable for skeletal function.

In its role as a signaling or messenger ion, Ca<sup>2+</sup> mediates vascular contraction and vasodilation, muscle contraction, nerve transmission, and hormone action. In response to a chemical, electrical, or physical stimulus, extracellular Ca<sup>2+</sup> enters the cell and increases intracellularly through release from internal stores such as the endoplasmic or sarcoplasmic reticulum (Awumey and Bukoski, 2006; Weaver, 2006). Increased intracellular Ca<sup>2+</sup>, often in the form of a Ca receptor protein called calmodulin, stimulates a specific response, for example, activation of a kinase to phosphorylate a protein that results in a physiological response (Weaver and Heaney, 2006a).

A number of enzymes, including several proteases and dehydrogenases, are activated or stabilized by bound Ca independent of changes in intracellular Ca<sup>2+</sup> (Weaver and Heaney, 2006a). These enzymes include glyceraldehyde phosphate dehydrogenase, pyruvate dehydrogenase, and  $\alpha$ -ketoglutarate dehydrogenase (Weaver and Heaney, 2006a).

About 99% of total body Ca is found in bones and teeth. Bone crystals have a composition similar to hydroxyapatite  $[Ca_{10}(PO_4)_6(OH)_2]$ , which contains about 39% Ca. The crystals, which have the ability to resist compression, are arrayed in a protein matrix, which has the ability to withstand tensile loads. Alterations in either the inorganic (hydroxyapatite) or organic (protein matrix) components can result in changes in bone strength (Rubin and Rubin, 2006). The skeleton must undergo continuous remodeling throughout life (it is replaced every 10 to 12 years) to adapt its internal microstructure to changes in its mechanical and physiological environment. Additionally, bone is renewed continuously to repair micro damage to minimize the risk of fracture.

*Consequences of Deficient Intakes.* The maintenance of extracellular Ca<sup>2+</sup> by mobilization of skeletal Ca stores means that nutritional Ca deficiency almost never manifests itself as a shortage of Ca<sup>2+</sup> in critical cellular or physiological processes

(Heaney, 2006). However, a low Ca intake may increase circulating 1,25(OH),vitamin D to a point that it opens Ca channels in some cells (e.g. muscle and adipocytes), resulting in increased intracellular Ca (Weaver and Heaney, 2006a). The increased intracellular Ca may contribute to the development or severity of disorders such as those associated with obesity. However, for most healthy individuals, the main concern about Ca intake is an amount that will maintain bone health. If bone renewal during remodeling or turnover is slower than bone loss, osteoporosis may occur. If bone repairing is slower than micro damage accumulation, stress fractures may occur. In a large case-controlled study of hip fracture risk in women in Europe, fracture risk declined until Ca intake rose to an estimated 500 mg (12.5 mmol)/day (Dawson-Hughes, 2004). Calcium supplementation alone of individuals consuming more than 500 mg (12.5 mmol) Ca/day apparently does not decrease fracture risk (Dawson-Hughes, 2004; Shea et al., 2002; Jackson et al., 2006; Cumming and Nevitt, 1997). This finding is consistent with reports that the Estimated Average Requirement (EAR) for adults, based on well-controlled balance studies, is between 700 and 800 mg/day (Hunt and Johnson, 2007; Uenishi et al., 2001), but lower than the recent EAR of 1,000 mg/day established by the U.S Food and Nutrition Board (2010). The EAR is an estimated intake that will meet the requirement of 50% of individuals in a life stage and gender group. Most studies with adults showing a positive influence of high dietary Ca in decreasing bone loss or fracture risk also had supplemental vitamin D as an experimental co-variable.

#### **Dietary Recommendations**

Calcium intake recommendations vary widely worldwide (Looker, 2006), with the United States among the highest where the Recommended Dietary Allowance (RDA) for adults aged 19 to 50 years was recently set at 1,200 mg (25 mmol)/day (Food and Nutrition Board, 2010). The RDA is an average daily intake that is sufficient to meet the requirement of nearly all (97% to 98%) individuals in a life stage and gender group. The official United States RDA is similar to the RDA of 1,035 mg (25.8 mmol)/day for adult men and women suggested by an analysis of primary Ca balance data from tightly controlled metabolic feeding studies (Hunt and Johnson, 2007). The Ca Dietary Reference Intake (DRI; reference values that can be used for planning and assessing diets for healthy populations) in the United Kingdom is much lower; 700 mg (17.5 mmol)/day for adults aged 19+ years (Francis, 2007). In India, the recommended dietary allowance for Ca is only 400 mg (10 mmol)/day for adults (Harinarayan et al., 2007). Several countries and organizations, including the United States and European Community have established 2,500 mg (62.4 mmol)/day as the Tolerable Upper Limit (UL) for Ca (Looker, 2006). The UL is the highest daily intake that is likely to pose no risks of adverse health effects to almost all individuals in the general population. DRIs established by the U.S. Food and Nutrition Board (2010) for adolescents and adults are shown in Table 1.

The Food and Nutrition Board of the U.S. Institute of Medicine (2010) recently concluded that, with a few exceptions, most North Americans are consuming

**Table 1.** U.S. Daily Estimated Average Requirement (EAR), and RecommendedDietary Allowance (RDA) or Adequate Intake (AI) for Ca, Mg, and K(Food and Nutrition Board, 1997; 2005; 2010).

Life Stage	Ca, mg		Mg	K, g	
Group	EAR	RDA	EAR	RDA	AI
Males, age					
9-13	1,100	1,300	200	240	4.5
14-18	1,100	1,300	340	410	4.7
19-30	800	1,000	330	400	4.7
31-50	800	1,000	350	420	4.7
51-70	800	1,000	350	420	4.7
>70	1,000	1,200	350	420	4.7
Females, age					
9-13	1,100	1,300	200	240	4.7
14-18	1,100	1,300	300	360	4.7
19-30	800	1,000	255	310	4.7
31-50	800	1,000	265	320	4.7
51-70	1,000	1,200	265	320	4.7
>70	1,000	1,200	265	320	4.7

enough Ca. Based on intake data (Looker, 2006), most Europeans also are consuming enough Ca. However, inadequate Ca intakes may be a significant concern in other countries where the diet is refined grains or grains low in Ca (e.g. rice), and very little milk products are consumed. Examples of these countries are Bangladesh (Combs et al., 2005) and Nigeria (Thacher et al., 2009).

#### **Human Calcium Intake Factors**

*Calcium Content in Foods.* The most Ca-dense foods in Western diets are milk products; they contain about 300 mg (7.5 mmol) Ca per serving (e.g. 8 ounces of milk or yogurt; 1.5 ounces of cheddar cheese). Grains are not particularly rich in Ca, but when consumed in large quantities, they can provide a substantial portion of dietary Ca. For U.S. adults, dairy products provide 78%, grain products about 11%, and vegetables and fruits about 6% of dietary Ca (Weaver and Heaney, 2006a). The Ca contents of some foods of plant origin are shown in **Table 2**.

*Interactions with Other Nutrients.* In addition to content, bioavailability of Ca from foods is an important consideration for the provision of adequate needs. About 32% of Ca in milk and dairy products is absorbed (Weaver et al., 1999). Calcium bioavailability from foods from plants is typically lower than milk be-

Food	Ca	Mg	К	Food	Ca	Mg	К
Fruits <sup>†</sup>				Vegetables <sup>†</sup>			
Apple	6	5	107	Lettuce	18	7	141
Orange	40	10	181	Celery	40	11	260
Banana	5	27	358	Tomato	10	11	237
Peach	6	9	190	Carrots	33	12	320
Strawberry	16	13	153	Onions	23	10	146
Pear	9	7	119	Peppers	10	10	175
Grapes	10	7	191	Potato§	8	20	328
Plums	6	7	157	Lima Beans <sup>§</sup>	32	74	570
Grapefruit	12	8	139	Navy Beans <sup>§</sup>	69	53	389
Cherries	13	11	222	Peas§	27	39	271
Avocado	12	29	485	Soybeans	145	60	539
Grains <sup>‡</sup>			Nuts <sup>†</sup>				
Barley	50	150	470	Almond	264	268	705
Corn	30	140	370	Brazil nut	160	376	659
Oats	70	140	440	Cashew	370	292	660
Rice, white	30	120	150	Pecan	70	121	410
Rye	70	140	520	Pistachio	105	121	1,025
Wheat	40	160	420	Walnut	98	158	441

**Table 2.** Calcium, Mg, and K (mg/100 g) content in selected foods as served of plant origin.

† Values from USDA Nutrient Database (2010)

*‡ Values are for whole grains (not food as served) as reported by McDowell (1992)* 

§ Boiled, with salt

cause of the presence of oxalate and phytate. For example, only about 5% of Ca in spinach, which is high in oxalic acid, is absorbed (Heaney et al., 1988). However, plants from the *Brassica* genus are unusual in that they do not use oxalic acid to detoxify excess Ca that would cause cell death. Thus, fractional absorption of Ca from vegetables such as broccoli (61%), bok choy (54%), and kale (49%) is higher than from milk (Weaver et al., 1999). These foods also are moderate sources of Ca with reported values ( $\mu$ g/g) of 493 for broccoli, 718 for kale and 929 for bok choy (Weaver and Heany, 2006b).

Phytic acid is the storage form of P in seeds. The amount of phytic acid or phytate in seeds, which only modestly inhibits Ca absorption, depends upon the P content of the soil that is growing plants (Weaver and Heaney, 2006b). Only foods with high phytate content, such as wheat bran and dried beans, significantly reduce Ca absorption (Weaver and Heaney, 2006b). Interestingly, food products from soybeans, rich in oxalate and phytate, have relatively high Ca bioavailability (Heaney et al., 1991). In addition, a study with Nigerian children indicated that Ca absorption was enhanced by increased phytate in a meal containing maize porridge (Thacher et al., 2009).

Because Ca and Na share the same transport system in the kidney proximal tubule, Na can have a negative effect on Ca metabolism. Every 1,000 mg (43 mmol) of Na excreted by the kidney results in an additional loss of 26.3 mg (0.66 mmol) of Ca (Weaver, 2006). This additional loss apparently is not offset by changes in Ca absorption because a high Na intake results in bone loss (Weaver, 2006).

Dietary protein increases urinary Ca loss, apparently through an increased urinary acid load caused by the presence of acids from the breakdown of S-containing amino acids (Cao and Nielsen, 2010). However, dietary protein does not decrease Ca retention because of offsetting changes in the absorption of Ca (Cao and Nielsen, 2010). Dietary P in forms other than phytate, such as that found in meat and carbonated beverages, also increases urinary Ca loss, apparently through increasing urinary acid load (Cao and Nielsen, 2010). However, similar to increased S amino acid intake, the increased P does not decrease Ca balance or retention (Heaney, 2008; Cao and Nielsen, 2010).

High intakes of Al, such as those resulting from the consumption of Al-containing antacids, can increase Ca loss. Therapeutic doses of such antacids can increase daily urinary Ca excretion by 50 mg (1.25 mmol) or more (Heaney, 2008).

Some non-digestible oligosaccharides (e.g. inulin) enhance Ca absorption and bone mineralization (Coudray et al., 1997; Abrams et al., 2005).

#### **Plant Nutrition Impacts on Food Calcium**

Nutritional Importance for Plants. Although usually classified as a secondary macronutrient, Ca may be considered a micronutrient for plants because their requirement for this element is small (Wallace et al., 1966; Marschner, 1995). The Ca requirement is much lower for monocotyledons than dicotyledons. For example, in well-balanced flowing nutrient solutions, maximal growth was achieved with 2.5  $\mu M$  by ryegrass and with 100  $\mu M$  by tomato, a factor of 40 difference (Marschner, 1995). Calcium is essential for the maintenance of plasma membrane integrity that facilitates ion uptake. Calcium also functions as a structural component in cell walls and as a second messenger in cellular signaling (Marschner, 1995). Calcium deficiency rarely occurs in non-legume plants grown on soils with appreciable cation-exchange capacity and a pH higher than 5.3 because the amount of Ca in soils is large compared to plant requirements (Barber, 1984). However, inadequate Ca for growth may occur on soils that are highly weathered, low in pH, and low in cation-exchange capacity. Crops with high Ca requirements, such as legumes, may need a high pH for some soils to supply sufficient Ca (Barber, 1984).

Soil solution and exchangeable Ca are the main forms that are absorbed by plant roots. Calcium in soil solution is balanced by soluble anions such as sulphate and carbonate. Calcium is the dominant exchangeable cation in many soils. Exchangeable Ca is in equilibrium with soil solution Ca (Barber, 1984). Alkaline soils containing Na, acid soils high in H<sup>+</sup> and Al, and serpentine-derived soils high in Mg have other dominant exchangeable cations (Barber, 1984). Exchangeable Ca is increased in acidic soils by liming, which precipitates out Al. Adding soluble Ca to acidic soils is not suitable for increasing exchangeable Ca because it displaces Al from cation exchange sites, resulting in an increase in Al solubility and toxicity, instead of precipitating Al into an insoluble form.

*Factors Affecting Calcium Content in Foods from Plants.* Assuring Ca requirements for plant growth assures that foods from plants will always contain some Ca, which can vary by soil Ca availability. Increasing the availability of Ca to the root increases the amount of Ca in plants. Increasing the amount of the cations Mg, K,  $NH_4^+$  decreases the uptake of Ca by plants. However, plant species variation in uptake of Ca is the most significant factor in amount of Ca provided by foods from plants. For example, when exposed to similar solution Ca concentrations, uptake of Ca by tomato was the greatest; soybean and lettuce were intermediate, and wheat was lowest (Halstead et al., 1968). In general, soybeans, nuts, and *Brassica* foods are high in Ca, some legumes and vegetables are moderate sources of Ca, and cereal grains, especially without the bran component, and fruits are poor sources of Ca. The variation in the Ca content of foods from different plant species is displayed in **Table 2**.

# Magnesium

#### **Nutritional Importance**

**Biochemical and Physiological Functions.** Magnesium is needed for enzymatic reactions vital to every metabolic pathway (Rude and Shils, 2006; Volpe, 2006). These reactions include those involving DNA, RNA, protein, and adenylate cyclase synthesis, cellular energy production and storage, glycolysis, and preservation of cellular electrolyte composition. Magnesium has two functions in enzymatic reactions. It binds directly to some enzymes to alter their structure or to serve in a catalytic role (e.g. exonuclease, topoisomerase, RNA polymerase, DNA polymerase). Magnesium also binds to enzyme substrates to form complexes with which enzymes react. The predominant role of Mg is involvement in ATP utilization. An example of this role is the reaction of kinases with MgATP to phosphorylate proteins. Magnesium exists primarily as MgATP in all cells. Magnesium at the cell membrane level regulates intracellular Ca and K, and thus, is a controlling factor in nerve transmission, skeletal and smooth muscle contraction, cardiac excitability, vasomotor tone, blood pressure, and bone turnover.

*Consequences of Deficient Intakes.* Based on dietary intake recommendations, subclinical or marginal Mg deficiency (50% to <100% of requirement) commonly occurs throughout the world (Nielsen, 2010). Yet, pathological conditions

attributed specifically to dietary Mg deficiency alone are considered rare. However, epidemiological and correlation studies indicate that a low Mg status is associated with numerous pathological conditions associated with aging, including atherosclerosis (Ma et al., 1995; Abbott et al., 2003), hypertension (Ma et al., 1995; Touyz, 2003), osteoporosis (Rude et al., 2009), diabetes mellitus (Barbagallo et al., 2003), and some cancers (Dai et al., 2007; Leone et al., 2006).

The pathological conditions associated with a low Mg status have been characterized as having a chronic inflammatory stress component (Hotamisligil, 2006; Libbey, 2007). Human studies indicate that a low Mg status often is associated with increased inflammatory and oxidative stress. C-reactive protein (CRP) is a well-documented indicator of low grade or chronic inflammation (Ridker, 2007). Several studies have found that Mg intake was inversely related to elevated serum or plasma CRP (King et al., 2005; King et al., 2007; Bo et al., 2006; Song et al., 2007; Chacko et al., 2010, Nielsen et al., 2010). Low serum Mg concentrations also have been associated with elevated CRP (Rodriguez-Morán and Guerrero-Romero, 2008; Almoznino-Sarafian et al., 2007). Animal experiments, however, suggest that Mg deficiency in humans may play more of a contributory role than a primary causative role in pathological disorders characterized by chronic inflammation (Nielsen, 2010). Although severe Mg deficiency (feeding less than 10% of requirement) results in an inflammatory response (Mazur et al., 2007), moderate-to-marginal or subclinical Mg deficiency alone apparently does not markedly affect variables associated with chronic inflammatory stress in animal models (Vormann et al., 1998; Kramer et al., 2003). However, animal experiments indicate that moderate Mg deficiency can enhance the inflammatory or oxidative stress induced by other factors (Nielsen, 2010). Thus, based on the dietary recommendations given below, Mg deficiency may be a significant nutritional concern under conditions that cause oxidative or inflammatory stress, such as obesity and high dietary intakes of sucrose or fructose, that lead to chronic diseases associated with aging (Nielsen, 2010).

In addition to contributing to the risk for some chronic diseases, controlled metabolic ward studies indicate that subclinical or marginal Mg deficiency also can affect physical performance and heart function. Heart rate and oxygen consumption increased significantly during sub-maximal exercise when untrained postmenopausal women were fed 150 mg (6.17 mmol) compared to 320 mg (13.16 mmol) Mg/day (Lukaski and Nielsen, 2002). Postmenopausal women fed marginal Mg-deficient diets also exhibited heart arrhythmias and changes in K metabolism (Nielsen, 2004; Nielsen et al., 2007 Klevay and Milne, 2002).

#### **Dietary Recommendations**

The lack of usable data has been the basis for the difficulty to establish sound recommendations for Mg by various policy groups. The Mg RDAs for adolescents and adults set by the U.S. Food and Nutrition Board (1997) are shown in **Table 1**. The RDAs for adult men and women between ages 30 and 60 years were set at 420 and 320 mg (17.28 and 13.16 mmol)/day, respectively (Food and

Nutrition Board, 1997). These RDAs are consistent with the recommendation of 6 mg (0.25 mmol)/kg body weight/day suggested by Seelig (1981) and Durlach (1989). The U.S. RDAs were based almost exclusively on findings from one poorly controlled balance study performed in 1984 (Lakshmanan et al., 1984). In that study, subjects consumed self-selected diets in their home environment and were responsible for the collection of their urine, feces, and duplicate diet and beverage samples used in the balance determinations. The study design resulted in the finding of much overlap in Mg intakes that gave negative and positive Mg balances. Because of the tenuous nature of the data used, the North American Mg RDAs have been appropriately questioned. The Food and Agriculture Organization/World Health Organization (FAO/WHO, 2002) concluded that evidence was lacking for nutritional Mg deficiency occurring with the consumption of diets supplying a range of Mg intakes sometimes considerably less than the RDAs for the United States and Canada. Thus, the expert consultation subjectively set Recommended Nutrient Intakes (RNIs) for Mg at 220 and 260 mg (9.05 and 10.69 mmol)/day for women and men respectively.

There are reports suggesting that the RNIs set by the FAO/WHO are more appropriate than the RDAs of the United States and Canada. These include the findings of impaired physical performance and energy use and heart arrhythmias in postmenopausal women fed slightly less than 200 mg (8.23 mmol) Mg/day under controlled metabolic ward conditions (Lukaski and Nielsen, 2002; Klevay and Milne, 2002), which suggest that intakes less than the RNIs set by FAO/WHO probably would result in Mg deficiency. Balance data from 27 different tightly controlled metabolic ward studies found that neutral Mg balance, without considering surface losses, occurred at an intake of 165 mg (6.79 mmol)/day with a 95% prediction interval of 113 and 213 mg (4.65 and 8.76 mmol)/day (Hunt and Johnson, 2006). These latter findings suggest that adults should strive for dietary Mg intakes of over 220 mg (9.05 mmol)/day. The U.S. Food and Nutrition Board (1997) determined that Mg ingested as a naturally occurring substance in food would not exert any adverse effects. Thus, the adult UL for Mg was set at 350 mg (14.6 mmol) of *supplementary* Mg.

In the U.S., data from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) indicated that about 60% of all adults do not meet the Mg RDA set by the Food and Nutrition Board (1997). It is estimated that about 10% of adults older than 19 years have Mg intakes from food and water that are about 50% of the RDA, an intake that may be insufficient according to balance data from controlled metabolic unit studies (Hunt and Johnson, 2006). In either case, intake data suggest that a significant number of adults do not consume adequate amounts of Mg.

#### **Magnesium Intake Factors**

*Magnesium Content in Foods.* Table 2 shows that green leafy vegetables, whole grains, legumes, and nuts are rich sources of Mg (Volpe, 2006). Milk and milk products provide moderate amounts of Mg. Fruits, tubers, meats, and highly
refined cereal grains are poor sources of Mg. Corn flour, cassava and sago flour, and polished rice flour are very low in Mg.

Interactions with Other Nutrients. Several dietary substances, including Ca, P, Zn, protein, vitamin B, and short-chain oligosaccharides may affect Mg metabolism. High dietary P was found to decrease Mg absorption (Rude and Shils, 2006). The decreased absorption may have been caused by the formation of insoluble Mg-phosphate. However, the decreased absorption was counterbalanced by decreased excretion such that Mg balance did not change. Magnesium absorption also may be decreased through binding with phosphate groups of phytate in high-fiber foods (Coudray and Rayssiguier, 2001). An increase in renal acid load, which might be induced by a high P or protein intake, may decrease Mg retention through increased renal loss (Rylander et al., 2006). On the other hand, a low protein intake also results in decreased Mg absorption and retention (Schwartz et al., 1973), which is consistent with the finding of decreased Mg balance (Hunt and Schofield, 1969) with a low protein intake. High Zn intakes of 142 mg (2.17 mmol)/day (Spencer et al., 1994) and 53 mg (0.81 mmol)/day (Nielsen and Milne, 2004) decreased Mg balance in adult males and postmenopausal women, respectively. Young women depleted of vitamin B<sub>6</sub> exhibited negative Mg balance because of increased urinary excretion (Turnland et al., 1992). Inulin (Coudray et al., 1997) and two fermentable polyols (Coudray et al., 2003) were found to increase the intestinal absorption of Mg.

#### **Plant Nutrition Impacts on Food Magnesium**

*Nutritional Importance for Plants.* Magnesium is an essential nutrient for plants, in which the relative abundance of Mg is less than N, K, and Ca and is similar to S and P (Wilkinson et al., 1990). The Mg requirement for optimal plant growth is in the range of 0.15 to 0.35% of the dry weight of the vegetative parts (Marschner, 1995). Magnesium is the central atom of chlorophyll and is needed for the aggregation of ribosome units for protein synthesis. Similar to animals and humans, plants have many enzymes that require Mg or have MgATP as a substrate. Thus, Mg is involved in DNA, RNA, protein, lipid, and carbohydrate formation and/or function and cellular energy production and storage. Intense cultural practices may increase the frequency of Mg deficiency in crop production, and the concentration of Mg in various parts of plants is affected by Mg fertilization (Wilkinson et al., 1990).

Magnesium is taken up by plants in the water soluble form  $(Mg^{2*})$  from soil solution. The presence of this form is influenced by the exchangeable fraction of Mg in soils. The availability of Mg in acidic soils is reduced by Al and Mn and in alkaline soils is reduced by Ca, K, and Na (Wilkinson et al., 1990). Acid-forming N fertilizers  $(NH_4^{+})$  are antagonistic to Mg uptake by plants, and may increase soil acidity, which increases exchangeable Al to compete with Mg (Wilkinson et al., 1990). Most Mg deficiencies in cultivated crops are the result of excessive K fertilization or concentrations in the soil (Wilkinson et al., 1990). Magnesium deficiencies also commonly occur in plants grown in severely weathered, wet,

acid, or sandy soils (Wilkinson et al., 1990). The first symptom of Mg deficiency in plants is loss of chlorophyll (chlorosis), in which Mg is critical for its light-gathering function for photosynthetic C reduction.

*Factors Affecting Magnesium Content in Foods from Plants.* Because Mg is involved in many cellular functions, it is distributed throughout the plant. About 10% is bound to chlorophyll, 75% is associated with the structure and function of ribosomes, and 15% is bound to enzymes and other cation-binding sites (Wilkinson et al., 1990). The concentration of Mg in the food portions of plants is influenced by factors that affect the availability of Mg from the soil and Mg fertilization. For example, Mg fertilization (134 kg/ha) of sweet corn increased Mg in grain by 33%, and of snapbeans increased Mg in pods by 31% (Than, 1955). Fortunately for animal and human nutrition, Mg apparently preferentially accumulates in grain when availability of Mg to plants is low. When Mg supplies approach adequacy, vegetative structures then become storage sinks for Mg (Wilkinson et al., 1990). These findings indicate that the values for foods from plants, especially foods made from vegetative plant parts, can vary depending upon the environment in which they were grown.

# Potassium

#### **Nutritional Importance**

**Biochemical and Physiological Functions.** Potassium is an activator or cofactor in some enzymatic reactions. These reactions include pyruvate kinase in carbohydrate metabolism that yields ATP, and Na<sup>+</sup>, K<sup>+</sup>-ATPase that is responsible for the active transport or pumping of Na<sup>+</sup> and K<sup>+</sup> in opposite directions across plasma membranes. This pump results in K being the major intracellular cation and Na the major extracellular cation. Potassium is the ion that neutralizes high concentrations of intracellular anions (e.g. proteins, phosphates, and Cl<sup>-</sup>). In addition, a major function of K is membrane polarization, which depends upon the concentrations of intracellular and extracellular K (Preuss, 2006). The roles of K result in it being involved in acid-base regulation, osmotic pressure maintenance, nerve impulse transmission, muscle contraction, and carbon dioxide and oxygen transport (National Research Council, 2005; Preuss, 2006).

**Consequences of Deficient Intakes.** Because of its role in membrane polarization, the major effects of both hypokalemia (<3.5 mmol/L plasma) and hyperkalemia (>5.5 mmol/L plasma) involve changes in membrane function, which are particularly significant in neuromuscular and cardiac conduction systems (Sheng, 2006; Preuss, 2006). Potassium deficiency caused by low dietary intake rarely occurs because K is usually consumed in amounts required for obligatory losses and maintenance of tissue levels. Depletion occurs only when intake is inadequate during prolonged fasting or with severe dietary restriction (Sheng, 2006). Adverse effects of hypokalemia include cardiac arrhythmias, muscle weakness, and glucose intolerance. Cardiac arrest caused by abnormal electrical conduction is the most serious clinical manifestation of hyperkalemia.

Chronic low K intakes not resulting in hypokalemia have been associated with hypertension and its related cardiovascular disorders such as stroke. Numerous studies have shown that K supplementation may lower blood pressure, especially in salt-sensitive individuals (Suter, 1998; He and MacGregor, 2001; Food and Nutrition Board, 2010). A low K diet may cause Na retention and augment hypertension in hypertensive individuals (Krishna and Kapoor, 1991), and increase blood pressure in healthy individuals (Krishna et al., 1987). However, hypertensive individuals are more likely to respond to K supplementation than non-hypertensive individuals (Siani et al., 1991).

Chronic low K intakes not resulting in hypokalemia also have been associated with bone loss. This association is thought to occur through inadequate K intakes resulting in a disordered acid-base metabolism. Modern diets generally are high in acid-producing NaCl, P, and proteins (contain acid-producing S amino acids), and low in fruits and vegetables containing acid-balancing K and bicarbonate, which results in a metabolic acidosis. This acid-base imbalance has been associated with bone loss that may lead to osteoporosis (New et al., 2004; MacDonald et al., 2005). The suggestion that K deprivation adversely affects bone maintenance through disordered acid-base balance is supported by the finding that K citrate prevented increased urine Ca excretion and bone resorption induced by a high NaCl diet in postmenopausal women (Sellmeyer et al., 2002). However, some K supplementation trials have not shown significant positive effects on bone maintenance in older men and women (Dawson-Hughes et al., 2009; MacDonald et al., 2008). These conflicting findings indicate that further studies are needed to determine the significance of K intake in the relationship between metabolic acidosis and bone maintenance.

#### **Dietary Recommendations**

The U.S. Food and Nutrition Board (2005) were not able to establish an EAR or a RDA for K because of insufficient dose-response data. Instead, an Adequate Intake (AI) of 4.7 g (120 mmol)/day was set for all adults. The AI is set when there are insufficient data to set a RDA and based on estimates of an average intake by a group of healthy people. The Food and Nutrition Board (2005) stated that available evidence indicated that the AI should help blood pressure, reduce the risk of kidney stones, and possibly reduce bone loss. The Food and Nutrition Board (2005) noted that the beneficial effects of K mainly were associated with forms of K with bicarbonate precursors, which are the forms found naturally in foods such as fruits and vegetables.

Dietary intakes of K of adults in the U.S. and Canada generally are lower than the AI (Food and Nutrition Board, 2005). The median intake in the U.S. was found to be about 2.9 to 3.2 g (74 to 82 mmol)/day for men and 2.1 to 2.3 g (54 to 59 mmol)/day for women, and in Canada, 3.2 to 3.4 g (82 to 87 mmol)/ day for men and 2.4 to 2.6 g (62 to 66 mmol)/day for women (Food and Nutrition Board, 2005). Based on NHANES III data, the K intakes of only 10% of men and less than 1% of women in the U.S. are  $\geq$  the AI (Food and Nutrition Board, 2005).

#### **Potassium Intake Factors**

**Potassium Content in Foods.** Because K is the principal intracellular cation in animals and plants, it is widely distributed in foods. Thus, a severely K-deficient diet is very unlikely. The richest plant sources of K are fruits and vegetables. Milk and meat products also are good sources of K. In one population study (Rafferty and Heaney, 2008), fruits and vegetables provided 44%, and dairy, meat, and cereal grains provided 56% of the K in the diet. Refined sugars and fats are very low in K. The K contents of some foods of plant origin are shown in **Table 2**.

*Interactions with Other Nutrients.* The beneficial effects of K supplementation usually are found when K is associated with bicarbonate precursors or organic anions such as citrate and malate (Demigné et al., 2004), which results in greater alkalizing potency; these are the major forms of K in fruits and vegetables. The K in cereals and animal products is chiefly associated with phosphate and Cl<sup>-</sup> (Demigné et al., 2004), which are acidogenic; these foods also are high in acidogenic S amino acids (Food and Nutrition Board, 2005).

As indicated above, K interacts with Na and Cl<sup>-</sup> to maintain acid-base balance and electrical and chemical gradients in the body. Also indicated above, is the fact that K blunts the effect of NaCl on blood pressure. The relationship between K and Na suggests the need for K may be increased by high intakes of Na.

Magnesium status also can affect the K metabolism. Magnesium depletion can cause hypokalemia (Whang et al., 1994). Both severe (Shils, 1980) and moderate (Nielsen et al., 2007) Mg deprivation increases urinary K excretion. Intracellular K is decreased during Mg depletion, which is enhanced by the inability of the kidney to conserve K (Rude and Shils, 2006). Repletion of the K deficit caused by Mg deprivation by supplemental K does not occur unless Mg is simultaneously supplemented (Rude and Shils, 2006).

#### **Plant Nutrition Impacts of Food Potassium**

*Nutritional Importance for Plants.* Next to N, K is the mineral nutrient required in the largest amount by plants (Marschner, 1995). Potassium in plants activates or stimulates numerous enzyme reactions, including pyruvate kinase, phospho-fructokinase, starch synthase, and membrane-bound proton-pumping ATPases. Potassium is involved in protein synthesis, photosynthesis, and osmoregulation (Marschner, 1995). The K requirement for optimal plant growth is in the range 2 to 5% of dry weight of vegetative parts, fleshy fruits, and tubers (Marschner, 1995).

Potassium in soil occurs in soil solution, as exchangeable K, difficultly exchangeable K and as a mineral (Barber, 1984). Soil solution K is considered the primary source of K absorbed by the plant root. Soil solution K varies with weathering, past cropping practices, and fertilization (Barber, 1984). Exchangeable K held by negative charges on clay and organic matter usually ranges from 40 to 500 mg/kg soil; 150 mg/kg is considered enough to ensure optimal plant growth (Barber, 1984). Because plants may absorb a large fraction of available soil K, and K movement from the difficultly exchangeable pool to the exchangeable pool does not readily occur in some soils, K fertilization of crops is important for production. Growth of plants is retarded in K deficiency, and in severe deficiency, plant leaves and stems become chlorotic and necrotic. Loss of turgor and wilting are symptoms of K deficiency when the soil water supply is limited. Potassium deficient plants are more susceptible to lodging, frost damage, and fungal attack (Marschner, 1995).

*Factors Affecting Potassium Content in Foods from Plants.* Increasing the supply of K to roots increases the K content of all organs of plants except grains and seeds, which maintain relatively constant K content of 0.3% of the dry weight (Marschner, 1995). Soybeans may be considered an exception, with K contents as high as 1.9% on a dry-matter basis. Cereal grains are the only plant food group that consistently yields noncarbonic acid precursors in excess of bicarbonate precursors (Food and Nutrition Board, 2005). Thus, increasing soil K to plants apparently will have limited impact on the amount and form of K in foods based on seeds or cereal grains. However, increasing K to roots may increase the K content of fruits and vegetables, which are characterized by having K associated with alkalizing bicarbonate precursors.

# **Summary and Conclusion**

Calcium, Mg, and K are essential nutrients for humans. Foods of plant origin are good sources to help meet human requirements for these elements. Legumes or pulses (especially soybeans), nuts and some vegetables can provide significant amounts of Ca, which may be increased by available soil Ca. Green leafy vegetables, whole grains, legumes and nuts are rich sources of Mg. The Mg content in these foods can vary depending upon the environment in which they were grown. The richest sources of K are fruits and vegetables, but grains may provide significant amounts to the human diet. Increasing the supply of K to roots increases the K content of all organs of plants except grains and seeds. Because the Ca, Mg, and K contents of foods of plant origin vary with the amount and availability of these minerals in the soil, fertilization practices may have an impact on how well these foods contribute to meeting the requirements for Ca, Mg, and K. **FCHH** 

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# Protein, Carbohydrate and Oil Composition of Food Crops

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# Abstract

Fertilizer use in crop production is often focused toward optimizing crop yield and profitability for the producer. However, it also affects the chemical composition of crops, potentially influencing the quality of food products made from them. Major crop quality factors include protein, carbohydrate, and oil; both the relative amounts and their specific composition and bioavailability can be important. To produce cereal grains with high levels of valuable protein, N must be made available at levels higher than those necessary for optimum yields. Management tools such as late foliar applications or controlled-release technologies can increase N availability for protein production while keeping losses of surplus N to a minimum. Applying other nutrients, particularly S, in balance with N is important to optimize protein for bread-making quality. Nitrogen applications increase the hardness of rice grains, reducing breakage during milling, and improving the amino acid balance from a nutritional standpoint. However, moderate rather than high levels of N optimize starch composition for improved cooking and eating quality. Higher levels of N increase protein in maize and potato, but reduce its biological value owing to lower ratios of the limiting amino acids lysine and tryptophan. However, opaque-2 cultivars of corn bred for high quality protein maintain high biological value at higher levels of N. Adequate K can help minimize acrylamide formation in fried potatoes. Enhancing S supply to soybeans during grain-filling can improve protein composition by increasing the ratio of the limiting amino acids methionine and cysteine. Where S limits yield in canola, the depressed yield can lead to increased oil concentration. In general, fertilizing for optimum yields does not differ greatly from fertilizing for optimum quality for most of the world's major food crops. In the long term, ensuring that soil fertility is maintained is important to avoid the major declines in crop yield and nutritional quality that can be seen when crops are grown on highly depleted soils.

Abbreviations specific to this chapter: ALA =  $\alpha$ -linolenic acid; CVD = coronary vascular disease; GI = glycemic index; HMWG = high-molecular-weight glutenins; LMWG = lowmolecular-weight glutenins; QPM = Quality Protein Maize. For symbols used commonly throughout this book see page xi.

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# Introduction

Effective nutrient management is critical to optimize crop yield and profitability and to reduce the risk of negative environmental effects from fertilizer use. However, nutrient management also plays an important role in crop quality. Major crop quality factors include protein content and composition, oil content and fatty acid profile, and carbohydrate composition. The relative importance of these factors depends on the final end use of the crop. This chapter discusses the influence of fertilizer management on the composition of the major food crops rice, maize, wheat and potatoes, and the oilseed crops, soybean and canola (rapeseed), and how such changes in composition influence the nutritional and functional quality of the crops.

# **Quality Considerations for Food Crops**

The three major staple food crops grown in the world are the cereals rice, maize and wheat (FAOSTAT, 2010). Cereals are an important source of energy, carbohydrate, protein and fiber in the human diet (McKevith, 2004). However, cereals are often consumed in a processed form, such as in breads and pasta, so quality of the product is often defined on the basis of the effect on the functional properties for processing, rather than solely on nutritional quality (Shewry, 2009).

Potatoes also make a major contribution to human nutrition, ranking fourth in world production volume after rice, wheat and maize (FAOSTAT, 2010). While considered primarily a source of starch, a potato crop can produce as much as 800 kg protein/ha, and its protein has been shown to have high nutritive value in comparison to other sources of plant protein including wheat, rice, maize, bean and soybean (Wang et al., 2008).

The two major annual oilseed crops produced in the world are soybean (*Glycine max* L.) and rapeseed (canola) (*Brassica spp.*), while palm oil, a product of the perennial oil palm tree (*Elaeis guineensis*) ranks between these two annual crops in terms of global oil production. Cottonseed, sunflower, peanuts and maize (corn) are also important sources of edible oil (USDA-FAS, 2010). In crops such as canola, soybean, sunflower, or flaxseed where the primary end product is oil, optimizing the concentration of extractable oil in the seed is desirable. The fatty acid composition of the oil is also important, affecting stability and health aspects. The meal that remains after the oil is removed is also important as a source of protein, and therefore, protein content is also a quality consideration for the oilseed crops. Soybean dominates the market both as a source of oil and meal and is a major protein source for vegetarian diets and in some Asian countries, including Japan.

#### Protein

Protein content and composition affects both the nutritional and functional properties of foods. In the UK, cereals contribute about 23 to 27% of the dietary intake of protein (McKevith, 2004), with the proportion likely to be higher in

countries where animal protein consumption is lower. In wheat, protein is mainly stored as gliadins and glutenins, in rice as glutelin (oryzenin) and in maize as prolamin (zein) (Dewettinck et al., 2008). The nutritional quality of a protein source depends largely on the essential amino acid content as well as the concentration of protein in the processed grain (Gatel, 1994). Bioavailability or digestibility of the protein is also a factor. Cereal proteins in general tend to be low in essential amino acids such as lysine and to a lesser degree threonine, but contain relatively high amounts of cysteine and methionine. However, the glutelins in rice tend to have higher lysine content than the prolamins in maize and wheat, increasing its nutritional value (Juliano, 1999; Souza et al., 1999).

Depending on the end-use, cereals often undergo milling to extract the endosperm, leading to removal of the seed coat and aleurone layers, which contain much of the trace element content of the seed (**Figure 1**). However, proteins are located around the starch granules in the endosperm (Piot et al., 2000) as well as in the outer layers of the kernel, and although protein content decreases with milling, the decrease is not as great as that for the trace elements and vitamins (Batifoulier et al., 2006; Dewettinck et al., 2008; Greffeuille et al., 2005).



#### Figure 1. General structure of a cereal grain (from WHEAT: THE BIG PICTURE, http://www.wheatbp.net/cgi-bin/display.pl?image=Graindiag; accessed 20 January 2011).

Oilseed crops may also contribute to protein composition of the human diet, either directly, as a food source, or indirectly after utilization as a livestock feed. The meal left after oil is extracted from soybean or canola is high in protein and is commonly used as a protein supplement. Soybean is also used in the human diet, mainly as a protein source in infant formula, meat substitutes, soy milk, tofu and baked goods, being especially important in vegetarian diets. Soybean has a relatively well-rounded amino acid profile for a plant protein, containing adequate quantities of most of the essential amino acids, with the exception of methionine and cysteine. Soybean is particularly high in lysine, and combining cereals such as rice, wheat or maize with soy protein improves the protein composition of the diet (Erdman and Fordyce, 1989; **Table 1**).

	Soybean (meal) <sup>†</sup>	Tofu <sup>‡</sup>	Wheat <sup>‡</sup>	Maize <sup>‡</sup>	<b>Rice<sup>‡</sup></b>
Dry matter	92	30	88	89	88
Crude protein	44	16	14	9	7
Amino acids:					
Methionine	0.59	0.20	0.22	0.20	0.17
Cysteine	0.67	0.22	0.33	0.17	0.15
Lysine	2.70	1.04	0.35	0.27	0.26
Threonine	1.72	0.64	0.39	0.35	0.26
Tryptophan	0.60	0.25	0.17	0.07	0.08
Arginine	3.29	1.05	0.60	0.47	0.59
Isoleucine	2.02	0.78	0.52	0.34	0.31
Leucine	3.39	1.20	0.95	1.16	0.59
Valine	2.11	0.80	0.62	0.48	0.44

**Table 1.** Concentration (g/100g) of dry matter, protein and selected amino acids in soybean meal, wheat, maize and rice.

† from Fontaine et al. (2000).

*‡ USDA-ARS (2010). National nutrient database values for tofu (raw, firm), wheat (mean of durum and hard red), maize (yellow), and rice (raw, white, long-grain).* 

#### Carbohydrate

Carbohydrates comprise about 40 to 80% of the energy in the human diet, with the greatest proportion occurring in the developing countries. Starch accounts for about 20 to 50% of the energy in countries where the total carbohydrate intake is high (FAO, 1998). Cereal crops provide over 50% of the carbohydrate consumed, followed by sugar crops, root crops, fruits, vegetables, and pulses. Carbohydrates are classified according to their degree of polymerization into three major groups, sugars, oligosaccharides and polysaccharides (**Table 2**).

Carbohydrate quality is affected by a number of factors, with glycemic index (GI) being of particular importance. The GI measures the rate at which starch is digested to release energy, with low GI associated with a more prolonged supply of energy and a more stable blood sugar level over time. Glycemic response is affected by the nature of the monosaccharide components, the nature of the starch, the cooking and processing that the food undergoes, and other food components present in the diet.

As well as being a source of energy, carbohydrates provide an important source of dietary fibre in the form of inulin, cellulose, hemicelluloses, resistant starch and other components (Gebruers et al., 2008). Dietary fibre is associated with a reduced risk of chronic diseases such as cancer, coronary heart disease, and diabetes.

Class (Degree of Polymerization)	Sub-Group	Components	
	Monosaccharides	Glucose, galactose, fructose	
Sugars (1-2)	Disaccharides	Sucrose, lactose, trehalose	
	Polyols	Sorbitol, mannitol	
	Malto-oligosaccharides	Maltodextrins	
Oligosaccharides (3-9)	Other oligosaccharides	Raffinose, stachyose, fructo-oligosaccharides	
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches	
i orysaccharlices (22)	Non-starch polysaccharides	Cellulose, hemicellulose, pectins, hydrocolloids	

Table 2. Th	he major dietary	carbohydrates	(adapted from	FAO, 1998).
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#### Oil

In crops such as canola, soybean, sunflower, or flaxseed where the primary end product is oil, optimizing the concentration of extractable oil in the seed is desirable. A secondary consideration is the effect on oil quality. In oils destined for human consumption, such as canola, maize, sunflower or soybean oil, it is desirable to maximize the mono-unsaturated fatty acids to improve the health aspects.

Oil is important to human health, particularly in developing countries, where it is in short supply and can potentially limit the absorption and retention of critical nutrients like vitamin A. While they noted that the amount of dietary oil required for optimal bioavailability of carotenoids from plants is not clearly defined, Brown et al. (2004) found that salad dressings containing canola oil markedly improved carotenoid absorption from salads consisting of spinach, romaine lettuce, cherry tomatoes, and carrots.

High levels of saturated fatty acids in the diet are associated with an increased risk of coronary vascular disease (CVD). In contrast, omega-3 polyunsaturated fatty acids (n – 3 fatty acids) have been associated with a reduced risk of coronary heart disease. While fish oils are among the best sources of preformed long-chain omega-3 polyunsaturated fatty acids,  $\alpha$ -linolenic acid (ALA), an essential shorter-chain omega-3 fatty acid, is present in flaxseed, soybean, and canola oils and is also of interest for CVD prevention (Jung et al., 2008).

# Wheat

Wheat is a major component of the human diet throughout much of the world. Approximately 95% of wheat grown in the world is hexaploid bread wheat (*Triticum aestivum*, L.), used for a wide range of baked goods, including bread, cookies, cakes and biscuit, with most of the remaining 5% being tetraploid durum

wheat (*Triticum durum*, Desf.) commonly used for pasta and noodles, couscous and bulgar. Protein content and composition is a critical quality factor in the production of these products (Shewry, 2009).

#### Protein

The protein content of wheat grain varies between approximately 10% and 18% of the total dry matter (Belderok, 2000). Nutritionally, wheat is a good source of many of the essential amino acids, although the prolamins that are the major storage protein in wheat are deficient in lysine, threonine and tryptophan (Shewry, 2009; **Table 1**).

Nitrogen is the major nutrient influencing protein concentration in grain. Nitrogen is a major component of protein (Olson and Kurtz, 1982), with approximately 17% of protein being composed of N. Numerous studies over the years have demonstrated the relationship between increasing soil N concentration and increasing grain yield and protein concentration (Fowler, 2003; Kindred et al., 2008; Miao et al., 2006; Miao et al., 2007; Olson et al., 1976; Terman et al., 1969). Nitrogen assimilation by the grain is more source-limited than dry matter yield (Gooding et al., 2007). If the amount of available N from the soil through the growing season is low relative to the crop yield potential, crop yield and protein concentration usually both increase with application of N fertilizer (Campbell et al., 1997; Fowler, 2003; Gauer et al., 1992; Halvorson and Reule, 2007). If crop yield is increased by factors such as beneficial weather, improved crop cultivars, or improved management practices, while the N supply remains constant, protein concentration often decreases by biological dilution (Fowler, 2003).



Figure 2. Yield and protein of wheat respond to applied N fertilizer.

The pattern of response of yield and protein content to N dose reflects the supply of available N relative to the crop yield potential. If N supply is restricting to yield, low doses of N fertilizer tend to increase grain yield preferentially over protein (Fowler, 2003). There may be a decrease in protein concentration with small applications of N, as the yield increase dilutes the protein with biomass (**Figure 2**). After an initial lag, protein concentration increases to an optimum—usually greater than the optimum for yield—and then levels off.

In semi-arid climates, where moisture is frequently the environmental factor that limits yield, moisture supply interacts with N in affecting protein concentration. Where moisture is ideal and yield is increasing substantially in response to N applications, protein increases are moderate until higher N rates are applied and the yield response begins to decline. With moderate moisture and N supply, both yield and protein increase with increasing N application rate. With moisture stress, yield is restricted and the major effect of increasing N application is to increase protein (**Figure 3**). In moisture-limited environments, changes in moisture supply essentially shift the protein-N response curve, so that with increasing moisture supply a greater amount of available N is required to produce a specific protein concentration in the grain (Gauer et al., 1992). As the available N increases and the N use efficiency of the crop decreases. Therefore, applying N at rates greater than those required for optimum crop yield in order to increase protein content may lead to lower NUE and increased potential for N losses.



Figure 3. Grain yield and protein concentration of hard red spring wheat under moderate and high moisture conditions (adapted from Gauer et al., 1992).

Timing of N supply also influences grain protein concentration. Nitrogen available to the plant early in the growing season tends to stimulate vegetative growth and increase crop yield, while later applications have a smaller effect on yield but a larger effect on protein concentration (Fowler, 2003; Fowler et al., 1990). Yield potential is largely affected by pre-anthesis conditions that determine the grain sink in terms of spikelets and florets, and the biomass of stems and leaves that provide a source for subsequent re-translocation of N (Peltonen, 1993). Application of N after anthesis normally has a greater influence on protein accumulation than on starch accumulation (Souza et al., 1999; Wuest and Cassman, 1992). Nitrogen in the grain comes from post-anthesis uptake and from remobilisation and transport of N from the vegetative parts of the plant to the grain. In wheat, as much as 70 to 100% of N uptake may occur by heading (Boatwright and Haas, 1961; Malhi et al., 2006). Often, the N in wheat grain is primarily derived from translocation from leaves, stems and chaff rather than from further absorption from the soil.

With winter wheat, application of N in the spring or split with a higher proportion applied in the spring tends to produce higher protein concentration than fall application (Grant et al., 1985; Kelley, 1995; Vaughan et al., 1990). In no-till winter wheat grown in Saskatchewan, Canada, early spring applications of ammonium nitrate and urea-based fertilizers were effective at enhancing grain yield. Delaying the application by three weeks reduced grain yield response and grain protein yield, but increased grain protein concentration (Johnston and Fowler, 1991). Delays in the availability of N as a result of late spring applications of N on winter wheat or prolonged dry periods following spring fertilization can limit grain yield potential (Fowler et al., 1990). Then, if accessed later, the fertilizer N may be in excess of requirements for optimal yield, so grain protein concentration can increase. Use of enhanced-efficiency fertilizers, such as polymer-coated products, that delay the release of N into the soil solution may be effective at increasing protein concentration of crops by releasing the N for uptake later in the growing season (Grant and Wu, 2008).

Late applications of N can be effective at increasing protein content, particularly where environmental conditions promote losses of N prior to crop uptake. Extra N applied during the post-anthesis phase can increase the rate of grain protein synthesis, if the pre-anthesis N supply is low and the crop is able to absorb the late-applied N (Peltonen, 1993). Uptake of N fertilizer applied at anthesis, as indicated by increase in grain protein per unit of N applied, was more efficient than uptake from pre-plant additions in irrigated hard red spring wheat in California (Wuest and Cassman, 1992). In this study, level of pre-plant N addition had little influence on post-anthesis N uptake, while N applied at anthesis increased N uptake markedly. Soil analysis showed little difference in soil N content among the pre-plant N applications, indicating that much of the pre-plant N fertilizer was lost by leaching, denitrification or immobilization. Application of irrigation water after the late season N application likely contributed to the efficient uptake of the late N application.

In rainfed cropping systems, uptake of late-applied N from the soil may be impaired when conditions are dry. Foliar applications of N fertilizer have been investigated for decades as a method of providing N during grain formation to enhance grain protein concentration in cereal crops (Bly and Woodard, 2003; Gooding and Davies, 1992; Gooding et al., 2007; Souza et al., 1999). Uptake of N from late-season foliar applications would be less dependent on soil conditions and could be effective for protein enhancement. In studies with winter wheat, foliar applications of urea after anthesis were more rapidly translocated to the grain than soil applications of ammonium nitrate (Gooding et al., 2007).

Foliar N applications are more likely to increase protein content when N supply is low relative to the crop yield potential (Bly and Woodard, 2003; Finney et al., 1957; Gooding and Davies, 1992; Gooding et al., 2007). Gooding and Davies (1992) reported that yield responses to foliar applications of urea decrease as application is delayed beyond flag leaf emergence. While soil applications of N fertilizer are more effective at improving grain protein concentration when applied before anthesis, foliar applications seem to be more effective when applied after anthesis. Reduced root activity after anthesis may limit uptake of soil applied N. The optimum timing for foliar urea application to increase protein concentration is usually from anthesis through the following two weeks. This may be in part because at these later timings there is less likelihood of a yield increase and so less dilution from increased carbohydrate. Applications later than two weeks after anthesis tend to be less effective in increasing grain protein concentration, possibly because of the limited green tissue to intercept the spray and restricted incorporation and translocation of the N in the plant (Gooding and Davies 1992).

Phosphorus does not normally appear to have a direct influence on protein concentration in cereals under field conditions, but may indirectly decrease protein concentration through dilution when P fertilization causes a significant increase in grain yield (Halvorson and Havlin, 1992; Holford et al., 1992; May et al., 2008; Porter and Paulsen, 1983). Phosphorus may also influence protein accumulation through effects on N uptake and metabolism. In solution culture, grain protein concentration increased with increased P supply (Porter and Paulsen, 1983). Phosphorus fertilization increased N uptake of wheat from emergence to tillering, indicating that the beneficial effect of P on N absorption occurred early in plant development. Under field conditions, adequate P in combination with N may stimulate better root development so that more N can be absorbed by the plants (Boatwright and Haas, 1961). Since the effects of P on protein content tend to be indirect, P fertilization often has no significant effect on protein content (Brennan and Bolland, 2009b; McKercher, 1964).

Potassium is important in N relations within the plant. In nutrient solution culture, K increased the rate of amino acid translocation into wheat grain as well as the conversion of amino acids into grain proteins (Blevins et al., 1978). Higher K appeared to increase the transport rate of amino acids from the vegetative plant parts into the grain (Mengel et al., 1981). Protein synthesis in the grain was also promoted by improved K nutrition, likely indirectly through the improved accumulation rate of amino acids into the grain. Under highly K-deficient conditions, K fertilization can increase both protein yield and protein concentration (Bakhsh et al., 1986). However, under field conditions of moderate to low deficiency, K normally has little effect on protein concentration of wheat (Brennan and Bolland, 2009a; Campbell et al., 1996; May et al., 2008).



#### Figure 4. The relation between loaf volume and protein content of the flour for three wheat varieties of good (▲), intermediate (○) and poor (●) baking quality (Belderok, 2000).

Baking quality of wheat flour generally increases with increasing grain protein content, with loaf volume increasing as protein concentration increases (**Figure 4**).

Albumins and globulins are small, physiologically active proteins, comprising about 25% of the total grain protein and concentrated in the seed coat, aleurone cells and the germ, with a lower concentration in the endosperm. The remaining 75% of grain protein consists of storage proteins in the endosperm, gliadins and glutenins that have no enzymatic activity but are important in the functional quality of the wheat for bread-making (Belderok, 2000). These components absorb water during dough mixing and form gluten.

Gluten creates an elastic structure during mixing that holds the  $CO_2$  formed by the yeast during fermentation in the bread-making process, making the bread rise. The more elastic and extensible the gluten structure, the more the dough rises. When the dough is baked, the protein structure sets, forming the crumb texture and loaf volume. The quality and quantity of gluten is the major factor affecting bread-making quality and is largely determined by crop genetics and N supply. Hard, high protein "strong" wheat generally has high gluten strength and is suitable for raised breads. Soft wheat flour has lower protein and forms softer gluten as compared to hard wheat. Soft wheat is not suitable for bread-making, but gives good results for biscuits and cookies. In pasta, both protein content and composition are important in determining cooking quality, with a high glutenin content encouraging good cooking quality (Porceddu, 1995). While N fertilization increases all protein fractions in bread wheat, it has a proportionally greater effect on the gluten fractions, specifically low-molecular-weight glutenins (LMWG) and gliadins as compared to albumins and globulins and the high-molecular-weight glutenins (HMWG) (Kindred et al., 2008; Tea et al., 2004). Similarly, in durum wheat, N application increased the amount of LMWG at the expense of the HMWG (Lerner et al., 2006). Increases in protein content due to N fertilization may not improve nutritional value to the same extent because of the proportionally higher increase in the lysine-poor gluten proteins (Shewry, 2009).

The N:S balance is important in ensuring protein quality. Sulphur is an important component of protein in wheat, being a constituent of the SH-containing amino acids that contribute the enhanced quality of protein for baking (Zhao et al., 1999a; Zhao et al., 1999b; Zhao et al., 1999c). Sub-units of the gliadins and glutenins which comprise gluten are rich in S. The S-containing sub-units are important in determining the elasticity of dough because the inter-chain disulphide bands stabilize the polymer network formed by the gluten molecules (Shewry et al., 2002). Therefore, adequate amounts of S must be present for protein quantity and quality (Flæte et al., 2005; Thomason et al., 2007; Zhao et al., 1999b; Zhao et al., 1999c). Where soil S levels are deficient, both grain yield and grain quality can increase with S fertilization. Even where S availability is adequate for optimum yield, protein quality may be affected by S application (Flæte et al., 2005; Thomason et al., 2007).

Sulphur deficiency leads to a change in the composition of protein in wheat. Amounts of S-poor proteins such as  $\omega$ -gliadins increase and S-rich proteins such as  $\gamma$ -gliadins and low molecular weight subunits of glutenin reduce with S deficiency (Tea et al., 2007; Tea et al., 2004; Wieser et al., 2004). The reduced proportion of S-rich compounds leads to tough, less extensible dough and lower bread volume even with a comparable protein content (Reinbold et al., 2008; Thomason et al., 2007; Zörb et al., 2009; **Figure 5**). Similarly, in durum wheat, on a site that was not S-deficient, S application increased the amount of LMWG at the expense of the HMWG (Lerner et al., 2006).

Sulphur does not remobilize from vegetative tissue to the grain as much as N does, therefore an adequate supply of S must be obtained through grain-fill to optimize crop quality. Under highly S-restricted greenhouse conditions, late S fertilization resulted in a higher loaf volume than early S fertilization (**Figure 5**; Flæte et al., 2005; Zörb et al., 2009. Application of high levels of N fertilizer to S-deficient areas may widen the N:S ratio, aggravating S deficiencies. Gooding et al. (1991) reported that a lack of improved loaf quality after urea application could sometimes be related to insufficient grain S concentrations, high N:S ratios and associated changes in the proportions of protein fractions. They suggest that more consistent effects of urea on bread-making quality might be achieved if S nutrition could be improved. In studies with wheat in England, application of N in the absence of S produced lower N concentration than applications of N with



**Figure 5.** Effect of different S fertilization rates on the baking quality of two wheat cultivars as measured by a microscale baking test using 10 g of wholemeal flour. (A) Images of micro bread slices of the cultivar Batis at a comparable scale. (B) Histograms of bread volumes; different letters represent significant differences of the mean values. Error bars represent (standard errors of five independent pot replicates. Statistical significance (p<0.05) is indicated by small letters for the S rates and capitals for the cultivars (Zörb et al., 2009).

S, consistent with a need for a balance between N and S to support protein synthesis (Godfrey et al., 2010). Dough from grain grown with high N, but without applied S showed lower dough strength than samples grown with S (Godfrey et al., 2010; Wooding et al., 2000). Sulphur may also interact with foliar N applications, with a greater increase in protein occurring with foliar N fertilizer when applied to wheat that had previously received S applications (Thomason et al., 2007).

#### Carbohydrate

Starch is the major carbohydrate present in wheat and is used as a source of energy in the human diet. Starch is composed of amylose and amylopectin, both polymers of glucose, amylose being fairly linear and amylopectin being highly branched (Cornell, 2003). A notable difference between amylose and amylopectin is in gel formation. Amylopectin does not form gels, while amylose forms gels in mixtures with water. Starch forms paste upon heating in water. Viscosity increases as gelatinization begins and continues to increase until gelatinization is complete, then decreases as the structure breaks down. This property is important where starch is used as a thickener. Excess  $\alpha$ -amylase in the grain

increases breakdown of starch and causes a reduction in the viscosity of starch paste. This leads to sticky doughs that are difficult to process and to poorly structured and discoloured loaves (Kindred et al., 2005). Low Hagberg falling number (HFN) indicates high  $\alpha$ -amylase in the dough and hence a weaker dough.

The amount of water absorbed by flour to give the best mixing also depends on the starch properties. Small granules give a higher water absorption than large granules and mixing time seems to be longer for large than for small granules (Eliasson, 2003). Amylose appears to be responsible for setting of the crumb structure. Bread goes stale largely because of recrystallisation of amylopectin and amylose appears to increase this recrystallisation.

Starch content also influences the quality of pasta produced from durum wheat. Normally starch content is negatively related with cooking firmness of pasta, although this may be a reflection of the negative relation between starch and protein content of grain (Porceddu, 1995).

Fiber in diets can reduce the risk of developing a range of diseases including coronary heart disease, stroke, hypertension, diabetes, obesity and some gastrointestinal problems (Anderson et al., 2009). The cellulose content of wheat can be an important source of dietary fiber, especially in whole wheat products. White wheat flour has about 0.6% cellulose while whole wheat flour contains over 2.4% cellulose due to the inclusion of the bran (Anderson and Bridges, 1988). Bran contains about 9% cellulose and about 30% other non-starch polysaccharides, such as the pentosans. Pentosans are hemicelloses that yield pentones upon hydrolysis. Arabinoxylans are pentosans in wheat grain that are composed primarily of D-xylose and L-arabinose, with the xylose unit backbone linked together in a  $\beta$ -1,4 linkage, and arabinose units linked to the main backbone as single unit sidechains (D'Applonia, 1980). As well as being important fibre components, pentosans such as arabinoxylan contribute to water absorption of flour and viscosity of doughs and batters and can increase loaf volume and improve crumb and crust characteristics (Buksa et al., 2010; D'Appolonia 1980).

Increasing protein content by application of N fertilization can decrease starch concentration in the kernel (Erbs et al., 2010; Kindred et al., 2008). Low N supply also reduced soluble  $\beta$ -amylase. Nitrogen fertilizer increased both protein content and Hagberg falling number (Kindred et al., 2005), related to a higher  $\alpha$ -amylase activity in the absence of N fertilizer application.

# Rice

Rice (*Oryza sativa* L.) is the cereal crop produced in the largest quantity worldwide and the most important crop as a human food. Before consumption, rice is dehulled to remove the lemma and palea and expose the brown rice, which consists of the embryo, endosperm and bran layers. The majority of rice is also polished before use, removing the seed coat and aleurone layer to varying degrees, leaving white rice. Polished rice grain is composed of up to 95% starch, 5 to 7% protein and 0.5 to 1% lipid (Fitzgerald et al., 2009), as well as a wide range of trace elements and components present in low concentrations. Quality traits for rice include physical appearance (i.e. shape, uniformity, and translucence of grains), cooking and sensory properties (gelatinization temperature, gel consistency, fragrance, taste) and nutritional value (Fitzgerald et al., 2009; Yang et al., 2007).

#### Protein

Although its protein concentration is low, rice contributes approximately 29% of the protein for human consumption in developing countries. This nutritional benefit could be improved by increasing the protein concentration and/or reducing the anti-nutritional components such as phytic acid (Ning et al., 2009).

Protein also affects the functional quality of rice such as texture, pasting capacity and sensory characteristics (Ning et al., 2010). Surface hardness of cooked rice is related to protein content. Rice grain breakage during milling can be a problem, because breakage reduces the amount of grain recovered during milling. In rice, proteins occupy the space between starch granules and may act as a binder. Therefore, increasing protein content may increase the resistance of the grain to breakage (Borrell et al., 1999). Percentage of unbroken rice is positively related to the storage protein content of the lateral region of the endosperm, so in cultivars susceptible to breakage, application of N to increase protein content can reduce breakage (Borrell et al., 1999; Leesawatwong et al., 2005). The increased protein increases hardness in the rice grains and increases the resistance to breakage during milling.

Protein content of rice increases most when N is applied at heading (Borrell et al., 1999; Perez et al., 1996). In a study with 31 cultivars of rice in Nanjing China, both the average total protein content, and the protein quality in terms of glutelin:protein ratio, increased with increasing N level (Ning et al., 2009). Nitrogen fertilizer also decreased the concentrations of phytic acid, an anti-nu-tritional factor that decreases the bioavailability of both protein and trace elements (Ning et al., 2009). Rice that received 340-55-375 kg/ha of N-P-K before transplanting had substantially higher protein content than rice that received no fertilizer (Champagne et al., 2009).

Timing of N application also affects protein content in rice. In studies conducted in Louisiana, protein content was increased by 90 and 130 kg N/ha fertilizer applications, with the greatest protein content found where all the N was applied subsurface at seeding or where the N was split into equal applications at seeding and at the 2-mm panicle stage (Patrick et al., 1974). Protein content was lower when all N fertilizer was broadcast prior to first flood, or with half broadcast at first flood and half at first joint.

In the studies with 31 rice cultivars in China, applying moderate amounts of N (185 kg/ha) equally as basal before transplanting and top-dressed at panicle initiation produced a higher yield than applying at the same times in a 80:20 ratio, and a yield equal to application of a higher N rate (300 kg/ha) (Ning et al., 2009). With the right timing, moderate N levels were sufficient to optimize grain yield and nutritional quality. Nitrogen fertilizer applied at anthesis significantly increased protein concentration of rice and increased the percentage of unbroken kernels in cultivars susceptible to broken kernels (Leesawatwong et al., 2005). Nitrogen fertilizer application at flowering consistently increased rice protein and translucency, under both wet and dry conditions, at the International Rice Research Institute farm in the Philippines (Perez et al., 1996), while foliar application 10 and 20 days after anthesis increased rice protein concentration without decreasing grain yield (Souza et al., 1999).

Nitrogen management can also influence the protein distribution in rice. Nitrogen fertilization can increase the protein located in the outer cell layers of the endosperm, which can lead to extra water absorption, affecting the texture of the cooked rice (Champagne et al., 2009). Nitrogen fertilization had a greater effect than genotype on the prolamin and glutelin fraction of the rice protein while albumin and globulin were affected more by genotype than by N treatments (Leesawatwong et al., 2005; Ning et al., 2010). Prolamin is concentrated in the outer layers of rice while glutelin is proportionally higher towards the centre of the grain, therefore glutelin is more likely be retained in the finished product during the milling process than prolamin (Leesawatwong et al., 2005; Ning et al., 2010). In addition, glutelin has a higher proportion of lysine than does prolamin (Souza et al., 1999).

The degree of polishing may affect the amino acid balance of rice protein. Polishing removes the outer layers and the protein that they contain, therefore the protein in the finished product is primarily derived from the endosperm (mainly glutelin and prolamin). In studies conducted by Leesawatwong et al. (2005), polishing decreased the albumin-globulin concentration but increased the glutelin concentration in some cultivars, with the change being affected by the distribution of storage protein in the different layers of the grain. Like other cereal crops, rice tends to be low in the essential amino acid lysine, but is relatively rich in cysteine and methionine (Juliano, 1999; Ning et al., 2010).

Ning et al. (2010) reported that N application increased the concentration of most amino acids in both brown and milled rice, but that methionine in brown rice and lysine and methionine in milled rice were not significantly affected by N. However, lysine was increased by N application in brown rice and tended to increase in milled rice, reflecting the increase in the high-lysine glutelin proteins (Ning et al., 2010). Therefore, N fertilizer may increase both total protein content and protein quality, and may have an important effect on dietary protein contribution from rice in the diet (Leesawatwong et al., 2005).

#### Carbohydrate

Nutritionally, rice is mainly a supplier of energy in the form of starch. Starch characteristics have a dominating effect on rice quality (Champagne et al., 2009). Sensory quality of rice is largely determined by the amylose and amylopectin content and structure, with the firmness of cooked rice increasing with the amylose content and the number of long chains in the amylopectin (Fitzgerald et al.,

2009). Generally, a higher ratio of amylose relative to amylopectin leads to harder cooked rice grains. Rice with low or intermediate amylose content is relatively dry and fluffy and retains a soft texture even after cooling (Yang et al., 2007). However, Bhattacharya (2009) noted that more recent data since the mid-1980s firmly attribute end-use quality mostly to amylopectin chain structure. Higher abundance of extra long chains of amylopectin correlates positively to quality. The long chains lead to strong and resilient starch granules that resist swelling and breakdown during cooking.

Amylose content showed a nonsignificant decreasing trend with N applications in studies conducted by Yang et al. (2007), while in studies by Champagne et al. (2009) amylose content decreased with N-P-K application. Stickiness and cohesiveness of the rice decreased with increases in amylose, while hardness increased. Fertilizer application affected rice flavour and texture, with initial starchy coating, slickness and stickiness between grains reduced by fertilization, and roughness, hardness and moisture absorption increasing with fertilization.

Gelatinization consistency was greatest at moderate N applications, decreasing with high or low N applications, while gelatinization temperature (related to cooking time requirement) was not affected by N application (Yang et al., 2007). Overall, grain quality was less responsive to N than was crop yield, showing trends of higher chalkiness and worse eating/cooking quality at high compared to moderate or low N levels. Quality may be improved by increasing the proportion of N applied after panicle initiation, to improve milling quality, appearance and protein content (Yang et al., 2007).

# Maize

Maize or corn (*Zea mays* L.) is grown primarily for its high starch production and is a major energy source for animal feed (McKevith, 2004), and more recently for fuel ethanol production. The majority of maize produced in 2008 in the United States, the world's largest producer of maize, was used to feed livestock (45%), with the next largest proportion going for ethanol production (30%) (Iowa Corn, 2010). About 10% of maize production in the United States enters the human diet, as starch, corn oil, sweeteners, flour, meal and grist, or to produce beverage alcohol, with an additional 15% being exported outside of the country. While the majority of maize grown in the United States is used for non-food purposes, it is an important food in Asia, Africa, Latin America and parts of the former Soviet Union. For use in feed and food, high protein and hard endosperm are the most desirable. The standard maize cultivars commonly grown in the United States contain about 7 to 10% protein, 68 to 74% starch and 3 to 5% oil (Dado, 1999).

#### Protein

Maize is relatively low in protein compared to wheat, and maize protein is low in the essential amino acids lysine and tryptophan. Consumption of maize with legume crops such as soybean improves the nutritional quality of the diet, as the amino acid profiles of maize and soybean are complementary. Physical quality of maize is also affected by protein content because kernel hardness (vitreousness or translucence) increases and kernel breakage susceptibility decreases with increasing protein content (Mason and D'Croz-Mason, 2002). Increases in crude protein in maize would increase the value of maize in the diet considerably (Johnson et al., 1999). Protein quality can be improved though the production of opaque-2 maize cultivars that have a higher level of lysine and tryptophan than conventional cultivars (Mason and D'Croz-Mason, 2002). However, some of the opaque-2 cultivars have lower zein, leading to softer, flourier endosperms (Misra, 2009). Quality Protein Maize (QPM) cultivars have also been bred to have higher contents of lysine and tryptophan and reduced concentrations of zein (Sullivan et al., 1989), thus increasing the biological value of the protein. However, newer QPM lines have been developed with harder endosperm (Sullivan et al., 1989).

In maize, as in most crops, there is a general inverse relationship between protein content and starch content (Mason and D'Croz-Mason, 2002). Therefore, factors that increase grain yield tend to increase starch concentration and decrease protein concentration. However, grain protein concentration and crop yield both tend to increase with increasing N availability, if N levels are initially deficient (Genter, 1956; Miao et al., 2006; Miao et al., 2007; Riedell et al., 2009; Singh et al., 2005). Differences in factors affecting N supply relative to yield potential, such as field variability, hybrid, and growing season, affect the response of protein to N application (Mason and D'Croz-Mason, 2002). Miao et al. (2007) evaluated variable-rate N management for effects on the protein content and test weight of two maize hybrids, in one field in 2001 and two fields in 2003, in Illinois. They found that the N rate to maximize grain protein concentration was 45 to 50 kg/ha higher than the optimal rate for yield. Protein content was more sensitive than yield to N supply and required more available N to attain a maximum (Miao et al., 2007; Singh et al., 2005). Attempting to address the variability in soil N supply using variable-rate N application did not, however, reduce variability in protein content.

As protein concentration of maize increases with higher rates of N application, the proportion of the protein composed of zein in the endosperm increases as well (Sauberlich et al., 1953; Tsai et al., 1992). Isoelectric focusing analysis showed that increases in zein were primarily due to a quantitative increase in alpha- and gamma-zein polypeptides (Tsai et al., 1992). As zein is important for kernel hardness but is low in lysine and tryptophan (Wang et al., 2008), increases in protein concentration in conventional maize cultivars due to N applications may lead to harder, more vitreous kernels, but decrease the proportions of lysine and tryptophan. Therefore, increases in the protein content of maize with N application may not improve nutritional performance as much as raw protein content because of the low content of the limiting amino acids. In contrast, application of N fertilizer to opaque-2 cultivars maintained or increased the lysine and tryptophan concentration of the kernel (Tsai, 1983). The reduced biological value of maize protein in conventional but not opaque-2 cultivars caused by higher rates of N has been demonstrated through rat feeding experiments (Blumenthal et al., 2008).

As in wheat, a significant amount of seed-filling in maize is due to remobilization of N accumulated in the stalks, leaves and roots during the pre-anthesis period (Bennett et al., 1989; Weiland and Ta, 1992). Newer maize hybrids, however, tend to take up a greater proportion of their N after anthesis, but do not translocate more N to the grain (Ma and Dwyer, 1998). Nitrogen taken up by the crop after canopy formation is less likely to be immobilized in structural components than N taken up earlier in growth and therefore may be more incorporated into grain protein (Gooding and Davies, 1992; Gooding et al., 2007; Powlson et al., 1987).

Phosphate and K fertilization had no significant effect on maize protein content in studies conducted in Virginia, even when significant yield increase occurred with fertilization (Genter, 1956).

#### Carbohydrate

Maize grain is mainly used to provide energy in the form of fermentable carbohydrate. Increases in grain yield normally increase starch concentration and decrease protein concentration (Mason and D'Croz-Mason, 2002). However, N fertilization under conditions of N deficiency can increase yield, protein content and test weight, and decrease oil, starch content and extractable starch content (Miao et al., 2007; Singh et al., 2005; Riedell et al., 2009). Protein and oil content tend to be inversely related to starch content, so increases in these components would tend to decrease the fermentable energy from carbohydrates in maize (Dado, 1999). With the exception of protein, maize quality parameters tend to respond less to N applications than does yield, with starch content and test weight responding least and oil content responding moderately (Miao et al. 2007).

#### Oil

The oil in maize is an important energy source in feed and is highly unsaturated, hence a good oil for human consumption (Mason and D'Croz-Mason, 2002). More than 90% of the kernel oil is located in the germ in maize and oil concentration increases as the germ to endosperm ratio increases. Therefore, oil concentration is normally negatively related to starch concentration (Riedell et al., 2009). Agronomic practices have only a minor effect on the oil concentration of maize grain (Mason and D'Croz-Mason, 2002). In studies conducted in Illinois, oil content increased with N application, but to a lesser extent than protein content (Lang et al., 1956). Similarly, in studies conducted in Virginia, N, P and K fertilization had only minor and inconsistent effects on oil content (Genter, 1956). Percentage oil was shown to increase slightly with N, P and K fertilization, possibly because fertilization increased the ratio of oil-rich germ relative to endosperm (Riedell et al., 2009; Welch, 1969). Fertilization frequently increased grain yield as well, so total oil yield was increased to a greater extent by fertilization.

### **Potatoes**

Eppendorfer and Eggum (1994) reported a comprehensive analysis of potato protein and starch qualities in response to application of mineral fertilizers. While their results were based on outdoor pot studies, making comparison to rates used in field production difficult, they found that nutrient addition levels producing maximum yields generally resulted in high protein and starch levels as well (**Table 3**). They reported that N applied to the soil strongly increased crude protein content of potato, but reduced its biological value. As crude protein increased, the proportion of asparagine increased while that of essential amino acids declined. Nevertheless, the reduction in biological value was smaller than the increase in crude protein, and thus the total production of bioavailable essential amino acids increased with N application, even beyond the rate of application required for maximum yield. Increasing levels of P and K reduced crude protein but increased its biological value. Sulphur deficiency strongly reduced biological value of protein as well, owing to reductions in methionine and cysteine.

Nutrient Level		vel	Potato yield, Starch content,		Crude protein, %		
N	Ρ	к	s	g/pot	%	Content	Biological value
2	3	3	3	124	70	8.3	89
4	3	3	3	317	72	12.9	80
6	3	3	3	266	69	15.9	75
4	1	3	3	134	68	14.9	74
4	4	3	3	454	74	10.3	81
4	3	1	3	50	59	22.9	65
4	3	4	3	332	68	11.5	82
4	3	3	0	173	65	14.7	45

**Table 3.** Effects of level of mineral nutrients applied to soil on yield, starch and protein characteristics of potato (Eppendorfer and Eggum, 1994).

Starch content of potatoes can be reduced by either deficiency or excess of nutrients. Deficiencies of P, K and S reduced the starch content in boiled potatoes, but levels of N, P, and S that maximized yield also provided the highest starch levels (**Table 3**). When K input was increased to very high levels, small increases in yield were offset by small decreases in starch content. Westermann et al. (1994) reported reductions in potato tuber starch content with increasing levels of N and K in field-grown irrigated potatoes in Utah, USA. Henderson (1965) found that sulphate of potash "gave a drier potato with higher content of dry matter and higher specific gravity than muriate, and also a mealier product when cooked" but also noted that these differences were not fully consistent, and were smaller than the variation among the 11 site-years of the study, conducted in Scotland. In India, Kumar et al. (2007a) found that the sulphate form of K was more suited for crisping potatoes than either  $KNO_3^-$  or KCl, since it increased tuber dry matter percentage and crisp yield and also decreased crisp oil percentage.

Working with high-yielding irrigated potatoes in Washington, USA, Davenport and Bentley (2001) reported no difference in specific gravity between sulphate and chloride forms of K fertilizer, applied at high rates, in either granular or liquid form, whether applied pre-plant or partially in-season.

Kyriacou et al. (2009) found no effect of increasing N rates over a range of 0 to 300 kg/ha on quality of chipping potatoes grown in Cyprus, concluding "Completion of physiological crop senescence of the spring potato crop under Mediterranean climatic conditions seems to mitigate the potential interference of preplanting N fertilization with tuber maturation and subsequently cold storage performance, reconditioning potential and processing quality." For processing-grade potatoes in India, Kumar et al. (2007b) found that specific gravity and tuber dry matter percentage increased with increasing N rates from 0 to 360 kg/ha, while crisp color and reducing sugars were unaffected.

Acrylamide is a compound formed when high-carbohydrate foods are cooked and has been associated with adverse health effects. Gerendas et al. (2007) reported that the highest acrylamide contents were observed in French fries processed from potatoes grown with high N and low K supply. Ensuring adequate K may thus help reduce health risks associated with acrylamide.

# Soybean

In soybean, both the oil content and the protein content of the seed are of value. Soybean contains high concentrations of protein (**Table 4**), with methionine and cysteine being the limiting amino acids. Generally, as the protein concentration of the seed increases, the oil concentration decreases.

As a legume crop, soybean normally fixes its own N in a symbiosis with *Brady-rhizobium* bacteria. However, application of P, K, and S fertilizers can influence seed yield, and protein content and composition. Soybeans show greater response in nodule activity and N-fixation capacity than in root or shoot growth when P is applied to P-deficient soils (Cassman et al., 1980; Israel, 1987; Brown et al., 1988). Thus P application could potentially increase protein levels in soybeans, and has been reported to do so in P-deficient soils of India (Majumdar et al, 2001; Tanwar and Shaktawat, 2003).

Effects of P and K fertilization on oil and protein content in soybean have been variable. Greenhouse studies using a P-deficient soil showed that P fertilization increased protein concentration under a range of water regimes (Jin et al., 2006). Potassium fertilization increased oil concentration but reduced protein concentration in soybean seed produced on soils with low to medium soil test levels (Gaydou and Arrivets, 1983), while fertilization with P or dolomite (a Mg-containing lime) increased both oil and protein along with yield.

In Ontario, Canada, Yin and Vyn (2003) reported that protein concentration declined from 43% to 42%, and that oil concentration increased from 21.5% to 21.8%, in response to band (but not broadcast) application of K fertilizer. In these studies, conducted on soils with low to medium K fertility, Yin and Vyn (2004) showed that oil concentration increased with increasing leaf K concentration, reaching a maximum when leaf K reached 2.2 to 2.5%.

Older research in Virginia also showed that K could decrease soybean protein in situations where it increased yield (**Table 4**). Since yield was increased more than protein was decreased, K still substantially increased protein production (Jones, 1976).

P₂O₅, kg/ha	K₂O, kg/ha	Yield, kg/ha	Protein concentration, %	Protein production, kg/ha
0	0	1,710	41.8	716
135	0	1,770	41.8	741
0	135	3,130	39.2	1,227
135	135	3,680	39.2	1,443

**Table 4.** Applied P and K fertilizer increased yield and protein production, even though the concentration of protein was reduced (two-year average, Virginia; soil test P and K were approximately 17 and 39 ppm, respectively, following five years of zero application; Jones, 1976).

Summarizing results from 112 field trials on soil and foliar P and K fertilization conducted across Iowa from 1994 to 2001, Haq and Mallarino (2005) concluded, *"Fertilization that increases soybean yield has infrequent, inconsistent, and small effects on oil and protein concentrations but often increases total oil and protein produc-tion.*" In other Canadian research, applied K only slightly reduced protein, and slightly increased oil and sugars (Zhang, 2003; **Table 5**). Similarly, effects of P and K fertilization on protein content of soybean were minimal in trials in Quebec, Canada, on soils with moderate to high initial fertility (Seguin and Zheng, 2006). In soils testing high or very high in P and K, applied P and K had little if any impact on qualities such as specific weight, visual quality, 100-seed weight, seed protein and oil content (Tremblay and Beausoleil, 2000).

Applied K <sub>2</sub> O, kg/ha	Protein, %	Oil, %	Sugar, %
95	41.9	21.6	11.0
0	42.3	21.4	10.9

**Table 5.** Potassium increased oil and sugar but decreased protein slightly in soy-<br/>beans. Means of five cultivars over four years, 1999-2002 (Zhang, 2003).

Sulphur may also influence the nutritional quality of soybean protein. Glycinin (11S) and  $\beta$ -conglycinin (7S) account for about 70% of the storage protein in soybean (Sexton et al., 1998). Glycinin contains about 3.0 to 4.5% S-amino acids while  $\beta$ -conglycinin contains less than 1%, so a higher ratio of 11S to 7S proteins indicates higher cysteine and methionine content and hence higher protein quality. Synthesis of low-S  $\beta$ -conglycinin is stimulated by an abundance of N and inhibited by an abundance of methionine, so the nutritional quality of soybean protein can be influenced by the relative N and S status of the plant (Imsande and Schmidt, 1998; Paek et al., 1997; Paek et al., 2000). Nitrogen appears to be remobilized more efficiently than S from vegetative material to the seed during pod filling; therefore increased tissue N may increase the N:S ratio in the seed (Imsande and Schmidt, 1998). Under hydroponic conditions, the 11S/7S ratio, and hence protein quality, increased substantially with provision of S during seed-filling, although protein concentration was not greatly affected (Sexton et al., 1998). Provision of S during vegetative growth had a much lower effect on the 11S/7S ratio than provision of S during seed filling. Therefore, an adequate supply of S through seed filling is important to ensure both optimum protein concentration and quality.

# Canola (Rapeseed)

Canola (rapeseed) oil is viewed as healthy because it contains a relatively low proportion of saturated fatty acids compared to other vegetable oils. Canola is one of the richest sources of mono-unsaturated fatty acids and is a good source of ALA (Harland, 2009). Reducing saturated fatty acid intake with mono-unsaturated fatty acids can reduce total cholesterol and low-density lipoprotein cholesterol, potentially improving human health.

As with other crops, there is generally a negative relationship in canola between protein and oil concentration. Therefore, increased protein content due to application of N fertilization commonly results in a reduced oil concentration (Asare and Scarisbrick, 1995; Brennan and Bolland, 2009b; Gao et al., 2010; Malhi and Gill, 2007; Rathke et al., 2005; Rathke et al., 2006). Increasing N fertilization has also been reported to affect the oil composition, although results were variable: increasing the proportion of oleic acid and decreasing the concentration of linolenic, linoleic and erucic acids in some studies (Behrens, 2002 as cited by Rathke et al., 2006) and decreasing the concentration of oleic acid and increasing the concentration of linoleic acids in others (Gao et al., 2010).

Phosphorus and K appear to have little to no effect on oil content of canola (Brennan and Bolland, 2007; Brennan and Bolland, 2009a). However, concentration of oil in canola seed has been reported to increase with S application when S was deficient (Brennan and Bolland, 2008; Grant et al., 2003; Malhi and Gill, 2002; Malhi and Leach, 2002; Malhi and Gill, 2006; Malhi and Gill, 2007; Malhi et al., 2007; Nuttall et al., 1987) but no effects occurred in other studies (Asare and Scarisbrick, 1995; Malhi and Gill, 2007). Sulphur fertilization did not have a consistent effect on fatty acid composition or oil content in studies in Turkey (Egesel et al., 2009).

### Summary

Proper nutrient management in crop production is important not only for improving crop yield and profitability, but also in optimizing the quality of crop-based food products. Protein, carbohydrate, and oil content composition and bioavailability can all be influenced by nutrient management. Adequate and balanced applications of N, P, K and S, managed in an efficient manner are critical to optimize the functional and nutritional quality of the staple crops wheat, rice, maize and potatoes and the major oilseed crops soybean and canola. In general, fertilizing for optimum yields does not differ greatly from fertilizing for optimum quality for most of the world's major food crops. In the long term, ensuring that soil fertility is maintained is important to avoid the major declines in both crop yield and nutritional quality that can be seen when crops are grown on highly depleted soils.

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# Fertilizer Application and Nutraceutical Content in Health-Functional Foods

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## Abstract

Fruits and vegetables are rich sources of nutrients and phytochemicals. Phytochemicals—such as polyphenols (flavonoids, anthocyanins), isoprenoids, S-containing compounds, soluble and insoluble fibre, etc.—are some of the agents that help to combat certain diseases, such as cancer and cardiovascular disease in humans. A growing body of research shows that fruits and vegetables are critical to disease prevention and promotion of good health. With an increasing consumer interest in healthy food, there is a need to improve the content of phytochemical and nutraceutical components in crop products. Fertilizers have been generally used to increase crop yields and improve crop quality. In the last decade, increased attention has been given to the use of fertilizers for enhancing nutraceutical content. The objective of this review is to summarize the effects of plant mineral nutrition on functional food components and nutraceuticals in crop products. Overall, fertilizers have diverse effects on the biosynthesis of health-promoting phytochemicals. Some have positive effects, but others have negative or inconclusive outcomes. Nonetheless fertilizers could play an important role in increasing the levels of nutraceuticals and functional food ingredients in crop plants, although more studies are required to optimize some of the reported successes.

## Introduction

In this chapter, the term fertilizers relates to chemical fertilizers that are manufactured products used in agriculture for the supply of plant nutrients. These include N, P, K, S, and combinations of them. Before the introduction of mineral fertilizers in the 19<sup>th</sup> century, soil fertility was maintained mostly by the recycling of organic materials and crop rotations that included N-fixing leguminous crops. The results

Abbreviations specific to this chapter: APX = ascorbate peroxidase; CAT = catalase; DNA = deoxyribonucleic acid; FOSHU = Foods for Specified Health Use; GSH = glutathione; LDL = low-density lipoprotein; POX = guaiacol peroxidase; SOD = superoxide dismutase. For symbols used commonly throughout this book see page xi.

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were insufficient food production for the increasing world population. There was a particular concern at the beginning of the 20<sup>th</sup> century about the availability of adequate quantities of N fertilizers. This issue was resolved through the industrial fixation of atmospheric N. Improved agricultural productivity through the use of fertilizers and novel productive varieties of food crops resulted in increased food production, that to date satisfies the food requirements in several countries. World fertilizer use has increased almost five-fold since 1960. Smil (2002) estimated that fertilizer N has contributed an estimated 40% to the increases in per-capita food production in the past 50 years, and Erisman et al. (2008) noted that this contribution continues to increase, reaching 48% by 2008.

Consumers are increasingly interested in the health benefits of foods and have begun to look beyond the basic nutritional benefits of food to disease prevention and health-enhancing compounds contained in many foods. This, combined with a more widespread understanding of how diet and lifestyle affect disease development and health-care costs, has created a market for functional foods and natural health products. Functional foods and nutraceuticals provide an opportunity to improve health, reduce health care costs and support economic development. They also offer a way for some producers to diversify their agriculture and marine-based resources. The global functional food and nutraceutical market is growing at a rate that is outpacing the traditional processed food market.

While fertilizers are mainly used for their primary purpose of increasing yield and improved quality, more emphasis is being given to increase nutraceuticals in foods. An increasing amount of research is being conducted to investigate the effects of various fertilizers on nutraceuticals such as lycopene, isoflavone, flavonoids, and organosulphur compounds. The focus of this chapter is to critically review the effects of fertilizers on nutraceuticals and functional foods.

## **Crop Quality**

## **Factors Influencing Crop Quality**

Quality is defined in various terms depending on the crop, ranging from how seed storage proteins of wheat define bread-making quality of wheat flour, to the long list of native antinutrients or phytotoxins found in many crops, particularly beans and other legumes. Nonetheless, quality is made up of many attributes, both intrinsic and extrinsic (Jongen, 2000). These attributes will vary depending on the expectations and memory of the consumer. Intrinsic features of the product include external attributes such as color, shape, size, and freedom from visible defects. In addition, internal attributes include texture, sweetness, acidity, aroma, flavor, shelf-life, and nutritional value. These are important components of the subjective approach used by the consumer in deciding what to purchase. Extrinsic factors refer to the production and distribution systems. These factors include chemicals used during production, package types and their recycling capability, sustainability of production and distribution in relation to energy utilization. These extrinsic factors are increasingly influencing consumer decisions to purchase.

#### **Nutraceuticals and Health-Functional Foods**

#### Nutraceuticals

The term nutraceutical was originally defined in 1989 by Dr. Stephen L. DeFelice, founder and chairman of the Foundation for Innovation in Medicine, Crawford, New Jersey. The word "nutraceutical" is formed by connecting the words "nutrition" and "pharmaceutical" and is a nutritional product—a single entity or combination which includes special diets—that reasonable clinical evidence has shown to have a medical benefit. However, the manufacturer or a physician cannot proclaim its benefits unless approved by the regulatory agencies [e.g. United States Food and Drug Administration, Health Canada] (Kalra, 2003; Cohen 2008). Since the term was coined by Dr. DeFelice, its meaning has been modified by Health Canada, which defines nutraceuticals as a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease development.

### Functional Foods

A functional food, on the other hand, is similar in appearance to, or may be, a conventional food, but is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. The general category of functional foods includes processed food or foods fortified with health-promoting additives, like "vitamin-enriched" products. Fermented foods with live cultures are considered as functional foods with probiotic benefits. The study of functional foods is an emerging field in food science owing to increasing popularity of these foods among health-conscious consumers because of their health benefits. The term functional food was first used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU) as the need arose for different age groups.

#### **Health Benefits of Food**

Centuries ago our ancestors proclaimed and believed in the medicinal properties of foods. The use of plants and foods for healing was used in the traditional Ayurvedic practices in India appearing in the Vedic texts, and as traditional medicine in China over 3,500 years ago. In Europe, the Greek physician and philosopher Hippocrates has been linked to the understanding of the healing properties of foods, and with the proclamation "Let food be thy medicine and medicine be thy food." He is considered to be the father of modern medicine (Kochhar, 2003). The Sumerians (4,000 BC) realized the medicinal use of licorice, opium, thyme, and mustard. Later, the Babylonians further developed the plant formulary to include saffron, cinnamon, coriander, and garlic. The modern concept of functional foods is attributed to the Japanese government's move to consider the limitations and cost-effectiveness of curative medicine's contribution to a healthier society. The term functional food as used in Japan refers to processed foods containing ingredients that aid specific bodily functions, in addition to being nutritious. To date Japan is the only country that has a legal definition of functional foods (Kochhar, 2003).

The Japanese FOSHU concept is based on knowledge concerning the relationship between particular foods or food components and certain expected health beneficial properties. A widely accepted definition of functional foods can be found in the consensus document produced by the European concerted action on functional food science in 1999. According to this definition, a particular food is functional if its beneficial effects on one or more targets in the body, beyond adequate nutritional effects, can be demonstrated (e.g. specific functional foods for infants and other functional foods for elderly adults). This must lead to an improved state of health and well-being and to a reduction in disease risk. Functional foods must remain as foods and achieve their effects in amounts consumed normally in the diet. Functional foods are not pills or supplements (Kochhar, 2003).

#### **Biochemistry of Functional Foods**

According to Ferrari (2004) aging is associated with mitochondrial dysfunctions, which trigger leakage across membranes, release of reactive species from oxygen and N, and subsequent induction of peroxidative reactions. Peroxidative reactions result in damage to the biological macromolecules. Free radicals induce neuronal cell death, which could be associated with a loss of memory. These pathological events are involved in cardiovascular, neurodegenerative and carcinogenic processes. Dietary bioactive compounds from different functional foods, herbs, and nutraceuticals (ginseng, ginkgo, nuts, grains, tomato, soy phytoestrogens, curcumin, melatonin, polyphenols, antioxidant vitamins, carnitine, carnosine, ubiquinone, etc.) can ameliorate or even prevent diseases. Protection from chronic diseases by nutraceuticals involves antioxidant activities, mitochondrial stabilizing functions, metal chelating activities, inhibition of apoptosis of vital cells, and induction of cancer cell apoptosis. Functional foods and nutraceuticals constitute a great promise to improve health and prevent aging-related chronic diseases (Ferrari, 2004; Bates et al. 2002).

### Protecting Pathways

Flavonoids such as quercetin, kaempferol, and luteolin as well as polyphenols (from grapes and wine), vitamin E, chlorophyllin (water-soluble chlorophyll analogue) and other phenols can protect membrane polyunsaturated fatty acids from oxidation, avoiding mitochondrial and other biomembrane disruptions (Brown et al. 1998; Frankel 1999; Terao and Piskula 1999; Boloor et al. 2000; Ferrari, 2004). Dietary  $\omega$ -3 fatty acids improved mitochondrial membrane lipids, decreasing calcium release (apoptosis trigger), and pyruvate dehydrogenase activity (Pepe et al. 1999). Recently, it was observed that the antioxidant N-acetylcysteine prevented Bcl-2 down-regulation increasing cell survival and life span (Kumazaki et al. 2002). Ebselen, (C<sub>13</sub>H<sub>9</sub>NOSe) an organoselenium compound, can significantly abrogate apoptosis of myocardial cells exposed to ischemic injury (Maulik et al. 1998). Namura et al. (2001) observed that ebselen decreased cytochrome-c release from mitochondria and increased survival of stroke-induced brain cells.

Tocopherol, glutathione (GSH) and idebenone abrogated the oxidative decay of complex III, but only GSH blocked damage to complexes II and V (Ferrari, 2004). Aged rats have decreased brain and plasmatic levels of dopamine, serotonin and some of their metabolites (Lee et al. 2001). Melatonin reversed alcohol-induced hepatic mitochondrial DNA strand-breaks and massive DNA degradation possibly by its antioxidant actions (Mansouri et al. 1999). Melatonin administration to Adriamycin patients improved cognitive functions, decreased nocturnal activity and prolonged sleep period (Asayama et al. 2003). Ubiquinone also improves mitochondrial respiration and enhances post-ischemic myocardial contractile function and decreases myocardial damage (Rosenfeldt et al. 2002). L-Carnitine is a mitochondrial membrane fatty acid transporter and stabilizer in aging cells and neurons (Hagen et al. 1998; Binienda 2003; Virmani et al. 2003), improving heart-related conditions and encephalomyopathy (Mahoney et al. 2002). Lipoic acid supplementation decreased heart mitochondrial DNA oxidation (Suh et al. 2001), hence it has many free radical scavenging activities (Pioro 2000). Carnosine stabilizes mitochondrial structure of stressed cells (Zakharchenko et al. 2003), blocking membrane permeability transition, cytochrome c leakage and subsequent events that lead to cell apoptosis (Kang et al. 2002, Ferrari, 2004).

### Anti-aging Mechanisms

By modulating many biological mechanisms in mammalian body and cells, functional foods can exert general health benefits and specific anti-aging benefits. Based on extensive literature review regarding normal aging and chronic diseases of aging (Ames et al. 1993; Mahoney et al. 2002; Reiter et al. 2002; Driver, 2003; Ferrari, 2004), the following anti-aging mechanisms of functional foods could be proposed: (1) Stabilizers of mitochondrial membranes and enhancers of mitochondrial function, agents that avoid cell death by apoptosis (programmed cell death) or necrosis (accidental cell death); (2) Metal chelating activities of functional foods; (3) Antioxidants that decrease cell injury, including those that stimulate antioxidant cell defense systems, protect DNA from oxidation or even inhibit apoptosis of target cells in vital organs; and (4) Inducers of apoptosis of preneoplastic and neoplastic cells.

### Antioxidant Activities

Consumption of fruit and vegetable is beneficial to human health, since numerous studies have shown that they reduce the risk of developing cancer and cardio-vascular disease (Hertog et al., 1993, 1995; Steinmetz and Potter, 1996; Shi et al., 2002; Bao and Fenwick, 2004; Dorais, 2007). Phytochemicals that possess antioxidant characteristics are believed to contribute to the overall health-protective effects of fruits and vegetables. They are thought to be protective against oxidative stress resulting from mitochondrial respiration and lifestyle factors such as smoking, exposure to environmental pollutants and solar radiation, all of which lead to disease development and aging process. The antioxidant properties of the most abundant types of phytochemicals found in fruits and vegetables such as vitamin C, carotenoids and phenolics result from their electron-rich structure in the form of oxidizable double bonds and hydroxyl groups. Antioxidant vitamins can counteract the

oxidizing effects of lipids by scavenging oxygen free radicals that have been found to be major agents of cardiovascular disease, certain cancers, neurodegenerative disease, diabetes, rheumatoid arthritis, cataract, and others (Shi et al., 2002). The antioxidant activity of fruits and vegetables varies according to species.

Many authors reported that aging impairs mitochondrial function resulting in oxidative imbalance and increased peroxidation biomarkers (lipid, protein, DNA), inducing heatshock proteins, and depleting antioxidant defense enzymes [catalase (CAT), SOD, GSH, glutathione-S-transferase] (Lucas and Szweda, 1998; Yang et al., 1998; Brack et al., 2000; Hall et al., 2001; Sandhu and Kaur, 2002; Rattan, 2003, Ferrari, 2004). This deleterious phenotype can be reversed by overexpression of SOD and CAT extending life span of *Drosophila melanogaster* and *Caenorhabid-itis elegans* (Larsen 1993; Sohal et al. 1995). Higher levels of vitamin A and E were found in healthy human centenarians (Mecocci et al. 2000), reinforcing the theory of an antioxidant–life span relationship. Rather than directly increasing life span, antioxidants' benefits are related to the control of free radicals that negatively influence healthy aging (Le Bourg 2003), saving antioxidant enzymes and performing the following protective mechanisms:

- Antioxidant gene expression ginsenoside Rb2 found in panaxadiol (*Panax ginseng* fraction) induced expression of SOD-1 gene, but total saponins and panaxatriol did not affect SOD-1 expression (Kim et al. 1996). Propolis was also able to induce SOD production in rats (Sforcin et al. 1995).
- Protection of LDL cholesterol from oxidation (Frankel 1999).
- Antiapoptotic protection of liver, brain and heart, preserving tissues (Green and Kroemer 1998; Ferrari 2000).

## Fertilizer Impacts on Nutraceuticals and Functional Foods Flavonoids in Apple

Plant flavonoids constitute one of the largest groups of naturally occurring phenolics, possessing chemical structures that can act as antioxidants, free radical scavengers and metal chelating agents (Rice-Evans et al. 1997). Apple fruits are rich in quercetin glycosides, catechin, epicathechin, procyanidins, dihydrochalcones such as phloretin and phloridzin, as well as anthocyanins in blushed cultivars, all of which are generally concentrated in the skin (Awad et al., 2001).

Awad and Jager (2002) investigated the relationship between fruit nutrients (N, P, K, Mg, and Ca) and concentrations of flavonoids and chlorogenic acid in "Elstar" apple skin and concluded that the most important variable in predictive models for the anthocyanin and total flavonoids concentration was N concentration in the fruit. The result suggested that the concentration of flavonoids in the fruit skin could be increased by optimizing fertilization, especially that of N. Paliyath et al. (2002) studied the effect of soil and foliar P supplementation on the post harvest

quality of apples (*Malus domestica* Borkh. cv. 'McIntosh' and cv. 'Red Delicious') and found that P fertilization increased the percentage of red skin on both varieties at harvest. They have also found that fruit from sprayed sides of the trees subjected to foliar treatments with P and Mg or P and Ca from blossom until a week before commercial harvest had increased red color compared to those from the non-sprayed side.

#### Lycopene in Tomatoes

Tomato fruit pigment is derived largely from the carotenoids lycopene and beta-carotene. Unlike anthocyanin pigments, which can be stimulated by increasing carbohydrate reserves, increasing carotenoid content seems to depend more on up regulating the general plant health, and by increasing phytoene, the carotenoid substrate needed to form lycopene. Abiotic and biotic factors often mask these effects, especially when studies are moved from greenhouse to field conditions. For instance, high air temperatures can shift carotenoid biosynthesis by oxidizing lycopene to beta carotene.

Potassium fertilization has been reported to stimulate lycopene production in tomato (Trudel and Ozbun, 1970, 1971; **Table 1**). Ramírez et al. (2009) found that increasing K increased the level of lycopene with a concomitant decrease in the level of  $\beta$ -carotene in greenhouse grown tomato fruits (**Table 2**). Carotenes change very rapidly as the tomato fruits mature, and thus nutrient effects on maturation rate can interact with their effect on carotene content. Hartz (1991) demonstrated that a higher concentration of K in field soil directly increased carotenoid biosynthesis enzyme activities and subsequently increased lycopene content. Taber et al. (2008) found that tomato cultivars with higher lycopene responded more to high rates of KCl applied in field conditions than low-lycopene cultivars. In field conditions, lycopene content increased by as much as 22% and  $\beta$ -carotene fell by as much as 53% in response to added K.

K levels, mmol/L	Total carotenes	Phytoene	Phytofluene	Beta- carotene	Lycopene
0	72	11.8	4.1	3.5	36.8
1	75	12.7	4.1	3.6	41.9
2	91	16.2	5.4	3.1	53.6
4	92	15.2	4.9	2.8	52.7
6	110	14.7	5.0	2.8	59.3
8	111	15.1	4.8	2.6	61.5
10	104	16.3	5.3	2.4	52.4

# **Table 1.** Carotenoid content (mg/kg fresh mass) of fresh market tomato fruit inresponse to various levels of K in the nutrient solution (adapted fromTrudel and Ozbun, 1970, 1971).

**Table 2.** Tomato fruit concentrations of carotenoids (mg/kg fresh mass) as affected by cultivar and K nutrient solution level in the greenhouse (fruit was harvested at 7 days after breaker-stage of development (n=111) (adapted from Taber et al., 2008).

Cultivars	Total carotenes	Phytoene	Phytofluene	Beta- carotene	Lycopene
Mountain Spring	2,056	9.8	5.8	5.6	50.5
Florida 91	2,067	11.2	6.7	6.0	51.7
Fla.8153	2,088	14.4	8.8	2.7	70.5
SED	NS	0.7	0.38	0.34	2.78
K levels, mmol/L	Total carotenes	Phytoene	Phytofluene	Beta- carotene	Lycopene
,		Phytoene 9.5	Phytofluene 5.9		<b>Lycopene</b> 51.3
mmol/L		-	-	carotene	
<b>mmol/L</b>		9.5	5.9	carotene 5.0	51.3
mmol/L 0 2.5		9.5 11.5	5.9 7.0	<b>carotene</b> 5.0 4.8	51.3 55.9

SED-standard error of difference for comparison among cultivars.

Regression analysis in which NS-not significant; L-linear; Q-quadratic.

\*, \*\*¬significance at p < 0.05 and p < 0.01 respectively.

Other researchers have reported contradictory results on the effects of fertilizers on the levels of lycopene and vitamin C. Lycopene production in tomato fruits depends not only upon K ion concentration in cytoplasm and vacuoles (Taber et al., 2008), but also upon other limiting factors, such as temperature and watering regime (Oded and Uzi, 2003; Dumas et al. 2002, 2003).

Addition of P has been reported to increase vitamin C and with variable effect on lycopene in tomato (Zdravković et al., 2007). Ahn et al. (2005) investigated the effects of P fertilizer supplementation on antioxidant enzyme activities in tomato fruits such as superoxide dismutase (SOD), guaiacol peroxidase (POX), and ascorbate peroxidase (APX). The results suggested that antioxidant enzyme activities may be influenced by the availability of P, but are subject to considerable variation depending on the developmental stage and the season. Oke et al. (2005) studied the effects of P fertilizer supplementation on processing quality and functional food ingredients in tomato and did not find any significant increase in lycopene, vitamin C, and flavor volatiles. Also, Dumas et al. (2002, 2003) did not find a positive influence of mineral nutrition on the levels of health-beneficial components. In other studies, the level of P, S, Mg, vitamins, total minerals, lycopene, and  $\beta$ -carotene did not show a dependency on soil K levels, but increased tomato

fruit yield (both size and number of fruit) (Fontes et al., 2000, Zdravković et al., 2007). Toor et al. (2006) reported that a  $NO_3^{-}$ -dominant fertilizer produced tomato fruit with lower acidity than  $NH_4^{+}$ -dominant or organic fertilizers. They also found higher phenolic and ascorbic acid content in tomatoes grown using chicken manure and grass-clover mulch as compared to mineral sources of N, but the lycopene content was 40% lower in tomatoes grown with either high Cl<sup>-</sup> fertilizers or grass-clover mulch.

#### **Isoflavones in Soybeans**

Soybean [*Glycine max* (L.) Merr.] seeds contain isoflavones that have several positive impacts on human health. Soybean-based foods have been implicated in the prevention of chronic diseases including cancer, heart disease and osteoporosis, as well as menopausal symptoms (Caragay, 1992, Hasler, 1998 and Messina, 1995) due to the regulation of estrogen-related functions (Kitts et al. 1980, Naim et al. 1976). Isoflavones are also antioxidants (Akiyama et al. 1987) and tyrosine protein kinase inhibitors (Vyn et al. 2002). Total isoflavone concentration in soybean seeds has been reported to range between 276 and 3,309  $\mu$ g/g, across a range of studies with different cultivars and environmental conditions (Carrao-Panizzi et al., 1999; Hoeck et al., 2000; Wang et al., 2000; Lee et al., 2003; Seguin et al., 2004). Total and individual isoflavone concentrations in soybean seeds are both genetically and environmentally determined. Cultivars differ widely in their isoflavone concentrations, with variations of up to 220% having been reported between cultivars grown in the same environment (Seguin et al., 2004).

Several abiotic and biotic factors have been found to affect soybean isoflavone concentrations, including air temperature, soil moisture level, and soil fertility (Tsukamoto et al., 1995; Wilson, 2001; Nelson et al., 2002; Vyn et al., 2002). Vyn et al. (2002) reported that in soils containing low to medium levels of K, isoflavone concentration in seeds may be increased up to 20% by K fertilization compared with an unfertilized control. Wilson (2001) reported that N fertilization negatively affected soybean isoflavone concentration, with a 90 kg/ha rate causing an almost ten-fold reduction in total isoflavone concentration compared with a 10 kg/ha rate. Seguin and Zheng (2006) investigated the effect of P, K, S, and B fertilization on soybean isoflavone content and other seed characteristics for two years and found that across years and cultivars, no fertilizer treatment effects were observed for most variables. This overall lack of response to fertilizers was attributed to the relatively high initial fertility of the sandy loam and sandy clay loam soils used.

### Organosulphur Compounds in Brassicaceae

Brassicaceae members are major vegetables in the diets worldwide. *Brassica oleracea*, for example, includes the following staple food cultivars: cabbage, broccoli, cauli-flower, kale, kohlrabi, and Brussels sprouts. Brassicaceous plants are also known for their production of the S-containing secondary plant metabolites, glucosinolates. Moderate intake of glucosinolate-containing plants is associated with a decreased risk of cancer (Gross et al., 2000; Hecht, 2000; Zhang and Talalay, 1994). When consumed, glucosinolates are hydrolyzed to isothiocyanates, which in turn

stimulate the activities of the anticarcinogenic phase II human enzymes. The production of glucosinolates in brassicaceous plants is influenced by a number of factors, including plant nutrition. A plant nutrient of specific interest is Se. Selenium is similar to S in both size and chemistry, and therefore often substitutes for S in physiological and metabolic processes. Charron et al. (2001) found that total glucosinolate production in rapid-cycling *Brassica oleracea* decreased when grown in the presence of sodium selenate.

Toler et al. (2007) studied the extent of Se impact on S uptake and glucosinolate production in *Brassica oleracea* L. and found that Se increases S uptake and regulates glucosinolate metabolism. They also demonstrated that it may be possible to produce a crop of *B. oleracea* vegetables that not only have preexisting anticarcinogen-inducing health benefits from glucosinolates, but also have the added health benefit of appropriate amounts of Se.

## **Concluding Remarks**

Fertilizers are important for human nutrition through their direct effect on producing healthy plants for food and through indirect effects of altering the nutraceuticals and other anti-aging and disease preventing compounds in plants. Fertilizer effects on nutraceuticals in plants include anthocyanin content of apples, carotenoid content of tomato, and grapefruit, glucosinolates in *Brassica*, and isoflavones in soybean. The plasticity of plant responses has made conclusive demonstrations of fertilizer effects difficult, as soil pH, season, moisture level, temperature, cultivars, and type of fertilizer can strongly influence outcomes. The increasing and aging global population underscores the need for fertilizer and a more tailored approach to optimize plant nutrition effects on key nutraceuticals and functional foods to prevent chronic disease and maintain good health. **ECHH** 

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# Fertilizer Use and Functional Quality of Fruits and Vegetables

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## Abstract

The crucial role that fertilizers can play in addressing the global food security problem is quite obvious since fertilizer input and crop productivity are directly related. Many initiatives aimed at securing an adequate food supply have focused primarily on improving crop productivity and market quality, thereby missing the opportunity to capture the nutritional and health benefits of foods. Consumers are increasingly aware of the health benefits of diets rich in fruits and vegetables or "Functional Foods," since these are excellent sources of essential nutrients and plant secondary compounds (phytonutrients) that have been linked with disease prevention and promotion of good health and well being. Consumption of functional foods often falls short of recommended guidelines, especially in developing economies where inadequate food supply and low phytonutrient densities of staple foods are prevalent. Besides genetics, pre-harvest farming practices, particularly fertilizer management, have a strong influence on the functional properties of foods and thus provide a sustainable and inexpensive approach for improving these attributes. Scientific evidence from numerous sources has demonstrated that judicious fertilizer management can increase productivity and market value as well as the health-promoting properties of foods. Low input or organic production systems could benefit the most from these findings since

For symbols used commonly throughout this book see page xi.

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they often lack access to expensive, high-yielding, nutrient-fortified hybrid varieties. This chapter summarizes key findings from studies where fertilizers have impacted phytonutrient content of fruits and vegetable crops, with examples drawn from studies highlighting viable fertilization practices that can be useful in guiding policy interventions for addressing the food security and nutrition problem.

## Introduction

Plant-derived foods are major components of staple diets around the world, and play a crucial role in the well being of human beings. They provide the essential nutrients (water, carbohydrates, protein, fats, minerals, and vitamins) that serve as substrates for energy, growth, and development (Lester, 1997; Kushad et al., 2003; Liu et al., 2003). Plants also synthesize and/or accumulate a diverse collection of minerals and complex secondary metabolites collectively known as phytonutrients, which have been associated with good health, disease prevention, and well being (Croteau et al., 2000; Kim et al., 2010; Prior and Cao, 1999). Phytonutrients include mineral elements, organic and inorganic compounds with known health benefits such as the essential nutrients (K, Fe, Ca, Mg, Zn) and vitamins (ascorbic acid or vitamin C, vitamin E, pro-vitamin A carotenoids), as well as trace elements (e.g. Se and Si) and secondary metabolites such as phenolic compounds, alkaloids, terpenes, and glycosides (Bramley et al., 2000; Cassidy et al., 2000; Milner, 2000; Mithen et al., 2000; van den Berg et al., 2000; Tsao and Akhtar, 2005; Winkels et al., 2007). Many of these plant secondary metabolites are synthesized in small amounts and used to attract pollinators (Croteau et al., 2000), or for protection against pathogens, herbivores, and environmental stresses (Heldt, 2005; Bray et al., 2000; Croteau et al., 2000). The vast diversity of defense-related phytonutrients that plants synthesize in response to environmental cues is a reflection of the high level of phenotypic plasticity which is essential for stress tolerance. This high degree of plasticity indicates that management practices can be manipulated to maximize the synthesis and accumulation of specific phytonutrients in target crops.

The role of nutrition in disease prevention and healthy living is receiving considerable attention, in part because of the need for alternatives to conventional strategies of disease management, and the potential for reducing health care costs through nutrition-based disease prevention programs (Milner, 2000; Bidlack, 1996; Tucker and Miguel, 1996). The current state of knowledge indicates that fruits, vegetables, and minimally-processed whole foods are excellent sources of phytonutrients and also constitute a superior delivery mechanism for dietary intake of these compounds compared to intake of supplements (Wahlqvist and Wattanapenpaiboon, 2002; USDA, 2005; WHO-FAO, 2003; Winkels et al., 2007). The awareness that plant food components have health benefits is one possible explanation for the expanding use of supplements by apparently healthy people (Eliason et al., 1997; Greger, 2001; Milner, 2000). However, purified phytonutrient compounds contained in dietary supplements often lack the synergism among multiple compounds found in whole foods (Salucci et al., 1999; Pignatelli et al., 2000; Freedman et al., 2001; Liu, 2003; Winkels et al., 2007). These problems with supplements clearly demonstrate that the greatest benefits may be obtained only through intake of a diversified diet with fruits and vegetables as major components.

In many societies, the current per capita consumption of fruits and vegetables is very low (Johnston et al., 2000), in part due to the fact that only 20 plant species, of the approximately 7,000 described edible species, make up 90% of plant-derived human foods (FAO, 2009). Such dependence on a narrow genetic base makes the world's food supply vulnerable to disease or sudden climatic change and puts food sustainability at risk. The food security problems in many parts of the world are also compounded by limited arable land, and the fact that subsistence agriculture is still the easiest and dominant method of sustaining many families (Sanchez, 2010). Soils in these regions of the world are also highly weathered and deficient in important macro- and micronutrients that are essential for plant growth and productivity as well as for basic human nutrition (Wild, 1993). Continuous intensive cropping and inadequate replacement of nutrients removed with harvested crops have resulted in reduced productivity and nutritional quality of foods (Sanchez, 2010; Lal, 2006). This trend has also contributed to persistent chronic malnutrition in many developing countries (Sanchez and Swaminathan, 2005; Stein, 2010). Nutrient mining and the associated reductions in productivity and quality are predicted to worsen with continued population growth and climate change (Sanchez, 2010; St. Clair and Lynch, 2010; Lal, 2004; Bohle et al., 1994). Various interventions to alleviate global malnutrition such as crop fertilization and/or breeding for increased nutrient use efficiency or micronutrient contents continue to focus heavily on crop yields (Sanchez, 2010; Stein, 2010; Sanchez and Swaminathan, 2005), with little regard for consumer preferences or functional properties. It has also been suggested that some of the yield gains brought about by the Green Revolution through the use of improved cultivars, fertilization, irrigation, and mechanization might have been achieved at the expense of essential nutrient and phytonutrient contents (Davis, 2009). Such potential tradeoffs are unfortunate since billions of people globally are malnourished in mineral nutrients and vitamins (Welch, 1997). Besides genetics (cultivar or variety), farming practices (including production location, planting date, planting density, irrigation, fertilization, maturity stage at harvest) and environmental factors can have a strong influence on the composition and diversity of phytonutrients in foods (Mozafar, 1993; Dixon and Paiva, 1995; Lester and Eischen, 1996; Lester and Crosby, 2002; Crosby et al., 2003, 2008). These factors also influence consumer preference characteristics such as taste (sweetness), texture, size, color, aroma, year-round availability, ease of processing, and absence of defects. Advances in farming techniques, including fertilizer management, and the diversity of underutilized edible fruits and vegetable crops hold promise for achieving food security as well as improving health and well being (Crosby et al., 2006, 2008, 2009; Welch and Graham, 2004).

The remainder of this review will focus on the important role that fertilizer management can play on the phytonutrient content of foods. Examples are drawn from studies highlighting key fertilization practices that have so far shown promise as viable strategies for improving the phytonutrient content of fruits and vegetables. Where appropriate, a discussion of how fertilizer management can guide policy interventions for addressing the nutrition security problem (i.e. intake of essential nutrients, including proteins, minerals, vitamins, and phytonutrients) is included.

## **Nutrient Management**

The impacts of fertilizer management on crop productivity and basic nutritional quality parameters (proteins, minerals, vitamins, and essential oils) are well documented (FAO, 1981; Marschner, 1995; Havlin et al., 2005; Stewart et al., 2005). However, information on the effects of mineral nutrients on phytonutrient compounds is limited. The potential for improving the bioactive properties of foods through fertilizer management strategies is very attractive since many mineral nutrient elements are either structural constituents of phytonutrients or participate in processes involved in phytonutrient synthesis and accumulation.

The available data suggests that fertilizers can have varying effects on phytonutrient profiles depending on the modulating effects of other environmental conditions on plant growth and development. For instance, Lester and Crosby (2002) found that vitamin C (ascorbic acid) and folic acid contents of green-flesh honeydew muskmelons were higher when grown on a clay loam compared to a sandy loam soil. Differences in the nutrient supply capacities of the different soil types probably accounted for the observed results. These modulating effects will be discussed in the succeeding sections and should be taken into account when developing fertilization strategies to improve phytonutrient contents.

## **Nitrogen Fertilization**

Nitrogen is an essential component of nucleic acids (DNA, RNA), amino acids, proteins, and enzymes that are needed to support growth and development. Close correlations between N fertilization, leaf N concentration, leaf soluble proteins, photosynthesis and productivity have been documented for many crops (FAO, 1981; Millard, 1988; Marschner, 1995; Havlin et al., 2005; Stewart et al., 2005). Increased leaf area and photosynthetic carbon dioxide assimilation associated with adequate N supply ultimately represents a source of carbon skeletons for phytonutrient synthesis.

Depending on crop cultivar and the harvested portion, N fertilization can have varying effects on the phytonutrient composition and nutritional quality of foods. In a detailed review of the literature on the impact of N fertilization on vitamin contents in plants, Mozafar (1993) noted that the concentrations of carotenes and vitamin  $B_1$  tend to increase with N fertilization whereas the concentration of vitamin C decreases. Recent studies have also confirmed these trends. For instance, Barickman et al. (2009) reported positive correlations between N supply amount and concentrations of antioxidant carotenoids (beta-carotene, lutein,

neoxanthin and zeaxanthin) in watercress (*Nasturtium officinal* R. Br.). Similarly, Kopsell et al. (2007a) found linear increases in carotenoid concentrations (lutein, beta-carotene, and chlorophyll pigments on a dry mass basis) in leaf tissues of kale in response to N fertilization. Lutein and beta-carotene are potent antioxidant pigments and play an important role in eye health. Together with vitamins A, C, and E, they can help lower the risk of developing, or slow down the progression of age-related macular degeneration (AMD), which is one of the leading causes of blindness (AREDS, 2007).



Fertilizer N source and form can also influence phytonutrient composition (Chance et al., 1999; Errebhi et al., 1990; Xu et al., 2001). For instance, Kopsell et al. (2007a) found that increasing the NO<sub>2</sub>:NH<sub>4</sub><sup>+</sup> ratio in fertilizer solutions resulted in significant increases in both the dry and fresh mass concentrations of lutein and beta-carotene in kale leaves. Similarly, Toor et al. (2006) found that total phenolic and ascorbic acid concentrations were highest in tomato fruits grown on grassclover mulch (29%) compared to fruits grown on soils fertilized with chicken manure (17%) or mineral nutrient solutions containing NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. This result may form the basis for some of the quality differences often reported

between organic and conventional production systems (Rosen and Allan, 2007; Lester and Saftner, 2011). Species and cultivar sensitivities to variations in N form should, therefore, be considered when designing fertilizer management strategies for phytonutrient enhancement.

In contrast to the enhancement effects of N fertilization on carotenoids, some studies have reported negative correlations between elevated N fertilization and vitamin C (ascorbic acid) concentrations in fruits and vegetables including some of the most widely consumed foods such as citrus, potatoes, spinach, cauliflower, tomato, and lettuce (Nagy, 1980; Lee and Kader, 2000; Lisiewska and Kmiecik, 1996; Sørensen, et al., 1995; Mozafar, 1993; Abd El-Migeed et al., 2007). Excessive N fertilization has also been associated with reductions in the concentrations of naringin and rutinoside in grapefruits (Patil and Alva, 1999; 2002), anthocyanin in apples (Awad and Jager, 2002), and polyphenolic compounds and antioxidant activity in basil (Nguyen and Niemeyer, 2008).

Nitrate and ammonium are the two major N forms available for crop uptake from the soil. Nitrate-N is highly soluble and is subject to leaching and groundwater contamination under certain conditions (Havlin et al., 2005). High  $NO_3^{-1}$  levels

in harvested plant products, especially fresh vegetables, have been the subject of health concerns, since consumption of  $NO_3^-$ -enriched foods is sometimes linked to illnesses such as methemoglobinemia (or the blue baby syndrome) (Correia et al., 2010; Greer and Shannon, 2005), and since nearly 80% of dietary nitrates are derived from vegetables. In a survey of 34 vegetables (different varieties of cabbage, lettuce, spinaches, parsley, and turnips) collected at several locations of intensive crop production in Europe, Correia et al. (2010) found that  $NO_3^-$  and  $NO_2^-$  levels ranged from 54 to 2,440 mg  $NO_3^-$ /kg and 1.1 to 57 mg  $NO_2^-$ /kg, respectively. Since the maximum acceptable daily intake (ADI) limits for these compounds were not exceeded, the authors concluded that consumption of these vegetables would still be beneficial for human health.

## **Phosphorus Fertilization**

Phosphorus is one of the primary essential macronutrients required for crop growth, productivity, and quality. It is involved in numerous biochemical reactions as a substrate and/or catalyst, and is a key component of many structural compounds such as phospholipids, DNA, and RNA (Marschner, 1995). Oxidative phosphorylation, the process that produces adenosine triphosphate (ATP), depends on adequate supply of P. Phosphorus is also a key substrate in reversible phosphorylation of proteins which regulates many metabolic processes in plants and animals (Marschner, 1995; Cohen, 2001). Phosphorus is a well-known yieldlimiting macronutrient, especially in highly-weathered soils (Cramer, 2010; Sanchez, 2010). In such soils, most of the available P is sorbed by various soil constituents and taken out of the soil solution. Hence, P must be supplied from external sources, including commercial fertilizers, plant and animal manures, wastes, and P-containing parent materials in soils (Oberson et al., 2006; Havlin et al., 2005; Brady and Weil, 2001).

Few studies have investigated the role of P fertilization on phytonutrient content of foods. The available data are often inconsistent or sometimes contradictory. Paliyath et al. (2002) reported that supplemental soil and foliar P fertilization (with superphosphate, Hydrophos®, and Seniphos®) increased the intensity of red color in peels of 'Red Delicious' apple fruits. This suggests that P fertilization can increase the concentration of anthocyanin and other flavonoids such as proanthocyanidins and flavonols which determine peel coloration in red-skinned apples. The authors speculated that the increase in color intensity may be related to activation of the pentose phosphate pathway, from which the precursor for flavonoid synthesis (erythrose-4-P) is derived (Bruulsema et al., 2004). Anthocyanins give fruits and vegetables the characteristic purple and red color appearances and have been shown to protect tissues from oxidative damage (Kushad et al., 2003; Croteau et al., 2000; Close and Beadle, 2003; Chalker-Scott, 1999). Foliar application of P-containing substances namely, ethephon (2-chloroethyl phosphonic acid) and a fertilizer that contained P, Ca, and N, has also been shown to enhance red peel color, and an increase in the concentration of flavonoids in 'Fuji' apples (Li et al., 2002). In an investigation of the effects of N, P, and K supply on the growth and chemical properties of celery, Gurgul et al. (1994) reported an increase in the activities of key antioxidant enzymes (peroxidase, catalase, and acid phosphatase) in response to P fertilization. In contrast, Ahn et al. (2005) found no consistent effects of soil and foliar P supplementation on the activities and levels of superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase in tomato fruits. Effects of P fertilization on functional quality seem to be highly variable and dependent on production location, season, crop maturity stage, weather conditions, and other environmental factors during growth (Oke et al., 2005; Ahn et al., 2005). Bruulsema et al. (2004) also noted that weather conditions modulated flavonoid responses to P fertilization. Warm sunny days and cool nights are known to stimulate anthocyanin production (Close and Beadle, 2003).

Numerous reports have linked P deficiency with increased anthocyanin content in plant tissues (Jiang et al., 2007; Close and Beadle, 2003; Stewart et al., 2001). Reddish-purple discoloration of leaves is a common symptom of such P-deficient plants. Stewart et al. (2001) found that P deficiency elicited an increase in flavonol content in early (mature green) stages of tomato fruit ripening, but not in the later (breaker and red) stages. They speculated that induction of flavonols in the skins of young tomato fruits may be important to protect fruit tissues and developing seeds from potentially damaging UV-B radiation. Anthocyanin accumulation during P deficiency has been linked to reduced gibberellins (GA) activity, or an increase in tissue concentrations of GA antagonists such as ethylene and abscisic acid (ABA) (Jiang et al., 2007; Saure, 1990). Besides P starvation, other biotic and abiotic stresses such as herbivory, temperature, and radiation stress have also been shown to elicit anthocyanin accumulation and purple coloration of leaves (Close and Beadle, 2003; Saure, 1990; Chalker-Scott, 1999). Due to their high antioxidant capacity, flavonoids, including anthocyanins, are believed to help plants cope with environmental stresses (Close and Beadle, 2003; Chalker-Scott, 1999). Increased dietary intake of flavonoids is also associated with potential health benefits (Pietta, 2000). These established relationships between P availability and flavonoid accumulation indicate that the functional quality of fruits and vegetables can be enhanced by altering P fertilizer management strategies. Further research is needed to characterize the magnitude of any potential tradeoffs between yield and enhanced functional quality.

In addition to its role in crop growth and yield, P fertilization has been associated with the synthesis and accumulation of phytic acid (or phytate, its salt form) (Marschner, 1995; Kumar et al., 2010). Phytic acid is the principal storage form of P in many plant tissues, especially high-fiber foods such as nuts, seeds, grains, and other foods including soy products, oatmeal, corn, peanuts, kidney beans, whole wheat, and rye (Kumar et al., 2010; Sotelo et al., 2010). Because of its tremendous affinity for dietary mineral elements, especially Fe, Zn, Ca, and Mg, phytic acid, and phytates have been the subject of intense nutritional scrutiny. They interfere with the bioavailability of proteins, lipids, and essential vitamins

and minerals (Kumar et al., 2010; Sotelo et al., 2010). This effect is worsened if the Zn, Fe, or Ca content of the diet is low, as is the case in many developing countries, where unrefined cereals and/or pulses constitute the staple foods (Bouis and Welch, 2010). Phytate concentrations in raw and cooked potatoes were found to range from 1.1 g/kg to 2.6 g/kg dry weight among eight potato varieties, and were 1.74, 0.95, and 2.05 g/kg in french fries, potato chips, and dehydrated potato flakes, respectively (Phillippy et al., 2004). In a survey of representative staple foods from Sidama, Southern Ethiopia, for phytate, Zn, Fe, and Ca contents, Abebe et al. (2007) found that local oilseeds (nyjer, Guizotia abyssinica, and sesame, *Sesamum indicum*) had the highest phytate contents (approximately 1,600 mg/100 g), whereas fermented foods prepared from enset (*Ensete ventrico*sum, a root crop) and tef [Eragrostis tef (Zucc.) Trotter; a grain crop] had low phytate, phytate:Zn, and phytate:Fe molar ratios, whereas unleavened corn bread, kidney beans, sesame, and nyjer seeds had higher molar ratios. They concluded that the phytate content of staple foods such as enset and tef is unlikely to limit the availability of essential mineral elements unless such foods are consumed together with other high-phytate foods such as corn bread, legumes, and oil seeds.

Contrary to its status as an anti-nutrient, there is increasing evidence that dietary phytates have beneficial health effects, such as protection against a variety of cancers, heart-related diseases, diabetes, and renal stones. They can also act as antioxidants in preventing the formation of free radicals, thereby reducing oxidative stress and preventing related diseases such as cardiovascular disease, kidney and other cancers (Graf and Eaton, 1990; Hanson et al., 2006; Kumar et al., 2010; Prieto et al., 2010). More research is needed to characterize the phytate profiles of staple and underutilized crops, their responses to P fertilization, production system (conventional versus organic) and potential anti-nutritional interactions prior to implementing fertility programs as a strategy to alleviate malnutrition and food insecurity.

## **Potassium Fertilization**

Together with N and P, K is one of the primary essential macronutrients involved in numerous physiological processes that control plant growth, yield, and quality (Marschner, 1995; Lester et al., 2005). Even though K is not an integral part of any plant structures, it plays a key regulatory role in many physiological processes. Other documented K-mediated processes include enzyme activation, osmoregulation, regulation of stomatal opening/closing, photosynthesis and transpiration, phloem transport, and fruit sugar accumulation (Usherwood, 1985; Geraldson, 1985; Kafkafi et al., 2001; Pettigrew, 2008; Marschner, 1995; Mengel and Kirkby, 1987). Many market quality and consumer preference traits such as taste, texture, and appearance are positively correlated with K availability (Usherwood, 1985; Lester, 2006). The positive effects of K nutrition on quality development are largely related to the promoting effects on enzyme activation, photosynthesis, and assimilate transport to storage sink organs such as fruits (Jifon and Lester, 2009; Pettigrew, 2008; Lester et al., 2006; Marschner, 1995). As in plants, K is important for animal physiology in maintaining a constancy of the internal environment (homeostasis), thus allowing normal functioning of vital processes such as enzyme activation, nerve impulses, heartbeat, and muscle activity. Inverse associations between K intake and the incidence of cardiovascular diseases such as stroke and coronary heart disease have been reported (He and MacGregor, 2008). Most fruits and vegetables including muskmelons (cantaloupes and honeydew melons), watermelons, squash, pumpkins, tomatoes, broccoli, orange juice, potatoes, bananas, avocados, peaches, pears, apples, soybeans, and apricots are good sources of dietary K (USDA, 2010; Lester, 1997). Insufficient consumption of fruits and vegetables has been implicated as the reason for low dietary K intake, currently at ~2 g/day, compared to the recommended 3-5 g/day (He and MacGregor, 2008; US Institute of Medicine, 2005).

Insufficient consumption of fruits and vegetables and hence K intake is partly linked to poor market and consumer preference quality characteristics such as taste, flavor, and texture, which are directly related to K availability during plant growth. In many species, K uptake occurs mainly during the vegetative stages when root growth is not inhibited by carbohydrate availability. Competition for photoassimilates between developing fruits and vegetative organs during reproductive growth stages can limit root growth/activity and K uptake (Ho, 1988). This creates an apparent K deficiency that can limit photoassimilate translocation to developing seeds and fruits, potentially reducing yield and quality.

Muskmelons (*Cucumis melo L.*), tomato (*Solanum lycopersicum*), citrus (Citrus spp), and banana (*Musa sapientum*) are commercially important horticultural fruit crops that are widely consumed for their nutritional benefits, and more recently for their functional properties. They are also excellent examples of the positive influence of K fertilization on yield and functional quality parameters, as briefly highlighted in the following examples.

### Muskmelons

Muskmelons (*Cucumis melo L.;* including the Reticulatus and Inodorus or honeydew Groups) are rich sources of K and other phytonutrients such as ascorbic acid,

beta-carotene, and folic acid. In the U.S., muskmelons are one of the few fruits that have experienced a significant increase (>2fold) in consumer demand within the last three decades (Lester, 2006) thanks to preference traits such as sweetness and year-long availability.

Compared to nine other highly consumed fresh fruits, muskmelons ranked among the top



five foods in Dietary Reference Intake (DRI) levels for beta-carotene (pro-vitamin A), ascorbic acid, K, and folic acid (Vitamin B9) (Lester, 2006). This high diversity of phytonutrients in muskmelon makes it an excellent dietary component for healthy living. However, there is considerable variability in the levels of these phytonutrients in muskmelon fruits due to cultivar differences as well as environmental factors (Jifon and Lester, 2009; Lester and Crosby, 2002; Lester and Eischen, 1996). One of the critical environmental factors regulating muskmelon phytonutrient contents is K availability (Lester, 2006). As discussed in preceding sections, however, soil-derived K is not always optimal during the critical fruit development period, and this is partly responsible for poor fruit quality, including phytonutrient contents.

Controlled-environment and field investigations have shown that supplementing soil-derived K with foliar applications can alleviate this deficiency and enhance quality traits such as sweetness, texture, color, vitamin C, beta-carotene, and folic acid contents (Jifon and Lester, 2009; Lester et al., 2005, 2006). These quality improvements were generally greater with S-containing K sources [e.g. potassium thiosulphate  $(K_2S_2O_2)$  and potassium sulphate  $(K_2SO_2)$  and amino acid-K chelates (e.g. Metalosate® Potassium) compared to standard mineral K sources (KCl and KNO<sub>2</sub>). Plausible mechanisms for the promoting effects of K fertilization include a combination of improved photosynthesis, assimilate translocation from leaves to fruits, improved leaf and fruit water relations, increased enzyme activation, and substrate availability for biosynthetic pathways (Marschner, 1995). Among several K salts studied, late-season foliar KNO<sub>2</sub> application consistently resulted in non-significant effects on fruit quality, perhaps due to its tendency to stimulate vegetative growth (mainly stems and leaves) at the expense of roots, fruit yield and quality development. Foliar fertilization with KNO, would be more beneficial during the vegetative growth stages when N is most needed for canopy development to establish a high photosynthetic capacity. Foliar fertilization is generally not meant to replace soil fertilization. In environments where soil K uptake is limited, carefully-timed, soil and late-season foliar K fertilization can improve the market, and functional quality of fruits. Late-season

foliar K fertilization is a readilyapplicable practice that growers can easily adopt to improve the functional properties of foods.

#### Tomato

Tomato (*Solanum lycopersicum*) is one of the most important horticultural fruit crops globally, and is an important source of phytonutrients such as lycopene, beta-carotene, ascorbic acid, phenolics, flavonoids, and vitamin E (Clinton, 1998;



Kaur et al., 2004; Dorais et al., 2008). Differences in shape, size, color, and maturity stages have been shown to influence tomato phytonutrient contents; for instance, the small-fruited (cherry) types generally have higher lycopene and antioxidant activities (Cox et al., 2003; Kaur et al., 2004; Wold et al., 2004; Passam et al., 2007).

Tomatoes require large amounts of K for optimum yield and quality; K deficiency results in slow and stunted growth, blotchy ripening, puffiness, hollow fruits, misshapen fruits, poor color development and a reduction in yield and the proportion of marketable fruits (Usherwood, 1995; IFA, 1992; Geraldson, 1985). Various effects of K fertilization on fruit lycopene and other carotenoids have been reported (Saito and Kano, 1970; Trudel and Ozbun, 1971; Dumas et al., 2003; Passam et al., 2007). Tomato yield improvements in response to K fertigation (Hartz et al., 2005) as well as improvements in fruit soluble solids, titrable acid, and ascorbic acid contents (Peyvast et al., 2009), in response to foliar K applications have also been reported. As with K fertilization of muskmelons (Jifon and Lester, 2009), additional factors such as timing and source of K fertilization can intensify or mask the beneficial effects of K fertilization on fruit quality (Hartz et al., 2001). For instance, late-season foliar fertilization with Ncontaining K sources such as KNO<sub>2</sub> can have the undesired effect of stimulating vegetative development and delay fruit development and maturation (Neuweiler, 1997; Jifon and Lester, 2009). Such factors should, therefore, be taken into consideration in fertilization programs for phytonutrient improvement.

### Citrus

Citrus fruits (including oranges, grapefruit, tangerines limes, lemons, and others) are produced in many countries, and are among the richest sources of a variety of phytonutrients including ascorbic acid, lycopene, beta carotene, limonoids, and flavonone glycosides such as naringin, narirutin, and hesperidin (Somasundaram et al., 2009; Murthy et al., 2009; Park et al., 2009; Harris et al., 2007). Global consumption of citrus products continues to increase, thanks, in part to increasing consumer awareness of its health benefits, as well as improved quality, year-round availability and affordability.

Potassium fertilization is a critical factor for citrus fruit quality development (Koo, 1985). It is estimated that approximately 2 kg of K is removed per ton of harvested citrus fruit, which exceeds the amount of any other nutrient element removed and reflects the high K content of citrus juice (IFA, 1992; Koo, 1985). Citrus phytonutrients, particularly ascorbic acid, and other quality factors such as fruit juice content, soluble solids and acid concentrations, soluble solids/acid ratio, fruit size, and color, fruit size, shape, and rind thickness are all impacted by K nutrition (Koo, 1985). In pink grapefruit, supplemental foliar K resulted in increased lycopene, beta-carotene, and vitamin C concentrations (Patil and Alva, 2002). However, higher levels of soil-applied K resulted in lower fruit total ascorbic acid levels (Patil and Alva, 2002) perhaps highlighting the effects of timing as well as application method on K uptake and metabolism. The positive effects

of K fertilization are probably related to improved enzyme activity, carbohydrate assimilation, transport, and sugar metabolism (Marschner, 1995).

#### Banana

Bananas (*Musa spp*) are cultivated in over 130 countries, and are one of nature's best known sources of K and one of the most convenient and nutritionally dense food items. They are also good and inexpensive sources of vitamins A, C, B6, and minerals (Robinson, 1996). In addition to the well-known effects of K in lowering the risk of developing diseases such as heart attack and strokes, functional compounds in banana are reported to relieve constipation, heartburn, ulcers, and have been linked to prevention of anaemia by stimulating the production of haemoglobin in the blood (Robinson, 1996).

Banana productivity and quality are strongly influenced by K nutrition. According to IFA (1992), bananas are among the highest accumulators of fertilizer K, with uptake/removal amounts ranging from ~20 kg K/t whole bunch (for Cavendish type varieties) to 50 kg K/t in other cultivars. Von Uexküll (1985) estimated that a banana plantation yielding 50 t/ha requires approximately 1,625 kg K/ha with most of the K being absorbed during bunch growth. Several studies have reported positive correlations between K nutrition and banana fruit quality parameters such as total soluble solids, reducing sugars, non-reducing sugars, total



sugars and ascorbic acid, and negative correlations with fruit acidity (Al-Harthi and Al-Yahyai, 2009; Kumar and Kumar, 2008; Hongwei et al., 2004). Investigations by Kumar and Kumar (2008), comparing the impact of fertilizer K source (KCl versus  $K_2SO_4$ ) on banana quality have also confirmed the positive effects of K supply on fruit quality

parameters. This study also demonstrated that the beneficial effects of K fertilization were greater with  $K_2SO_4$  than with KCl, which is similar to results obtained for muskmelon fruit by Jifon and Lester (2009).

Many current fertilizer recommendations are designed to optimize crop yields, while quality attributes are assumed to depend on other cultural practices such as variety selection or timing of produce harvest. It is apparent from the preceding discussion that carefully-designed fertilizer management strategies can play a key role in quality enhancement. With respect to K management, the current evidence indicates that supplementing soil K with foliar K fertilization during



the fruit development and maturation period can improve consumer preference attributes and functional quality of fruit and vegetable crops. However, in order to develop K fertilizer recommendations for improving the functional quality of foods, information regarding crop nutrient removal amounts, production season, fertilizer source, and soil properties is required. This information is useful in determining nutrient amounts that must be applied to sustain yields and quality while maintaining soil fertility. This information can also be useful in selecting cultivars for specific sites based on their nutrient accumulation/removal capacities.

# Sulphur and Selenium Fertilization of Allium and Brassica Crops

Allium crops including onion, garlic, leeks, and chives, have been cultivated and used in human diets for centuries for their unique flavors. More recently, their benefits to human health including antiplatelet activity, anticarcinogenic properties, antithrombotic activity, antiasthmatic and antibiotic effects have been reported (Turner et al., 2009; Havey, 1999). The functional flavor components of Allium crops are organo-S compounds that are synthesized from a common precursor, the S-alk(en)yl cysteine sulfoxides (ACSOs) (Yoo and Pike, 1998). Sulphur is directly involved in the synthesis of the ACSOs, and is a major constituent of the flavor compounds; higher available S in the soil generally results in greater flavor intensity and also alters the composition of ACSOs (Randle et al., 2002; Randle et al., 1995; Coolong and Randle, 2003; Randle and Brussard, 1993; Bloem et al., 2005; McCallum et al., 2005).
*Brassica* crops, including broccoli, brussels sprouts, kale, and radish, are also excellent sources of S-containing phytonutrients such as glucoraphanin, which have been associated with numerous health benefits (Cartea and Velasco, 2008; Johnson, 2002; Osmont et al., 2003). Positive correlations between S fertilization and the concentrations of these phytonutrients have been reported (Barickman et al., 2009; Kopsell et al., 2007b; Aires et al., 2007; Finley, 2007; Bloem et al., 2007).

Selenium fertilization of crops, especially Brassicas, has recently gained considerable attention, in part because Se is an essential trace element involved in protein synthesis, and has shown antioxidant, anti-inflammatory, and anti-carcinogenic properties (Ip et al., 1992). Although trace amounts of Se are necessary for cellular function, adverse health effects associated with Se deficiency or excess supply have been observed, in part due to the relatively narrow range for optimal Se requirements (Jackson-Rosario and Self. 2010). Selenium is also closely related to S, and may be substituted for S in metabolic pathways (Young 1981; Mäkelä et al., 1993; Goldman et al., 1999; Arthur, 2003; Finley, 2007). Selenium concentration in foods is directly related to the soil content where the crops were grown (Arthur, 2003). Several studies have demonstrated that Se fertilization increases crop uptake (Kopsell et al., 2009; Kopsell et al., 2007b; Finley, 2007; Toler et al., 2007; Charron et al., 2001; Barak and Goldman, 1997), and may alter the relative levels of individual S-containing phytonutrients (Charron et al., 2001). Many countries have now established Se-fortification programs aimed at increasing intake rates to recommended sufficiency levels (Lintschinger et al., 2000; Mäkelä et al., 1993).

### **Concluding Remarks and Future Perspectives**

The central role that fertilizers play in addressing the global food security problem is irrefutable. While fertilizer use in crop production has been instrumental in increasing food production in many societies, lack of access (availability and affordability) to foods with health benefits (functional foods) is still a global problem. Previous policies to eliminate hunger by increasing access to food have focused heavily on improving crop productivity. The compelling evidence linking diet and health presents a unique opportunity for redefining global agricultural food policies to promote the production of foods rich in a wide variety of phytonutrients. This has the potential to improve food supplies and reduce disease incidences and health care costs globally.

The link between fertilizer management and phytonutrient concentrations in fruits and vegetables is becoming much stronger. Fertilizer management represents a sustainable and inexpensive complement to conventional breeding and biotechnology for improving the human-health properties of foods. However, major gaps still exist in the knowledge regarding interactive effects among fertilizers, and among phytonutrients in foods. For instance, the relationships between P fertilization, phytate accumulation, and micronutrient bioavailability discussed earlier, clearly demonstrate that alternative P management guidelines

are needed to maintain an optimal balance between crop growth, micronutrient and phytonutrient contents, and bioavailability. Fertilizer management strategies should take into account the potential for significant tradeoffs among biosynthetic pathways and functionality of target phytonutrients. Human nutrition guidelines that emphasize consumption of balanced/diversified diets, containing a wide variety of fruits, vegetables, and whole-grain products, can ensure optimal bioavailability of essential nutrients and phytonutrients, and minimize some of the observed negative interactions. Given the rapid global increase in consumer demand for organically-grown foods, research documenting the relative effects of fertilizers on the functional properties of foods derived from conventional or organic production systems is warranted. Research is also needed to characterize the caloric and phytonutrient contents of the vast majority of edible, non-staple food crops, and farmers should be encouraged to focus not only on yield, but also on nutritional and health benefits of produce. Nevertheless, enhancing the human-health quality of foods through carefully-planned fertilizer management practices can be an effective dietary approach for enhancing the health, well being, and productivity of human beings. **FCHH** 

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Chapter 9

# Plant Nutrition and Health Risks Associated with Plant Diseases

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### Abstract

Seventeen of the 25 essential elements for humans are also essential for all plants. Three are essential or beneficial for some plants, and most of the remaining five can be found in plants. The availability of a mineral element for plant growth is not only dependent on its abundance in soil, its form and solubility, or the presence of competing or toxic entities, but also on microbial associations, the assimilative capacity of the plant, and environmental factors such as pH, moisture, and temperature. Plant diseases and pests are common causes of disturbed mineral nutrition and limit crop production efficiency, food safety, and nutrient quality. Nutrition has always been a primary component of disease control. Cultural practices such as crop sequence, tillage, organic amendment, soil pH adjustment, and water management often influence disease through nutrient interactions. Nutrient management is important to minimize the impact of diseases on the quantity, nutritional quality, and safety of crops for food and feed. Not only can the severity of many diseases be reduced, but also the chemical, biological, and genetic control of many plant pathogens can be enhanced by proper nutrition. A healthy plant is more efficient and able to balance its nutritional needs more effectively from the limited resources available. This, in turn, results in a greater nutrient sufficiency of the crop and enhances its beneficial use for food and feed purposes.

### Introduction

Plants provide the primary source of minerals and other nutrients for animals and humans. Thus, the sufficiency of human nutrition is dependent in large measure on the availability and nutrient sufficiency (quality) of the plants consumed directly, or indirectly through animals. Healthy plants promote healthy people. A varied diet is required to provide a full complement of the essential nutrients since no single plant source contains all of the essential carbohydrates, fats, amino acids, minerals, vitamins, etc. required in their proper ratios or concentrations. Traditionally, each self-sustaining society has cultivated crops and animal sources to provide the energy (carbohydrate), protein, and fat nutrients required

For symbols used commonly throughout this book see page xi.

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in the largest quantity; with the essential minerals and vitamins also generally being available through these three primary food groups.

In plants, each element has specific functions as part of an intricate system of delicately balanced physiological interactions. A deficiency or excess of one element greatly influences the activity of others and sometimes can exert catastrophic effects as secondary and tertiary consequences reverberate throughout the entire metabolic network of the plant (Evans et al., 2000). A deficiency of Mn or Mg that limits photosynthesis reduces the efficiency of those physiologic systems requiring energy, while a deficiency of K or Mg essential for sugar movement in the plant results in the accumulation of sugars in photosynthetic tissues and limits the desired accumulation of carbohydrate and protein in developing reproductive structures. The individual elements are required in varying amounts depending on whether they become structural or metabolic components, or function primarily as regulators of metabolic pathways. Nitrogen, P, K, S, Ca, and Mg are required in the largest amounts. However, B, Co, Cu, Cl-, Fe, Mn, Mo, Ni, and Zn are required in much smaller quantities. Sodium is considered essential for halophytic plants and Si for some grasses and horsetails. Plant essential C, H, and O are supplied through air or water while the remaining elements come from solubilization of various salts and minerals in soil or water. Various crops have different nutrient requirements for optimal growth and productivity (Marschner, 1995).

There are large differences among crops in their efficiency for the uptake and utilization of specific mineral nutrients so that a particular crop species, or even cultivar, may be more or less adapted to a certain environment than another. For example, a soil with 0.5 ppm available Cu may be adequate for pea, corn, rye, and cole crops, but severely limiting for the less efficient wheat, barley, and flax which need 3 to 4 times this level for sufficiency and optimum production (Evans et al., 2000). Root configuration, production of selective root exudates, and physiological efficiency influence whether a plant will be nutrient sufficient or deficient in a particular environment. Generally, as yields have increased, the need for available nutrients also has increased to support the increased physiological activity of the plant and to compensate for the larger amount of nutrients removed with the harvested crop. A different nutritional regime may be required to produce specific crop components such as protein in cereal grain or soybean compared with oil in canola (oilseed rape), soybean, or sunflower. If any one nutrient becomes limiting, yield and quality of the harvested component is often disproportionately reduced because of the intricate interrelationship of each nutrient with physiological processes (Rengel, 1999).

Plant nutrient deficiency is a major limitation to crop production efficiency and nutritional quality, and a predisposing factor for disease. Plant nutrient deficiencies can reduce both the quantity and quality of nutritive components of plants. When plants become deficient in a particular nutrient, other nutrients also may be affected so that the vitamins, protein, carbohydrate, fat and other essential nutritional components that plants are grown for will be affected. As primary food and feed sources, plants must provide nutrients in adequate quantity, safety and nutritional quality. Factors that result in a nutrient deficiency for plants also affect their nutrient value or nutrient availability for animals or man. Major causes of nutrient deficiency are an inadequate supply, lack of access to forms of nutrients available for absorption, or disease denial of nutrients necessary to maintain plant health and nutrient quality. Nutrient deficiencies can be overcome by increased availability, more efficient plant uptake, increased physiological efficiency, and improved disease control. Benefits of nutrient sufficiency of the plant are achieved through increased production efficiency and greater productivity of more nutritious and safer food. A healthy plant will be more efficient and able to meet its nutrient needs more effectively from the generally limited resources available. The focus of this chapter is on the role of plant nutrition in the management of plant diseases that affect the safety and nutritional value of food crops.

### **Causes of Plant Nutrient Deficiency**

Plant nutrient deficiency is caused by an insufficient level of an essential element at a critical time to maintain normal plant function. This may result from negligible amounts of an element in soil, inaccessibility (solubility, form), unfavorable abiotic soil conditions (pH, aeration, compaction) that limit root function, microbial-induced deficiency in the soil, or the effect of infectious plant disease and impaired physiological function. Balanced nutrition may be as important as the presence of any particular element since the intimate effects of nutrient deficiency occur at the cellular level, but may be manifest in altered growth and nutritional quality of the whole plant. Both abiotic and biotic factors can influence nutrient availability and disease severity.

#### Abiotic Factors Affecting Plant Nutrition and Disease

Compaction, pH, moisture, temperature, tillage, and biological activity largely determine the availability of nutrients contained in soil, affect root growth, and can predispose plants to various diseases. Soils between pH 6 and 7.5 are considered optimum for most cultivated crops although most crops can be grown well outside this pH range, and some crops are specifically adapted to either acid or alkaline soils. Iron, Mn, Cu, and Zn may reach toxic concentrations in tissues when soil pH is below 5 and be severely deficient at pH above 7.5. Highly acid soils can be limed to reduce toxicity from excess Al, Cu, and Mn, while flooding or applications of S to alkaline soils will increase the availability of B, Cu, Fe, Mn, and Zn that otherwise might be unavailable for plant uptake.

Drought reduces root growth, the solubility of nutrients for plant absorption, and the uptake of nutrients that are poorly mobile in soil. Nitrogen and carbohydrate metabolism and other plant physiological functions are impaired under drought stress, but are impacted less if nutrient sufficiency can be maintained.  $NO_3^-$  and  $NO_2^-$  reductases are especially impaired by moisture stress and high temperature to result in lower protein and high levels of tissue  $NO_3^-$  under otherwise fertile conditions. High  $NO_3^-$  in forages fed to ruminant animals

can pose a health hazard for them. Symbiotic N-fixation of legumes is reduced under drought stress because Mn and Ni needed for ureide synthesis become less available, and the soil organisms involved in their solubility are less active. In contrast to moisture deficit, excessive moisture limits oxygen in the root zone to inhibit active nutrient uptake.

Coarse-textured (sandy) and highly organic soils pose special situations for maintaining nutrient sufficiency in plants. Coarse-textured soils are subject to leaching and movement of nutrients below the area of active root absorption while organic soils sequester (chelate) cationic nutrients to reduce their availability for plant uptake. Availability of Cu, Mn, and Zn can be disproportionately affected. Although cereal plants can absorb both NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in cool soils, a visible plant response is faster with the more readily translocated NO<sub>3</sub><sup>-</sup> than with the NH<sub>4</sub><sup>+</sup> that is translocated primarily as amino acids (glutamine, glutamate, etc.).

Tillage and cultivation mix nutrients in the root zone, break compaction for better aeration, stimulate mineralization, and increase plant access to limited nutrients by facilitating root growth. Reduced-tillage agricultural systems frequently have limited availability of some essential nutrients because of localized distribution (stratification), immobilization in residue, limited root growth, and modified microbial activity affecting nutrient availability. High levels of some nutrients may competitively inhibit the uptake of other elements. Repeated applications of large quantities of organic manures (high in P) can immobilize Cu, Mn, and Zn to reduce their availability for plant uptake from soil. Copper used to control bacterial or fungal diseases can accumulate to toxic levels in soils over years of application.

### **Biological Factors Affecting Plant Nutrition and Disease**

Soil microorganisms, as activators in biological nutrient cycles, modify mineral availability and play a critical role in either inducing or alleviating mineral deficiencies and thereby affecting plant resistance and pathogen activity. Biological mineralization of residues releases minerals from their bound state in residues and organic matter, but can also result in a transient immobilization of certain nutrients such as N that are preferentially acquired for microbial growth and activity during decomposition of residues low in N. The dynamics of nutrient cycling can be changed by variations in microbial, environmental and plant factors. Thus, time of nutrient application may be crucial because of changes in the interactions involved. Biological oxidation of N (nitrification) can lead to extensive losses of this essential element through subsequent denitrification or leaching. A characteristic of climax ecosystems is inhibition of nitrification to preserve N for plant availability (Rice, 1984). The biological oxidation of reduced Fe and Mn makes them unavailable for plant uptake, while plants can only use the oxidized form of S. Various synergistic interactions of soil microbes with plants can greatly increase nutrient uptake and reduce disease severity. Examples of the dynamic interactions influencing nutrient availability are the biological fixation of atmospheric N to plant available forms by soil microbes, increased P and Zn uptake by mycorrhizae, biological reduction of Fe and Mn in plant rhizospheres, and the biological oxidation of S.

### **Effect of Nutrient Deficiency on Plant Disease**

Increased susceptibility to infectious disease is a common result of nutrient deficiency (Datnoff et al., 2007; Evans et al., 2000; Huber, 1991; Huber and Graham, 1999). Increased cell permeability from Zn or B deficiency can result in the loss of nutrients through root or leaf exudation to attract various pathogens or enhance infection (Cakmak and Marschner, 1988). This nutrient-enriched rhizosphere environment provides the chemical stimulation and attraction for soilborne pathogens such as *Pythium* and *Phytophthora* (Huber, 1978).

Foliage diseases such as powdery mildew (*Erysiphe graminis*) are more severe on Cu-deficient cereals because maturity of the plants is delayed to extend the infectious period. Copper deficiency predisposes gramineaceous plants to ergot because of pollen sterility that causes the glumes to open so that stigmas are exposed to infection by *Claviceps purpurea*, the cause of ergot. Application of Cu



Application of Cu fertilizer (CuSO₄ crystal on right) has been an effective treatment in ergot-prone soils.

fertilizers can greatly reduce severity of this disease (Evans et al., 2000). Zinc deficiency predisposes plants to Rhizoctonia solani and other cortical pathogens because inhibitory carbohydrates are reduced and the energy required for defense is lacking. Early Zn deficiency predisposes winter cereals to winterkill by Rhizoctonia cerealis because adequate carbohydrate reserves necessary for resistance are not retained throughout extended periods of snow cover or low photosynthesis. Early seeding of winter wheat to reduce winterkill facilitates root colonization by mycorrhizal fungi and increases Zn uptake to provide a plant that is more resistant to the causal fungus (Bockus et al., 2010). Potassium deficiency predisposes potato and tomato to leaf blight caused by Alternaria solani. Manganese deficiency predisposes cereal plants to take-all root and crown rot (Gaeumannomyces graminis), potato to common scab (Streptomyces scabies), many plants to Verticillium wilt (Verticillium dahliae), rice to blast (Magnaporthe griseae) and numerous other plants to severe debilitating plant diseases because the defense compounds regulated by Mn that inhibit these pathogens are not produced (Huber and Wilelm, 1988; Thompson and Huber, 2007).

In addition to the lower amino acid content and protein from a deficiency of N, N deficiency will also predispose plants to early senescence and diseases caused by toxigenic *Fusarium* species and other pathogens. Stalk rot describes a symptom of one of the most destructive diseases of maize. It is often referred to as a disease of stress or senescence since actively growing plants at nutrient sufficiency are seldom lodged from infection by *Gibberella zeae* or other stalk rotting pathogens. Stalk rot is especially severe when the available N from the soil reservoir or by

recycling stored N compounds from vegetative tissues is inadequate to meet demands of the developing kernels. To meet the needs of developing kernels, most plants will cannibalize photosynthetic enzymes (Rubisco, PEP carboxylase) and glycoproteins (structural proteins) in vegetative tissues as a source of N to meet demands of the developing kernels. This loss of photosynthetic capacity induces early senescence and exposes tissues to maceration by extra-cellular proteolytic, pectolytic, and cellulolytic enzymes of stalk-rotting pathogens (Huber et al., 1986). Maintaining sufficiency of N and other essential elements throughout the grain-fill period is an important control for this disease.

Agricultural herbicides and other biocides (nitrification inhibitors, fungicides, plant growth regulators, etc.) that have chelating properties can immobilize specific secondary and micronutrients (Ca, Cu, Fe, Mg, Mn, Ni, Zn) in the soil or plant to reduce plant uptake, physiological function, or accumulation in reproductive parts of target or non-target plants and predispose plants to various pathogens (Huber, 2010). Fenoxaprop-p-ethyl (Puma<sup>®</sup> super), trifensulfuronmethyl (Harmony<sup>®</sup>), clodinefop-propargyl, and picloram (Tordon<sup>®</sup>) are a few examples of Cu-chelating herbicides that can induce a Cu deficiency in non-target food plants and predispose plants to ergot. Several herbicides that induce a Zn deficiency in plants also predispose plants to winter injury by *Rhizoctonia cerea*lis. In contrast to most agricultural compounds that chelate with a single or few metal species, the extensively used herbicide glyphosate (N-(phosphonomethyl) glycine) is a broad-spectrum metal chelator of both primary, secondary, and micronutrients (Ca, Co, Cu, Fe, K, Mg, Mn, Ni, and Zn) and was first patented as such (Stauffer Chemical Co., 1964). This broad-spectrum chelating ability that makes glyphosate a good herbicide and selective anti-microbial agent (Ganson and Jensen, 1988) can also immobilize numerous essential plant nutrients to predispose plants to disease and reduce their mineral content in food or feed products by as much as 45% (Eker et al., 2006; Huber, 2010; Johal and Huber, 2009; Zobiole et al., 2010). Reduced content of amino acids and altered polyunsaturated fatty acids in seeds of food crops also are observed after glyphosate treatment of glyphosate-tolerant crops (Zobiole et al., 2010). The list of over forty diseases and mineral deficiencies affected by glyphosate is increasing as growers and pathologists recognize the cause-effect relationship with this strong mineral chelator (Johal and Huber, 2009). The accumulation of glyphosate in root tips (meristematic tissues) reduces root growth and further limits plant access to soil nutrients (Huber, 2010). The effect of glyphosate on root growth and nutrient efficiency is similar for genetically modified (glyphosate-tolerant) plants as for their normal parental lines since there is nothing in the genetic engineering technology that impacts glyphosate itself. The technology merely inserts another gene for the EPSPS enzyme that is not sensitive to glyphosate in mature tissues (Huber, 2010).

Infectious diseases intensify nutrient deficiency of plants and further reduce their nutritional value for food or feed. Severe outbreaks of many diseases are an indication of low nutrient availability or mineral deficiency (Datnoff et al., 2009; Englehard, 1989; Evans and Huber, 2000; Huber, 1978, 1980, 1991; Graham and Webb, 1991; Huber and Graham, 1999; Marschner, 1995). Thus, adequate nutrition is an important means of reducing many diseases as will be discussed later.

### **Disease as a Cause of Plant Nutrient Deficiency**

Disturbed mineral nutrition is one of the most common effects of infectious disease. Many primary and secondary symptoms associated with various toxicants and infectious diseases are similar to those expressed by mineral deficiencies and it is not always possible to clearly distinguish symptoms of infectious from noninfectious (abiotic) disease (Huber, 1978). Stunting, chlorosis, wilting, mottle, rosette, witches' broom, dieback, leaf spot, abnormal growth, and other symptoms of specific mineral deficiency are also caused by plant pathogens. Nutrient relationships with disease have been derived from: a) observed effects of mineral amendments on disease severity; b) a comparison of mineral concentrations in resistant compared with susceptible plants or diseased compared with disease-free tissues; c) the correlation of conditions known to influence mineral availability with disease incidence or severity; or d) a combination of these (Huber, 1989; Huber and Haneklaus, 2007). Pathogens impair nutrient uptake (root rots), translocation (vascular wilts), distribution (galls, cankers, and microbial sinks), and utilization (necrosis and toxin producers). Efficient nutrient use by plants is impaired when there is an accumulation of nutrients around the infection site, direct usage of nutrients by pathogens, or a chemical change to render nutrients more or less available for uptake in the rhizosphere or at the infection site (Table 1). All of these effects of disease can reduce the nutrient density and quality of edible plant parts.

Disease type Effect on nutrition	
Microbial growth in soil*	Immobilize (N, Fe, Mn, and S); toxicity (Mn)
Root rots, soil insects, nematodes	Immobilization, absorption, distribution, plunder
Macerating, rotting diseases	Distribution, nutrient sinks, depletion, plunder
Vascular wilts, leaf spots, blights	Translocation, distribution, metabolic efficiency
Viruses, spiroplasmas	Distribution, nutrient sinks, metabolic efficiency
Galls, 'brooms,' over growths	Distribution, nutrient sinks, usage, efficiency
Fruit and storage rots	Nutrient sinks, usage, distribution, low reserves
Toxigenic pathogens	Function, distribution, absorption, safety

#### **Table 1.** Effect of plant disease on plant nutrition.

(after Huber, 1978; Datnoff, et al., 2007; Evans et al., 2000; Huber and Graham, 1999) \*Referred to as microbially-induced "deficiency" or "toxicity" diseases.

#### **Reduced Nutrient Uptake and Translocation**

Plants absorb nutrients throughout the soil profile by an enormous root system. Destruction of the absorptive capacity of this system through necrosis, malfunction, or reduced growth can severely impair plant functions throughout the entire plant. Soil-borne fungal pathogens, viruses, and nematodes reduce the amount of functional absorptive tissue and have a disproportional effect in reducing uptake of Ca, Mn, P and other relatively immobile elements which require an extensive functional root system to access them. Foliar pathogens (bacteria, fungi, viruses) that affect photosynthesis (rusts, mildews, blights) can create a shortage of energy needed for root growth and mineral uptake. A healthy plant can produce siderophores and other compounds in root exudates to solubilize soil minerals and increase their availability and uptake. Root-rotting pathogens not only severely limit the area of the soil available for nutrient extraction, but also interfere with the production of these root exudates.

Gums, gels, cellular slimes, and other vascular occlusions associated with diseases caused by fungal, bacterial, and viral pathogens interrupt translocation of minerals and water to starve tissues at a distance from the blockage. Deficiencies of N, P, or K that are necessary in large quantities become especially obvious. Absorbed minerals may accumulate below a blockage but are of little benefit unless translocated to all parts of the plant. Pathologically redirected movement and accumulation of sugars, amino acids, and minerals toward infection sites induce a nutrient deficiency in cells that would normally receive those nutrients, and can affect the physiology of the entire plant even though the total quantity of nutrients in the plant may be unchanged. Vascular blockage can have a direct effect or be indirect through imbalances created in various nutrients and localized shortage of water for physiological functions.

#### **Impaired Nutrient Utilization**

Plant pathogens impair nutrient utilization through immobilization, alteration of cell permeability, or competitive inhibition. Alteration of cell permeability by pathogen-produced toxins (pericularin, victorin, Corynebacterium toxins) can regulate nutrients available for a pathogen at the infection site. These toxins are also strong mineral chelators that can render specific micronutrients, such as Mn, unavailable to the plant, but mobile toward the infection site where they can accumulate to the benefit of the pathogen (Cheng, 2005). Cells adjacent to injured or necrotic tissues become depleted of Mn essential for photosynthesis to create a chlorotic halo around the infected tissue. Cell walls become impermeable to nutrients in some plant-pathogen interactions, while increased cell permeability is observed following infection by obligate (rusts, mildews, etc.) and tumor-inducing (crown gall, fasciations, etc.) pathogens (Huber, 1978). Six of the seven biosynthetic systems unblocked in autonomous tumor cells are activated by specific mineral ions. Tumor tissues are high in auxin and contain high concentrations of micronutrients that activate metabolic systems involved in autonomous growth. The addition of Zn causes a rapid rise in auxin, and large amounts of auxin accumulate in Cu- and Mn-deficient plants because of decreased oxidative enzyme activity.

Modification of cellular permeability contributes to the accumulation of nutrients around an infection site to create a 'sink' effect that immobilizes a nutrient and prevents its normal reuse several times during growth of the plant. Necrosis associated with localized infections common with apple scab (Venturia inaequalis), eye spot of wheat (Pseudocercosporella herpotrichoides), Rhizoctonia canker of potato and cotton, or citrus canker (Xanthomonas citri); defoliation by citrus variegated chlorosis (Xylella fastidiosa); or impaired translocation makes nutrients accumulated in some areas inaccessible to new growth or other parts of the plant. Obligate pathogens create very powerful nutrient sinks where minerals and plant metabolites are mobilized to the infection site as permeability and metabolic activity are increased (Horsfall and Cowling, 1978). Pathogen metabolism may also maintain a concentration gradient and higher osmotic pressure to ensure a continuous flow of materials to the infection site and thereby deprive the rest of the plant of those essential nutrients. Nitrogen, P, S, other elements and plant metabolites accumulate in plant tissues at the infection site of viruses, rusts, mildews, and potato late blight (Phytophthora infestans) (Huber, 1978; Horsfall and Cowling, 1978).

The ability to induce a localized mineral deficiency can be a virulence factor for some pathogens. Only isolates of Gaeumannomyces graminis (take-all root and crown rot of cereals), Streptomyces scabies (common scab of potato), and Magnaporthe grisea (rice blast) that can oxidize Mn from the reduced, plant-available form to the oxidized, non-available form are able to cause disease. By immobilizing Mn in plant tissues at the infection site, these pathogens turn off plant defense mechanisms regulated through the shikimate pathway (Cheng, 2005; Huber and Thompson, 2007; Thompson and Huber, 2007). Nutrients accumulating around infection sites are unavailable to the plant as are those that accumulate in hyperplasia (growths from an abnormal increase in cells) induced by certain bacteria, fungi, and nematodes. Restricted root growth from necrosis or girdling can directly reduce nutrient absorption and predispose plants to more severe infection or susceptibility to other pathogens. A malfunctioning vascular system or changes in membrane permeability can induce a systemic or localized nutrient deficiency. All of these effects of infectious diseases can reduce the mineral content, nutritional quality, and safety of the food or feed products produced.

### **Effect of Plant Pathogens on Food Safety**

In addition to causing a direct loss in yield and nutritional quality, some plant pathogens produce chemically diverse toxins that threaten food safety. These toxins can cause gastrointestinal disturbances, intestinal necrosis, hemorrhage, vomiting, cancer, kidney damage, liver damage, hepatic changes, reduced feed and production efficiency, immune suppression, infertility, and death in animals and man. They can be produced during crop development prior to harvest, in transport, in storage, and during processing so that raw or processed foods and feeds may contain them (CAST, 1989). Factors influencing microbial toxin production include crop substrate, moisture, temperature, pH, drought, disease, nutrient stress, damage by other pests, and several commonly used agricultural chemicals. Some of the major crops affected include barley, maize, cotton, peanuts, wheat, and nut crops. The primary toxin-producing fungi include various species of *Aspergillus, Acremonium, Claviceps, Fusarium, and Penicillium*.

### **Risks Due to Pathogen-Produced Toxins**

Contamination of milk, eggs, and meat can result if animals consume mycotoxin-contaminated feed. Since mycotoxins are naturally occurring compounds, some level of mycotoxin is unavoidable in various crops such as peanuts, cotton, corn, wheat, and nut crops. The usual route of exposure to mycotoxins is through ingestion of contaminated feed or food; however, dermal or inhalation exposure also may be significant (CAST, 1989). Wheat straw used as bedding for pigs or cattle can leave them infertile from absorption or consumption of estrogenic zearalenone produced by *Fusarium graminearum* (*Gibberella zeae*) or other toxins produced by *Fusarium* species that cause cereal head scab/blight, root and crown rot, stalk rot, or ear rot (Rottinghaus et al., 2009).

The presence of a potential toxin-producing fungus in the crop does not automatically establish the presence of mycotoxins, just as the absence of the fungus from the harvested product does not ensure absence of a toxin. Both deoxynivalenol and zearalenone produced by *Fusarium* in cereal root and crown tissues are translocated and accumulate in the grain and other plant parts (Rottinghaus et al., 2009). A few mycotoxins, such as those associated with ergot, are produced exclusively in the field, while many other mycotoxins also are produced in storage or later in processed food products (CAST, 1989).

### Factors Influencing Mycotoxin Production

During plant growth, any condition favoring fungal infection can predispose to mycotoxin production. Abiotic stress (chemical, moisture, temperature), reduced vigor from nutrient deficiency, weed competition, and insect or other wounds are especially favorable conditions for toxin production. Heavily lodged grain is often damaged more by fungal and bacterial pathogens (also dense planted crops) because of the more conducive microenvironment. Post-harvest production of toxins is favoured by high temperature and moisture during storage or processing (CAST, 1989). Many pests predispose plants to infectious disease as vectors or by providing avenues for entry that breach natural defense barriers.

Aflatoxins - Infection of grain, peanuts, and cottonseed by *Aspergillus* species, and their subsequent production of highly carcinogenic aflatoxins, is associated with insect damage and environmental stress. Drought, high temperature and other environmental stresses weaken a plant's resistance to infection and provide an environment conducive for infection. Use of insecticides to control seed infesting insects can prevent fungal infection and toxin production in fruiting structures.

**Fusarium toxins** - The production of trichothecene, zearalenone, and other toxins by various species of *Fusarium* is favoured by nutrient deficiency, environmental stress, and insect damage. Control of root and stem insects, and maintaining structural integrity through proper plant nutrition can reduce pest damage to improve both the quality and quantity of the crop produced. Less dense populations, wide rows that permit air circulation, and full nutrient sufficiency also can provide a less conducive environment for infection or pathogenesis (disease development).

**Ergot** - Ergot (*Claviceps* species) is the oldest recognized mycotoxicosis of man. The ergot toxins are produced during grain growth as the fungus replaces the grain (seed) with a sclerotium containing the mycotoxins. Infection occurs by wind blown spores of *Claviceps* species in open-pollinated cereals and has limited the production of hybrids of self-pollinated species when glumes are opened to receive outside pollen. Late spring frosts that kill the anther, or Cu deficiency of cereals grown on low Cu soils or induced by certain Cu-chelating herbicides, can result in heavy infection and contamination by ergot sclerotia. Application of adequate Cu fertilizers for physiological plant sufficiency provides a significant measure of control of ergot (Evans et al., 2000).

Agricultural chemicals - Although of significant benefit in reducing pest damage, some agricultural chemicals can reduce crop nutritional quality and predispose plants to pathogens and toxigenic organisms (Johal and Huber, 2009). Fusarium species causing head blight (also referred to as scab) are common root and crown rot pathogens of cereals everywhere; however, Fusarium head blight (FHB) has generally been a serious disease of wheat and barley only in warm temperate regions of the world. Fusarium head blight and the mycotoxins produced by these fungi are greatly increased with the extensive use of the glyphosate herbicide (N-(phosphonomethyl)g|ycine) that is a strong micronutrient chelator. With the extensive use of glyphosate, FHB is now of epidemic proportions and prevalent throughout most of the cereal producing areas of the world. Canadian research has shown that the application of glyphosate one or more times in the three years previous to planting wheat or barley was the most important agronomic factor associated with high FHB in wheat, with a 75% increase in FHB for all crops and a 122% increase for crops under minimum-till where more glyphosate is used (Fernandez et al., 2005, 2007, 2009). The most severe FHB occurs where a glyphosate-tolerant crop precedes wheat or barley in the rotation for the same reason. Glyphosate-altered plant physiology (C and N metabolism) increases susceptibility of wheat and barley to FHB and increased toxin production from heading to maturity of the crop.

The increased FHB with glyphosate results in a dramatic increase in tricothecene 'vomitoxins' (deoxynivalenol and nivalenol) and estrogenic (zearalenone) mycotoxins in grain. The high concentrations of mycotoxin in grain are not always associated with *Fusarium* infection of kernels. Quite often overlooked is the increase in *Fusarium* root and crown rot with glyphosate usage and the production of mycotoxins in root and crown tissues that are subsequently translocated to stems, chaff, and grain. Caution has been expressed in using straw and chaff as bedding for pigs, or roughage for cattle, because of mycotoxin levels that can exceed clinically significant levels for animal infertility and toxicity (Sweets and McKendry, 2009).

#### **Other Health and Safety Concerns**

Potential pest and disease damage have necessitated the use of various chemical biocides (herbicides, insecticides, fungicides, etc.) to reduce their economic impact on agriculture. Many of these products can be of great benefit in reducing nutrient competition, crop damage and toxin production, but may pose a toxicological health risk of their own if they accumulate in the harvested product used for feed or food. Such concerns have been expressed most recently with the unrestricted use of the herbicide glyphosate because of relatively high concentrations of this micronutrient-chelating herbicide that can accumulate in many foods and food products to enter the food chain directly (Watts, 2009). The level of glyphosate in feed and foods has increased significantly with the use of glyphosate tolerant crops and the direct application of glyphosate to food and feed plants (Antoniou, et al., 2010; Watts, 2009). In addition to increased mycotoxins and chemical residues in feed, food, and water, glyphosate reduces the content of essential micronutrients (Co, Cu, Fe, Mn, Ni, and Zn) in the harvested food product (Bellaloui, 2009; Huber, 2010; Zobiole et al., 2010). Recent studies have found levels of glyphosate in manure from chickens fed grain of glyphosate-tolerant crops equivalent to one-tenth the labeled herbicidal rate of this material per ton (Dr. M. McNeil, personal communication).



Figure 1. Interacting components of plant, environment, and pathogen affecting disease and nutrient quality.

### Interacting Factors of Nutrition and Disease

Infectious plant disease is the expression of the interaction of the plant, a pathogen, and the environment over time (**Figure 1**). Disease control is most effectively achieved when the interacting factors of these three primary components are recognized and understood to make them less conducive for disease development. All interactions between the plant, pathogen, and environment are affected by nutrition, and all of the essential mineral elements are reported to influence disease incidence or severity (Datnoff et al., 2007; Englehard, 1989; Huber, 1980, 1991; Huber and Graham, 1999; Huber and Haneklaus, 2007). Although nutrients, as a component of the environment, influence the plant's resistance, and a pathogen's virulence, each of the three primary components also influences the availability of nutrients. As previously discussed, nutrition of the plant can be drastically altered by many disease organisms through their effect on the uptake, translocation and distribution, or utilization of nutrients, and it is frequently difficult to clearly differentiate between the biotic and abiotic factors that interact to "cause" a plant nutrient deficiency or excess.

#### **Recognizing Plant Nutrient Deficiency**

All nutrients have specific metabolic functions, and impaired nutrient status of a plant may be indicated by symptoms associated with the malfunction of particular metabolic pathways. The manifestation of nutrient deficiency may be very subtle ('hidden hunger') or quite pronounced and distinct depending on the level of deficiency or severity of disease. Visible symptoms, however, are often late manifestations of metabolic disruptions that occurred much earlier. Detailed descriptions and colour photographs of mineral deficiency symptoms (and toxicity) are available and useful for this purpose (Bennett, 1993; Grundon, 1987; Plank, 1988). Reduced productivity as measured by yield or quality may have multiple causes such as weather, management practices, infectious diseases and the soil environment so that correcting the problem may require multiple approaches. With multiple deficiencies, some symptoms may be alleviated only after all elements are available in sufficient quantity. Analytical tests of soil or plant tissue provide a basis to prevent or remedy potential deficiency conditions. Nutrient amendment (fertilization) or modification of the soil environment influencing a particular nutrient's availability are important techniques to provide nutrient sufficiency to plants. The root cause of many nutrient deficiencies may be in the roots, and a plant will balance many nutritional needs if it has access to nutrients through a fully functional and healthy root system.

When a nutrient is deficient, its content in the plant is usually reduced. Soil and tissue analysis can be used as a general guide to the availability and nutrient status of plants. Chemical analyses for the mineral nutrients provide a quantitative evaluation of nutrient status capable of revealing mild deficiencies or excesses in a range where symptoms are not expressed. A number of analytical techniques have been developed to establish the relative availability of nutrients in soil or water, or the sufficiency status of a plant (Page et al., 1982; Mills and Jones, 1996). Most commercial laboratories use standardized procedures to provide consistency between laboratories; however, interpretation and recommendations from the data differ widely between areas because of innate differences in environment, local experience, or sales incentives. These techniques can help to achieve optimal conditions since internal requirements for most essential nutrients (critical levels) have been determined for many crop plants (Graham, 1983; Mills and Jones, 1996; Plank, 1988). Such diagnostics indicate the current nutritional status of the plant, but do not necessarily give a prognosis for sufficiency through to harvest because they are limited in scope to the time of sampling. For example, an assay of ear-leaf tissue N at tasseling may be accurate for predicting N sufficiency of maize hybrids which absorb 90-95 % of their required N prior to tasseling, and then recycle this N during grain formation; but grossly underestimate N required for 'high yielding' hybrids which are dependent on 40-50 % of their N uptake after flowering, or for cultivars that recycle little of their vegetative N sources to grain ("stay green" hybrids) (Tsai et al., 1983). Tissue analysis also gives little indication of the dynamics of microbial intervention in nutrient availability and uptake.

# **Managing Nutrition to Control Plant Diseases**

Each of the 14 plant-essential mineral elements and several functional elements are known to influence disease severity. Disease suppression through manipulation of nutrient availability may be achieved by direct application of a nutrient to enhance resistance, by cultural practices which modify abiotic and biotic environments influencing nutrient availability, and by modifying the plant genotype relative to its nutrient uptake or interaction with the abiotic or biotic environment. A well-balanced nutrition program, integrated with other crop production practices, permits a broad utilization of this cultural disease control, and generally provides the best opportunity for maximum disease suppression.

### Strategies to Reduce Disease through Nutrient Interactions

Six key strategies to manage disease through nutrition include: 1) selection of cultivars with the highest genetic disease resistance and nutrient efficiency; 2) provide a balanced nutrition for full nutrient sufficiency; 3) apply a form of nutrient that is not conducive to disease; 4) apply the nutrient at a time when conditions for disease are least conducive; 5) use nutrient sources which suppress rather than enhance disease; and 6) integrate nutrition with other management practices that influence nutrient availability or function in the agricultural production system (Datnoff et al., 2007; Graham and Webb, 1991; Huber and Graham, 1999; Huber and Haneklaus, 2007). The greatest response to nutrition in reducing disease is generally observed when going from deficiency to sufficiency and excess application may increase sensitivity to some diseases.

**Selection of adapted, nutrient-efficient cultivars.** The availability of genetic resistance to disease has permitted the production of many crops in areas that would otherwise be non-profitable because of certain diseases; however, nutrient sufficiency is a primary component for the full expression of genetic resistance. Resistance of wheat and flax to rust, and maize to Stewart's wilt may be lost under K-deficient conditions (Huber and Arny, 1985). Cultivars that are resistant or tolerant to disease are generally more responsive to nutrient manipulation than highly susceptible cultivars. Rye is resistant to take-all by its high efficiency for the uptake of Mn and other micronutrients essential for resistance to this pathogen that is mediated through the shikimate pathway. In contrast to rye, wheat is inefficient in micronutrient uptake and highly susceptible to take-all. Rye-wheat interspecific hybrid lines (triticale) that contain rye's efficiency for nutrient uptake are as resistant to take-all as the rye parent, while lines that do not contain this genetically controlled nutrient efficiency are as susceptible to take-all

as wheat (Huber and McCay-Bius, 1993). Oats that are resistant to gray speck (Mn deficiency) produce root exudates that inhibit Mn-oxidizing organisms in the rhizosphere to increase Mn availability in soil and are resistant to take-all compared to gray speck susceptible varieties (**Table 3**). This change in soil biology to increase the availability of Mn also protects a subsequent wheat crop from take-all while rye, as a preceding crop to wheat, has no effect in reducing susceptibility of the wheat that follows it. (Huber and McCay-Bius, 1993).

Provide a balanced nutrition for full nutrient sufficiency. The greatest response to nutrition is observed when going from deficiency to plant sufficiency. The needs and uptake of nutrients depend on the stage of plant growth, availability of nutrients in the soil, time of application, microbial activity, and general health of the plant. The severity of take-all and tan spot of wheat and stalk rot of corn decrease as N approaches full sufficiency (Bockus and Davis, 1993; Bockus et al., 2010; Huber et al., 1986, 1987). A similar effect is observed with Alternaria leaf blight of potato and tomato as rates of K approach physiologic sufficiency (Prabhu et al., 2007). When disease decreases with rates above physiological sufficiency for the plant, it is usually because of changes in the environment or interactions with other elements. Nutrient imbalance may be as detrimental to plant growth and disease resistance as a deficiency. Excess N can increase stalk rot because physiological sufficiency of other nutrients is not in balance (Huber et al., 1986). Potassium decreases take-all of wheat if N and P are sufficient, but increases this disease if they are deficient (Huber and Thompson, 2007). Fusarium wilt of cotton, clubroot of crucifers and late blight of potato have been correlated with the ratio of K to Mg and N rather than the actual amount of either element individually (Engelhard, 1989). An excess of some nutrients is toxic and can predispose plants to disease while a disease may be reduced by the same nutrient up to the sufficiency needs of the plant. Liming to reduce toxicity and availability of Mn can provide effective control of hyperplasia (gall) diseases.

Use a form of nutrient that is not conducive to disease. Different forms of some nutrients often influence disease differently because of differences in plant uptake, physiological pathways involving specific defense mechanisms, or pathogen activation. Elements such as N, Fe, Mn, and S are readily oxidized or reduced in most soils by soil microorganisms to affect their availability for plant uptake. Both the cation  $(NH_{4}^{+})$  and the anion  $(NO_{3}^{-})$  forms of N may be assimilated by plants, but they frequently have opposite effects on disease (Table 2) because they are metabolized differently. Practical control of some diseases can be achieved by manipulating the environment to favor one or the other forms of N. Application of NO<sub>3</sub><sup>-</sup> and liming has proven to be a practical control of *Fusarium* wilt diseases of melons, tomatoes, and other crops, while NH<sub>4</sub><sup>+</sup> decreases the severity of takeall of cereals, Verticillium wilt of potato, common scab of potato, and rice blast. Diseases that are reduced by  $NH_{4}^{+}$  are also reduced by environmental conditions that slow or inhibit nitrification and increase the availability of Mn (**Table 3**). In contrast, diseases such as *Fusarium* wilt of fruit and vegetable crops, clubroot of crucifers, and Rhizoctonia canker that are reduced by NO<sub>3</sub><sup>-</sup> also are less severe with supplemental Ca and environmental conditions that favor nitrification.

Сгор	Disease	Pathogen			
Diseases decreased by NO <sup>3-</sup> fertilization and alkaline pH:					
Asparagus	Wilt	Fusarium oxysporum			
Bean (Phaseolus vulgaris)	Chocolate spot	Botrytis			
	Root and hypocotyls rot	Fusarium solani			
	Root and hypocotyls rot	Rhizoctonia solani			
Beet	Damping off	Pythium species			
Cabbage	Club root	Plasmodiophora brassica			
	Yellows	Fusarium oxysporum			
Celery	Yellows	Fusarium oxysporum			
Cucumber	Wilt	Fusarium oxysporum			
Ornamental plants	Crown gall	Agrobacterium tumefaciens			
Pea (Pisum sativum)	Damping off	Rhizoctonia solani			
Pepper	Wilt	Fusarium oxysporum			
Potato	Stem canker	Rhizoctonia solani			
Tobacco	Frenching	Bacillus cereus			
Tomato (and others)	Gray mold	Sclerotinia sclerotiorum			
	Sclerotium blight	Sclerotium rolfsii			
	Wilt	Fusarium oxysporum			
Wheat	Eye spot	Pseudocercosporella herpotrichoide			

### Table 2. Some diseases influenced by the form of N and pH.

### Diseases decreased by $NH_{\!_{\!\!4}}^{+}$ fertilization and acid pH:

Root rot	Thielaviopsis basicola	
Root knot	Meloidogyne	
Root rot	Sclerotium rolfsii	
Wilt	Fusarium oxysporum	
Stalk rot	Gibberella zeae	
white rot	Sclerotium rolfsii	
Root rot	Pythium species	
Common scab	Streptomyces scabies	
Wilt	Verticillium dahliae	
Virus	Potato virus X	
Blast	Magnaporthe oryzae	
Southern wilt	Pseudomonas solanacearum	
Anthracnose	Colletotrichum	
Wilt	Virticillium dahliae	
Virus	Potato virus X	
Take-all	Gaeumannomyces graminis	
	Root knot Root rot Wilt Stalk rot white rot Root rot Common scab Wilt Virus Blast Southern wilt Anthracnose Wilt Virus	

(After Huber and Graham, 1999)

Condition or	Effect on:			
cultural practice	Nitrification	Mn availability	Disease severity*	
Low soil pH	Decrease	Increase	Decrease	
Green manure crops (some)	Decrease	Increase	Decrease	
Oat pre-crop		Increase	Decrease	
Ammonium fertilizers	Increase	Increase	Decrease	
Irrigation (some)	Decrease	Increase	Decrease	
Firm seedbed	Decrease	Increase	Decrease	
Nitrification inhibitors	Decrease	Increase	Decrease	
Soil fumigants	Decrease	Increase	Decrease	
Metal sulfides	Decrease	Increase	Decrease	
Glyphosate	Increase	Decrease	Increase	
High soil pH	Increase	Decrease	Increase	
Liming the soil	Increase	Decrease	Increase	
$NO_3^-$ fertilizers		Decrease	Increase	
Animal manure	Increase	Decrease	Increase	
Low soil moisture	Increase	Decrease	Increase	
Loose seed bed	Increase	Decrease	Increase	

Table 3. Some conditions affecting N form, Mn availability, and disease severity.

\*Common scab of potato, take-all of cereals, rice blast, maize stalk rot (after Huber and Haneklaus, 2007)

Apply nutrients at a time when conditions for disease are least conducive. Mineral nutrients are applied to meet the potential needs for efficient crop production in an economically and environmentally sound manner. The time of fertilization is important to minimize periods of irreversible nutrient deficiency without stimulating pathogenic activity. Fall application of N to winter wheat (under non-leaching conditions) can provide full sufficiency throughout the crop season without affecting eyespot (*Pseudocercosporella herpotrichoides*), whereas N applied in the spring increases disease severity by stimulating growth and virulence of the pathogen. Sharp eyespot and winter-kill (*Rhizoctonia cerealis*) of wheat are increased when N is applied during cool, wet conditions favorable for disease, but not if applied later to actively growing wheat under less conducive conditions for disease (Bockus et al., 2010). Liquid N increases these diseases more than granular fertilizers because of enhanced contact with the pathogen to increase its virulence.

**Use nutrient sources that suppress disease.** The associated ion applied with a fertilizer salt or in an organic fertilizer may have an effect on disease independent of the primary ion. Zinc in barnyard manure is primarily responsible for reduced *Rhizoctonia* spring blight rather than the more abundant N, P, or K that

are also available with this source of nutrient. Stalk rot of maize, take-all of cereals (*Gaeumannomyces tritici*), northern leaf blight of maize (*Setosphaeria turcica*), and rusts (*Puccinia* spp.) on wheat are reduced by high rates of KCl, but not with  $K_2SO_4$ , possibly as a result of the Cl<sup>-</sup> decreasing nitrification and increasing Mn availability (Christensen et al., 1986; Elmer, 2007).

**Integration of nutrition with management systems.** Many of the cultural practices used to control plant diseases function through their influence on plant nutrition (Table 3). Crop rotation, green manure cover crops, and fallowing practices have made crop production efficient in many areas of the world by increasing the supply of readily available nutrients and controlling weeds that compete for nutrients and moisture. Long-term monocropping can provide biological stability in soil and reduce diseases such as take-all of wheat through a phenomenon referred to as take-all decline involving enhanced N conservation and increased Mn availability (Hornby et al., 1998). Maize is a preferred crop to precede potatoes because it provides almost twice the available Mn as other cereals (Smith, 2006) and suppresses Verticillium wilt (Thompson and Huber, 2007). Integration of nutrient amendment with cultural practices such as tillage, seeding rate and date, and pH adjustment can accentuate the benefits of nutrient amendment by modifying the environment for plant growth or microbial activity. Tillage distributes nutrients in the root zone for easier access, prevents nutrient stratification in soil, facilitates root growth, and changes microbial activity that affects specific nutrient forms or availability.

Time of tillage and seed bed preparation are important because of their effect on soil microorganisms involved in mineralization of residues and nutrient availability. Seed bed preparation and uniform planting depth for quick emergence can shorten the infection period for seedling diseases and establish a vigorous plant with a well developed root system that can sustain the nutrient needs of the plant throughout vegetative growth and reproduction. A firm seedbed provides an environment conducive to Mn reducing microbes to increase availability of Mn for plant uptake (Huber and McCay-Buis, 1993). This has been a long-standing recommendation for reducing take-all root and crown rot of cereals (Hornby et al., 1998) and results in 9-15 ppm higher tissue Mn at the tillering stage of growth to enhance resistance to take-all through callose production around penetration hypha of the fungal pathogen.

The first 30 days after planting is an especially critical period to ensure stand establishment, early vigour, and optimum physiological function for subsequent growth and reproduction. An early deficiency of nutrients can stress the plant to cause irreversible effects on crop yield and nutrient quality so it is important that adequate levels of the essential nutrients are available throughout the various stages of crop growth in order to maintain disease and stress resistance as well as optimum nutrient content in harvested crop parts. Seed treatments can minimize early root damage from seed-borne pathogens and protect tender tissues from early colonization and deleterious effects of soilborne pathogens. Root configuration (architecture) can also impact the nutrient efficiency of a cultivar. A deep taproot provides access to deeper soil moisture for drought tolerance, but may be less efficient for uptake of micronutrients located near the top of the soil profile. Soil temperature influences root configuration of crops like maize so that early planting in cooler soils produces a shallower, more fibrous root system efficient for nutrient uptake from shallow soils or with nutrient stratification as occurs with non-tillage practices.

Inoculation of seed or soil with N-fixing, mycorrhizae, and plant growth promoting rhizosphere (PGPR) organisms can increase nutrient availability and sufficiency for many plants, with the reduction in disease as an additional benefit. Soil fumigation may be required to reduce the population of plant parasitic nematodes and other soilborne pathogens. Most chemical soil fumigants also inhibit nitrification so that selection of the fertilizer N may be used to enhance the control of other diseases through the form of N. Success of the nutritional program for disease control will depend on its integration with the overall management practices to maximize crop productivity and quality. A well-balanced nutrition program, integrated with other crop production practices, permits a broad utilization of this cultural disease control.

## Mechanisms of Disease Control with Nutrition

Disease resistance is a property of the plant that describes the relative incompatibility of the plant-pathogen interaction, while tolerance describes the ability of the plant to produce even though diseased (compatible plant-pathogen relationship). Virulence is a characteristic of the pathogen to cause disease, and disease escape refers to environmental conditions that are not conducive to disease even though the pathogen and plant might be present (**Figure 1**). Nutrition influences all of these interactions. Nutrients suppress disease by maximizing the inherent genetic resistance of plants, by facilitating disease escape and shortening the infection period, increasing tolerance through stimulating plant growth and yield in the presence of a pathogen, and by modifying the abiotic or biotic environment to reduce the survival or activity of pathogens (**Table 4**).

Nutrient mechanism	Effect on disease
Compensate for disease damage	Restore mineral nutrient quantity and quality
Facilitating disease escape	Increased root and leaf growth, shorter infective period
Increasing tolerance to disease	Compensate for reduced efficiency or disease damage
Increasing physiologic resistance	Less susceptible tissue, production of physical and chemical defenses to limit damage
Modifying the environment	Less conducive environment for disease, nutrient com- pensation, enhance rhizosphere biological interactions
Reducing pathogen activity	Reduce survival, growth, virulence, pathogenesis

#### Table 4. Effects of plant nutrition on disease severity and nutritional quality.

#### **Nutrient Effects on Plant Resistance**

Mineral elements are directly involved in all mechanisms of a plant's defense to disease as integral components of cells, substrates, enzymes, and electron carriers; or as activators, inhibitors, and regulators of metabolism. Resistance to disease is generally a dynamic process involving the principles of metabolic regulation by substrate feedback, enzyme repression, and enzyme induction that are all controlled through mineral factors (Datnoff et al., 2007; Graham, 1983; Huber, 1980, Huber and Graham, 1999). Nutrient-sufficient plants contain preformed antimicrobial compounds and have active response mechanisms where inhibitory phytoalexins, phenols, flavonoids, proteins, and other defense compounds accumulate around infection sites. An adequate supply of Mn, Cu, and other nutrients is important in most of the defense mechanisms mediated through the shikimate pathway. Production of glycoproteins (lectins) associated with disease resistance also requires Mn. Calcium and Mg suppress tissue-macerating diseases caused by bacteria and fungi by increasing the structural integrity of the middle lamella, cell wall components, and cell membranes to resist the extra-cellular enzymes produced by these pathogens. Silicon, combined with other components, gives cell walls greater strength as a physical barrier to fungal penetration (Datnoff et al., 2007). The rapid walling off of pathogens around a wound or infection site can limit potential damage by various pathogens.

All aspects of disease resistance are intimately related to the nutritional status of the plant and reflect either a modified nutritional environment for a pathogen or the production or accumulation of compounds inhibitory to pathogenesis. The nutritional environment is especially critical for obligate pathogens and the concentration of many viruses is inversely proportional to the growth status of the plant. Resistance based on regulation of amino acid or protein synthesis is greatly affected by the Cu, N, Mg, Mn, Ni, and Zn status of the plant. Resistance of potato to *Phytophthora* is associated with the K-induced accumulation of fungistatic levels of arginine in leaves while the decreased levels of glutamine and glutamic acid in leaves is associated with resistance to *Alternaria, Cercospora*, and *Sclerotinia* (Huber and Graham, 1999). Physiological responses may deny obligate pathogens of essential metabolic intermediate compounds needed for pathogenesis, survival, or reproduction. Providing adequate N throughout the grain-fill period minimizes cannibalization of physiological and structural proteins that are required for stalk rot resistance of maize plants (Huber et al., 1986).

Nutrient sufficiency provides a general form of disease resistance by maintaining a high level of inhibitory compounds in tissue, and energy for a quick response to invasion by a pathogen. Nutrient seed treatments can promote a well-established, vigorous seedling with an efficient root system for maximum nutrient uptake and expression of resistance.

#### **Nutrient Effects on Disease Tolerance**

Disease severity, and subsequent yield loss, may be limited by supplying sufficient nutrient quantity to offset the deleterious effects of a pathogen. Phosphorus, N, and Zn stimulate root growth of cereal plants to compensate for tissue lost through root rots such as take-all. Increased availability of nutrients can compensate for reduced uptake efficiency caused by soilborne pathogens. Although N rates required for nutrient sufficiency can increase powdery mildew (*Blumeria graminis*) in cereal plants by 10-20%, yield is increased 50% to show that the vigorous, N-fertilized plants are able to tolerate the increased disease burden (Huber and Thompson, 2007; Last, 1962). Phosphorus, N, and Zn stimulate root growth to promote more efficient nutrient uptake and translocation to promote disease resistance.

### **Nutrient Effects on Disease Escape**

A response to fertilization by increased growth may constitute a form of disease escape, especially if a susceptible growth stage is shortened for some plant-pathogen interactions. Plants adequately fertilized with B and Zn have fewer root and leaf exudates to break spore dormancy (fungistasis) or stimulate fungal pathogens (Marschner, 1995).

### **Nutrient Effects on Pathogen Survival and Virulence**

Mineral nutrients may reduce the ability of a pathogen to cause disease by inhibiting germination, growth, virulence or survival directly or through plant exudates. The need for an external source of nutrients for saprophytic growth of fungi prior to infection is common. *Botrytis cinerea, Typhula* species, *Fusarium* species, *Sclerotinia*, and *Armillaria mellea* infect healthy plants slowly unless an external source of nutrients is available from soil or decaying organic matter. Exogenous C and N are required for germination of dormant *Fusarium* chlamydospores. Zinc is required for appressorium formation of *Puccinia coronata* on oat leaves and infection of broadbean by *Botrytis*. Leaf exudation of arginine from K and N sufficient plants inhibits germination of *Phytophthora infestans* sporangia, and the levels of arginine generally increase as the sufficiency for K increases. Calcium suppresses extra-cellular macerating enzymes of pathogens required for pathogenesis. Iron, Mg, Mn, and Zn also suppress macerating pathogen enzymes (Huber, 1980). By reducing tissue maceration, there also are fewer nutrient sources available to pathogens.

Detailed discussion of specific nutrient interactions with plant disease can be found in Datnoff et al. (2007), Englehard (1989), Graham and Webb (1991), Huber (1978, 1980, 1991), Huber and Graham (1999), Huber and Haneklaus (2007), Johal and Huber (2009), Marschner (1995), and Rengel (1999).

# Importance of Pest and Disease Control on Nutritional Quality and Food Safety

Economic forces operating over a long period of time have produced a highly efficient agricultural system. The benefits to society through efficient crop production include lower prices, reliable supplies, employment opportunities, environmental improvements, and higher nutritional quality food and feed. Nutritional quality is markedly reduced by disease and pest damage, and sometimes before a yield reduction is observed. Greatest losses are sustained in protein, vitamin, and mineral composition, and least in carbohydrates. The need for increased processing required to compensate for pest losses or contamination may of itself reduce nutritional value. Mycotoxin production initiated during crop production can continue in storage to expose large segments of a population to highly toxic or carcinogenic compounds.

Good animal and human health is dependent on healthy plants that are only available from fertile soils. Disease resistance is genetically controlled but mediated through physiological or biochemical processes interrelated with the nutritional status of the plant, pathogen, and environment. The nutritional status of a plant determines its histological or morphological structure and properties, the function of tissues to hasten or slow penetration and disease development, and its nutritional value for feed or food. The severity of most diseases can be greatly decreased by proper nutrient management (Datnoff et al., 2007). It is not possible to generalize the effects of any particular nutrient on all plant diseases because it is the sum of many interacting factors of the plant, pathogen, and environment over time that determine how a specific disease is affected by nutrition. The disease response may be independent of vigour or other generalized growth responses since nutrients can limit pathogenesis or toxin production through passive and active mechanisms of defense that are activated through effective nutrient management.

Pest and disease damage are generally greatest with plants that are nutritionally or environmentally stressed. A balanced nutrition increases plant vigour, competitive advantage, and ability to successfully respond to limit infection. Disease control by cultural practices—crop rotation, organic amendment, irrigation, liming to adjust soil pH, and tillage—frequently influences disease through effects of these practices on nutrient availability, and this often involves altered microbial activity (Table 3). Disease and pest control are integral aspects to reducing mycotoxin contamination during crop production and storage. Since crop residues provide the primary inoculum for non-soil inhabiting and mycotoxigenic fungi, removal or management of crop residues to hasten their decomposition can reduce mycotoxin levels significantly. Tillage that buries infected crop residues not only hastens their demise as inoculum sources, but also makes nutrients contained in them available for subsequent crops much sooner. Thus, crop rotation or tillage to facilitate degradation of infected residues and lower inoculum of plant pathogens can supplement other control practices. Nutrient availability for microbial activity is especially important for surface decomposition of plant residues. Increased levels of genetic resistance or chemical controls are required when residue burial is limited by the need to reduce soil erosion. Aflatoxin contamination of both maize and peanuts is more severe with prolonged late-season drought. Adequate Ca nutrition can minimize aflatoxin contamination of peanut but requires moisture for plant uptake. Control of pink bollworm and stinkbug damage that predispose cottonseed to aflatoxin is important in reducing levels of this carcinogen in feed. Although A. flavus can infect corn silks at temperatures above 30°C, it is more likely to colonize insect-damaged kernels. In contrast to

the warm conditions that favour *Aspergillus* infection, cool, wet conditions during grain fill favour infection by toxigenic *Fusarium* species. (CAST, 1989)

The advent of readily available inorganic fertilizers has brought about the demise of many diseases through improved plant resistance, disease escape, altered pathogenicity, or microbial interactions influencing these. Efficient fertility programs can enhance plant resistance to pathogens, reduce the impact of environmental stress, and increase the nutritional quality of the food and feed that are produced. Effective disease and pest management improves crop quality and quantity to result in surplus food production, lower prices for consumers, and an abundance of quality food products. Ensuring nutrient sufficiency to maintain resistance to pathogens and abiotic stress is necessary to provide food safety, abundance, and nutrient quality. An abundant supply of affordable, safe and nutritious food and feed is essential to meet society's needs.

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Chapter 10

# Human Health Issues Associated with Nutrient Use in Organic and Conventional Crop Production

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#### Abstract

In recent years, there have been intensive discussions about agricultural systems that can produce sufficient amounts of nutritious and healthy food. This chapter focuses on crop quality of organic and conventional systems in relation to human health. A number of field studies and national agricultural statistics clearly indicate that organic crop production cannot provide sufficient food for the current and growing population in the world. Crop yields are too low, mainly due to lack of nutrient supply, especially of N. We reviewed crop quality variables related to N supply. The compilation showed that contents of protein, NO<sub>3</sub>, and A and B vitamins were often increased in conventionally grown crops by the use of mineral N fertilizer but vitamin C contents were slightly higher in organically grown crops. The results are in agreement with knowledge based on plant physiology. Higher levels of NO<sub>2</sub> in conventionally grown crops should not be misinterpreted as poor quality since a beneficial effect of NO<sub>3</sub><sup>-</sup> for the human immune system was discovered. The hypothesis of the founders of organic agriculture that NPK fertilizers may lead to a dilution of non-added minerals in crops seems plausible. However, earlier reviews and recent studies do not indicate that concentrations of trace elements in organically and conventionally grown crops differ systematically. A recent theory that enhanced levels of secondary metabolites in crops are a quality indicator is doubtful, considering that they are non-essential and can also be harmful. Published reports on mycotoxin contents in crops from organic and conventional systems revealed no differences. Our review provided no evidence that organically grown crops are of superior quality or that the use of mineral fertilizers deteriorates food quality. In contrast, controlled application of

Abbreviations specific to this chapter: EU = European Union; FAO = Food and Agriculture Organization of the United Nations; IFOAM = International Federation of Organic Agriculture Movements; SCB = Statistics Sweden; UN = United Nations. For symbols used commonly throughout this book see page xi.

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plant nutrients in mineral form enables improvement of crop quality. Despite the great interest in food quality among supporters of organic agriculture, we conclude that focussing on food supply and dietary composition is most important for human health.

## **Principal Considerations**

Cropping systems can affect human health in several ways: through production of insufficient amounts of food and products of insufficient quality. If the amounts of crops produced by agricultural systems are not sufficient to cover human needs, this will result in malnutrition, starvation, and ultimately shortened lifespan. This aspect is so fundamental that humans who are able to buy sufficient amounts of food may not necessarily consider this a major health concern in the world. However, lack of food is a reality in many developing countries worldwide (e.g. Sanchez and Swaminathan, 2005). According to the world summit on food security (FAO, 2009), about one billion people suffer from malnutrition, a main cause of disease and disability of children (World Health Organization, 2000). Food shortage is definitely one of the most important aspects when discussing health issues relating to food, although excessive diets, over-consumption and obesity can be a main cause of untimely death in rich countries.

The other major issue is the quality of the food produced. Crops may contain too little of the nutrients essential for animal and human well-being, reduced levels of protective antioxidants and anticancer compounds, high levels of unwanted elements, pesticide residues, toxic microorganisms or high concentrations of natural toxins. It should be borne in mind that organic agriculture was founded on the conviction that this type of agriculture assures superior food quality (Steiner, 1924; Balfour, 1943; Rusch, 1978).

In this chapter, we compare food supply and food quality issues in organic and conventional cropping systems, the former considered to be superior by many. The main difference between organic and conventional cropping systems is the principal exclusion of processed inorganic N and P fertilizers and synthetic pesticides in organic agriculture. The impact of these systems on food quantity and quality is reviewed. Quality refers to nutrients essential for the human body.

# **Food Supply**

#### **Crop Yields in Organic and Conventional Systems**

Providing sufficient and healthy food for everyone is probably one of the most important survival issues for mankind in the future. Already today, the global production of food is unable to keep pace with consumption and the demand for food, feed, fuel, and fibre will greatly increase during coming decades (Evans, 1998; FAO, 2007), driven by a growing population (Bruinsma, 2003; GeoHive, 2007). Human population has doubled over the last 40 years, to around 6.5 billion people in 2006, while food plus feed production has tripled during the same period (FAO, 2007). By 2030, the global population may reach 8 to 9 billion, of which 6.8 billion may be living in developing countries (Bruinsma, 2003). As the projected increase will mainly take place in developing countries (i.e. Africa would need to increase food production by 300%, Latin America by 80%, Asia by 70%) still even North America would require a 30% increase. Assuming that the additional population consumes only vegetarian food, a minimum of 50% more crop product would be needed by 2030 to ensure sufficient food supply.

The recommended daily intake of total protein is 63 g per person per day (National Research Council, 1989) but opinions differ on the minimum intake of animal proteins required for a satisfactory diet. However, as protein of animal origin has an amino-acid composition closer to human physiological need than vegetable proteins, an optimal diet has been defined to consist of 40 g animal protein per person per day (see overview by Gilland, 2002). As diets throughout the world are changing with the rise in income towards more meat and dairy products irrespective of culture, there will be a need to actually increase food plus feed production by 60 to 70%. For example, meat consumption in developing countries amounted to 71 g per person per day between 1997 and 1999 and is projected to further increase to 100 g per person per day in 2030 (Bruinsma, 2003). In developed countries, meat consumption is estimated to be 180 g per person per day in 2030. Since the largest proportion of meat consumption is expected to come from pork, poultry, and aquaculture, meeting future demand will depend on achieving increases in cereal yields (Bradford, 1999). A doubling in cereal yields may be necessary by 2030 if one does not want to increase cultivated area, which, however, is hard due to many different constraints.

Food production is coupled to a moral imperative, as adequate food supply is a cornerstone of human welfare. Development of agricultural practices ensuring food sufficiency is a basic human requirement, a precondition for satisfactory social conditions and a necessity for civilizations to flourish. Lack of food, on the other hand, is a tragedy leading not only to suffering and loss of life but also to inhumane behaviour, political instability and war (Borlaug, 1970). In fact, eradication of famine and malnutrition has been identified as the most important task on Earth (UN Millennium Project, 2005). Thus, when discussing different forms of crop production, it is of the utmost importance to examine without prejudice the forms of agriculture that can contribute to food sufficiency and security, at present and in the future. Separation of facts and wishful thinking is absolutely necessary and only an unbiased review of the scientific literature can provide objective answers to the questions put forward below.

In terms of crop yields, a number of reviews have shown that organic crop yields are consistently lower than conventional yields (e.g. Badgley et al., 2007; Kirchmann et al., 2008a; Korsaeth, 2008; Goulding et al., 2009). However, the magnitude of the yield decrease attributed to organic cultivation differs. For example, in the review by Badgley et al. (2007), a biased selection of experimental data from a limited number of studies with small yield differences were chosen, which is not representative. The conclusions have been criticized by Connor (2008) and Goulding et al. (2009), pointing out that if one considers the whole existing literature it is obvious that organic agriculture cannot feed the world.

To avoid reliance on experimental data only, crop yields given in national statistics of organic and conventional agriculture were examined. One may argue that national statistics may be biased because organic farming may be carried out mainly on land with lower production potential and thereby not representative for arable soils in general. However, the Swedish experience seems not to corroborate this view. The majority of organic farmers in Sweden are milk and meat producers maintaining grass/clover leys in rotation and having access to manure. This means conditions to maintain reasonable crop yields in organic production in Sweden are therefore given.



Figure 1. Official yield data for organically and conventionally grown crops in Finland and Sweden, 2005. \*Only the first of two or three cuts is represented by the data.

A search in agricultural statistical databases of EU countries, the USA, Canada, and Australia revealed that information on organic crop yields is very scarce. No crop yield data were found except for Sweden and Finland. Official Swedish statistics (SCB, 2008) reveal that yields of organically grown crops are 20 to 60% lower than those of conventionally grown crops. Yields of organically grown legumes (peas and beans) and grass/clover leys are, on average, 20% lower (**Figure 1**), as they are sufficiently supplied with N, whereas yields of cereals are 46% lower and yields of potatoes as much as 60% lower than in conventional production. National statistics for Finland (Finnish Food Safety Authority, 2006; Statistics Finland, 2007) show a similar picture with yields of organically produced cereals being 41% lower and yields of potatoes 55% lower (**Figure 1**).

Organic farming requires large areas for legume production (N-fixing crops); for example in Sweden, the area used for the legumes such as peas and beans is twice as high in organic as in conventional production. Also the proportion of agricultural land used for forage production (grass/clover leys) is much higher in organic than conventional production systems, 69 versus 49%, respectively (see **Table 1**). However, the production of digestible energy for ruminants is lower in grass/clover leys than in winter wheat or in alfalfa than in maize. Thus, increasing legume production substituting cereals means that productivity of land is reduced.

	Portion of agricultural land, %			
Сгор	Conventional	Organic		
Forage	49	69		
Legumes	1.2	2.3		
Cereals	43	27		
Oilseed rape	3.8	1.0		
Potatoes	1.2	0.3		

# **Table 1.** Proportion of crops grown in conventional and organic agriculture inSweden (SCB, 2008).

In summary, there is on average a 40% reduction in crop yields through conversion from conventional to organic crop production according to the statistics. This estimate is in good agreement with the assessment of Smil (2001), who claimed that industrial N-fixation (N fertilizer production) provides the means for 40% of global food production. This implies that the greatest part of the yield difference between organic and conventional production is due to N. In fact, insufficient N supply to crops has been identified as being the main yield-limiting element in organic crop production (Kirchmann et al., 2008b). Deficiency of K has also been pointed out as a reason for decreased yield especially in potato (Torstensson et al., 2006). Thus, the exclusion of synthetic fertilizers and lack of approved and cheap nutrients for organic agriculture limit organic yields to such an extent that shortage of food, or alternatively, the conversion of new land to agricultural production, will be the immediate consequence of large-scale adoption of organic crop production.

Some advocates of organic agriculture claim that the lower yields produced in organic systems can be compensated for by a shift to a more vegetable-based diet (e.g. Woodward, 1995; Tudge, 2005). In other words, it is proposed that the shortcoming of organic production to supply sufficient food for humans should be resolved through a change to a more vegetarian diet. However, it must be borne in mind that if such a shift is recommended due to benefits for human health, the most efficient solution would be to grow conventional crops and use the surplus set-aside land for other forms of production or return to natural land including forest.

# Lower Yields in Organic Crop Production Due to Non-Scientific Fertilization Rules

Low yields of organically grown crops are the result of the organic farming approach to supply the soil with nutrients but not to feed the crop with soluble nutrients and the fact that total input of nutrients is often too low for high-yielding crops. In organic systems, plant nutrients are added in organic forms or as untreated minerals with low solubility and plants are expected to obtain balanced nutrition through the actions of roots and soil microbes and through weathering of minerals. Furthermore, there is a widespread conception, as proposed by Steiner (1924), that organic and self-sustaining farms constitute the real core of sound agricultural production. The need to import nutrients to a farm is considered a sign of failure of the system (Steiner, 1924). A common view is that on-farm recycling of nutrients, with any small losses balanced by soil weathering, will maintain soil fertility (IFOAM, 2006). However, the view of a farm as a self-sustaining unit contradicts the 'law of nutrient replacement', where nutrient removal must be restored to maintain soil fertility and avoid nutrient mining of soils.

In contrast, in conventional agriculture, the focus is on crop demand and on optimizing the nutrient supply. Since von Liebig (1840) showed that plant roots take up nutrients in the form of ions dissolved in water, the exclusion of water-soluble mineral fertilizers in organic agriculture is contrary to these basic findings and to modern plant nutrient research. Even though plant nutrients are added as organic manures or untreated minerals in organic crop production, uptake by crops is mainly in the form of ions, dissolved or mineralized into the soil solution from minerals or organic manures. In other words, the source of plant nutrients does not affect the uptake mechanism but nutrient sources of low solubility cannot increase yields.

For optimal growth, crops require a minimum of nutrients consisting of macroand microelements. A number of these elements are supplied through inorganic fertilizers to complement nutrient release from soils. Complete and sufficient nutrient supply to crops is the rationale to apply macro- and micronutrients in conventional agriculture. In addition, organic manures, recycled wastes, and mineral fertilizers are used in conventional agriculture to cover crop demand. A further difference between organic and conventional agriculture is that plant nutrients removed through sale of crops and animal products from the farm are principally replaced in conventional agriculture - the 'law of replacement' is adopted. If the nutrients lost or sold are not replaced, the soils used in any cropping system will become depleted and soil fertility will decline.

Although organic farmers recognize farm animal manures as a valuable source of nutrients and place much emphasis on proper use of manures in a crop rotation, the rules given by the founders of organic agriculture have to be followed, even when they are not necessarily in accordance with best management practices. For example, in biological dynamic agriculture (Steiner, 1924) farmers must compost animal wastes. However, losses of N in the form of NH<sub>3</sub> are higher during composting than when manure or slurry is properly stored under anaerobic conditions (Kirchmann, 1985). In biological organic farming (Rusch, 1978), surface application of manure or green manure crops is prescribed, also leading to high losses of NH<sub>3</sub>-N. These practices, which are central to two main schools of organic agriculture in Europe, lead to less efficient recycling of N and organic matter compared with other forms of manure treatment such as anaerobic storage of solid or liquid manure, protection against rain during storage, and incorporation of animal wastes into soil directly upon spreading.

#### Different Proportions of Crops in Organic and Conventional Systems and Implications for Human Diet

One of the most distinct differences between organic and conventional agriculture is the type of crops grown in a crop rotation. According to official Swedish statistics (SCB, 2008), the production of forage crops for ruminants (grass/clover leys) increases considerably in organic systems as pointed out above (**Table 1**). Why are proportionally more forage crops grown in organic than conventional rotations? The reason is that N-fixing forage crops show less yield reduction than other organically grown crops and can fulfil several functions: N supply, weed control, and manure production. There is simply lack of other organic methods to produce reasonable yields. In other words, organic agricultural systems in many parts of Europe will be mainly based on forage-ruminant systems.

In addition, the proportion of legumes (peas and beans) in organic rotations is almost doubled due to their ability to fix N, and similar yields are obtained as compared to conventionally grown legumes (**Table 1**). A logical consequence of growing more N-fixing crops in organic rotations is that other crops decrease proportionally. The biggest change when converting to organic agriculture in Sweden is the decline of cereal, potato, and oilseed rape production. According to figures in **Table 1**, only 63% of cereals and 25% of potato and oilseed rape would be produced as compared to conventional agriculture. In addition, assuming yield reductions shown in (**Figure 1**), supply of these products will be further reduced. Actually only 13% of present production of oilseed rape and potatoes would remain.

If one assumes that the statistical data (**Table 1**) are representative even for a fullscale conversion to organic agriculture in Sweden, some changes of the dietary composition are foreseeable. One probable consequence of large-scale organic production is that the increase in forage production (from 49 to 69% on arable land substituting cereal growth) may not lead to a large decline in milk and red meat supply. Furthermore, reduced cultivation of cereals and oilseed rape from 47 to 28% on organically managed land, will reduce the fodder supply for pigs and poultry. Simply, less eggs, poultry, and pork will be produced.

Differences in the proportion of crops grown and decline in yields in organic production systems can have a great impact on food supply and the composition of the human diet. Despite many uncertainties, some general conclusions can be drawn. Firstly, it is not likely that organic agriculture will result in a vegetarian diet. Organic food production in cold temperate climate is mainly based on a forage-ruminant system, which seems to be the only system proved that can maintain reasonable organic yields through a large proportion of legumes in rotation and control of weeds. Secondly, less pig and poultry products in the diet requires replacement through other food. However, it is not likely that ruminant products will substitute eggs, poultry, and pork as ruminant production is less energy-efficient and requires more water per calorie unit. The constraint put on human diets through organic agriculture to cover protein and energy need is likely to be overcome through an increased intake of vegetable proteins and carbohydrates (i.e. more legumes and cereals in the diet). A third consequence can be that the domestic demand for these staple legumes and cereals will be difficult to cover through organic crop production and shortage of some vegetable products is possible.

## **Food Quality**

A large number of environmental factors influence food quality. They include parent material of soils, geographical location, climate, weather conditions, exposure to industrial, traffic or natural emissions, etc. Crop variety, storage, refinement and food amendments may further impact quality. None of these factors were considered in the present review. Our focus was solely on the effect of organic and conventional agricultural practices on crop composition. The aim of this literature compilation was to answer the ultimate question whether organic products contribute to health benefits for the consumer.

One objective of this review was to quantify the extent of changes in nutrient composition in crops caused by conventional and organic practices. Another objective was to discuss how apparent differences in crop nutrient composition can be coupled to organic and conventional practices and which mechanisms that may be responsible for the differences found. A third objective was to put crop nutrient composition in perspective to the nutritional demand of humans.

#### The View on Food Quality in Organic Agriculture

The founders of organic agriculture stressed the superior nutritional quality of organically produced food. For example, Steiner (1924) stated that use of mineral fertilizers would degrade crop quality to such an extent that food would become a worthless filler of the abdomen. The view of the founders of the Soil Association (Howard, 1947; Balfour, 1943) was that only a healthy soil produces healthy food. Howard (1947) believed that only plant nutrients made available through a kind of living bridge between life in soil and plants can feed plants properly.

Today, organic farming organizations (e.g. Soil Association; Organic Food Information Net; the Organic Center) are of the opinion that organic food is of higher quality. The common understanding within organic agriculture is that addition of mineral fertilizers enhances plant growth, which means production of more sugar, proteins, and fats, but yield increases are not accompanied by corresponding increases in mineral uptake, vitamin, and antioxidant production. Only the exclusion of synthetic fertilizers will guarantee high contents of trace elements, vitamins, and beneficial non-nutrient compounds. Benbrook et al. (2008) conclude in their report that organically grown plants are better for health, having about 25% more nutrients than conventionally grown plants. A decline in nutrient density of crops over time (Mayer, 1997; Davis et al., 2004) is sometimes mentioned as a proof for the 'dilution' of nutrients through mineral fertilizers.

#### **Existing Comparative Studies**

Numerous studies comparing the nutritional quality of organic and conventional food have been published since 1924. The intention of this chapter is not to re-examine the existing literature but to understand the consistent differences found in existing review papers and discuss the possible principles that may explain these differences. Food quality is a poorly defined concept—for instance, taste and appearance are personal assessments—and our purpose was to focus only on the nutritional composition of food.

Reviews by Woese et al. (1997), Bourne and Prescott (2002), Magkos et al. (2003), and Dangour et al. (2009) show that there are consistent differences in the composition of organically and conventionally produced food (**Table 2**). Woese et al. (1997) found clear evidence of higher  $NO_3^-$  concentrations in vegetables and higher protein content in cereals if grown conventionally, whereas vitamin C was higher in vegetables grown organically. Bourne and Prescott (2002) found only a significant difference in  $NO_3^-$  contents being higher in conventional food. Magkos et al. (2003) found higher levels of vitamin C and lower protein contents in organically produced vegetables. Finally, the review by Dangour et al. (2009) found significantly higher N contents in conventional crops and higher contents of titratable acid (i.e. vitamin C) and P in organic crops. A similar summary was presented by Tinker (2000).

# Differences in Crop Composition Due to Organic and Conventional Cultivation

Organic agriculture excludes synthetic fertilizers, and it is well-known that different nutrient supply can cause major shifts in plant composition, especially N application. Nitrogen is the most important plant nutrient in terms of increasing crop yields (Mengel and Kirkby, 2001). Furthermore, N fertilizer application stimulates growth-enhancing shoot elongation and increasing shoot/root ratios, and alters plant composition more than any other mineral nutrient (Marschner, 1995). An increasing supply of N to crops stimulates the synthesis of crop

# **Table 2.** Compilation of significant differences in crop composition reported inreviews between organically and conventionally grown crops and possible mechanisms.

Significant differences found in reviews	Possible mechanisms	
Woese et al. (1997)		
Higher NO <sub>3</sub> <sup>-</sup> content in conventionally grown vegetables	More plant-available N in soil	
Higher protein content in conventionally grown cereals	More plant-available N in soil	
Bourne and Prescott (2002)		
Higher NO <sub>3</sub> <sup>-</sup> content in conventionally grown crops	More plant-available N in soil	
Magkos et al. (2003)		
Higher dry matter content in organically grown crops	Smaller cells with proportionally less water	
Higher protein content in conventionally grown crops	More plant-available N in soil	
Higher vitamin C content in organically grown crops	More light per unit leaf area (less mutual shading)	
Dangour et al. (2009)		
Higher N content in conventionally grown crops	More plant-available N in soil	
Higher titratable acid content in organically grown crops	More light per unit leaf area (less mutual shading)	
Higher P content in organically grown crops	More light per unit leaf area (less mutual shading)	

biomass, which leads to more chloroplast formation (part of leaf that contains chlorophyll) and higher concentrations of the constituents in chloroplasts such as chlorophyll, proteins, and lipids. An increase in total N in crops or crude protein (total N multiplied with 6.25) representing the sum of proteins, amino acids, amides, and  $NO_3^-$  (often not fully included in total N analysis) is normally found. Nitrogen fertilizer application can also increase the biosynthesis of carotene and B vitamins in crops (Marschner, 1995).

Dry matter contents are normally somewhat lower in N-fertilized crops probably because of larger cells that store proportionally more water than smaller cells of unfertilized crops. In many studies, no moisture-adjusted concentrations of nutrients and vitamins are used. For instance, declines of nutrient contents in food over time are less significant if increases in water content are made as shown in recalculation of data by Davis et al., (2004).

Changes in crop composition due to exclusion and application of N fertilizer are discussed in detail below.

#### Total Nitrogen and Nitrate

In organic agriculture, exclusion of N fertilizer and reliance on biological N-fixation as a N source has resulted not only in yield losses but also lower N and  $NO_3^-$  contents in crops (**Table 2**). Organic farming organizations (e.g. Benbrook et al., 2008) regard lower N and  $NO_3^-$  contents in organically grown crops to be a quality indicator. The general argumentation is that there is less but higher quality of N compounds in organic products. Elevated  $NO_3^-$  contents are regarded to be undesirable for plants and humans.

Nitrogen is an essential component of amino acids, the building blocks of proteins. Protein concentrations in crops are highly dependent on N supply and high protein concentrations, for example in grains, and are associated with good nutritional and commercial quality. The ten essential amino acids that cannot be synthesized by humans (arginine, histidine, valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine, tryptophan) must be part of the diet and must at least partially be obtained from ingested crops. Application of N fertilizer during late stages of crop growth can be effective to increase protein concentrations.

However, late application of N fertilizer to barley and wheat can also increase the content of non-essential proteins, which improve baking properties but not the nutritional value. On the other hand, late application of N to oats increased the nutritional quality of protein (Mengel and Kirby, 2001).

Studies on protein quality showed that the portion of essential amino acids of the total protein content was not significantly different in organically and conventionally grown cereals (e.g. Dloughý, 1981) or vegetables (Eppendorfer and Bille, 1996). As there is no scientific evidence that the content of essential amino acids and protein quality is reduced through N fertilizer, but only additional proteins and amino acids are formed, lower protein contents in organically grown cereals are of no advantage. A review by Wang et al. (2008) summarizes information on

the effects of inorganic N fertilizer on crop quality, and generally finds them to be positive.

There is still a misunderstanding on the NO<sub>3</sub><sup>-</sup> issue in crops requiring a comprehensive explanation. NO<sub>3</sub><sup>-</sup> is abundant in our diet, and levels found in many vegetables range from as low as 1 mg/kg in peas (Pisum sativum L.) and Brussels sprouts (Brassica oleracea L.) to as high as 4,800 mg/kg in rucola (Eruca sativa L.), see review by Lundberg et al. (2004) and EFSA (2008). High  $NO_3^{-1}$  contents in crops have traditionally been considered to be a health risk as NO<sub>2</sub><sup>-</sup> was thought to be a potential cancer-causing chemical in the gastro-intestinal tract. Consequently, lower NO<sub>3</sub><sup>-</sup> contents in organically grown crops were believed to be a nutritional advantage. This perspective is still dominating among many scientists including interest organizations for organic food (e.g. Benbrook et al., 2008). However, in 1994 the standpoint on NO<sub>3</sub><sup>-</sup> started to change. It was observed that the human stomach contains large amounts of nitric oxide (NO) and that the gas was able to kill bacteria in the stomach (see overview by Minkel, 2004). Bacteria in the mouth were found to convert NO<sub>3</sub><sup>-</sup> to NO<sub>3</sub><sup>-</sup>, and when swallowed, production of NO in the stomach was induced. The  $NO_3^{-}$  -  $NO_2^{-}$  - NO pathway in physiology was detected and the role of NO3<sup>-</sup> as an important mammalian resistance mechanism against infectious diseases was discovered (see review by Lundberg et al., 2008). In addition, no epidemiological evidence for an increased risk of gastric and intestinal cancer in population groups with high NO3<sup>-</sup> intake was found (Duncan et al., 1997) and positive effects of dietary NO<sub>3</sub><sup>-</sup> were reported (Leifert and Golden, 2000). The European Food Safety Authority concluded in a review (EFSA, 2008) that "Overall, the estimated exposures to NO<sub>3</sub><sup>-</sup> from vegetables are unlikely to result in appreciable health risks, therefore the recognized beneficial effects of consumption of vegetables prevail."

In summary, to consider lower protein and  $NO_3^-$  contents in crops to be of nutritional advantages in the human diet has no scientific basis. From the view point of human health, it is of great importance to increase the content of essential nutrients in crops.

#### Vitamin A

According to plant physiological knowledge, an increasing supply of N enhances the synthesis of proteins and chloroplasts in crops (e.g. Marschner, 1995; Mengel and Kirkby, 2001). An increase of chloroplasts means that also chloroplast constituents such as chlorophyll and carotenoids (for instance  $\beta$ -carotene the precursor for vitamin A) increase in crops. Carotenoids are integrated together with chlorophyll into membrane proteins forming light-harvesting centers. Carotenoids act as light interceptors for more energy-rich radiation than chlorophyll. The most frequently occurring carotenoid in higher plants is  $\beta$ -carotene. Carotenoids are lipid-soluble pigments synthesized by plants, fungi, algae, and bacteria being responsible for the yellow, orange, and red colours of fruits and vegetables.

Over 600 carotenoids have been identified so far (Bendich, 1993) and significant differences in carotenoid accumulation among different vegetable crop species

have been reported (Kopsell and Kopsell, 2006). In the human diet, fruits and vegetables are the main sources of carotenoids (Rao and Rao, 2007) and the daily requirement for vitamin A is estimated to be 800 retinol equivalents (1 retinol equivalent = 12  $\mu$ g  $\beta$ -carotene). Carotenoids have received attention for their antioxidant properties.

A review by Mozafar (1993) compiling about 180 studies on the effect of mineral N fertilizer on vitamins in plants showed clearly that carotenoids in crops increased when fertilized with increasing rates of mineral N, which is in accordance with plant physiological understanding outlined above, and he found no contradictory studies. A compilation of some recent references (Table 3) show that mineral N fertilization can increase  $\beta$ -carotene concentrations in a number of crops. In comparative studies, organically grown crops had lower  $\beta$ -carotene concentrations than crops fertilized with mineral N fertilizer. However, apparently contradictory results published by Caris-Veyrat et al. (2004) are in fact in line with our understanding because N fertilization rates were 340 kg/ha in organic and 160 kg/ha in conventional production in this specific study. Increasing rates of green manure N applied to crops also resulted in significantly increasing β-carotene concentrations in carrots (Kaack et al., 2001). At very high N application rates, concentrations of carotenoids declined somewhat if expressed on a fresh-weight basis due to more water storage in plant cells (Kopsell et al., 2007a). However, on a dry-weight basis, concentrations of carotenoids continued to increase with increasing N fertilizer applications (Kopsell et al., 2007b; Lefsrud et al., 2007).

The above-cited studies corroborate that N supply is a major determining factor for carotenoid synthesis in crops. An earlier study by Trudel and Ozbun (1971) showed that also the supply of K fertilizer had a positive effect on carotenoid formation in tomatoes. Thus, increasing the nutrient supply to crops and thereby gaining higher yields is also followed by an increased production of carotenoids per unit crop biomass. As less N is normally applied in organic cultivation (see review by Kirchmann et al. 2008 a,b), a greater supply of nutrients to conventionally grown crops will result in at least similar or higher carotenoid contents than in organically produced ones.

#### **B**-vitamins

Humans and mono-gastric animals rely on the supply of B-vitamins with the diet. Microbial biosynthesis of B-vitamins is well-known and fermented food can be a significant source. Although comparative studies between organically and conventionally grown crops showed no significant differences concerning certain B vitamins (e.g. Woese et al., 1997; Bourn and Prescott, 2002), it is of interest to discuss how N supply may affect the synthesis of B vitamins in crops. The review by Mozafar (1993) included B vitamins and showed that application of N fertilizer resulted in higher vitamin concentrations in crops. According to Mengel and Kirkby (2001), there is a close relationship between protein concentrations in cereal grains and concentrations of B vitamins. Late application

of N to cereals increased concentrations of B vitamins. According to Marschner (1995) the content of lipids in green leaves is closely related to N supply. An enhancement of protein synthesis through N application also leads to an increase in the lipid layers of leaves. In the lipid metabolism, vitamin  $B_1$  (thiamine) plays a key role as thiamine pyrophosphate together with coenzyme A.

<b>Table 3.</b> Review of mean concentrations of $\beta$ -carotene in crops as affected by N
supply. References cover studies between 2001 and 2010.

Reference and crops	β-carotene concentration <sup>#</sup> , mg/kg fresh weight			Relative increase with
	Increase with N level	Mineral	Organic	N supply,
		fertilization		%
<i>Kaack et al. (2001)</i> Carrot (green manure, 10 rates, 2 yrs)	110-150	-	-	+10
<i>Caris-Veyrat et al. (2004)*</i> Tomato (320 kg organic N vs. 116 kg mineral N, 3 cultivars)	-	8.7	12.3	+70
<i>Chenard et al. (2005)</i> Parsley (N fertilizer, 5 rates)	39.7-78.5	-	-	+98
<i>Kopsell et al. (2007b)</i> Kale (N fertilizer, 5 rates, 3 cultivars)	61.6-65.3	-	-	NS
<i>Lefsrud et al. (2007)</i> Spinach (N fertilizer, 4 rates, 2 cultivars)	57.9-69.6 47.0-51.4	-	-	+15 NS
<i>del Amor (2007)</i> Sweet pepper (low organic N vs. high mineral N) <i>Kopsell et al. (2007a)</i> Watercress (N fertilizer, 3 rates)	- 3.3-9.3		a -	+24 +182
Juroszek et al. (2009) Tomato (3 paired farms, organic vs. conventional, 2 cultivars)	-	5.2	5.8	NS
<i>Behera and Rautaray (2010)</i> Durum wheat (N fertilizer, 2 rates)	4.0-4.7	-	-	+17
Mean difference				+42

# 12 µg of "dietary" beta-carotene correspond to 1 µg vitamin A being 1 retinol activity equivalent (RAE).

\* Note that more N was applied with organic manure than mineral fertilizer.

NS = not significant.

a = no absolute values given.

Although knowledge about the synthesis of several B vitamins in crops is limited, it seems that an enhanced protein formation is followed by the syntheses of B vitamins. It is therefore most likely that the content of B vitamins is equal or higher in conventionally, due to a higher N supply, than in organically grown crops. The B vitamin content in foods of plant origin would likely be relevant to human nutrition mainly in vegetarian diets, since in other diets, fish and animal products supply much larger relative amounts.

#### Vitamin C (Ascorbic Acid)

Humans cannot synthesize vitamin C in the body and an adequate and regular intake with the diet is therefore necessary. The recommended dietary intake of vitamin C is 75 mg per day for adults. In Western diets, vitamin C is mainly provided through fruits and vegetables (including potatoes) and deficiencies are rare.

Vitamin C is a water-soluble plant metabolite linked to carbohydrate metabolism formed from glucose as a precursor (Wheeler et al., 1998). A high production rate of glucose promotes vitamin C synthesis. Among factors determining the level of vitamin C in crops, radiation interception is the most important one. This means that intensive light and high photosynthetic activity favors the synthesis of vitamin C in crops (Mengel and Kirkby, 2001). Vitamin C is needed by the crop in three reactions during photosynthesis and is also protecting crops against oxidative stress (Smirnoff, 1996). However, N fertilizers at high application rates seem to decrease the concentration of vitamin C in fruits and vegetables. The review by Mozafar (1993) showed that the content of vitamin C decreased in plants at high N fertilization. Similarly, a review by Nagy (1980) on citrus fruits showed that an increasing application of N fertilizer resulted in higher N contents and lower vitamin C contents. The same finding is obvious from **Table 4**, which shows that higher total N contents in conventionally grown crops are followed by lower vitamin C contents and vice versa for organically grown crops.

A relevant question to ask is what mechanism may cause lower concentrations of vitamin C in plants with higher N supply? The most probable explanation is that application of N fertilizer results in denser crop canopies affecting the leaf area exposure to light and thereby the rate of photosynthesis. Knowing that N fertilization increases the amount of biomass produced and knowing that vitamin C formation increases with light intensity, it is most likely to assume that a dense crop canopy can cause mutual shading, which means reduced light penetration to certain parts of the crops. In other words, although total photosynthesis per plant or area increases due to more leaves, a larger and denser crop canopy can reduce photosynthesis per leaf area compared to sparse-leafed crops. As a result, a lower vitamin C production is related to dense canopies and vice versa. This mechanism can also explain instances where vitamin C concentrations do not decline with N fertilizer application (see **Table 3**). For example, vegetables grown in pots with sufficient light not affected through mutual shading did not show lower vitamin C content with increasing N supply (e.g. Müller and Hippe, 1987). Similarly, cabbage and sweet corn, which are most likely less affected by mutual shading

showed no decline in vitamin C contents between conventionally and organically grown plants (Warman et al., 1997, 1998) as well as peas (Fjelkner-Modig et al., 2000) to which no N fertilizer is applied.

An important question to be answered is how large declines in vitamin C that have been reported upon N application? An earlier study by Åberg and Ekdahl (1948) showed that the difference in vitamin C content was less than 10% between low and high N application rates for different plants. Lisiewska and Kmiecik (1996) found a reduction in the vitamin C content of 7% when increasing

Reference and crops	Vitamin C con mg/kg fresh	Organic relative to conventional,	
	Conventional	Organic	%
Leclerc et al. (1991)			
Carrot (6 farms, 2 yrs)	38	45	+18
Celeriac (6 farms, 2 yrs)	73	81	+11
Cayuela et al. (1997)			
Strawberry (11 sampling dates, 1 yr)	700	720	+3
Warman and Havard (1997)			
Carrot (3 yrs)	26	25	-4
Cabbage (3 yrs)	538	479	-11
Warman and Havard (1998)			
Potato (2 yrs)	275	262	-5
Sweet corn (3 yrs)	67	64	-4
Fjelkner-Modig et al. (2000)			
Cabbage (6 yrs)	376	370	-2
Carrot (6 yrs)	53	58	+9
Onion (6 yrs)	80	90	+12
Pea (2 cultivars, 6 yrs)	165	160	-3
Potato (3 cultivars, 4 yrs)	213	223	+5
Asami et al. (2003)			
Corn (1 yr)	28	32	+14
Caris-Veyrat et al. (2004)			
Tomato (3 cultivars, 1 yr)	121	154	+27
Chassy et al. (2006)			
Tomato (2 cultivars, 3 yrs)	168	203	+21
Bell pepper (2 cultivars, 3 yrs)	518	554	+7
Mean difference			+6.1

**Table 4.** Vitamin C contents in crops from comparative organic and conventional cropping.

fertilizer application from 80 to 120 kg N to cauliflower. Although these studies do not deal with the comparison of organic and conventional agriculture, it is interesting to note that the reported decline is of the same order of magnitude as in the review of Dangour et al. (2009), who found a 6.8% lower content of titratable acidity including ascorbic acid. The compilation of data in **Table 4** derived from paired studies over the last 20 years shows that the mean decline of vitamin C in conventional products is 6.1% as compared to organic ones. In summary, a small decline of vitamin C in conventionally produced crops can be expected. The dietary impact may not be very severe as the difference is small in comparison to the varying contents among fruits and vegetables of different species. Sparing application of N to fruits and vegetables will help to produce high vitamin C crops.

#### Trace Elements

Another important crop quality aspect is the trace element composition. Principally, plant availability of nutrients and trace elements in soil affects the composition of crops. If for example NPK fertilizers are applied to soil, a larger amount of these nutrients in soil solution enables crops to take up more of these and yields increase. However, if the addition of mineral NPK fertilizer may not be followed by a sufficient amount of trace elements, less trace elements in proportion to applied nutrients may be taken up. As a result, concentrations of trace elements in crops may become diluted (Jarrell and Beverly, 1981). Dilution of trace elements through application of mineral NPK fertilizers has been pointed out as one reason why mineral fertilizers could reduce crop quality. According to Rusch (1978), one of the founders of organic agriculture, high crop quality can only be achieved when easily soluble mineral fertilizers are excluded. In fact, decreasing contents of some plant nutrients in vegetables and fruits available on the market over a period of 50 years have been observed (Mayer, 1997; Davis et al., 2004).

The study by Mayer (1997) showed that there are significant reductions of Ca, Mg, Cu, and Na in vegetables; and Mg, Fe, Cu, and K in fruits over time. A similar study by Davis et al. (2004) in the USA revealed a decline in Ca, P, and Fe in garden crops although observations of sometimes increased levels of nutrients were found. However, the nutrients mentioned are both macro- (K, P, Mg, Ca) and micronutrients (Fe, Cu) and a systematic decline of only micronutrients was not obvious. Furthermore, one may not expect K, P, and Ca, which have been applied in agriculture as mineral fertilizer and lime on a regular basis, to be declining. Other factors than dilution due to fertilizer application were also pointed out by Davis et al. (2004). Selection of cultivars with a high yield potential, and unpredictable genetic variability of cultivars, were given as possible explanations (Davis et al., 2004). Comparisons of trace element contents in organic and conventional food based on recent publications reveal no definite results for a dilution effect in the latter (Gundersen et al., 2000; Lorhem and Slania, 2000; Ryan et al., 2004; Hajšlová et al., 2005; L-Bäckström et al., 2006; Kristensen et al., 2008). In most cases there were no differences in mineral contents and in the remaining cases both higher and lower concentrations can be found in organic and conventional food. For example, one study found that Cu concentrations were higher in conventional than organic food, while another study found the opposite and yet another study found no difference between the two (**Table 5**).

Trace element	1 Experiment, 18 maize samples	19 Farms, 190 pea samples	2 Experiments, 39 cereal samples	1 Experiment, 18 cereal samples	2 Experiments, vegetables
Cr	n.a.	org = conv	n.a.	n.a.	n.a.
Co	n.a.	org = conv	n.a.	org = conv	org = conv
Se	n.a.	org = conv	n.a.	org > conv	n.a.
Ni	n.a.	org = conv	n.a.	conv > org	org = conv
Mo	n.a.	org = conv	n.a.	n.a.	org > conv
Cu	conv > org	org = conv	org > conv	n.a.	org = conv
Fe	org > conv	org = conv	org = conv	org > conv	org = conv
Zn	org > conv	org = conv	org > conv	n.a.	org = conv
Mn	n.a.	org = conv	org = conv	n.a.	org = conv
Reference	Warman and Havard (1998)	Gundersen et al. (2000)	Ryan et al. (2004)	L-Bäckström et al. (2006)	Kristensen et al. (2008)

**Table 5.** Review of studies comparing concentrations of trace elements in organically and conventionally grown crops.

n.a. = not analyzed

Furthermore, absolute concentrations in crops differed more between studies than between organic and conventional systems in the same study. This means that location, soil, soil-crop management, etc. seem to have a major influence on the micronutrient composition of crops. As data in the literature are inconclusive or conflicting and do not support a mineral depletion hypothesis caused by NPK fertilizers, other factors may influence trace element concentrations in organically and conventionally grown crops, which are discussed below.

The major source for trace elements is agricultural soil. Native concentrations of trace elements in soil may vary due to differences in parent material. As a consequence, differences in the supply of trace elements from soil can have a greater impact on concentrations in crops than the type of cropping. For example, low Se content in a soil will lead to low Se contents in crops, regardless of the type of production. Furthermore, flows of micronutrients and trace elements to soils and crops will greatly affect crop composition. Micronutrients and trace elements are added to soil with mineral P fertilizers, untreated minerals, or purchased manures, or are applied as such to cover crop demand. Even purchased animal feed being enriched with mineral nutrients can lead to enrichment in soils that receive manure on a regular basis. Information on flows of trace elements must be provided and considered when comparing crops from different cultivation systems. Unfortunately, this information is often not available and therefore seldom mentioned.

Atmospheric deposition of trace elements can have a significant effect on contents in crops as shown by concentrations of unwanted elements (Pb and Cd) that have decreased in crops over the last two decades (Kirchmann et al., 2009) due to reduced emissions.

One may conclude that the hypothesis that organically produced crops have higher concentrations of trace elements than conventional crops is not supported by scientific data. In fact, use of synthetic fertilizers allows controlled application of trace elements to achieve defined concentrations in crops. Fertilization with Se in Finland since 1984 is an example of controlled application to achieve defined concentrations in crops, animal products and human blood (Eurola et al., 2003).

#### Non-essential Secondary Metabolites

Secondary crop metabolites (other than essential vitamins) consist of different groups of compounds such as phenolic acids, polyphenols, terpenoids, alkaloids, flavonoids, estrogens, glucosinolates, etc., in total 5,000 to 10,000 different substances whose role in plants is not fully known, including functions such as protection against light, control of oxidative stress, defence against insect and pathogen infestation, and herbivore grazing. Also their dietary role and function in humans is not well understood. The antioxidant function of some metabolites, reducing free radicals and their preventive role in cancer has been pointed out (Hasler, 1998). However, one may be reminded that if a secondary metabolite would be essential, the compound would be defined as a vitamin. Identified vitamins and micronutrients such as vitamin A, C, Se, etc. act also as antioxidants. In fact, polyphenols were proposed as vitamin P more than 70 years ago (Kroon and Williamson, 2005), but evidence for essentiality was lacking.

Some commonly occurring secondary compounds, for example solanine (alkaloid) in potatoes and cyanide in cassava are occurring at concentrations in crops harmful for humans. Some secondary metabolites have the potential to cause cancer (Ames, 1983; Ames et al., 1990). It seems that the large number of secondary compounds present in plants can make it difficult to know which are beneficial or harmful for human health. However, several epidemiological studies have shown that a higher daily intake of vegetables and fruits, being the major source of secondary metabolites, reduce the risk for cardiovascular disease (Ness and Powles, 1997) and cancer (Block et al., 1992).

Despite their non-essentiality for humans, supporters of organic agriculture consider secondary crop metabolites as key substances for human health (e.g. Lundegårdh and Mårtensson, 2003; Caris-Veyrat et al., 2004; Mitchell et al., 2007; Benbrook et al., 2008). In fact, the beneficial health effects of fruit and vegetable consumption have been attributed to a higher intake of secondary metabolites (Brandt and Mølgaard, 2001) and not of essential vitamins and micronutrients. However, the grounds for considering secondary metabolites an extremely important health component in the diet are not supported by scientific evidence. Ames and Wakimoto (2002) point out that the health-promoting and cancerreducing effect of a higher fruit and vegetable consumption is attributed to a sufficient level of vitamin and mineral intake avoiding suboptimal conditions and dietary deficiencies. To explain elevated levels of secondary crop metabolites as a crop quality indicator can be a misinterpretation.

It is therefore highly questionable to consider secondary metabolites (e.g. phenolic compounds in berries) (Asami et al., 2003), and chlorogenic acid (Caris-Veyrat et al., 2004) and kaempferol in tomatoes (Mitchell et al., 2007), as being as beneficial as contents of vitamin C. These compounds are not known to be essential and in addition have been shown in some studies to be harmful (Ames, 1983; Sahu and Gray, 1994).

Recent comparative studies with onions, carrots, and potatoes showed that organic or conventional production had no significant and systematic effects on polyphenol and flavonoid content (Sølhoft et al., 2010 b) or polyacetylene in carrots (Sølhoft et al., 2010 a)—a group of less abundant compounds in crops also ascribed a health promoting effect (Christensen and Brandt, 2006). Testing the source of N fertilizer (organic or inorganic N) on possible changes of the most common flavonoid in onion (quercitin) resulted in no significant differences (Mogren et al., 2007; 2008). A meta-analysis done by Koricheva et al. (1998) investigating six environmental factors on contents of some secondary metabolites in plants showed that phenols and terpenoid concentrations responded marginally to N fertilization, P fertilization, shading, drought, ozone and  $CO_2$ -enrichment.

In summary, based on the fact that our understanding of the reactions of secondary crop metabolites in human metabolism is poor, and that they can be both harmful and beneficial, it may be incorrect to interpret elevated levels as a quality improvement. In the discussion of secondary plant components, it may be useful to remember that humans only use a limited number of plants as food crops simply because many plants contain high contents of unwanted secondary metabolites making them unsuitable as food.

#### Mycotoxins in Organically and Conventionally Grown Crops

A further quality aspect of crops is the presence of toxins produced when fungi colonize food crops, the so-called mycotoxins. There are large numbers of mycotoxins synthesized by fungi, but only a few pose a potential health risk (Murphy et al., 2006). A distinction can be made between mycotoxins formed before harvest such as deoxynivalenol, zearalenone, and derivatives originating from *Fusarium* species; and those formed after harvest such as aflatoxins and ochratoxins originating from *Aspergillus* species. Thus, when examining the effect of cropping system on mycotoxin formation, only toxins produced before harvest are of interest. Our examination focuses solely on deoxynivalenol (DON), since it is the most frequently detected *Fusarium* toxin in wheat (Edwards, 2009).

In principal, inorganic N fertilization can increase the occurrence of fungi and mycotoxin formation as a result of higher moisture conditions in crops (Clevström et al., 1986; 1987). It is well-known that N fertilization results in denser crops, which can lead to less air flow and cause a higher relative humidity in crop canopies. Furthermore, use of N fertilizer can lead to higher water contents in crops as compared to unfertilized. In addition, fertilizer use can prolong the maturity period of crops and may also increase the risk for lodging. Fungal infections are favoured by high levels of moisture from flowering to the end of the maturation period. In other words, intensive conventional agriculture may indirectly increase the problem of fungi infestations.

While some studies showed no significant increase of mycotoxin contamination with N fertilization (e.g. Teich and Hamilton, 1985; Schaafsma et al., 2001; Blandino et al., 2008), others indicate an increase in deoxynivalenol contents (e.g. Lemmens et al., 2004; Heier et al., 2005; Oldenburg et al., 2007). Although high N fertilization can favour mycotoxin formation, a number of additional factors can also have an influence, such as infection pressure, weather conditions and the susceptibility of crop varieties. Furthermore, it seems that only very high N application rates exceeding crop demand increase the risk for mycotoxin contamination (Blandino et al., 2008). Main factors having a depressive effect on mycotoxin formation were K fertilization and a high pH in soil (Teich and Hamilton, 1985).

In summary, there is no clear evidence that conventional crops grown with inorganic N fertilizer will be more contaminated. In addition, the use of synthetic fungicides in conventional agriculture can be a powerful tool to control fungal infestation in crops and thereby maintain low mycotoxin levels. Whether organic or conventional crop production is favouring mycotoxin formation is reviewed below in which a number of experiments and field studies are compared.

Data on grain concentrations of deoxynivalenol were compiled (**Table 6**) to examine whether there are indications of the concentrations being higher in any particular system. Only grain samples prior to storage and processing were selected, while commercially available grain products (e.g. Malmauret et al., 2002; Schollenberger et al., 2003, 2005; Cirillo et al., 2003; Jestoi et al., 2004) were excluded in order to avoid the possible influence of different conditions related to processing.

Concentrations in grain showed large variations in organic crops (25 to 760  $\mu$ g deoxynivalenol/kg grain) and in conventionally grown crops (16 to 1,540  $\mu$ g dexonivalenol/kg grain). Mean dexonivalenol values of the data compiled (organic 225  $\mu$ g/kg grain and conventional 215  $\mu$ g/kg grain) were not significantly different. In some studies, variations between years were larger than variations between systems (Birzele et al., 2002; Champeil et al., 2004). Fungicide treatment of growing crops decreased the level of deoxynivalenol in the grain. However, the treatment was less effective for low to moderate disease infections than for a high infection rate (Birzele et al., 2002; Champeil et al., 2004). Minimum tillage generally resulted in higher levels of deoxynivalenol in grain than more intensive tillage (Champeil et al., 2004). From the data compilation above, we can conclude

that conventionally grown crops will not lead to a greater contamination than organically grown crops.

**Table 6.** Review of mean concentrations of the *Fusarium* mycotoxin deoxynivalenolmeasured in grain samples obtained from organic and conventional cropproduction.

Type of crop	No. of samples	Concentration of deoxynivalenol in grain prior to storage <sup>†</sup> , mean µg/kg grain		Reference
		Organic	Conventional	
Wheat	35 in total	_	54	Teich and Hamilton, 1985
Wheat	51 and 50	484	420	Marx et al., 1995
Rye	50 and 50	427	160	
Wheat	37 in total	26	20	Olsen and Möller, 1995
Rye	10 in total	20	15	
Wheat	n.g.††	50	16	Eltun, 1996
Oat	n.g.	36	19	
Wheat	169	_	16	Langseth and Rundberget, 1999
Oat	178	_	32	
Wheat	46 and 150	760	1,540	Döll et al., 2000
Wheat	47	111	—	Birzele et al., 2002
Wheat	58	280	—	
Wheat	n.g.	205	150	Birzele et al., 2002
Oat	9 and 14	25	24	Schollenberger et al., 2003
Wheat	24 and 36	126	394	
Wheat	8 and 8	123.5	37.5	Finamore et al., 2004
Wheat	75 and 75	500	450	Champeil et al., 2004
Wheat	31 and 40	160	200	Hoogenboom et al., 2008
Wheat	247 and 1,377	230	230	Edwards, 2009
Wheat	13 and 13	310	132	Solarska et al., 2009
Wheat	4 and 4	201	46	McKenzie and Whittingham, 2010
	4 and 4	201	323	
Mean v	alues	225	215	

† The maximum permissible deoxynivalenol concentration in grain is 500 μg/kg for direct human consumption and 100 μg/kg for infants and young children.

*†† Not given.* 

### **Concluding Remarks**

A number of representative reviews have shown that adopting organic agriculture on a world-wide scale would lead to severe shortages of food (Smil, 2001; Kirchmann et al., 2008a; Goulding et al., 2009). However, some advocates of organic agriculture have been quick to respond that this is not a problem as we should change to a more vegetarian diet. In other words, the world should adapt to organic systems that produce less food (Badgley and Perfecto, 2007). The recommendation given is that lack of food as a result of conversion to organic production should be compensated for by a change in diet. However, if the aim is to produce more vegetarian food owing to nutritional recommendations based on science, the most efficient and environmentally friendly way would be to cultivate crops through conventional practices. Large land areas used for agriculture today could then be re-converted to natural ecosystems, forests or used for production of bioenergy. The environmental impact of agriculture would be minimized.

Worryingly, it is foreseeable how organic food production on a large scale will endanger food security. Based on statistics from Sweden, organic crop production would imply a shift towards milk and red meat production requiring much more land for forage utilized by ruminants. Yield reductions of about 50% and declines of 75% less land used for organic potato and oilseed rape production would not be sufficient to cover demands in Sweden.

Since it was stated by the founders of organic agriculture that organic food is superior, quality has been a key argument to promote this type of agriculture. Numerous studies on food quality have been performed, but stringent reviews subjecting results to extensive comparisons or rigorous statistical procedures (e.g. Magkos et al., 2006; Dangour et al., 2009) reveal few differences showing that food quality of organic products is not necessarily better. In this review, an understanding of enhanced or decreased contents of vitamins, protein, NO<sub>3</sub><sup>-</sup>, and trace elements in crops due to different fertilization was aimed for. It seems that only vitamin C is reduced by conventional cropping (mutual shading in crop canopies fertilized with N), whereas the synthesis of other vitamins (A and B) are favoured by N fertilization. The literature data reviewed in this chapter provides no evidence for a systematic dilution of trace elements in conventionally grown crops and no difference in mycotoxin contents between the cropping systems were found. We conclude that the quality of organically grown crops does not seem superior.

#### Food Supply, Dietary Composition and Food Quality

Many people want to supply their body with the best food available and choose organic food. Lady Eve Balfour, the British founder of the Soil Association, wrote that she changed to a diet based on organically produced food (Balfour, 1943). However, she also shifted to whole grain instead of refined flour products, a high proportion of vegetables and fruits and abolishment of meat in her diet. She lived a long life. Her experience may lead to the conclusion that organically produced food supports a long and healthy life. However, to be able to evaluate health effects, the distinction between dietary composition and food quality is necessary.

Many human health problems in the world are the result of malnutrition or obesity (i.e. shortage of nutrition or excessive food consumption). Furthermore, an imbalanced dietary composition can cause health problems as a result of insufficient supply of essential nutrients. Finally, differences in food quality may have an impact on human health. In other words, one has to distinguish three aspects related to food intake: amount of food eaten, dietary composition, and nutritional quality.

Our analysis indicated that large-scale organic production will bring about two major changes-food supply may not be secured and shortage of certain foods will affect the dietary composition. Although claims about nutritional benefits are used as an argument for organic products, our review corroborated earlier studies also showing that organic products do not have a superior quality. Considering the dietary composition (i.e. proportions of carbohydrates to fats, intake of products with sugar and white flour, consumption of fruits and vegetables, amount of fish or meat eaten, etc.) has a great impact on human health (Willet, 1994; Taubes, 2001; Trichopoulou and Critselis, 2004). Focus should be on proper nourishment first of all. It seems that any possible differences in product quality may actually be of minor importance for health as long as the supply of essential nutrients is sufficient, which can be regarded to be a healthy diet (Ames and Wakimoto, 2002). For example, a daily intake of five conventionally grown fruits and no cake per day will supply more vitamins than two organically grown fruits plus cakes. We conclude that food shortage and consequences for the dietary composition are of uttermost importance when discussing health aspects of organic production rather than food quality.

#### Belief in the Superior Quality of Organic Food

A frequent statement of advocates of organic agriculture is that organically produced crops are more nutrient dense. A late example is a report from the organic center in Boulder, Colorado by Benbrook et al. (2008) stating that organic plantbased food contains 25% more nutrients than the same food produced conventionally. The evaluation of this report by Rosen (2008) showed that: i) the selection of references indicate exclusion of results favourable to conventional food; ii) the revised standpoint on dietary NO<sub>3</sub><sup>-</sup> actually being essential for the human immune system was completely ignored; and iii) the magnitude of 25% higher contents cannot be derived from the literature studies cited.

This recent example shows that conviction in the superiority of the nutritional benefits of organic food rather than search for the understanding of the complexity and factors controlling food quality seems to be a driving force for supporters of organic agriculture. The wide-spread belief that organic products must be superior is based on a view idealizing nature. The slogans 'nature knows best' and 'nature makes it good' are examples characterizing this view. However, not to idealize nature but to recognize the wholeness of nature is in accordance with natural science.

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Chapter 11

# Fertilization as a Remediation Measure on Soils Contaminated with Radionuclides <sup>137</sup>Cs and <sup>90</sup>Sr

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## Abstract

A wide range of countermeasures has been used to mitigate the consequences of the Chernobyl accident in affected regions of Belarus. Radical improvement of grassland, liming, fertilizers, and manure application are the most widespread, applicable, and effective countermeasures to restrict soil-to-plant radionuclide transfer. Efficiency of fertilization depends on radionuclide deposition, texture and chemical properties of the soils, and biological characteristics of plants. Soil fertility improvements through liming, manure, and NPK application are the basic remediation measures in the long-term period after the Chernobyl accident. This paper reviews existing experimental data on the efficiency of agrochemical countermeasures on land contaminated by <sup>137</sup>Cs and <sup>90</sup>Sr.

## Introduction

Radiocaesium (<sup>137</sup>Cs) and radiostrontium (<sup>90</sup>Sr), the important products of nuclear fission, have been introduced into the terrestrial environment by nuclear weapons testing, nuclear waste disposal, and by nuclear accidents such as those at Chernobyl and Fukushima. Following a large-scale release of radioactivity into the environment, inhabited land and food production systems may be contaminated for many years. Radiocaesium in the environment can affect human health following exposure via various pathways. The consumption of agricultural

Symbols and abbreviations specific to this chapter: Bq = Becquerel; BRISSA = BelarusResearch Institute for Soil Science and Agrochemistry; Cs = caesium; FYM = farmyard manure; LSD = least significant difference; PL = permissible level of radionuclide concentration; <math>RF = reduction factor; SD = standard deviation; Sr = strontium; Tag = Aggregated transfer factor [the ratio of the mass activity density (Bq/kg) in a specified object to the unit area activity density in  $Bq/m^2$ ] =  $m^2/kg$ ; Y = yttrium.

For symbols used commonly throughout this book see page xi.

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products contaminated with <sup>137</sup>Cs and <sup>90</sup>Sr represents the main long-term exposure pathway of the population (Alexakhin, 1993; Shaw and Bell, 1994). There is considerable variation in the soil-to-plant transfer of radionuclides. This is due to differences in soil pH, K status, and the contents of clay and organic matter (Absalom et al., 1995, Arapis and Perepelyatnikova, 1995, Sanzharova et al., 1996). Prister et al., 2003 proposed a comprehensive model for prediction of <sup>137</sup>Cs and <sup>90</sup>Sr transfer from soil to plants in relation to deposition, time of incubation, and main soil properties: pH, absorbing capacity of cations, content of organic matter, and exchangeable K and Ca.

An accident in the Chernobyl nuclear power plant on 26 April 1986 resulted in a massive radioactive contamination of territories in Republic of Belarus, Russian Federation, and in Ukraine. As a consequence, large-scale countermeasures in agriculture of the affected countries have appeared to be necessary. Such countermeasures have been intensively applied in the post-accident period. More details on implementation of remediation strategies in the areas affected by the Chernobyl accident—especially in terms of costs and averted doses, efficiency, and also the possibility to involve stakeholders in the remediation process—can be found elsewhere (Jacob et al., 2009). The primary aim of agrochemical countermeasures on contaminated land is to reduce radionuclide transfer into the human food chain. Liming and extra rates of K and P fertilizers are basic elements of plant production technology in radioactive contaminated land, which result in essential change of soil agrochemical properties and radionuclide behaviour in the soil-plant chain.

The aim of our work was to determine parameters of radionuclide transfer from soil to plants depending on soil fertility status and fertilizers, based on the background of our research experience in the post Chernobyl period in Belarus. These parameters are the basis for the development of protective measures in the long-term post-accident period to decrease the transfer of <sup>137</sup>Cs and <sup>90</sup>Sr in the food chain with minimal expenses, using conventional methods of fertilization.

The Chernobyl accident in Belarus has resulted in a radioactive contamination covering about 23% of the territory and affecting 2.2 M people. An area of 265,000 ha has been excluded from agricultural use due to deposition of <sup>137</sup>Cs over 1,480 kBq/m<sup>2</sup>, <sup>90</sup>Sr over 111 kBq/m<sup>2</sup>, and plutonium (Pu) isotopes over 3.7 kBq/m<sup>2</sup>. At present, agricultural production is conducted on 1.0 M ha of land contaminated by <sup>137</sup>Cs with deposition of 37 to 1,480 kBq/m<sup>2</sup>. A portion of this land, 0.34 M ha, is simultaneously contaminated with <sup>90</sup>Sr as well (6 to 111 kBq/ m<sup>2</sup>). The agricultural sector has been the area of the economy most affected by the accident. The Government has provided significant financial support for the rehabilitation of contaminated territories. However new efforts are needed for development of reliable experimental background for the choice of efficient remediation measures. In relatively favorable climatic conditions, the level of soil fertility is the most important parameter for limiting the contamination of harvested crop product.

# **Materials and Methods**

Studies on the influence of soil fertility on <sup>137</sup>Cs and <sup>90</sup>Sr transfer to plants were carried out with randomized plots 1 m<sup>2</sup> in size on crop fields of the Gomel region. The efficiency of fertilizers was studied on Luvisol loamy sand soil under conventional agricultural conditions. The soil agrochemical properties were as follows: 2.2% humus, 6.0 pH (KCl), 170 mg P2O5/kg, and 160 mg K2O/kg. Radionuclide deposition on soil (mean  $\pm$  SD) was rather high at 350  $\pm$  18.0 kBq <sup>137</sup>Cs/m<sup>2</sup> and 48 ± 5.2 kBq <sup>90</sup>Sr/m<sup>2</sup>. Treatments included rates of K fertilizer (60, 120, and 180 kg K<sub>2</sub>O/ha) along with 90 kg N and 60 kg  $P_2O_2$ , which were compared to no treatment. The fertilizer treatments (four replications) were laid out on 3 blocks receiving 0, 8, and 16 t/ha of manure within the crop rotation of maize, spring wheat, vetch-oat mixture, winter wheat, and lupin for grain. Associative N<sub>2</sub>-fixing bacteria (local strain *Azospirillum brasilense*) were tested in field trials by the inoculation of perennial grass seeds. The experiments in the Mozyr district of the Gomel region were conducted on poor Luvisol loamy sand soil with the following characteristics: 1.2% humus, 6.0 to 6.2 pH, 160 to 180 mg  $P_2O_5/kg$ , and 180 to 190 mg K<sub>2</sub>O/kg. Deposition of <sup>137</sup>Cs and <sup>90</sup>Sr was 185 kBq/ m<sup>2</sup> and 12 kBq/m<sup>2</sup>, respectively. Timothy grass (*Phleum pratense*), fescue (*Festuca* pratensis), brome grass (Bromus Inermus) and cocksfoot (Dactylis Glomerata) were grown on small experimental plots (6.4 m<sup>2</sup>) using four replications and background fertilization of 30-60-90 kg N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O/ha.

Soil analyses were conducted using conventional methods. Available  $P_2O_5$  and  $K_2O$  were determined by extraction in 0.2 M HC1 using a 1:5 soil to water ratio. <sup>137</sup>Cs activity concentration in plant and soil samples was measured using gamma spectrometry (HP-Ge detector Canberra GC4019). <sup>90</sup>Sr activity concentration was determined in plant and soil samples ashed at 600°C, using the oxalate method with separation of radioyttrium (<sup>90</sup>Y) (Cherenkov counting). Results were calculated for each soil sample both as Bq/kg and as kBq/m<sup>2</sup>. Then aggregate transfer coefficients of radionuclides from soil into crop production, Tag values (m<sup>2</sup>/kg x 10<sup>-3</sup>) for <sup>137</sup>Cs and <sup>90</sup>Sr were calculated. Conventional dispersive and regression statistical analysis for the experimental data was carried out using MS Excel (Clever and Scarisbrick, 2001).

## **Remediation Measures**

### Liming

Plants absorb Cs and Sr by the same uptake mechanisms as their competitor ions (K and Ca, respectively). Both K and Ca are important plant nutrients and are therefore actively taken up by the plant. Where their competitor ions are abundant and bioavailable, <sup>137</sup>Cs and <sup>90</sup>Sr accumulation by plants is expected to be relatively low.

Soil acidity influences the availability of dissolved nuclides and their uptake in plants. The liming of acid soils is an effective way of reducing <sup>90</sup>Sr transfer to plant products. This countermeasure, based on decreasing soil acidity and a relation of  $Sr^{2+}/Ca^{2+}$  in soil solution, was known a long time before the Chernobyl

accident (Wiklander, 1964). During the post-accident period numerous trials with Ca amendments were done. A priority was given to such chemical amendments to try to reduce the level of radionuclides in soil solution by increasing the concentration of competitive species, such as Ca and K (Alexakhin, 1993; Nisbet et al., 1993).

It was found that Ca addition may significantly reduce both the transfer of <sup>137</sup>Cs and <sup>90</sup>Sr to plant production only on acid soils, relatively low in exchangeable Ca (about 2 cmol of exchangeable Ca per kg soil) (Nisbet et al., 1993). The most appropriate liming material for coarse-textured soils of Belarus was finely-ground dolomite containing 22% Ca and 13% Mg.

The pH (KCl) values for maximum yield of growing plants on Podzoluvisol loamy sand soil were different: 6.7 for barley, 5.9 for potatoes, and 4.9 for lupine. The priority criterion for choosing a countermeasure treatment needs to consider the value of extra yield obtained (Bogdevitch, 2003). An example of combined radiological and economic justification is the optimal liming treatment for potato plants presented in **Table 1**.

Dolomite applied, t/ha	Fertilizer applied (N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O), kg/ha	Soil pH	Soil exchangeable Ca, cmol/kg	Yield of potato, t/ha	Net return, €/ha	<sup>137</sup> Cs activity RF (reduction factor)	<sup>9°</sup> Sr activity RF
0	0-0-0	4.9	2.5	16.2	-	1.0	1.0
6	0-0-0	5.9	4.2	17.6	67	1.6	-
18	0-0-0	6.7	5.8	15.4	-95	1.7	-
0	70-60-150	4.9	2.5	24.3	403	1.8	1.2
6	70-60-150	5.9	4.2	26.4	509	2.1	1.5
18	70-60-150	6.7	5.8	23.1	298	2.3	1.7
LSD <sub>0.05</sub>				1.4			

**Table 1.** Effect of liming on potato yield and radionuclide accumulation on Podzoluvisol loamy sand soil (Deposition of <sup>137</sup>Cs – 370 and <sup>90</sup>Sr – 37 kBq/m<sup>2</sup>, <sup>137</sup>Cs activity of potato tubers on Control treatment – 10.2 Bq/kg, <sup>90</sup>Sr activity of tubers – 11 Bq/kg).

The highest yield of potatoes in the experiment was achieved with application of 6 t of dolomite and an application of 70-60-160 kg of fertilizer  $N-P_2O_5-K_2O$  per ha. This treatment resulted in the highest net profit per hectare, while substantially reducing the plant accumulation of <sup>137</sup>Cs and <sup>90</sup>Sr. Increasing the rate of dolomite to 18 t/ha provided a neutral soil reaction (pH 6.7), slightly less plant accumulation of <sup>137</sup>Cs and <sup>90</sup>Sr, but resulted also in decrease of yield and net profit. It is well known that large additions of lime to acid soils that raise pH above 6.5 often lead to depressed yield owing to low availability of micronutrients such as Fe, Mn, Cu, and Zn (Bergmann, 1992). Combined applications of lime

and fertilizer were able to increase yields and profits while reducing plant accumulation of radionuclides.

Perennial grasses accumulate the highest concentrations of radionuclides for both <sup>137</sup>Cs and <sup>90</sup>Sr, which is most problematic for grazing cattle, especially for dairy stock. Soil acidity influences the availability of dissolved nuclides and their accumulation in plants. Our investigation on farm fields showed that liming changed the reaction of Luvisol soils from pH (KCl) 4.2-4.5 to pH 6.5-7.0, and strongly reduced <sup>90</sup>Sr accumulation in clover (*Trifolium pratense*) (**Figure 1**).



Figure 1. <sup>90</sup>Sr transfer (Tag m<sup>2</sup>/kg x 10<sup>-3</sup>) to clover biomass in relation to the reaction (pH KCl) of Podzoluvisol loamy sand soil.

The results of the present study can be simply described by an empirical regression using a negative power function. Similar relations have been established for other crops. Such negative power functions have been widely used in Belarus for the prediction of <sup>137</sup>Cs and <sup>90</sup>Sr plant uptake from soil. The mean reduction factors of <sup>137</sup>Cs and <sup>90</sup>Sr accumulation in plants due to liming of contaminated soils with different pH (KCl) in Belarus, Russia, and Ukraine varied from 1.3 to 2.6 times (Deville-Cavelin et al., 2001). However in the case of highly acidic Podzoluvisol and peat soils (pH < 4.0 to 4.5), liming in our experiments reduced radionuclide accumulation in perennial grass by up to 10 times.

The recommended lime rates were differentiated according to soil type, texture, and initial degree of acidity to achieve the optimal pH level for main crops grown on contaminated land (BRISSA, 2003). The target levels of pH (KCl) for Pod-zoluvisol texture groups were: clay and loam 6.0 to 6.7, loamy sand 5.8 to 6.2, sand 5.6 to 5.8. The target pH range for liming of drained Histosols (peat-boggy soils) was 5.0 to 5.3. Liming of contaminated acid soils is a mandatory prerequisite of agriculture practice in contaminated areas.

#### **Potassium Fertilizer**

The application of K fertilizer is a main agrochemical measure for restriction of <sup>137</sup>Cs accumulation in crop products. It is well known that K, as a chemical analog of Cs, could effectively inhibit the transfer of <sup>137</sup>Cs from soil to plants (Andersen, 1963; Evans and Dekker, 1963). However, the inhibitory effect is strongly dependent on the K concentration in soil solution, which determines the effect of K fertilization as a countermeasure to reduce the Cs contamination of crop products (Menzel, 1954; Shaw and Bell, 1991). The genotypic differences in <sup>137</sup>Cs uptake between the various crops are very important; however, they also depend on exchangeable K content in soil (Bogdevitch, 1999). Exchanges of K nutrition of plants result in essential change of intensity of accumulation both of <sup>137</sup>Cs and <sup>90</sup>Sr in plants. Insufficient soil K supply leads to the intensive involvement of <sup>137</sup>Cs in the biological chain on contaminated soils (Alexakhin, 1993; Prister et al., 1993; Fesenko et al., 2007).

Our field experiments were carried out on soil with three prepared blocks characterized by different levels of soil K supply. Increasing doses of K, balanced with NP fertilizers, were applied on each level of soil K supply. Data from the spring wheat experiment is shown in **Table 2**.

region				
Soil treatment <sup>†</sup>	Yield of grain, t/ha	Response to control, t/ha	<sup>137</sup> Cs Tag value, m²/kg x10 <sup>-3</sup>	Reduction factor
		3.2 mmol K/kg		
Control	3.24	-	0.028	1.0
$N_{_{70}}P_{_{60}}K_{_{80}}$	4.58	1.34	0.024	1.1
$N_{70}P_{60}K_{160}$	4.79	1.55	0.017	1.6
$N_{70}P_{60}K_{240}$	4.90	1.66	0.014	2.0
		5.3 mmol K/kg		
N <sub>70</sub> P <sub>60</sub> K <sub>80</sub>	4.90	1.66	0.014	2.0
$N_{70}P_{60}K_{160}$	4.90	1.66	0.010	2.7
$N_{70}P_{60}K_{240}$	5.00	1.76	0.009	2.8
		7.4 mmol K/kg		
N <sub>70</sub> P <sub>60</sub> K <sub>80</sub>	5.00	1.76	0.010	2.7
$N_{70}P_{60}K_{160}$	5.13	1.89	0.010	2.8
$N_{70}P_{60}K_{240}$	5.21	1.97	0.009	2.9
LSD <sub>05</sub>		0.22	0.0037	

Table 2. Effect of increasing K fertilizer doses (kg K20/ha) on yield and<sup>137</sup>Cs transfer to spring wheat grain under three different contents of<br/>exchangeable soil K in Podzoluvisol loamy sand soil of Belarus, Gomel<br/>region.

 $\pm Note$  values indicated for P and K application are actually  $P_2O_5$  and  $K_2O_5$ .

It was found that improvement of soil K supply level (exchangeable K) of a Podzoluvisol loamy sand soil from 3.2 to 5.3 mmol/kg significantly increased yields and reduced <sup>137</sup>Cs transfer from soil to grain by a factor of 1.7 to 1.6. High K fertilizer rates up to 160 to 240 kg K<sub>2</sub>O/ha are effective for crop cultivation on loamy sand soils with low K content. Only moderate K fertilizer rates are needed for soils with medium to high K supply (5.3 to 7.4 mmol/kg) to replace crop K removal.

Our investigation provided experimental background for K fertilizer application efficiency as affected by radionuclide deposition, soil agrochemical status, and genotypic differences of crops. For example, a close reverse correlation was observed between <sup>137</sup>Cs accumulation in clover (*Trifolium pratense*) biomass and available K content in soil (**Figure 2**).



**Figure 2.** <sup>137</sup>Cs transfer (Tag value, m<sup>2</sup>/kg x 10<sup>-3</sup>) to clover biomass depending on K supply of Luvisol loamy sand soil.

The relationship between <sup>137</sup>Cs transfer to clover plants and soil supply K level is well described by a negative power function that explains 62% of the variability of the data. A significant decrease of <sup>137</sup>Cs accumulation took place within the available K content range of 50 to 250 mg/kg, or 1.1 to 5.3 mmol/kg. Data indicates the optimal K content threshold in sod-podzolic loamy sand soil is about 5.3 mmol/kg for most field crops. Further increase in exchangeable soil K did not provide a significant reduction in radionuclide accumulation in plants, but strongly increased the cost of treatment. The efficiency of K fertilization was found to be higher after liming if soil acidity was significantly reduced. This result is in agreement with those obtained by Nisbet (1995) who concluded that K fertilizer could be beneficial in ameliorating the effects of <sup>137</sup>Cs contamination in both organic and mineral soils of low fertility in which exchangeable K is less

than 5 mmol/kg. Therefore our experimental data provided the quantified base for prediction of efficient use of K fertilizer in relation to K soil tests.

However, different soil properties can strongly influence the concentrations of <sup>137</sup>Cs and <sup>90</sup>Sr (and their competitor ions) in the soil water, and observed uptake to plants can vary within a wide range of values (Sheppard and Evenden, 1997). We studied the inhibitory effect of increasing doses of K, balanced with NP fertilizers, on <sup>137</sup>Cs and <sup>90</sup>Sr transfer to wheat grain in relation to three levels of FYM application within a long-term field experiment started in 1999. The levels of FYM (0, 8, and 16 t/ha) resulted in different levels of soil organic matter content in the topsoil layer, corresponding to 1.56, 1.94, and 2.40%. Application of 16 t/ha of FYM had a prolonged effect on crop rotation yield and it resulted in a spring wheat (*Triticum aestivum*) grain response of 0.96 to 1.14 t/ha. The accumulation of <sup>137</sup>Cs in the grain decreased by 1.3 to 1.4 times due to FYM application, while <sup>90</sup>Sr accumulation in grain was reduced by 2.0 to 2.6 times (**Figure 3**).





The major factor in the behavior of Sr in soils is the formation of organic complexes with humic substances (Arapis et al., 1997). Therefore additions of organic matter to coarse-textured soils can decrease <sup>90</sup>Sr plant uptake by increasing the holding capacity of soils for trace levels of radionuclides. The combined effect of higher rates of FYM and optimal fertilizer treatment ( $N_{90}P_{60}K_{180}$ ) allowed for a 4 times reduction in <sup>90</sup>Sr transfer to wheat grain (Tag value) from 1.11 to 0.28.

### **Phosphorus Fertilizer**

It is known that heavy dressings of P fertilizer can prevent or reduce plant uptake of toxic concentrations of trace elements (Bergmann, 1992) as well as of <sup>137</sup>Cs and <sup>90</sup>Sr radionuclides (Nisbet et al., 1993; Prister et al., 1993). The reduction of

<sup>90</sup>Sr transfer from soil to crop production was admitted to be due to formation of insoluble strontium phosphates. However application of unbalanced high doses of NP fertilizers on fertile soils has resulted in the enhancement of <sup>137</sup>Cs accumulation in plants (Sanzharova et al., 1996). Usually the agrochemical properties have close intercorrelations. In our experiment we had opportunity to measure the effect of different concentrations of available P in soil while keeping the level of other input factors equal (**Figure 4**).



**Figure 4.** Tag values (m<sup>2</sup>/kg x 10<sup>-5</sup> for <sup>137</sup>Cs and m<sup>2</sup>/kg x 10<sup>-3</sup> for <sup>90</sup>Sr) indicating flux into grain of spring wheat (means for 2005, 2006, and 2007 under the Control and N<sub>110</sub>P<sub>60</sub>K<sub>180</sub> treatments) in relation to different levels of available P content for a Luvisol loamy sand soil.

Four blocks with different content of available P in soil (mg  $P_2O_5/kg$ ) were prepared: I (67 to 72); II (110 to 124); III (189 to 211), and IV (388 to 398). Increasing available soil P content within the wide limits of 67 to ~400 mg  $P_2O_5/kg$  has been accompanied with an increase in grain yield on fertilized plots from 3.8 to 6.9 t/ha, as well as a decrease in accumulation of <sup>137</sup>Cs by a factor of 1.4 to 1.9, and by a factor of 1.5 to 1.6 for <sup>90</sup>Sr. Close relation of Tag transfer values of <sup>137</sup>Cs and <sup>90</sup>Sr to wheat grain (y) with increasing available P content in soil (x) was found to fit well with quadratic curves (R<sup>2</sup> = 0.91 and 0.77, P<0.01). The minimum accumulation of radionuclides in wheat grain is expected at an available P content of 300 to 320 mg  $P_2O_5$ /kg for loamy sand soil (Bogdevitch and Mikulich, 2008).

We also studied the inhibitory effect of increasing doses of K, balanced with NP fertilizers, on <sup>137</sup>Cs transfer to wheat grain in relation to different levels of soil P supply (**Figure 5**).



**Figure 5.** Transfer Tag values (m<sup>2</sup>/kg x 10<sup>-5</sup> for <sup>137</sup>Cs) indicating flux into grain of spring wheat (mean ± SD 2005 to 2007) in relation to increasing rates of K fertilizer at different levels of available P content for a Luvisol loamy sand soil.

The K status of our experimental field was close to optimal for cereal crops on loamy sand soil (4.7 mmol/kg of exchangeable K). The application of increasing rates of K fertilizer resulted in sufficient reduction of <sup>137</sup>Cs transfer from soil to wheat grain. Compared to the background application (kg/ha) of  $N_{90}P_{60}$ , addition of  $K_{90}$  reduced <sup>137</sup>Cs transfer by 10 to 16%,  $K_{120}$  by 14 to 27%, and  $K_{180}$  by 22 to 40%. The range of reduced <sup>90</sup>Sr transfer to grain under the influence of K fertilizer rates has been comparatively lower at 3 to 28%. The relative inhibitor effects of K fertilizer on radionuclide transfer from soil to wheat grain were similar for all studied levels of soil P supply. However the values of the transfer coefficients of <sup>137</sup>Cs had a clear tendency to decrease as the available P content in soil increased.

The Tag values for <sup>137</sup>Cs were low; therefore the grain activities varied for the treatments and P levels between 1.9 to 9.1 Bq/kg, which is much lower than the permitted level (PL) for food-grade grain (90 Bq/kg). The transfer of <sup>90</sup>Sr from soil to wheat grain was almost two orders of magnitude higher and the grain activities varied between 19.5 to 42.7 Bq/kg. The PLs for <sup>90</sup>Sr currently in force in Belarus are particularly low at 11 Bq/kg for food-grade grain and 3.7 Bq/kg for bread. For this reason the increase of the available soil P content up to an optimal level is very important because it permits the production of food-grade wheat in cases of <sup>90</sup>Sr deposition up to 16 kBq/m<sup>2</sup>. On soil with poor soil P status, food-grade wheat can only be permitted in cases of <sup>90</sup>Sr deposition below 11 kBq/m<sup>2</sup>. About 80,000 ha of arable land in Belarus are contaminated with <sup>90</sup>Sr higher than 11 kBq/m<sup>2</sup>.

For farmers in contaminated areas, it is important to be able to grow food-grade grain (instead of fodder) under the relevant PL values to maximize their income. The most economically efficient treatment was 110-60-120 kg N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O/ha. The profitability of fertilization has been increasing along with enhanced soil P supply, resulting in net returns of 99 and 252 €/ha, respectively, for food-grade grain produced at the 1st and 4th level of P input. Grain contaminated by <sup>90</sup>Sr above the PL and sold as fodder gave a lower net return, correspondingly, 28 and 105 €/ha. No stimulation effect on radionuclides <sup>137</sup>Cs and <sup>90</sup>Sr accumulation in wheat grain was observed due to increasing rates of N fertilizer because moderate rates (60, 90, and 110 kg/ha) were used. Moreover, the well-known biological "dilution" effect of radionuclide concentration was observed due to high wheat grain yield response to applied rates of N fertilizer.

#### Biofertilizer

Diazotrophic bacteria belonging to the genera *Azospirillum* has attracted much interest among agronomists and microbiologists. They were successfully used for inoculation of plants to achieve higher yields and production quality (Boddey and Dobereiner, 1995; Okon and Kapulnik, 1986; Kennedy et al., 2004).

The biofertilizer Azobacterin, containing a local strain of *Azospirillum brasilense* B-4485, was developed at the Belarusian Research Institute for Soil Science and Agrochemistry. The strain was found to possess high  $N_2$ -fixing activity, significant hormonal effect, and P solubilization activity as well. Azobacterin proved to be effective inoculant for barley, flax, and perennial grasses (Mikhailouskaya, 2006; Mikhailouskaya and Bogdevitch, 2009).

A series of experiments with the application of Azobacterin for perennial grasses inoculation were performed on Podzoluvisol loamy sand soil contaminated with radionuclides as a result of the Chernobyl accident. Our 6-year field experiments have revealed a significant yield increase of 11 to 17% as well as a reduction in radionuclide accumulation in perennial grasses (**Table 3**).

Indices	Bromus inermus	Dactylis glomerata	Phleum pratense	Festuca pratensis
Dry matter yield (Control), t/ha	5.2	4.7	4.1	3.5
Yield response to inoculation, t/ha	0.9*	$0.7^{*}$	0.7**	0.4*
LSD 05	0.39	0.43	0.4	0.35
<sup>137</sup> Cs Tag (Control)	0.35	0.27	0.30	0.21
<sup>137</sup> Cs Tag (Inoculated plants)	0.22	0.19	0.22	0.17
Reduction factor	1.6**	1.4**	1.3**	1.2*
<sup>90</sup> Sr Tag (Control)	2.33	3.08	1.67	1.33
<sup>90</sup> Sr Tag (Inoculated plants)	1.25	1.67	0.75	0.75
Reduction factor	1.9**	1.8**	2.2**	1.8**

**Table 3.** Perennial grass yield responses to Azospirillum brasilense inoculation and<br/>radionuclides transfer from soil to grass (Tag value,  $m^2/kg \ge 10^{-3}$ ).

Responses are significant at \*P < 0.05 and \*\*P < 0.01.

Cheap and environment-friendly biofertilizers may be accepted as an effective remediation measure. The beneficial effect of bacteria on reduction of  $^{137}$ Cs and  $^{90}$ Sr accumulation by a factor of 1.2 to 1.6 and 1.8 to 2.2, respectively, could be explained by a combination of the "dilution" effect due to increased yield, and also the effect of biosorption. This is supported by Russian scientists who report that bacteria of the genus *Azospirillum* are best for immobilization of  $^{137}$ Cs and  $^{90}$ Sr with biomass-medium distribution coefficients of 560 and 6,400, respectively (Belimov et al., 1996).

Land	Available K <sub>2</sub> O, mg/kg	Initial doses of K <sub>2</sub> O, kg/ha	Additional doses of K <sub>2</sub> O (kg/ha) according to deposition, kBq/m²			
		_	<sup>137</sup> Cs 37-184 <sup>90</sup> Sr 6-10	<sup>137</sup> Cs 185-554 <sup>90</sup> Sr 11-73	<sup>137</sup> Cs 555-1,480 <sup>90</sup> Sr 74-111	
Arable land	< 80	100	50	100	150	
	81-140	90	30	60	90	
	141-200	80	20	40	60	
	201-300	55	15	30	45	
	> 300	25	-	-	-	
Meadows/	< 80	80	40	80	120	
pastures	81-140	70	30	60	90	
	141-200	60	20	40	60	
	201-300	45	15	30	45	
	> 300	20	-	-	-	

 Table 4. Recommended annual doses of K fertilizer on Podzoluvisol soils contaminated with radionuclides <sup>137</sup>Cs and <sup>90</sup>Sr in Belarus.

Land	Available P <sub>2</sub> O <sub>5</sub> , mg/kg	Initial doses of P₂O₅, kg/ha	Additional doses of P2O5 (kg/ha) according to deposition, kBq/m2			
			<sup>137</sup> Cs 37-184 <sup>90</sup> Sr 6-10	<sup>137</sup> Cs 185-554 <sup>90</sup> Sr 11-73	<sup>137</sup> Cs 555-1,480 <sup>90</sup> Sr 74-111	
Arable land	< 60	45	15	30	45	
	61-100	40	10	20	30	
	101-150	35	5	10	15	
	151-250	20	-	5	10	
	251-400	10	-	-	-	
Meadows/	< 60	35	15	30	45	
pastures	61-100	30	10	20	30	
	101-150	25	5	10	15	
	151-250	10	-	5	10	
	251-400	-	-	-	10	

 Table 5. Recommended annual doses of P fertilizer on Podzoluvisol soils contaminated with radionuclides <sup>137</sup>Cs and <sup>90</sup>Sr in Belarus.

## Recommendations

The K fertilizer application system in combination with NP fertilizers, manure, and liming has been elaborated on soils contaminated after the Chernobyl accident in 1992 and then improved in 2003 (BRISSA, 2003). The economically acceptable rates of potash were found to ensure the stable level of soil fertility and minimization of the radionuclide uptake in crops and pastures. The recommended fertilizer doses were differentiated for soil types, levels of soil K content, and deposition density of <sup>137</sup>Cs and <sup>90</sup>Sr. The annual K doses for typical crop rotation on Podzoluvisol soils is shown in **Table 4**. The doses of P fertilizer have been differentiated in a similar manner according to available P soil test values and deposition of radionuclides (**Table 5**).

The recommended doses of NPK fertilizers have been widely implemented on all contaminated soils because the cost of PK fertilizers for farmers are totally subsidized by the State Programme for Overcoming the Consequences of Chernobyl Catastrophe. The costs of N fertilizer for farmers are also subsidized 30 to 60% by the Ministry of Agriculture and Food and by local budgets. The soil fertility in Belarus is commonly evaluated in terms of the properties monitored every 4 years (pH value,  $P_2O_5$ ,  $K_2O$ , Ca, Mg, and organic matter contents as standard practice, and also B, Cu, and Zn contents as required). The monitoring of soil fertility and recommendations for the efficient use of fertilizers are the responsibility of the Agrochemical Service under the methodical management of the Research Institute for Soil Science and Agrochemistry.

The high efficiency of countermeasures applied in Belarus is evident. The flow of <sup>137</sup>Cs to the food chain decreased by more than 12 times, <sup>90</sup>Sr up to 3 times during the post-accident period. All agriculture foodstuff that is produced in

large cooperative farms satisfied the requirements of National permissible levels PL-99 for radionuclide content. On the majority of agricultural land the optimal level of soil reaction and K status is achieved and maintained.

Experimental findings were implemented in cooperative farms as well as on private farmer's fields in the course of the EC project ETHOS (1996 to 2001). This pilot project was initiated in 1996 by a team of scientists from France with the objective of directly involving the population in the management of the radiological situation (Jullien, 2005; Lochard, 2007). The inhabitants of six villages of Stolin and Slavgorod districts during 2000 to 2007 years tested developed technology for growing potatoes that included seed selection of new potato varieties, application of fertilizers and plant protection means. As a result, average potato yields increased 1.6 times from an initial 15 to 20 t/ha and radionuclide concentration declined by 20 to 30% in comparison with control plots. Every 1  $\notin$  invested in the potato project provided 1.5 to 2.0  $\notin$  of net return. The ETHOS approach had been highly appreciated by local farmers and authorities on the State and Local levels.

## Conclusions

Soil fertility improvements through liming, manure, and NPK application are the basic remediation measures in the long-term period after the Chernobyl accident. Balanced NP fertilizers with K fertilizer rates up to 180 kg K<sub>2</sub>O/ha are profitable for crop cultivation on soils with low and medium K content. Increasing soil K supply in Podzoluvisol loamy sand soil from 2-3 to 5-6 mmol/kg allowed a yield improvement and a reduction in radionuclide <sup>137</sup>Cs transfer from soil to crops by a factor of 1.8 to 2. Only moderate K fertilizer rates are needed for high K soils to replace the crop K removal.

The rise of available P content of soil within the wide limits of 67 to ~400 mg  $P_2O_5$ /kg has been accompanied with an increase of the spring wheat grain yield from 3.8 to 6.9 t/ha as well as decreased radionuclide accumulation for <sup>137</sup>Cs (by a factor of 1.4 to 1.9) and for <sup>90</sup>Sr (by a factor of 1.5 to 1.6). The values of <sup>137</sup>Cs and <sup>90</sup>Sr transfer from soil to spring wheat grain were in close relation with available soil P content and were well described by downward concave quadratic curves. The minimum accumulation of radionuclides in wheat grain was calculated at available  $P_2O_5$  contents of 300 to 320 mg/kg in Podzoluvisol loamy sand soil.

Azobacterin containing  $N_2$ -fixing bacteria *Azospirillum brasilense* B-4485 may be used as an additional countermeasure. The inoculation of perennial grasses resulted in yield increase of 11 to 17% and the reduction of <sup>137</sup>Cs and <sup>90</sup>Sr accumulation in forage by factor 1.2 to 1.6 and 1.8 to 2.2, respectively.

The implementation of potato growing on private plots with modern technology as demonstrated in the ETHOS Project has a high social significance. The involvement of rural inhabitants in processes of self-rehabilitation and self-development is a way to improve people's life quality on radioactive contaminated territories as an example of common heritage. **FCHH** 

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