Fertilizing Crops to Improve Human Health: a Scientific Review

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Volume 1
Food and Nutrition Security

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Contents

Abbreviations and symbols commonly used throughout this publication

Al	Aluminum
B	Boron
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
$CaSO_4·2H_2O$	Calcium sulphate (gypsum)
CH_4	Methane
Cl^-	Chloride
Cu	Copper
$CuSO_4$	Copper sulphate
F	Fluorine
Fe	Iron
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
H_2O	Water
I	Iodine
K	Potassium
KCl	Potassium chloride (also muriate of potash or MOP)
K_2O	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
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
Ni	Nickel
NO_2^-	Nitrite
NO_3^-	Nitrate
N_2	Dinitrogen

NO_x	Nitrogen oxides (nitric oxide and nitrogen dioxide)
N_2O	Nitrous oxide
O_2	Dioxygen
P	Phosphorus
P_2O_5	Oxide form of P, used in trade to express P content of fertilizer
S	Sulphur
Se	Selenium
SO_4^{2-}	Sulphate
Zn	Zinc

Introduction/Executive Summary

Fertilizing Crops to Improve Human Health: a Scientific Review

By Tom W. Bruulsema, Patrick Heffer, Ross M. Welch, Ismail Cakmak, and Kevin Moran[1]

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 abbreviations and symbols used commonly throughout this book see page v.

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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."

This publication is to include three volumes. The first is on Food and Nutrition Security, with 4 chapters. The second will be a volume of 4 chapters on health-functional properties of foods. The third and final will focus on fertilizer impacts on selected health risks associated with plant production systems.

Volume 1: 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
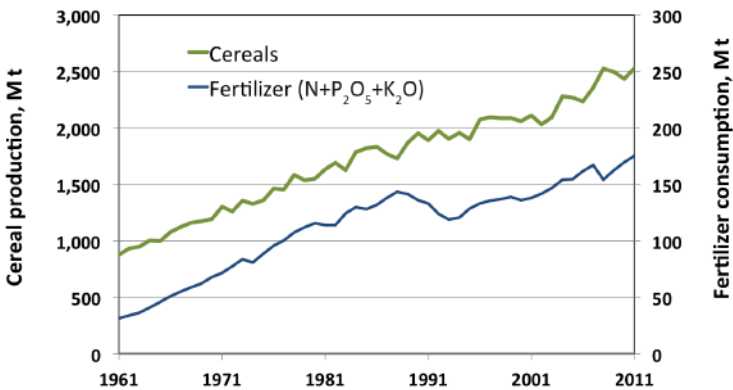

Figure 1. Global cereal production and total fertilizer consumption 1961-2011 (FAO 2012; IFA 2012).

to over 150 Mt. Without fertilizer use world cereal production would be halved (Erisman et al., 2008).

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 micronutrient. 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	%
N	85
P	73
K	55
B	31
Cu	14
Mn	10
Mo	15
Zn	49

Table 2. Global and regional estimates of the proportion of the population at risk of inadequate Zn intake (Hotz and 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

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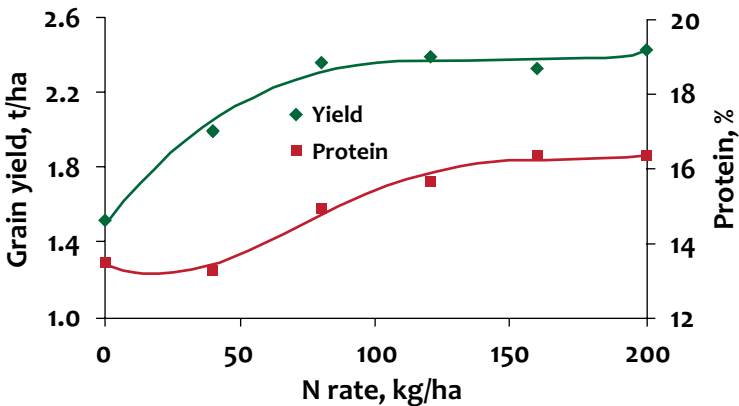
Figure 2. Yield and protein of wheat respond to applied N fertilizer.

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.

Volume 3: 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$_4$ 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 (^{137}Cs) and radiostrontium (^{90}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[1]

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 abbreviations and symbols used commonly throughout this book see page v.

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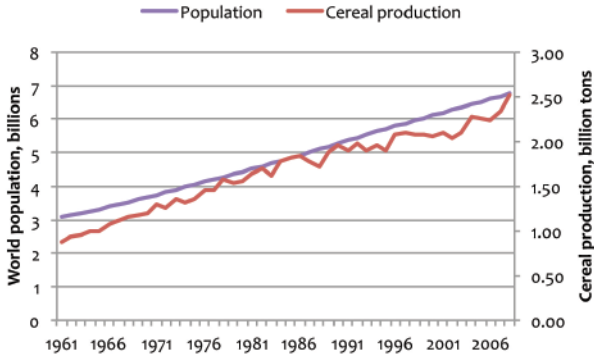
Figure 1. Change in global cereal production and population, 1961-2008.

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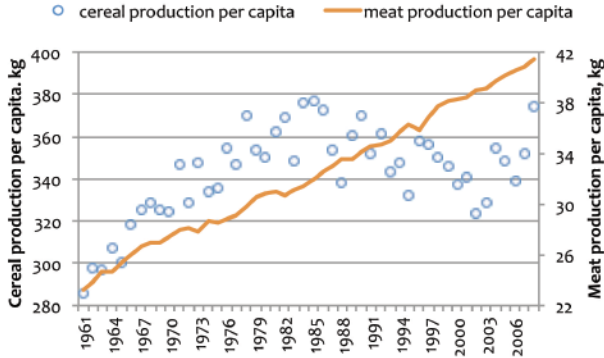
Figure 2. Change in cereal and meat production per capita from 1961-2008
(FAO, 2010b).

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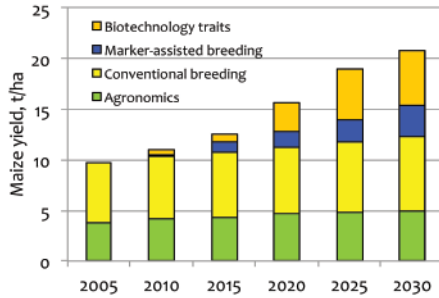
Figure 3. Anticipated impact of improvements in agronomics, breeding, and biotechnology on average maize yields in the USA (Edgerton, 2009).

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
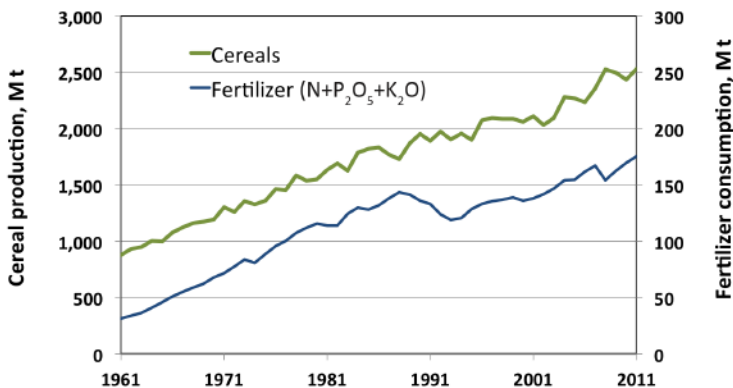

Figure 4. Global cereal production and total fertilizer consumption 1961-2011 (FAO 2012; IFA 2012).

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.

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

Crop	Estimated crop yield, t/ha		% reduction from no N
	Baseline yield	Without 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 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
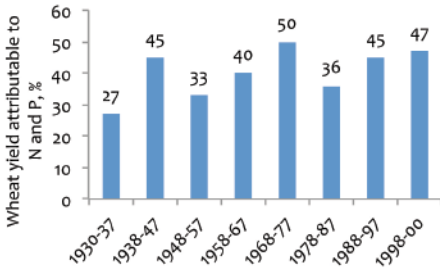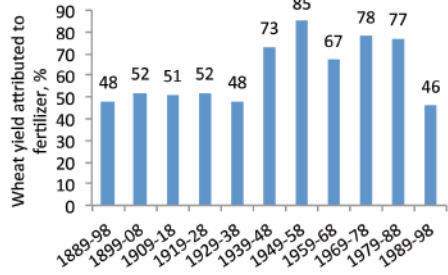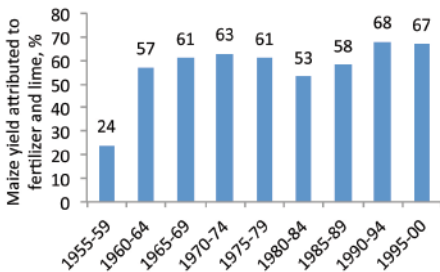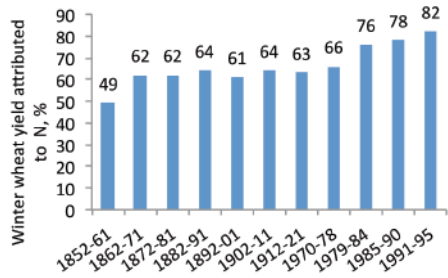
Figure 5. Yield attributed to fertilizer: **(A)** N and P from 1930 to 2000, in the Oklahoma State University Magruder plots; **(B)** N, P, and K from 1989 to 1998 in the University of Missouri Sanborn Field plots; **(C)** N, P, K, and lime from 1955 to 2000 in the University of Illinois Morrow plots; 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-yielding 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 on-farm 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.

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

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[1]	2.94	0	5	4	0
FYM (10 t/ha) + NPK[1]	3.57	0	1	5	3

[1]Fertilizer 50–50–25 (kg/ha N–P_2O_5–K_2O)

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%).
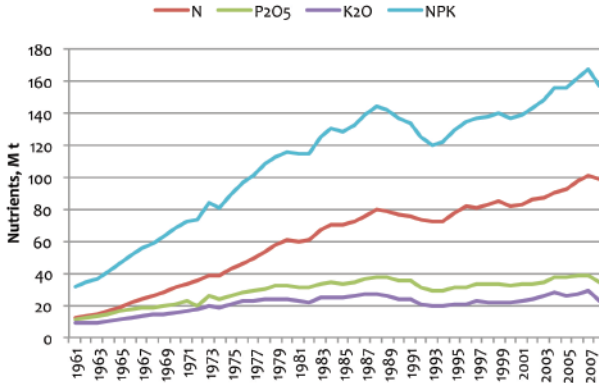
Figure 6. World fertilizer (N, P$_2$O$_5$, and K$_2$O) consumption from 1961 to 2008 (IFA 2010).

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
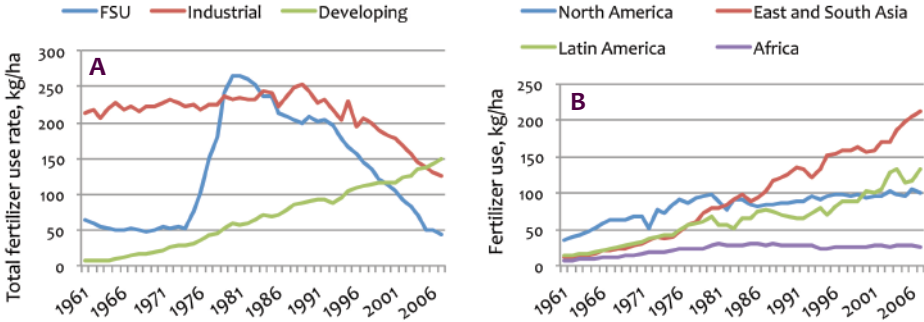
Figure 7. Regional trends in fertilizer use, 1961-2007 (FAO, 2010b).
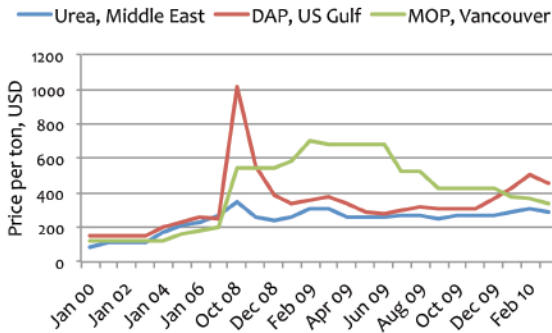
Figure 8. Average monthly price of urea, diammonium phosphate (DAP), and muriate of potash from January, 2000 to March, 2010 (Pike & Fischer, 2010).

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 21st 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. **F C H H**

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Chapter 2

Micronutrient Malnutrition: Causes, Prevalence, Consequences, and Interventions

Howarth Bouis, Erick Boy-Gallego, and J.V. Meenakshi [1]

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 abbreviations and symbols used commonly throughout this book see page v.

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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.[2]

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

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2 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.

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

Food Group	Bangladesh				Kenya					Philippines				
	Income Tercile			All House-holds	Income Quartile				All House-holds	Income Quartile				All House-holds
	1	2	3		1	2	3	4		1	2	3	4	
Per Capita Energy Intake														
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 Available**					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

public or private investments in agricultural research), which in turn will lead to more efficient production, faster supply growth rates, and eventually lower non-staple 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,
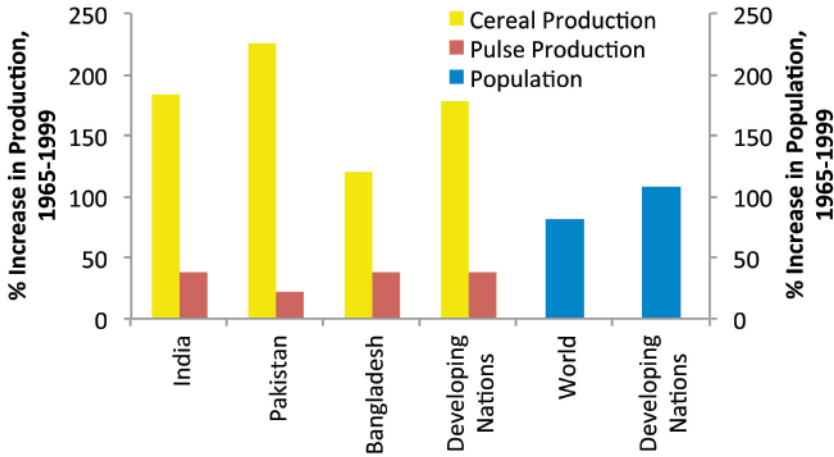
Figure 1. Percent changes in cereal and pulse production and in population, 1965-1999 (Graham et al., 2007).
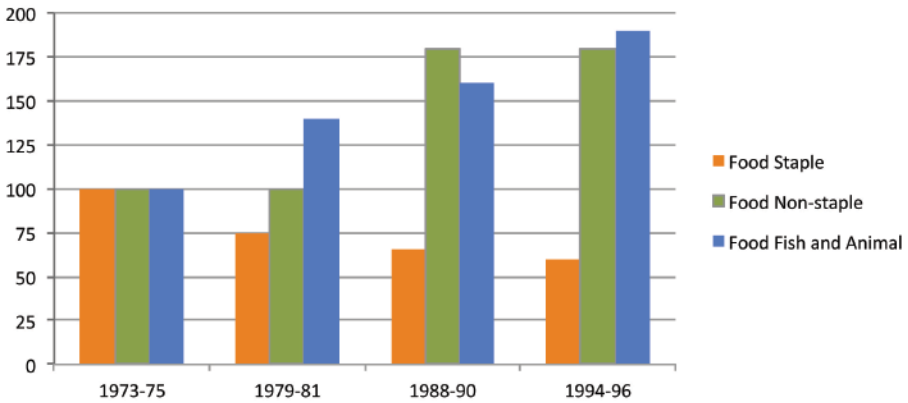
Figure 2. Indices of inflation-adjusted prices for Bangladesh 1973-75 = 100 (Bouis et al., 1998).

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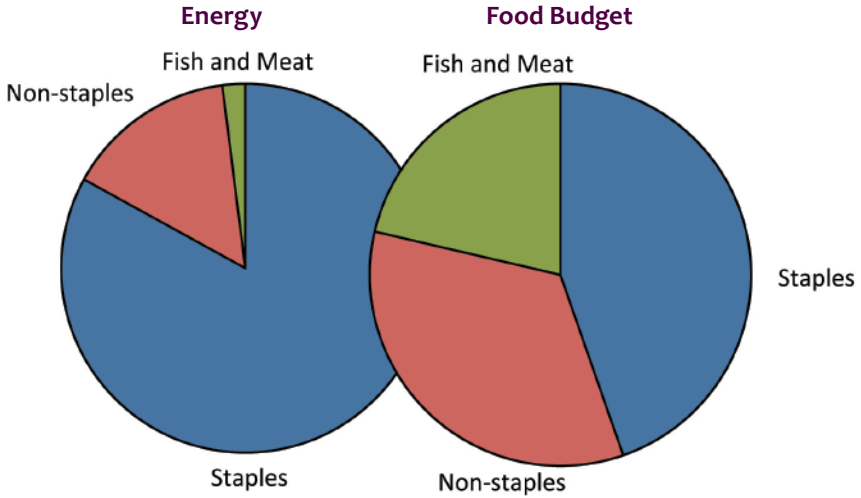
Figure 3. Share of energy source and food budget in rural Bangladesh (Bouis et al., 1998).

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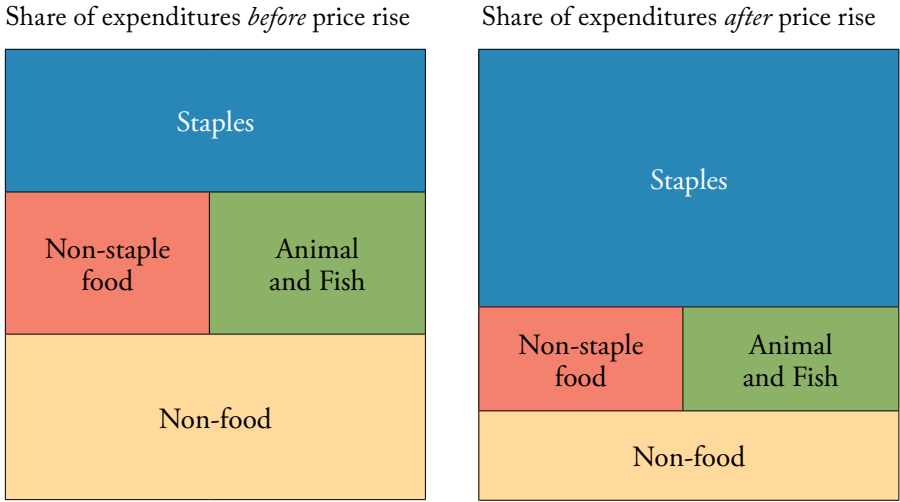
Figure 4. Share of food groups and non-food in total expenditures before and after rises in food staple prices (Bouis et al., 2011a).

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

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.

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

	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 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.
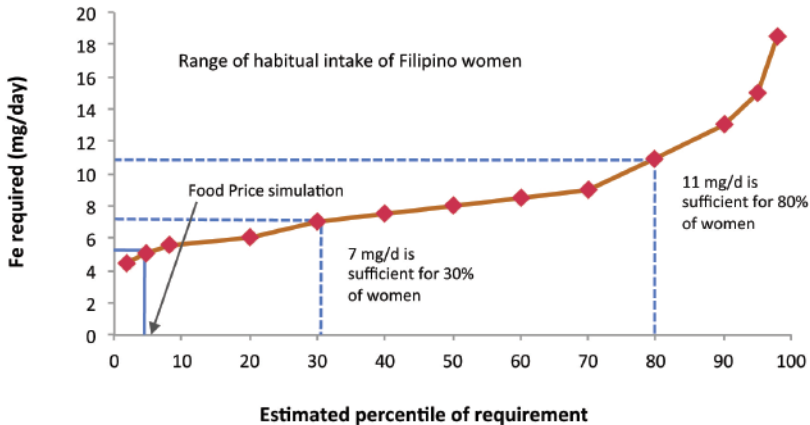
Figure 5. A 30% decline in iron intakes among Philippine women results in a decline in the proportion of women meeting iron intake requirements from 30% to 5%.

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?[3]

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

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3 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%.[4]

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-

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4 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+ new-born 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[5] (**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

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5 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).

6 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 mineral deficiencies.

WHO region	Vitamin A[1] Deficiency		Anemia (proxy for Iron deficiency[2])			Iodine[3]
	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 population 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 ± 15.0
Latin America & Caribbean	24.8 ± 12.0
USA and Canada	9.5 ± 1.3
Eastern Europe	16.2 ± 10.5
Western Europe	10.9 ± 5.2
South-East Asia	33.1 ± 5.9
South Asia	26.7 ± 9.4
China (+ Hong Kong)	14.1
Western Pacific	22.1 ± 8.2
Global	20.5 ± 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 auto-immune 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 ad-aptometry 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 de-velopment 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, neu-rological 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 suscep-tibility to nuclear radiation, iodine-induced hyperthyroidism, and neurological dysfunction of various degrees depending on the timing and duration of the in-sult (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 document evidence-based, effective large-scale micronutrient (MN) 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[7] 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.

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

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

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[2]	30	70
Iron fortification, 50% coverage[2]	27	43
Promising and emerging interventions		
Vitamin A biofortified sweet potato, 40% coverage[3]	9	
Vitamin A biofortified maize, 40% coverage[3]	11-18	
Zinc biofortifed rice, 60% coverage[3]		2-7
Zinc supplementation, 50% coverage[1]	476-823	7

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 child-health 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™) 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 develop-

ment 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.[8]

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.[9]

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

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8 An important part of the overall solution is to improve the productivity of a long list of non-staple 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.

9 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.
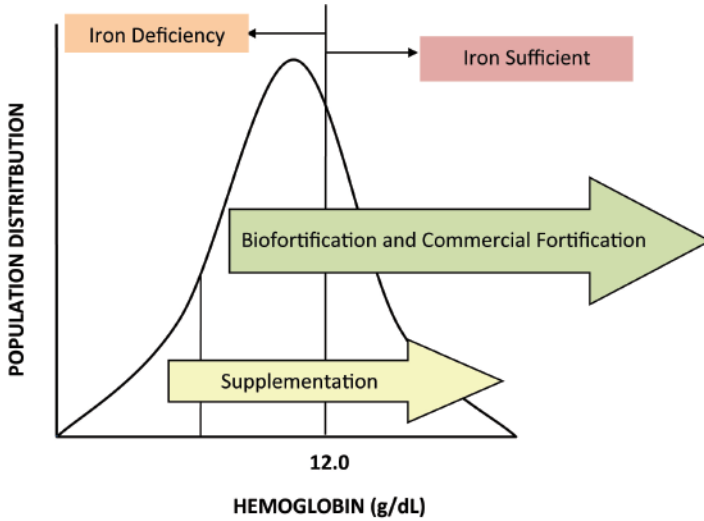
Figure 6. Biofortification improves status for those less deficient and maintains status for all at low cost.

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.[10]

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

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10 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. **FCHH**

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Chapter 3

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

Ross M. Welch and Robin D. Graham[1]

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 abbreviations and symbols used commonly throughout this book see page v.

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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⁻ 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.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo		Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Ce	Pr	Nd		Sm	Eu	Gd	Tb	Dy	Ho	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

Table 2. Concentration ranges of essential trace elements in common food crops (μg/g, dry weight) (Modified from Welch, 1995).

Element	Plant species, part	Deficient	Adequate	Toxic
Fe	Soybean (*Glycine max* 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 (*Lycopersicon esculentum* Mill.), leaf	5-6	70-400	—
	Wheat (*Triticum aestivum* L.), shoot	4-10	75	>750
	Sugar beet (*Beta vulgaris* 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	—
B	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	—	—
Cl	Potato (*Solanum tuberosum* L.), leaf	210	2580	>5000
	Sugar beet (*Beta vulgaris* L.), leaf	40-100	>200	—

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[†]	Soybean[‡]	Wheat[§]
Fe	25	30	25
Mn	15	20	30
Zn	15	15	15
Cu	5	5	5
Ni	—	<0.004[¶]	<0.1[#]
B	10	25	15
Mo	0.2	0.5	0.3
Cl[-††]	—	—	—

[†] *Leaf below ear at tasseling.*
[‡] *Youngest mature leaves and petioles after pod formation.*
[§] *Entire shoot at boot stage of development.*
[¶] *Entire shoot*
[#] *Mature grain*
[††] *The critical concentration of Cl^{-} 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 some deficiency implications, functions, estimated dietary needs and rich food sources[†] (Table from Welch, 2001).

Element	Deficiency and Function(s)	Human Dietary Need[‡]; 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
B	impaired Ca utilization in bone; more severe signs of vitamin D related rickets; decreased apparent absorption of Ca, Mg, and P; impaired metal functions in older women and men (>45 years old); *cis*-hydroxyl reactions with biomolecules; cell membrane integrity	0.5 - 1.0 mg/d (est.); non-citrus fruits, leafy vegetables, nuts, and pulses
Cr	impaired glucose tolerance; impaired growth; elevated 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; hypopigmentation of hair and skin; impaired bone formation with skeletal fragility and osteoporosis; vascular abnormalities; steely hair; metal cofactor in numerous metalloenzymes (e.g. cytochrome oxidase, caeruloplasmin, superoxide dismutase, etc.)	1.5 - 3.0 mg/d; organ meats, seafood, nuts and seeds
F	status as an essential trace element debated; beneficial element because of its effects on dental health	1.5 - 4.0 mg/d; tea, marine fish consumed with bones
I	wide spectrum of diseases including severe cretinism with mental retardation; enlarged thyroid (goiter); essential constituent of the thyroid hormones	150 µg/d; seafood, iodized table salt; milk; I concentrations in plant foods vary greatly depending on various environmental factors including the geochemical environment, fertilizer, food processing and feeding 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 function; 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 cartilage 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[‡]; 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 concentrations in plant foods can vary greatly depending on the available Se content of the soil where grown and the plant species grown
Si	depressed collagen content in bone with skull structure abnormalities; long bone abnormalities; decreased 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.); unrefined grains, cereal products; root and tuber crops
V	death proceeded by convulsions; skeletal deformities; increased thyroid weight; participates in oxidation 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 parturition; 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 membrane stability function	15 mg/d; animal products especially red meats, cheese, legume seeds and pulses

[†] *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 a given essential trace element that only presents fully when the deficiency of other nutrient elements is relieved.

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

Deficiency	N	P	K	B	Cu	Fe	Mn	Mo	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

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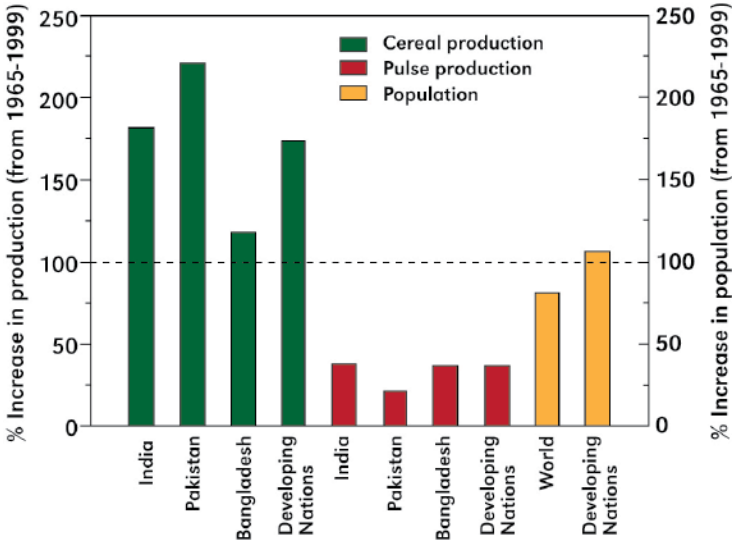
Figure 1. Percent change in cereal and pulse production and in human populations from 1965 to 1999 for select countries, developing nations and world population (Graham et al., 2007).

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 pulse-wheat-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^{2+} and Fe^{3+}], 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).

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

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)

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
  - o Up-regulates tumor suppressor genes (p53 and p21) in HT-29 human colon carcinoma cells
  - o 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
  - o Lowers serum cholesterol and triglycerides
  - o Natural antioxidant lowering lipid peroxidation
  - o 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

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

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
β-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

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^{2+} to Cu^{1+})]. It has been the most studied Fe promoter substance identified in plants (Lopez and Martos, 2004; Fairweather-Tait, 1992). The Fe^{2+} 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^{3+} to Fe^{2+} causes destabilization of Fe^{3+} 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^{3+} 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^{3+} and Zn^{2+} 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^{2+} and Fe^{2+} 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^{2+} and Fe^{2+} 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, and adding both limiting nutrients (assuming no others) causes a large 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 β-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 developing 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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<div align="center">*Chapter 4*</div>

Agronomic Biofortification of Food Crops with Micronutrients

<div align="center">

Graham Lyons and Ismail Cakmak[1]

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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

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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 biofortificaton 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^{2+} into plant-unavailable Fe^{3+} 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 $FeSO_4$ 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 vegetables 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 anti-pernicious 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 $CaCO_3$ (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 ($ZnSO_4$) 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 $ZnSO_4$ are similarly effective but in terms of their role in biofortification of food crops with Zn, $ZnSO_4$ 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 $ZnSO_4$ 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 $ZnSO_4$ 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 $ZnSO_4 \cdot 7H_2O$/ha) and without soil Zn application and foliar spray of 0.5 % $ZnSO_4 \cdot 7H_2O$ (approx. 4 kg $ZnSO_4 \cdot 7H_2O$/ha) at different growth stages in the Konya location (Cakmak et al., 2010b).

Soil Zn appl., kg/ha	Foliar Zn application stages	Zn concentration, mg/kg			
		Whole grain	Bran	Embryo	Endosperm
0	Control (no Zn)	11.7	20	38	8
	Stem + Booting	18.8	28	47	10
	Booting + Milk	26.9	35	62	15
	Milk + Dough	25.4	41	63	15
50	Control (no Zn)	21.7	33	52	11
	Stem + Booting	25.5	34	58	13
	Booting + Milk	29.3	45	69	16
	Milk + Dough	25.4	41	63	15
	$LSD_{0.05}$ for soil Zn application	1.8	3.0	3.4	1.0
	$LSD_{0.05}$ 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 $ZnSO_4$ 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 $ZnSO_4$) 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 $ZnSO_4$ 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 $ZnSO_4$ 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**).

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).

Treatments	Rice		Wheat	
	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 $ZnSO_4$	4.67	39	4.25	49
2% Zn as ZnO	4.95	43	4.39	49
2% Zn as $ZnSO_4$	5.15	48	4.53	51

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 Fe- and 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 fine root system, and enhanced root uptake and root-to-shoot translocation 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 μg 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 $ZnSO_4$ 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 twenty-first 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$_2$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 µg/kg on the clay soil, while 9 g basally applied raised it to just 77 µg/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 μg/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 μg/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
      Heart disease (especially in men) begins to decline

1984: National Se biofortification program commences

1987: Se in spring wheat grain increases from 10 (pre-1984) to 250 µg/kg
      Human Se intake trebles
      Human plasma Se level doubles (55 to 107 µg/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

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 cost-efficiency 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-methylselenocyste-ine 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. F CHH

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