CAN BIOFORTIFIED PLANTS ACCUMULATE TRACE ELEMENTS ESSENTIAL TO THE GROWTH AND DEVELOPMENT OF HUMANS?

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KEY WORDS

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ABSTRACT

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Micronutrient and trace element deficiencies are a problem affecting nearly two billion people globally. The people affected the most by these deficiencies are those living in poor and rural communities in the developing countries and thus cannot always afford the diverse diet as advocated by WHO and the FAO. Millions of these people living in the poor and developing countries die yearly, either directly or indirectly, as a result of micronutrient and trace element deficiencies. Thus, this study aimed to determine the nutrient content (Co, Cr, F, I, Se and V) of various vegetable based food items collected from the Cape Town area in the Western Cape Province of South Africa. This was done to determine which vegetable crops provided the highest concentrations of essential trace elements, and how much they contribute to the daily recommended intake (DRIs) of these trace elements. It also aimed to assess the effects of the addition of the trace elements (Co, Cr, F, I, Se, Si, Sn and V) on seed germination and root growth under controlled conditions in order to calculate their phytotoxicity, and then to biofortify four vegetable crop species, grown in sand culture, with a composite treatment of the trace elements to determine how the addition of these elements will affect the vegetable crops grown under these experimental conditions.

From this study, it was shown that trace element content in vegetable crops in the Western Cape Province of South Africa varied between different geographic locations and that certain trace elements were absent from several items collected from some areas. Although some crop species contained sufficient amounts of certain trace elements to satisfy our daily recommended intakes, most of the crops were found to contain insufficient amounts of many of the trace elements to satisfy our needs. Leafy vegetables and tubers were identified as the better vegetable types to biofortify with essential trace elements as they

already contain higher concentrations of several of the essential trace elements and should thus be assessed for their effectiveness as crops to be biofortified. When the trace elements were applied directly to cress and lettuce seeds, it was found that all the trace elements, as well as the composite treatments, exerted phytotoxic effects on cress and/or lettuce seeds when applied at high concentrations. Lettuce was found to be more prone to the effects of these elements. Seed germination was strongly inhibited by fluoride, while several elements affected root growth. When fluoride was left out of the composite treatment, phytotoxicity only occurred at high concentrations. The addition of the trace elements at the high concentrations to already established spinach, cabbage, lettuce and turnip plants were found to affect the uptake of several essential plant nutrients, but the concentrations of the elements affected generally remained higher than the concentrations needed for adequate growth of agricultural crops. Several of the trace elements supplied to the plants were also found to be retained in the roots of the vegetable crops however, as the concentrations supplied to the plants increased, so did the concentrations found in the edible portions of the crops.

Agronomic biofortification of vegetable crops with simultaneous additions of multiple trace elements, under these experimental conditions, was thus considered to be a viable option to increase the concentrations of essential mineral nutrients in the edible portions of vegetable crops. However, these modified nutrient fertilizers should only be given to established crops or without the addition of fluoride. Further research on a wider variety of seeds and vegetable crops, as well as research under field conditions is needed to determine whether these findings remain relevant under these conditions.

DECLARATION

I declare that "**Can biofortified plants accumulate trace elements essential to the growth and development of humans?**" is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Francuois Lloyd Müller

November 2013

Signed: _____

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Trace elements essential to humans but not to the plants we eat:

A Review

1.1 Introduction

All living organisms require specific elements essential for their growth, development and general wellbeing. Human beings and other mammals require more than 25 mineral nutrients while only 17 have been shown to be metabolically essential to plants for their growth and development (Table 1.1) (Marschner 1995, Hopkins and Hüner 2004, White and Broadley 2005a, White and Brown 2010). Some of these essential elements are required by plants and/or animals in large amounts (macronutrients), others in small amounts (micronutrients) and others only in trace amounts (Hirschi 2009). The term 'trace element' refers to elements which occur at very low concentrations, usually in micrograms per kilogram however, they are still essential to the proper functioning of various physiological systems of the organism (Marschner 1995, Senesei *et al.* 1999, Hopkins and Hüner 2004, Schulze *et al.* 2004).

Table 1.1: Essential mineral elements required by plants and animals

		Essentiality	
Element		**Plant**	**Animal**
Arsenic	As		**x**
Boron	B	**x**	**x**
Calcium	Ca	**x**	**x**
Carbon	C	**x**	**x**
Chloride	Cl	**x**	**x**
Chromium	Cr		**x**
Cobalt	Co		**x**
Copper	Cu	**x**	**x**
Fluoride	F		**x**
Hydrogen	H	**x**	**x**
Iodine	I		**x**
Iron	Fe	**x**	**x**
Magnesium	Mg	**x**	**x**
Manganese	Mn	**x**	**x**
Molybdenum	Mo	**x**	**x**
Nickel	Ni	**x**	**x**
Nitrogen	N	**x**	**x**
Oxygen	O	**x**	**x**
Phosphorous	P	**x**	**x**
Potassium	K	**x**	**x**
Selenium	Se		**x**
Silicon	Si		**x**
Sodium	Na		**x**
Sulphur	S	**x**	**x**
Tin	Sn		**x**
Vanadium	V		**x**
Zinc	Zn	**x**	**x**

The focus trace elements of this project are Co, Cr, F, I, Se, Si, Sn and V. These elements are essential to humans and other animals, but not to the plants they eat. All of these elements may however, have various beneficial effects on plant growth and development, and some are known as beneficial plant nutrients. Beneficial plant nutrients are mineral nutrients which are essential only to certain plant species or which, under certain conditions, stimulate growth, but are not essential to growth (Hopkins and Hüner 2004, Pilon-Smits *et al.* 2009). The different criteria for assessing an elements essentiality to plants and animals are summarised in Table 1.2.

Table 1.2: Criteria for assessing an elements essentiality to plants and animals (Moynahan and Jackson 1979, Salisbury and Ross 1992, Marschner 1995, Hopkins and Hüner 2004, Schulze *et al.* 2004).

	Criteria for assessing essentiality of elements to plants
1	The plant cannot complete its life cycle in the absence of the element.
2	The element must form part of an essential plant constituent or metabolite.
3	Within the plant, the element must act directly in the metabolism of the plant.
4	The element must not affect the availability of other elements or antagonise the effects of other elements within the plant.
5	Should the element be absent, deficiency symptoms will occur.
6	The deficiency symptoms must be corrected by the administration of the element.
	Criteria for assessing essentiality of elements to animals
1	The element must be present in all of the tissues of a given organism.
2	Its concentration in a given tissue must be fairly constant from one organism to another.
3	Taking the element out of an organism's diet must lead to a specific deficiency syndrome.
4	The deficiency syndrome must be associated with a definite biochemical change.
5	Both the syndrome and the biochemical changes must be prevented or corrected by the administration of the element.

1.2 Trace elements in the soil and plants

Despite the selectivity of plant roots to the uptake of mineral nutrients, both essential plant nutrients and non-essential nutrients are detected in their tissues (Robb and Pierpoint 1983, Moore and Chapman 1986, Marschner 1995, Hopkins and Hüner 2004). Since plants are the major accumulators of mineral nutrients in the terrestrial environment, they are the major sources of these essential mineral nutrients, either directly or indirectly, to the human diet (Nellessen and Fletcher 1993, White and Broadley 2005b).

To meet the nutritional needs of an increasing world population, estimated to reach 10 billion by 2050, and to feed some 868 million already malnourished people, two farming strategies were proposed. The first was to intensify land use by expanding agricultural land. However, expansion of agriculture is known to be one of the most significant anthropogenic activities changing the natural environment, and this led to concerns about

the long-term sustainability and increased environmental consequences. The second is to intensify crop production on the land already used for agriculture (Matson *et al.* 1997, Henao and Baanante 1999, Welch and Graham 2002, Graham *et al.* 2007, FAO *et al.* 2012). Although expansion of agricultural land and the increase in the production of certain staple foods on existing agricultural land seemed to be the answer to mitigating hunger, it has resulted in significant global nutrient depletion from agricultural soils (Table 1.3) (Fageria *et al.* 2002, Marler and Wallin 2006).

Table 1.3: Global nutrient depletion from agricultural soils (Marler and Wallin 2006)

Country	% Nutrients Lost
Africa	74
Asia	76
Canada	85
Europe	72
South America	66
United States	85

The depletion of trace elements and micronutrients from agricultural soils and vegetable crops is seen as one of the greatest flaws of the "green revolution" and the implementation of nitrogen, phosphorous and potassium (NPK) fertilizers which focussed only on the production and intensification of higher yielding crop varieties (Welch and Graham 2002). Because farmers only supply plants with the nutrients that the plants need to grow, coupled with the global nutrient depletion from agricultural soils, it is possible that by continual cropping these essential trace elements can become depleted from agricultural soils which, inevitably will result in food crops with insufficient nutrients to satisfy our needs. Agriculture is however, still considered to be the major nutrient supplier to humans and domestic animals (Drechsel *et al.* 2001, FAO and WHO 2001, Johnsson 2005, Legard 2005, White and Broadley 2005b, Garvin *et al.* 2006, Marler and Wallin 2006, Islam *et al.* 2007, Fan *et al.* 2008, Zhao *et al.* 2009). Table 1.4 provides an overview of the ways in which these focus trace elements are found in the soil, how they are taken up, where in the plant they are accumulated and the effects of high concentrations on the plants.

Table 1.4: Overview of the focus trace elements in the soil and in plants.

Element	Form in soil	Soil concentration (g/kg)	Plant concentration (g/kg)	Taken up as	Mobility	Site of accumulation	Negative effects of high concentrations in plants
Se	SeO_4^{2-} and SeO^{3-} **(1-3)**	≤ 0.01 **(1, 4-6)**	0.00001 – 0.1 **(7-13)**	SeO_4^{2-} or SeO^{3-} **(1-3)**	Good **(14,15)**	Roots and shoots **(16)**	Replaces sulphate in amino acids **(16,17)**
V	VO^{2+} and VO_4^{3-} **(18,19)**	0.004 – 4 **(20-24)**	0.0001 – 0.154 **(20,25)**	VO^{2+} or VO_4^{3-} **(18,19)**	Poor **(26)**	Roots **(26)**	Inhibits enzyme reactions, root growth, chlorophyll synthesis **(28-30)**
Cr	Cr^{3+} and Cr^{6+} **(31-33)**	0.005 – 5**(20,21,34-42)**	≤ 0.018 **(41,42)**	Cr^{6+} **(43-45)**	Poor **(42,46)**	Roots **(42,46)**	Reduces plant growth, germination and crop yield **(47-52)**
Co	Co^{2+} and Co^{3+} **(53-55)**	0.008 **(56)**	Up to 0.005 **(56)**	Co^{2+} **(57)**	Poor **(53,58,59)**	Roots and shoots **(53,58,59)**	Alter the number and structure of chloroplasts in leaves. Interveinal chlorosis and leaf fall. Inhibits Hill reaction and reduces shoot growth. Obstructs enzyme involved in CO_2 fixation in C4 and CAM plants.**(53,55,60)**
F	Combined with Al and Ca **(61-64)**	0.2 **(56)**	Up to 0.02 (56)	Combined with Al and Ca **(61-64)**	Poor **(65)**	Roots **(65)**	Interfere with metal-ion transport to leaves. Changes levels of enzymes, proteins and chlorophyll. Inhibits growth and reproduction **(66-69)**

I	IO^{3-} and I^{-} **(70)**	≤0.150 **(71,72-75)**	0.05 – 0.5 **(75)**	I^{-} **(76,77)**	Good **(78-79)**	Shoots **(78,79)**	Reduces biomass depending on chemical form of iodine **(79)**
Si	$Si(OH)_4$ **(1,80,81)**	330 **(56)**	0.2 – 10 **(56)**	H_2SiO_4 **(82)**	Good **(83)**	Roots and shoots **(84,85,86)**	No information
Sn	SnO_2 **(87,88)**	0.00089 – 0.2 **(88)**	≤ 0.004 – 0.3 **(88-92)**	/	Good **(92)**	Roots and Shoots **(92)**	Stunted growth, leaf chlorosis, reduced root size **(92)**

1.Marschner 1995 **2.**Ellis and Salt 2003 **3.**White *et al.* 2004 **4.**White and Broadley 2005a **5.**Sager 2006 **6.**Fordyce 2005 **7.**Wilber 1980 **8**.Wu *et al.* 1996 **9**.Galeas *et al.* 2007 **10.**Virupaksha and Shrift 1965 **11.**Davis 1972 **12**.0 **13.**Hamilton and Beath 1964 **14.**Shrift 1969 **15**.Terry *et al.* 2000 **16**.Harborne 1982 **17**.Sors *et al.* 2005 **18.**Poucheret *et al.* 1998 **19.**Mandiwana and Panichev 2004 **20.** Vinogradov 1959 **21.** Shacklette and Boerngen 1984 **22**. Anke 2004 **23.** Goc 2006 **24.** Poledniok and Buhl 2003 **25**.Hopkins *et al.* 1977 **26.**Furukawa *et al.* 1999 **27.**Serra *et al.*1990 **28.**Somasundaram *et al.* 1994 **29.**Hidalgo *et al.* 1988 **30.**Sklenar *et al.* 1994 **31**.Samantaray *et al.* 1998 **32**.Dayan and Paine 2001 **33.**Shrivastava *et al.* 2002 **34.**Shanker *et al.* 2005 **35.**Skeffington *et al.* 1976 **36.**Verry and Vermette 1991 **37**.Katz and Salem 1994 **38.**NAS 1974 **39.**Aubert and Pinta 1977 **40.** Smith *et al.* 1989 **41.** Pawlisz 1997 **42**.Samantaray *et al*. 1998 **43.**Cary and Kubota 1990 **44.** Cary *et al.* 1977a **45.** Cary *et al.* 1977b **46.**Lyon *et al.* 1970 **47.**Sharma and Sharma 1993 **48.**Hanus and Thomas 1993 **49**.Rout *et al.* 2000 **50.**Peralta *et al.* 2001 **51.**Mei *et al.* 2002 **52.**Shanker *et al.* 2005 **53**.Palit *et al.* 1994 **54.**De Boeck *et al.* 2003 **55.**Nagpal 2004 **56.**Larcher 2003 **57.**Gál *et al.* 2008 **58.**Barysas *et al.* 2002 **59.**Bakkaus *et al.* 2005 **60.**Chatterjee and Chatterjee 2003 **61.**Stevens *et al.* 1997 **62**.Stevens *et al.* 1998a **63.**Stevens *et al.* 1998b **64.**Madhavan and Subramanian 2002 **65.**Takmaz-Nisancioglu and Davison 1988 **66.**Kumar and Rao 2008 **67**.Rathore 1987 **68.**Rathore and Agrawal 1989 **69.**Gristan 1993 **70. 71.**Bowen 1979 **72**.Whitehead 1984 **73**.Coughtrey *et al.* 1983 **74**.Coughtrey *et al.*1985 **75.**Christiansen and Carlsen 1989 **76.**Zhu *et al.* 2003 **77**.Blasco *et al.* 2008 **78.**White and Broadley 2001 **79.**Roberts 2006 **80.**Yoshida 1975 **81.**Berthlesen *et al.* 2001 **82**.Zekri and Obreza 2013. **83.**Jones and Hendreck 1967 **84**.Epstein 1999 **85.**Savant *et al.* 1999**. 86.**Weber 1985 **87.**WHO 2005 **88.**Binzel *et al.*1988 **89.**IPCS 1980 **90**.Eckel and Langley 1988 **91.**Schafer and Femfert 1984**. 92.**Cohen 1940

1.3 Trace elements in humans and animals

In 2012, the Food and Agricultural Organisation (FAO) estimated that nearly 868 million people were undernourished, this representing nearly 12.5% of the human population (FAO *et al.* 2012). Of this total, 852 million (98.2 %) live in developing regions while only 16 million live in developed regions. Ninety-three percent of the undernourished people living in developing countries are found in Asia (65%) and Africa (28%) alone. Much of the available information on malnutrition tends to focus primarily on children under the age of five as the consequences of malnutrition here are more obvious (FAO 2010). There are approximately 177.7 million children under the age of five living in developing countries that are affected by malnutrition. Of this total, 130.7 million live in South Asia (41.5%) and Africa (32%) (Black *et al.* 2008, Horton *et al.* 2008). Nutritional deficiencies in childhood often result in excessive loss of cognitive abilities, and in severe conditions, high mortality rates (Horton *et al.* 2008). Table 1.5, taken from Calder and Jackson (2000), indicates the major causes of deaths among children younger than five years of age in developing countries in 1995.

However, due to the miniscule concentrations involved in regulating the proper functioning of various mammalian physiological systems, their deficiencies are often overlooked or, in many instances, only the symptoms caused by the deficiencies are treated. The World Health Organisation (WHO) in 1998 estimated that nearly 10 million children under the age of five die yearly in the developing countries. The majority of these deaths were however as a result of preventable illnesses, and over six million of these deaths were directly or indirectly as a result of malnutrition (WHO 1998).

The effectiveness of the immune defence system in protecting us from infectious diseases is determined by our nutritional status (Bhaskaram 2002, Calder and Kew 2002, Chandra 2002, Marcos *et al.* 2003). Immune responses can however be impaired by our dietary and nutritional status in two broadly classified ways. Immunocompetence can be impaired as a result of deficiencies in one or more essential nutrients, or as a result of very high or toxic concentrations of certain nutrients (Calder and Kew 2002, Marcos *et al.* 2003). Individuals that suffer from either deficiency or toxicity of one or more nutrients are at risk of increased infections. Infections in turn further compromise the nutritional status of individuals, resulting in a continuous cycle of morbidity which, if nothing is done to

correct the problem, could result in death (Calder and Jackson 2000, Chandra 2002, Marcos *et al.* 2003). Deficiencies in certain essential nutrients and various infectious diseases thus often coexist as these 'fuel' each other, especially in the poor, developing countries (Bhaskaram 2002).

Table 1.5: Major causes of deaths among children under five years of age in the developing countries in 1995 (Calder and Jackson 2000).

Cause of death	Deaths (millions)	Due to malnutrition (%)
Lower respiratory tract infections	2.1	44
Diarrhoea	2	70
Prematurity	1	40
Measles	1.1	65
Birth asphyxia	0.9	35
Malaria	0.7	40
Congenital abnormalities	0.5	30
Pertussis	0.4	50
Neonatal tetanus	0.4	20
Birth trauma	0.4	30
Neonatal sepsis and meningitis	0.4	30
Malnutrition	0.3	100
Tuberculosis	0.1	60
All other causes	0.2	40

As a result of the significant nutrient depletion from agricultural soils, and the lack of essential nutrients from fresh produce, the problem that we are facing today is not only to provide nutritional relief to the 868 million undernourished people, but to provide relief to approximately two billion people (*more than double the amount of undernourished people*) that are globally suffering from the 'hidden hunger', a term used to describe deficiencies in essential micronutrients and trace elements (Bouis and Islam 2011, FAO *et al.* 2012). The ranges between deficient, adequate and toxic concentrations of these elements to humans are very narrow due to the miniscule concentrations needed for the proper functioning of mammalian physiological systems. Adequate concentrations could thus easily be exceeded or deficiencies can easily be overlooked, negatively affecting various physiological systems in the human body (Farga 2005). The effects of these elements on human and animal lives are summarised in table 1.6.

Table 1.6: The essentiality, deficiency and toxicity of the focus trace elements.

ELEMENT	ESSENTIALITY, DEFICIENCY AND TOXICITY
Selenium (Se)	Selenium is incorporated into a series of selenoproteins and enzymes [1-4], and has been found to be an essential component of the antioxidant defence system, immune system and thyroid hormone metabolism [1, 3-6]. Various studies have also determined the anticarcinogenic properties [7-9] and protective properties against heavy metal toxicity [10-15]. Selenium deficiency is associated with reduced redox regulation, heart disease, reduced antioxidant protection, hyperthyroidism, reduced energy production as well as reduced immunocompetence. Due to the reduced immunocompetence, selenium deficiency often result in increased susceptibility to infections and other chronic, but preventable diseases [1-3, 16]. In China and Eastern Siberia selenium deficiency often results in Keshan and Keshan-Beck disease [17, 18]. Selenium toxicity results in selenosis characterised by hair loss, brittleness of nails, gastrointestinal disturbances, skin rash and abnormal functioning of the nervous system [19]. Other toxic effects include disruption of endocrine function, reduced immune functions and reduced synthesis of thyroid hormones and growth hormones [3].
Vanadium (V)	Vanadium is incorporated into the liver, heart, brain, kidney, muscles and bones [19-24]. It is important for the contraction of blood vessels as well as enhancing oxygen-affinity of haemoglobin and myoglobin. It also has anti-diabetic properties by lowering glucose, cholesterol and triglyceride levels as well as anticarcinogenic properties by reducing the occurrence of tumours [25-30]. Deficiency inhibits growth, impairs thyroid hormone metabolism, bone mineralization and disturbs lipid carbohydrate balance [31-33]. Toxic doses of vanadium results in haemolyses, reduced erythrocyte counts and reduced haemoglobin levels. Other toxic effects include a decrease in enzyme reactions, weight loss, weakness, nose bleeds, vomiting, diarrhoea, dehydration, morphological and/or functional damage to the liver, kidneys, bones, spleen and leucocytes, cognitive impairment as well as abnormalities in development and reproduction [30].

Chromium (Cr)	The essentiality of chromium to humans is still under discussion. Previous studies suggested that Cr^{3+} is required to promote the action of insulin by increasing insulin binding to cells, increasing insulin receptor numbers as well as activating insulin receptor kinases[34-37]. It is also suggested that chromium plays a role in glucose, lipid and protein metabolism[38-40]. No information was found relating to chromium deficiencies in humans due to the lack of deprivation studies. However, toxicity often result in blood and lung function problems, gene mutations and DNA lesions, suppressed immune responses and in severe cases, cardiac arrest[41-44].
Cobalt (Co)	Cobalt forms part of the vitamin B_{12} complex and is also a cofactor of various enzymes involved in DNA synthesis and amino-acid metabolism [6, 45-47]. Deficiency in cobalt results in vitamin B_{12} deficiency which is associated with the synthesis of DNA and the regulation of cellular division and growth[6]. In humans, toxicity is associated with polycythaemia and heart failure. Toxicity also negatively affects the male reproductive system, causes asthma, pneumonia as well as hyperthyroidism [48-54].
Fluoride (F)	Fluoride plays a major role in the development and strengthening of bones and teeth. The bacteria found in our mouths release acids, and the fluoride in our saliva reduces the amount of this acid, and by doing so, reduces the occurrence of dental caries. When exposure to high levels of this acid has affected teeth, fluoride also helps with enamel remineralisation which further protects the teeth against dental caries [50, 55-59]. Deficiencies in fluoride increase the occurrence of dental caries, cause growth retardation, osteoporosis, reduced fertility as well as resulting in anaemia [6, 60, 61]. Toxicity results in fluorosis, corrosion of the enamel of teeth, bone deformities, inhibited enzyme reactions, bone thickening and becoming ankylosed which results in reduced growth [62].

Iodine (I)	Iodine is an essential component needed for the synthesis of the thyroid hormones thyroxin and triiodothyronine [6, 63, 64]. Deficiencies affect carbohydrate metabolism, oxygen consumption and protein synthesis. It can also cause goitre and cretinism, and in severe cases physical and/or mental retardation [65-70]. Toxic concentrations affect the immune system through the depression of antibody responses, reducing phagocytosis and impairment of the responses by lymphocytes [41]. Toxicity can also impair reproductive functions, cause genetic mutations, heart problems, haemoptysis, cramping, bronchospasms and in severe cases, death [71-80].
Silicon (Si)	Dietary silicon is important for bone formation and bone health as well as for the initiation of cartilage calcification [81-83]. No silicon deprivation studies has been done on humans [83] however, animal studies have shown that deficiencies result in abnormal skull structures and poorly formed joints [81, 82, 84-88]. Toxic concentrations impair the immune system by depressing Tcell mitogen responses as well as reducing antibody production [41].

Tin (Sn)	No information on the essentiality of tin to humans was found however, studies done on rats made it possible to infer that tin is required for normal growth, and it was also found to improve pigmentation and incisor growth of the rats [6, 89]. No information was found on tin deficiency in humans or animals however, animal studies show that toxic concentrations negatively affect the central nervous system and spinal cord [90-100]. Toxicity in rats was found to reduce various levels of neurotransmitters in brains [101-104], lead to muscular weakness [105], anemia, as well as extensive damage to the liver and kidneys [106]. The toxic effects found in humans include muscular weakness and paralysis, vomiting, diarrhea, fatigue and headache [106, 107].

1: Brown and Arthur 2001 **2:** Ellis and Salt 2003 **3:** Navarro-Alarcon and Cabera-Vique 2008 **4:** Govasmak *et al.* 2010 **5:** Rayman 2000 **6:** Soetan *et al.* 2010 **7:** Clark *et al.* 1996 **8:** Reid *et al.* 2002 **9:** Whanger 2002 **10:** Caurant *et al.* 1996 **11:** Whanger 2004 **12:** Throne 2003 **13:** Cabanero *et al.* 2005 **14:** Kibriya *et al.* 2007 **15:** Mousa *et al.* 2007 **16:** Combs 2001 **17:** Hartikainen 2005 **18:** Li *et al.* 2007 **19:** Goldhaber 2003 **19:** Talvite and Wagner 1954 **20:** Ramandham *et al.* 1991 **21:** Fantus *et al.* 1995 **22:** Hammel and Duckworth 1995 **23:** Saponja and Vogel 1996 **24:** Wilsky *et al.* 2001 **25:** Rehder 1992 **26:** Thompson *et al.* 1993 **27:** Poucheret *et al.* 1998 **28:** Bishayee *et al.* 1999 **29:** Cam *et al.* 2000 **30:** Goc 2006 **31:** French and Jones 1993 **32:** Rojas *et al.* 1999 **33:** Moskalyk and Alfantazi 2003 **34:** Anderson 1989 **35:** Anderson 1997, **36:** Anderson 2000 **37:** Shrivastava 2002 **38:** Jirapinyo *et al.* 1985 **39:** Cobo *et al.* 1995 **40:** Mutuma *et al.* 1999 **41:** Chandra 1996 **42:** Dayan and Paine 2001 **43:** Shrivastava *et al.* 2002 **44:** Guertin 2004 **45:** Leonard and Leawerys 1990 **46:** De Boeck *et al.* 2003 **47:** Arinola *et al.* 2008 **48:** Haga *et al.* 1996 **49:** Cerklewski 1997 **50:** Murry *et al.* 2000 **51:** FSA 2003 **52:** ATSDR 2004 **53:** Nagpal 2004 **54:** USEPA 2005 **55:** Leone *et al.* 1955 **56:** Bernstein *et al.* 1966 **57:** Malhotra 1998 **58:** Rao 2003 **59:** Edmunds and Smedley 2005 **60:** Messer *et al.* 1997 **61:** Cerklewski 1998 **62:** Hays and Swenson 1985 **63:** Schomburg and Köhrle 2008 **64:** Gomez-Jacinto *et al.* 2010 **65:** McMichael *et al.* 1980 **66:** DeLange 1994 **67:** Marshall and Bangert 1995 **68:** WHO 1996 **69:** WHO 2002 **70:** Haldimann *et al.* 2005 **71:** Gessner *et al.* 1994 **72:** Cobra *et al.* 1997 **73:** DeLong *et al.* 1997 **74:** Penmann *et al.* 1997 **75:** Schulman and Wells 1997 **76:** Glinoer and DeLange 2000 **77:** Dunn and DeLange 2001 **78:** Jugdaohsing 2007 **79:** Ozsvath 2009 **80:** Gomez-Jacinto *et al.* 2010 **81:** Nielsen 1991 **82:** Seaborn and Nielesen 1994 **83:** Bisse *et al.* 2005 **84:** Carlisle 1972a **85:** Carlisle 1972b **86:** Brown *et al.* 1979 **87:** Carlisle 1982 **88:** Carlisle 1988 **89:** Schwarz 1974 **90:** Wender *et al.* 1974 **91:** Bouldin *et al.* 1981 **92:** Reiter *et al.* 1981 **93:** Chang *et al.* 1982a **94:** Chang *et al.* 1982b **95:** Chang *et al.* 1983 **96:** Chang and Dyer 1983 **97:** O'Callaghan *et al.* 1983 **98:** Bouldin *et al.* 1984 **99:** Chang *et al.* 1984 **100:** Fechter *et al.* 1986 **101:** Gerren *et al.* 1976 **102:** Graham *et al.* 1976 **103:** WHO 1980 **104:** Snoeij *et al.* 1987 **105:** JECFA 1982 **106:** Elliot *et al.* 1996 **107:** Poulter *et al.* 1990

1.4 Strategies to overcome nutritional deficiencies

Traditional strategies to provide the mineral nutrients lacking from human diets included food fortification programs, diversification of the diet and/or with the aid of dietary supplements (Table 1.7) (FAO and WHO 2001, Winkler 2011, FAO *et al.* 2012). Although few changes in the prevalence of micronutrient malnutrition can be attributed to dietary diversification programs (Faber *et al.* 2013), through inferential studies conducted in Guatemala, it was shown that micronutrient supplementation in childhood increased schooling, cognitive ability and reading comprehension. This was shown to be beneficial later in life as the test subjects were found to earn an annual income of approximately 14 – 28% higher than the control group (Hoddinott *et al.* 2008). It is thus clear that these strategies do work, and if nutrition is improved, the benefits to health as well as the financial situations in developing countries will be appreciable (Calder and Jackson 2000, Horton *et al.* 2008).

Table 1.7: Traditional strategies to improve nutrition (Kennedy *et al.* 2003)

Supplementation	Nutrients are delivered to humans by means of a syrup, nutritional shakes and/or tablets.
Fortification	The addition of nutrients to commonly consumed foods during
Diet Diversification	Consuming a wider variety of foods.

These strategies have however not always been successful, especially in the developing countries where they are needed the most (Mayer *et al.* 2008, FAO *et al.* 2012). Although the above mentioned strategies work, they can become costly. A diverse diet and pharmaceutical nutrient supplements in poor countries is a luxury that many of the people cannot afford. Fortification can cater for large populations however, the management of food fortification programs is time consuming and expensive, and in most cases the fortified foods are too costly for the poor. The biggest problem pertaining to these strategies is however the problem of reaching poor and rural populations that relies on the production of their own fresh produce. Recently biofortification has been proposed as a possible alternative to the above mentioned strategies (White and Broadley 2005a, Cakmak 2008, Winkler 2011).

1.5 Biofortification

Biofortification is the term used to describe the process of increasing essential mineral nutrients in the edible portions of crops (White and Broadley 2005ab, Winkler 2011). It is believed that biofortification is a more cost-effective approach, which means that there is a greater possibility of success in the developing countries (Nestel *et al.* 2006, Mayer *et al.* 2008). There are three different methods of biofortification. The first is through conventional breeding of superior crops, the second is through genetic modification and the third approach is through agronomic practices such as fertilization (White and Broadley 2005a, Zhao and Shewry 2010, Winkler 2011). All of these strategies are superior to the traditional methods however, these also have their flaws (Hirschi 2009).

Biofortification through conventional breeding relies on the natural genetic variation in the nutrient content of seeds as well as the ability of crops to take up essential nutrients from the soil. It is this genetic variation that allows breeding programs to improve the levels of essential nutrients in crops (Mehta *et al.* 2002, Welch 2002, Gelin *et al.* 2007, Cakmak 2008). The problem however is that there is very little genetic variation present in the gene pool of most modern crop varieties. In the absence of genetic variation in nutrient content in some crop species, biofortification through genetic modification and transgenetic approaches is used. Unlike biofortification through conventional breeding, genes can be manipulated, inserted or replaced, to not only improve the general uptake of nutrients, but also to target accumulation in the edible portions of crops (Hirschi 2009). The possibilities with selective breeding and genetic modification are seemingly endless, however, nearly all of these modified crops have patented or patentable technologies associated with them which brings extra costs and inhibits commercial applications thereof (Freese and Schubert 2004, Weil 2005, Johnson *et al.* 2007, Powell 2007, ISLI 2008). Also, although genetic modification of the plants seems to be a viable alternative, it does not address the underlying issues resulting in these deficiencies. The major reason for biofortification is to increase the concentrations of essential nutrients in the edible portions of crops. Genetically modifying crops to take up and store more nutrients from the soil only solves one half of the problem. The major issue is to replenish the nutrients lacking from the soil in order for these crops to take up and incorporate these nutrients in their tissues.

The third approach to biofortification is through fertilization. The addition of the trace elements to the fertilizer is fairly simple and inexpensive however, there are various complications associated with the method. These complications include the man power needed for the actual application of the fertilizers, the soil nutrient composition that needs to be actively monitored to ensure that the concentrations of the nutrients added do not exceed beneficial concentrations, determining whether the mineral nutrients supplied to the plants are mobile within the plant, and where the mineral nutrients are accumulated within the crops (Zhu *et al.* 2007). However, these are standard procedures of already existing agricultural strategies and even with all of these complications, there have been numerous successful applications of agronomic biofortification strategies (Delong *et al.* 1997, Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005, Broadley *et al.* 2010). Due to the fact that agronomic biofortification not only improves the uptake and incorporation of these essential elements into the edible portions of various crops, but also rejuvenates micronutrient reserves in the soil, it is argued here that biofortification through agronomic practices rather that biotechnology is the better option for increasing micronutrient concentrations in the human diet.

1.6 Objectives

Although biofortification clearly has a lot of potential, there is one criticism of biofortification as a whole. Only certain crops, mostly cereals and staple foods due to their wide availability and low cost in the developing and poor countries, and only certain elements, mostly iron, zinc, iodine and selenium, are targeted for biofortification (Welch and Graham 2002). The simple reason for this is that some deficiencies are more common and have more dire health consequences for the individuals affected by the deficiencies (Bouis *et al.* 2012). However, biofortification with a wider variety of essential mineral nutrients and a wider variety of crop species would however bring considerable benefits to human well-being across the world. Not all populations have the same diets, and it is no longer only the developing countries and the poor that have fallen prey to the "hidden hunger", but it is also becoming more prevalent in the developed countries (Graham *et al.* 2007, FAO *et al.* 2012).

Agronomic biofortification through fertilization is being proposed as possibly the best and most cost effective method to achieve suitable concentration levels of essential trace elements in agricultural crops (White and Broadley 2005b). The global production and use of macronutrient fertilizers to increase crop yields, makes these macronutrient fertilizers a convenient method to supply crops with micronutrients as well. Most of the available information on mineral nutrient deficiencies is however only from Europe, Asia and Central Africa. Here we try and determine whether these focus trace elements are deficient in the vegetable based food items in the Cape Town area in the Western Cape Province of South Africa. Previous studies have shown how certain of these elements decreased in concentration from 2000 to 2006 in the Philippi horticultural area, one of Cape Town's major fresh produce producers. It is thus important to determine whether there have been further decreases in these elements and others in the Cape Town area and how much does the element concentrations found in the fresh produce actually contribute to the daily recommended intake of these essential trace elements.

In the past, numerous papers have been published explaining the effects of the addition of single nutrient additions on plant growth and/or human health. However, with the significant nutrient depletion from agricultural soils, concurrent shortages of more than one element are likely to occur. Thus, we aim to assess the effects of eight trace elements on seed germination and root growth, as single nutrient additions as well as composite treatments, in order to calculate their phytotoxicity on seeds, and then to biofortify four vegetable crop species with a composite treatment of all eight these trace elements essential to the growth and development of humans. Thereafter, we aim to determine how the addition of these elements will affect plant growth, water content and the uptake of essential plant nutrients as well as the elements supplied to the plants under experimental conditions. From this we can then infer the effectiveness of agronomic biofortification with multiple trace elements, and suggest whether or not the research should now also be done under field conditions.

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CHAPTER 2

Essential trace element concentrations in vegetable crops collected from the greater Cape Town area in the Western Cape Province of South Africa

2.1 Abstract

Micronutrient deficiencies in agricultural crops have been receiving increasing attention internationally, but very little information is available about the micronutrient and trace element quality of fresh produce grown in South Africa. This study assessed the trace element nutritional quality of fresh produce and other food items collected in the Western Cape Province of South Africa. It was shown that trace element content in vegetable crops varied between different geographic locations and that certain trace elements were absent from several crop species collected from some areas. It also became clear that no one crop species contained sufficient concentrations of all the focus trace elements. Although some crop species contained sufficient amounts of certain trace elements to satisfy our daily recommended intakes, most of the crops were found to contain insufficient amounts of many of the trace elements to satisfy our needs. Leafy vegetables and tubers were identified as the better vegetable types to biofortify with essential trace elements as they already contain higher concentrations of several of the essential trace elements and should thus be assessed for their effectiveness as crops to be biofortified.

Key Words: trace elements, biofortification, malnutrition, hidden hunger

2.2 Introduction

In recent years, the primary focus of agriculture has been to increase the production of certain staple crops and cereals to feed an ever increasing human population, estimated to reach 10 billion by the year 2050. However, this increase in crop production has been done at the expense of the nutritional quality of not only staples and cereals, but many other crop varieties. Several researchers have indicated a decrease in the nutritional quality of the new crop varieties, and this is thought to primarily be due to the significant nutrient depletion from agricultural soils as a consequence of the increased production on agricultural lands which is exaggerated by the use of NPK fertilizers (Tan *et al.* 2005, White and Broadley 2005a, Garvin *et al.* 2006, Marler and Wallin 2006, Fan *et al.* 2008, Sands *et al.* 2009, Zhao *et al.* 2009, Bruulsema *et al.* 2012). This increase in the production of only a selected few crops had many unforeseen consequences. The most significant of these consequences is the increase in the prices of non-staple, more nutrient-dense crops, which in turn resulted in the more nutritious crops contributing a smaller proportion of the diets of the majority of the worlds poorer and rural communities (Temple and Steyn 2009, 2011, Temple *et al.* 2010, Bruulsema *et al.* 2012).

In South Africa, there are several barriers inhibiting the achievement of nutritional food security, including access to sufficient, safe and nutritious foods due to increasing food prices (Labadarios *et al.* 2011 a,b). Several studies that have been conducted in South Africa to assess food security and dietary diversity indicated that the majority of South Africans, especially those living in poor and rural communities, have a highly monotonous diet based primarily on starches and staples which are less nutritious than a diet rich in fresh produce (Labadarios *et al.* 2005, 2011a,b, Faber and Wenhold 2007, Steyn *et al.* 2008, Temple and Steyn 2009, 2011, Temple *et al.* 2010, Wolfhard 2010, Wenhold *et al.* 2012, Faber *et al.* 2013). However, it was also indicated that, even when healthier foods are available, the cost of a more nutrient-dense diet is up to 20 % more expensive than a diet based on cereals and other staples. The average South African household in rural and poor communities is comprised of four members. However, with a large proportion of South Africans living in poverty, earning less than R 1, 000 a month, it was estimated that approximately R 1, 146 is needed every month to produce a healthier, more nutrient-dense diet, for all four members in the households (Temple and Steyn 2009, Labadarios et al 2011b). It is for this reason that the majority of South Africans living in poor and rural

communities is forced to buy the cheaper, less nutrient-dense staples and cereals. This is also the reason why the majority of these individuals suffer from, or are at risk of developing multiple micronutrient deficiencies (Darmon and Drewnowski 2008, Temple and Steyn 2011, Labadarios *et al.* 2011a,b).

Millions of dollars are spent annually on food aid and fortification programmes in Africa including South Africa (Labadarios *et al.* 2011b). However, food aid programmes only cater for the neediest communities while food fortification programmes are limited in their effectiveness as diet preferences changes between different communities. For these programmes to work more effectively, various agricultural interventions should also be incorporated. Recently, home and community gardens have been identified as an important means to increase the nutrient status in poor and rural communities (Maunder and Meaker 2007, Faber *et al.* 2002 a,b, Faber and Laubscher 2008, Laurie and Faber 2008, Faber and Laurie 2011). However, these home and community gardens are only addressing certain micronutrient deficiencies as the nutritional quality of many crop species is insufficient to address all micronutrient needs. Although micronutrient deficiencies are more prevalent in rural and low-income communities, they are now also becoming more prevalent in urban and wealthier communities, and for this reason, other agricultural strategies are needed in order to deliver sufficient quantities of micronutrients to all. One of these strategies is agronomic biofortification through modified fertilizers, and this is being proposed as a possible means to enrich agricultural soils with several micronutrients at the same time, and thus, also all vegetable crops grown on these soils with these added essential trace elements. There have been several successful implementations of agronomic biofortification strategies globally (Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005, White and Broadley 2005b, Cakmak 2008, Broadley *et al.* 2010) and for this reason it is considered to be a viable option to try and mitigate micronutrient deficiencies in South Africa.

This study however, aims to determine the nutrient content (Co, Cr, V, Se, I and F) of various crop species collected from the Cape Town area in the Western Cape Province of South Africa. The primary goal of this study was to determine which vegetable crops provides the highest concentrations of essential trace elements, and how much they contribute to the daily recommended intake of these trace elements. From this information, it would be possible to determine which crops could be biofortified as well as with which

elements these crops should be biofortified in order to further increase the nutritional quality of the selected crops to supply a more nutrient-dense food source to those who cannot afford a diverse diet.

2.3 Materials and Methods

2.3.1 Sample collection

Samples (Table 1) were collected from four distributors from Strand, Somerset-West, Bellville/Parrow, Stellenbosch, Paarl/Wellington areas in the Western Cape Province of South Africa. Sampling was done based on the availability of the distributors at each of the sampling areas as well as the availability of the produce at each of the distributors. The samples were selected randomly from each distributor after which they were cut into slices or quartered. A 250 g (fresh mass) sample, of each of the items collected from each of the distributors and sampling areas, was oven-dried at 65 °C for 14 days after which it was milled using a stainless steel laboratory blender.

Table 2.1: Produce collected from the Cape Town area in the Western Cape Province of South Africa

Leafy vegetables	Tubers	Grains	Cereals
Spinach	Potato	Rice	
Cabbage	Turnip	Sweet Corn	Maize meal
Lettuce	Sweet Potato White	Green Beans	-
	Sweet Potato Orange	Samp	

2.3.2 Nutrient determination

2.3.2.1 Sulphuric peroxide digestion (Co, Cr, I and V determination)

A sulphuric peroxide digestion (Moore and Chapman 1986) was performed to determine the concentrations of Co, Cr, I and V in the samples. A 0.42 g amount of Se powder and 14 g of $Li_2SO_4.H_2O$ was added to 420 ml of vol. H_2O_2. The solution was mixed well and 350 ml conc. H_2SO_4 was carefully added to it. The mixture was cooled by placing it on ice during the addition of the acid. After use, the digestion mixture was stored at approximately 4 °C. A 0.4 g sample of the dry ground plant material was placed into a digestion tube. A 5 ml aliquot of the digestion mixture was then added. The mixture was digested in a heating block in a fume cupboard at 150 °C until the initial reaction was complete thereafter, the temperature was gradually increased to 380 °C until an almost

colourless solution was obtained. Glass funnels were used to cover the opening of the digestion tube to minimize the loss of fumes. After the digestion, the solution was transferred into a 50 ml volumetric flask after filtering, and diluted to volume with deionized water. Two blank solutions were prepared with each of the digestions.

I^- Determination

A 5 ml aliquot of the digested sample was diluted ten times with deionised water after the pH was adjusted to 8 using a concentrated NaOH solution (5 M). Iodine concentrations in the samples were then determined using a Spectroquant Pharo 300-M Unit spectrophotometer (Merc. (Pty) Ltd.).

Co, Cr and V Determination

These element concentrations were determined using a Unican M-series Pye Solar Atomic Absorption Spectrophotometer (Unicam Unlimited, Cambridge, UK). Cobalt concentrations were determined in flame mode while the rest were determined in furnace mode.

2.3.2.2 Nitric acid digestion (Se determination)

A 5 g sample of dry plant material was place in a digestion tube and digested with 10 ml of HNO_3 for approximately 30 minutes in a heating block in a fume cupboard at 120 °C. Glass funnels were used to cover the opening of the digestion tubes to minimize the loss of fumes. After cooling, 10 ml of deionized water and 5 ml HCL was added to the solution and boiled for another 10 minutes to convert Se (VI) to Se (IV). The solution was cooled down and transferred into a 50 ml volumetric flask after filtering. The mixture was thereafter neutralised with a 10 M NaOH solution and diluted to volume with deionized water after adding 5 ml of 5 % EDTA with a pH of 7. Two blank solutions were prepared with each of the digestions (Narayana *et al.* 2003, Mathew and Narayana 2006).

Preparation of standards

An aliquot of the solution containing 2 – 10 µg/ml of selenium was transferred into a series of 10 ml volumetric flasks. A volume of 1 ml 2% potassium iodide solution followed by 1 ml of 2 M hydrochloric acid was added to the solution and shaken gently until the

appearance of a yellow colour was observed. A (0.1 %) 0.5 ml solution of Azure B in methanol-water was added to the solution and shaken for approximately 2 minutes after which it was diluted to volume with deionised water. The solution was thereafter filtered through a syringe filter with a pore size of 0.45 µm and the absorbance was measured at 644 nm using a SHIMADZU (UV-160 A) UV-visible recording spectrophotometer. A reagent blank was also prepared and measured as zero concentration.

Se Determination

An aliquot (3 ml) of the digested solution prepared above was transferred into 10 ml volumetric flasks after which 1 ml of 2% potassium iodide and 1 ml 2M hydrochloric acid was added to it. The mixture was shaken gently until a yellow colour appeared which indicated the liberation of iodine. A 0.5 ml aliquot Azure B was added to this solution and the mixture was shaken for approximately 2 minutes after it was diluted to volume with deionized water. The mixture was filtered through a syringe filter with a pore size of 0.45 µm. The absorbance of the coloured solution was measured at 644 nm using a SHIMADZU (UV-160 A) UV-visible recording spectrophotometer.

2.3.2.3 Dry ashing (F determination)

A 2 g sample of the dry ground spinach, cabbage, potato, turnip, sweet potato, green beans and sweet corn, and a 3 g sample of maize, rice and samp were placed into nickel crucibles and ashed at 550 °C in a muffle furnace until a white ash was formed. After ashing, the samples were allowed to cool down for approximately 30 minutes, after which the ash was dissolved in 3 ml of NaOH (5 M). The mixture was filtered and acidified to a pH of 5.3 using glacial acetic acid after which it was diluted to 50 ml using deionized water (Mezghani *et al.* 2005).

F^- Determination

The extract (15 ml) was mixed 1:1 with TISAB II buffer with CDTA after which total fluoride was determined using an Orion Dual-Star pH and ISE benchtop meter with a fluoride ion specific electrode (9609BNWP and 960900 Fluoride combination electrode) (Mezghani *et al.* 2005) .

2.3.3 Determination of the contribution towards the daily recommended intake (DRI)

The amount of each trace element was calculated for one portion (40 g fresh mass or approximately 4 g dry mass) of each crop species. This was used to determine the percentage contribution that one portion of a specific crop would make to the daily recommended intake or in the absence of DRI, the tolerable upper limit (UL) of each element. The DRIs used in this study was obtained from http://search.nap.edu/napsearch.php?term=DRI.

2.3.4 Statistical analyses

The Statistical Package for the Social Sciences version 21 (SPSS Inc., Chicago IL) was used to perform an one-way Analyses of Variance (ANOVA) to determine whether there were statistically significant differences ($P \leq 0.05$) in trace element concentrations between the different crop species. After differences were found, a LSD Post Hoc test was done to determine between which crops these differences occurred.

2.4. Results and Discussion

Internationally, trace element concentrations in vegetable crops are receiving increasing attention due to their contribution to many of the worlds illnesses and deaths (Calder and Jackson 2000, FAO *et al.* 2012). In South Africa however, very little information exists pertaining to the micronutrient and trace element quality of fresh produce produced in the country. From the few studies that have been conducted in South Africa, it is clear that the nutritional quality of crops differs significantly between geographical locations (Steyn and Herselman 2005). In this study, it was found that in each of the sampling locations, there was a significant percentage of the fresh produce collected, that had no trace elements however, this differed between the sampling areas (Table 2.2). In the Wellington/Paarl and Stellenbosch areas, this was especially worrying as 47 and 53% of the produce collected from these areas respectively, had no detected chromium in the edible portions (Table 2.2). The high prevalence of fresh produce deficient in iodine and selenium is another worrying situation and a problem that is not only affecting South Africa, but also various other areas around the world. It is estimated that approximately 30 % of the worlds population is iodine deficient and 15 % is selenium deficient (White and Broadley 2009). The effects of a diet deficient in iodine and selenium have many adverse consequences and is summarised in table 1.6 (Chapter 1).

Table 2.2: The percentage of produce collected from different geographic locations in the Cape Town area in Western Cape Province of South Africa that contained no concentrations of each of the trace elements.

	Percentage crops with no trace elements					
	Cobalt	**Chromium**	**Vanadium**	**Iodine**	**Fluoride**	**Selenium**
Wellington/Paarl	26	47	33	25	2	36
Stellenbosch	26	53	29	29	0	29
Bellville	35	12	6	37	15	36
Somerset West	39	20	5	29	11	23
Strand	35	13	22	30	18	20

When the overall averages of each of the trace elements in each crop species were assessed, it became clear that no one single crop contained high enough concentrations of all the focus trace elements (Fig 2.1). Leafy and tuber crops, as well as green beans in some instances, seemed to contain higher concentrations of several of the focus trace elements, however, this was not always true for all leafy and tuber crops for all trace elements (Fig 2.1).

Maize meal, in most instances, contained the lowest or, one of the lowest concentrations, of several of the focus trace elements (Fig 2.1). This result is one that causes great concern as maize meal is a staple food in South Africa (Labadarios *et al.* 2005, Steyn and Herselman 2005). Results from the national food consumption survey of 1999 showed that between 78 and 95 % of the participants consumed maize meal the day before, or on the day of the survey. This was true for all South African provinces, except the Western Cape where only 31 % of the participants consumed maize meal (Labadarios *et al.* 2005).

A study done by Labadarios *et al.* (2011b) indicated that the four South African provinces with the highest prevalence for poor dietary diversity were the Eastern Cape, KwaZulu Natal, North West and Limpopo, while only 15.7 % of the participants in the Western Cape had a low dietary diversity. However, although dietary diversity was found to be higher in the Western Cape, results from this study indicates that trace element concentrations in fresh produce collected from the Western Cape are low and could still significantly affect the nutritional status of the people living there (Fig 2.1).

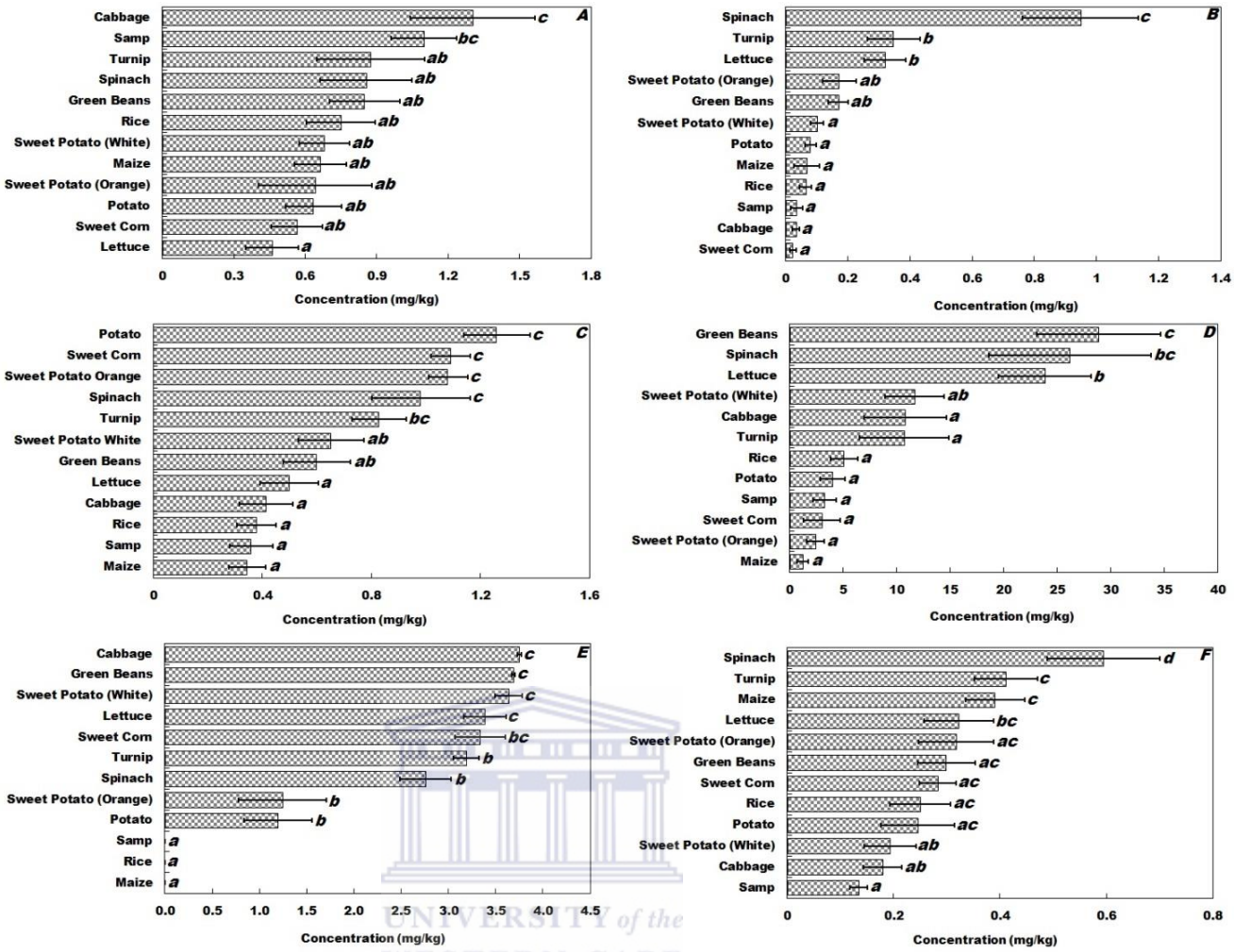

Figure 2.1: Trace element concentrations (mg/kg) in produce collected from the Western Cape Province of South Africa. Significant differences were found in cobalt (A: $F_{11} = 2.425$, $P = 0.010$), chromium (B: $F_{11} = 17.210$, $P < 0.001$), fluoride (C: $F_{11} = 8.509$, $P < 0.001$), iodine (D: $F_{11} = 6.923$, $P < 0.001$), selenium (E: $F_{11} = 44.445$, $P < 0.001$) and vanadium (F: $F_{11} = 3.193$, $P = 0.026$) concentrations between the different crops.

To determine whether the above statement is correct, the concentrations of the trace elements in each of the vegetable crops were assessed to determine the percentage they contribute to the daily recommended intakes (DRI) of each of the focus trace elements. Table 2.3 provides the daily recommended intakes of each of the focus trace elements however, in some instances, no DRIs were available and in these instances the upper tollerable limit (ULs) was used.

Tables 2.4 – 2.8 provides the contribution of each of the crops to the daily reccommended intakes of the focus trace elements. Leafy and tuber crops, and in some instances, grains (sweet corn and green beans) were found to contribute the most to the DRIs of chromium (Table 2.4), iodine (Table 2.5) and selenium (Table 2.6) while maize meal, which was found to be the most commonly consumed food item in rural and poor communities (Labadarios *et al.* 2005) only provided a small percentage of the DRI of each of the focus trace elements. All of the produce collected however, only contained a fraction of the DRI of fluoride (Table 2.7), and also of the upper limits of vanadium (Table 2.8). In each instance however, the group affected the most by the low nutrient content of the fresh produce, was lactating women as they require a higher DRI of each of the trace elements (Table 2.3).

From these results, it is thus clear that the contribution of certain of the vegetable crops to the DRI of some trace elements is sufficient however, several other vegetable crops contained insufficient concentrations of all trace elements. One should bear in mind that many of these elements can also be obtained from several other food sources such as meat, fish and dairy (Labadarios *et al.* 2011a). Also, no DRI or ULs were found for cobalt. Cobalt however forms part of the vitamine B_{12}-complex and is a cofactor of many enzymes (Table 1.6). Serious consideration is thus needed to determine DRIs for cobalt as deficiencies results in vitamine B_{12} deficiency which is associated with the synthesis of DNA and the also for the regulation of cellular division as well as growth (Table 1.6).

Table 2.3: Daily Recommended Intake (DRI) of essential trace elements.

	Age (Years)	Cobalt	Chromium (µg/d)	Vanadium (mg/d)*	Iodine (µg/d)	Selenium (µg/d)	Fluoride (mg/d)
Children	**1 to 3**	ND	11	ND	90	20	0.7
	4 to 8	ND	15	ND	90	30	1
Males	**9 to 13**	ND	25	ND	120	40	2
	14 to 18	ND	35	ND	150	55	3
	≥ 19	ND	35	1.8	150	55	4
Females	**9 to 13**	ND	21	ND	120	40	2
	14 to 18	ND	24	ND	150	55	3
	≥ 19	ND	25	1.8	150	55	3
Pregnancy	**14 to 18**	ND	29	ND	220	60	3
	19 to 50	ND	30	ND	220	60	3
Lactation	**14 to 18**	ND	44	ND	290	70	3
	19 to 50	ND	45	ND	290	70	3

ND: Not determined * Based on the upper tolerable limit as no DRI is available. DRIs available from: http://search.nap.edu/napsearch.php?term=DRI

Table 2.4: The percentage contribution that chromium concentrations in one portion of each of the vegetable crop provide to the daily recommended intake (DRI) of chromium.

Chromium	Percentage contribution to DRI											
	Children			Males			Females		Pregnancy		Lactation	
	1 to 3	4 to 8	9 to 13	14 to 18	≥ 19	9 to 13	14 to 18	≥ 19	14 to 18	19 to 50	14 to 18	19 to 50
Cabbage	1.22	0.89	0.54	0.38	0.38	0.64	0.56	0.54	0.46	0.45	0.30	0.30
Green Beans	6.18	4.53	2.72	1.94	1.94	3.24	2.83	2.72	2.34	2.26	1.54	1.51
Lettuce	11.63	8.53	5.12	3.66	3.66	6.09	5.33	5.12	4.41	4.27	2.91	2.84
Maize	2.45	1.79	1.08	0.77	0.77	1.28	1.12	1.08	0.93	0.90	0.61	0.60
Potato	2.87	2.10	1.26	0.90	0.90	1.50	1.32	1.26	1.09	1.05	0.72	0.70
Rice	2.34	1.72	1.03	0.74	0.74	1.23	1.07	1.03	0.89	0.86	0.59	0.57
Samp	1.29	0.94	0.57	0.40	0.40	0.67	0.59	0.57	0.49	0.47	0.32	0.31
Spinach	34.52	25.31	15.19	10.85	10.85	18.08	15.82	15.19	13.09	12.66	8.63	8.44
Sweet Corn	0.84	0.62	0.37	0.26	0.26	0.44	0.39	0.37	0.32	0.31	0.21	0.21
Sweet Potato (Orange)	6.26	4.59	2.75	1.97	1.97	3.28	2.87	2.75	2.37	2.30	1.57	1.53
Sweet Potato (White)	3.66	2.69	1.61	1.15	1.15	1.92	1.68	1.61	1.39	1.34	0.92	0.90
Turnip	12.63	9.26	5.56	3.97	3.97	6.61	5.79	5.56	4.79	4.63	3.16	3.09

Table 2.5: The percentage contribution that iodine concentrations in one portion of each of the vegetable crop provide to the daily recommended intake (DRI) of iodine.

Iodine	Percentage contribution to DRI											
	Children		Males			Females			Pregnancy		Lactation	
	1 to 3	4 to 8	9 to 13	14 to 18	≥ 19	9 to 13	14 to 18	≥ 19	14 to 18	19 to 50	14 to 18	19 to 50
Cabbage	47.89	47.89	35.92	28.73	28.73	35.92	28.73	28.73	19.59	19.59	14.86	14.86
Green Beans	128.33	128.33	96.25	77.00	77.00	96.25	77.00	77.00	52.50	52.50	39.83	39.83
Lettuce	105.98	105.98	79.48	63.59	63.59	79.48	63.59	63.59	43.36	43.36	32.89	32.89
Maize	5.34	5.34	4.00	3.20	3.20	4.00	3.20	3.20	2.18	2.18	1.66	1.66
Potato	17.70	17.70	13.27	10.62	10.62	13.27	10.62	10.62	7.24	7.24	5.49	5.49
Rice	22.53	22.53	16.90	13.52	13.52	16.90	13.52	13.52	9.22	9.22	6.99	6.99
Samp	14.46	14.46	10.84	8.67	8.67	10.84	8.67	8.67	5.91	5.91	4.49	4.49
Spinach	116.25	116.25	87.19	69.75	69.75	87.19	69.75	69.75	47.56	47.56	36.08	36.08
Sweet Corn	13.38	13.38	10.03	8.03	8.03	10.03	8.03	8.03	5.47	5.47	4.15	4.15
Sweet Potato (Orange)	10.72	10.72	8.04	6.43	6.43	8.04	6.43	6.43	4.39	4.39	3.33	3.33
Sweet Potato (White)	51.84	51.84	38.88	31.10	31.10	38.88	31.10	31.10	21.21	21.21	16.09	16.09
Turnip	47.51	47.51	35.63	28.51	28.51	35.63	28.51	28.51	19.44	19.44	14.74	14.74

Table 2.6: The percentage contribution that selenium concentrations in one portion of each of the vegetable crop provide to the daily recommended intake (DRI) of selenium.

Selenium	Percentage contribution to DRI											
	Children		Males			Females			Pregnancy		Lactation	
	1 to 3	4 to 8	9 to 13	14 to 18	≥ 19	9 to 13	14 to 18	≥ 19	14 to 18	19 to 50	14 to 18	19 to 50
Cabbage	75.08	50.05	37.54	27.30	27.30	37.54	27.30	27.30	25.03	25.03	21.45	21.45
Green Beans	73.74	49.16	36.87	26.81	26.81	36.87	26.81	26.81	24.58	24.58	21.07	21.07
Lettuce	67.74	45.16	33.87	24.63	24.63	33.87	24.63	24.63	22.58	22.58	19.35	19.35
Maize	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Potato	23.98	15.99	11.99	8.72	8.72	11.99	8.72	8.72	7.99	7.99	6.85	6.85
Rice	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Samp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spinach	55.14	36.76	27.57	20.05	20.05	27.57	20.05	20.05	18.38	18.38	15.75	15.75
Sweet Corn	66.70	44.47	33.35	24.25	24.25	33.35	24.25	24.25	22.23	22.23	19.06	19.06
Sweet Potato (Orange)	24.90	16.60	12.45	9.05	9.05	12.45	9.05	9.05	8.30	8.30	7.11	7.11
Sweet Potato (White)	72.76	48.51	36.38	26.46	26.46	36.38	26.46	26.46	24.25	24.25	20.79	20.79
Turnip	63.80	42.53	31.90	23.20	23.20	31.90	23.20	23.20	21.27	21.27	18.23	18.23

Table 2.7: The percentage contribution that fluoride concentrations in one portion of each of the vegetable crop provide to the daily recommended intake (DRI) of fluoride.

Fluoride	Percentage contribution to DRI											
	Children		Males			Females			Pregnancy		Lactation	
	1 to3	4 to8	9 to 13	14 to 18	≥ 19	9 to 13	14 to 18	≥ 19	14 to 18	19 to 50	14 to 18	19 to 50
Cabbage	0.24	1.50	0.08	0.06	0.04	0.08	0.06	0.06	0.06	0.06	0.06	0.06
Green Beans	0.34	1.47	0.12	0.08	0.06	0.12	0.08	0.08	0.08	0.08	0.08	0.08
Lettuce	0.29	1.35	0.10	0.07	0.05	0.10	0.07	0.07	0.07	0.07	0.07	0.07
Maize	0.20	0.00	0.07	0.05	0.03	0.07	0.05	0.05	0.05	0.05	0.05	0.05
Potato	0.72	0.48	0.25	0.17	0.13	0.25	0.17	0.17	0.17	0.17	0.17	0.17
Rice	0.22	0.00	0.08	0.05	0.04	0.08	0.05	0.05	0.05	0.05	0.05	0.05
Samp	0.21	0.00	0.07	0.05	0.04	0.07	0.05	0.05	0.05	0.05	0.05	0.05
Spinach	0.56	1.10	0.20	0.13	0.10	0.20	0.13	0.13	0.13	0.13	0.13	0.13
Sweet Corn	0.62	1.33	0.22	0.15	0.11	0.22	0.15	0.15	0.15	0.15	0.15	0.15
Sweet Potato (Orange)	0.62	0.50	0.22	0.14	0.11	0.22	0.14	0.14	0.14	0.14	0.14	0.14
Sweet Potato (White)	0.37	1.46	0.13	0.09	0.07	0.13	0.09	0.09	0.09	0.09	0.09	0.09
Turnip	0.47	1.28	0.17	0.11	0.08	0.17	0.11	0.11	0.11	0.11	0.11	0.11

Table 2.8: The percentage contribution that vanadium concentrations in one portion of each of the vegetable crops provide to the tolerable upper limits (ULs) of vanadium.

Vanadium	Percentage contribution to UL											
	Children			Males		Females			Pregnancy		Lactation	
	1 to 3	4 to 8	9 to 13	14 to 18	≥ 19	9 to 13	14 to 18	≥ 19	14 to 18	19 to 50	14 to 18	19 to 50
Cabbage	ND	ND	ND	ND	0.04	ND	ND	0.04	ND	ND	ND	ND
Green Beans	ND	ND	ND	ND	0.07	ND	ND	0.07	ND	ND	ND	ND
Lettuce	ND	ND	ND	ND	0.07	ND	ND	0.07	ND	ND	ND	ND
Maize	ND	ND	ND	ND	0.09	ND	ND	0.09	ND	ND	ND	ND
Potato	ND	ND	ND	ND	0.05	ND	ND	0.05	ND	ND	ND	ND
Rice	ND	ND	ND	ND	0.06	ND	ND	0.06	ND	ND	ND	ND
Samp	ND	ND	ND	ND	0.03	ND	ND	0.03	ND	ND	ND	ND
Spinach	ND	ND	ND	ND	0.13	ND	ND	0.13	ND	ND	ND	ND
Sweet Corn	ND	ND	ND	ND	0.06	ND	ND	0.06	ND	ND	ND	ND
Sweet Potato (Orange)	ND	ND	ND	ND	0.07	ND	ND	0.07	ND	ND	ND	ND
Sweet Potato (White)	ND	ND	ND	ND	0.04	ND	ND	0.04	ND	ND	ND	ND
Turnip	ND	ND	ND	ND	0.09	ND	ND	0.09	ND	ND	ND	ND

ND: Not determined

2.5. Conclusion

The findings of the present study are a cause of serious concern. With a low dietary variety, consisting primarily of staples and cereals (Labadarios *et al.* 2005, 2011a,b), coupled with the low nutritional status of several crops, including that of maize meal, found in the current study, a nation-wide intervention to address these issues is urgently needed. The food based dietary guidelines that is being promoted by the Department of Health (DOH) in South Africa, which states that plenty of fruits and vegetables, and plenty of dry beans, lentils and soya should be eaten regularly (Love *et al.* 2001) is something that many South Africans cannot follow due to the cost of these healthier food items. However, even when these items are available to those who can afford it, this study indicates that most of the produce collected in the Western Cape area does not, or contains very low concentrations of certain of the essential trace elements.

Although food-based approaches such as food aid- and fortification programmes are ideal to address these issues over a short time period, long term agricultural interventions are needed to permanently address these issues. Agronomic biofortification through modified fertilizers could thus be a viable, more cost effective long term approach with almost immediate results. The addition of the trace elements to fertilizers not only addresses the issue of the nutrient provision to agricultural soils, but also provides more nutrient-dense crops which mean that those individuals who cannot afford a large variety of fresh produce could attain their DRI from fewer crops. From this study, leafy vegetables and tubers seem to be the better vegetable types to biofortify as they already contain higher concentrations of several of the essential trace elements and should thus be assessed for their effectiveness as crops to be biofortified.

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CHAPTER 3

The effects of the addition of eight trace elements on cress and lettuce seed germination and root growth.

3.1 Abstract

Biofortification through fertilization has been proposed as a means to enrich agricultural soils with trace elements and micronutrients lacking from human diets. The early stages of plant development could however be significantly affected by the addition of these elements as they do not form part of the 17 elements deemed metabolically essential to plants. This study investigated the effects of eight trace elements on seed germination and root growth of lettuce and cress seeds under experimental conditions. Seeds were germinated under controlled conditions in 90 mm petri dishes on filter paper using treatments containing the trace elements as single nutrients and as a composite treatment of all eight elements added to a nutrient solution. All eight trace elements, as well as the composite treatments, exerted phytotoxic effects on cress and/or lettuce seeds when applied at the highest concentrations. Lettuce was found to be more prone to the effects of these elements. Seed germination was strongly inhibited by fluoride, while several elements affected root growth. When fluoride was left out of the composite treatment, phytotoxicity only occurred at the higher concentrations. Further studies on a wider variety of seeds are needed as it was shown here that different species are affected differently by the addition of these trace elements. Further research under field conditions is also needed as the availability and possibly also the phytotoxicity of many of the trace elements might change under those conditions.

Key words: Biofortification, Fluoride, Home gardens, Malnutrition, Phytotoxicity

3.2 Introduction

There are several elements that are metabolically essential to humans and other mammals, but not to the plants they eat (Table 1.1, Chapter 1) (Marschner 1995, Hopkins and Hüner 2004, White and Broadley 2005a, White and Brown 2010). Because plants are the major sinks for mineral nutrients in the terrestrial environment, they are the primary sources of these nutrients to the human diet. However, because these elements do not form part of the 17 elements deemed metabolically essential to plants for their growth and development, their deficiencies in agricultural soils and crops often go unnoticed (Tables 1.1 and 1.6: Chapter 1). This has been one of the major causes for various health problems, either directly or indirectly relating to deficiencies in essential trace elements and micronutrients, further up the food chain (Table 1.5. and 1.6: Chapter 1). Recently biofortification through modified fertilizers has been proposed as a possible means to enrich agricultural soils with the essential nutrients lacking from human diets. This in turn is believed to result in increased concentrations of these elements in the edible portions of the crops grown on these soils, which, in turn, should supply sufficient quantities of these essential nutrients to human diets (White and Broadley 2005b).

Various results as to the effects of single nutrient additions on the growth of already established crops exist (Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005, White and Broadley 2005b, Cakmak 2008, Broadley *et al.* 2010). However, vegetables are grown from seeds, and the early stages of plant development are more prone to the possible phytotoxic effects of chemicals and other substances (Carlson *et al.* 1991, De Vere Burton and Cooper 2005, Gupta *et al.* 2009, Hema and Subramani 2013). The addition of fertilizers containing additional trace elements and micronutrients to agricultural soils, that are not essential to plants, could thus have adverse consequences on the growth and development of the new seeds sown on these soils. Although agricultural businesses generally germinate seeds under controlled conditions before transplanting, crops that are grown in home and community gardens are primarily sown directly on soils (De Vere Burton and Cooper 2005). Due to the fact that the majority of under and malnourished people live in the poor, developing countries, and cannot afford to buy fortified foods and nutrient supplements (Table 1.7, Chapter 1), home and community gardens play a crucial role in the acquisition of mineral nutrients by these individuals (Faber *et al.* 2002, 2007, Bolaane 2006, Faber *et al.* 2013).

The purpose of this study was thus, to determine the effects of eight trace elements (Co, Cr, F, I, Se, Si, Sn and V) on seed germination and root growth, as single nutrient additions, and as a composite treatment of the eight trace elements, added to a commercial fertilizer. This was done to subsequently determine which of these elements will influence germination and root growth, and at what concentration they exert phytotoxicity on the seeds. In many countries, nutrient depletion from agricultural soils is extensive (Table 1.3: Chapter 1), and thus concurrent shortages of more than one trace element are likely to occur, which is why the composite treatment was tested.

3.3 Materials and Methods

3.3.1 Growing conditions

Twenty five seeds of two crop species, lettuce (*Lactuca sativa* L. var. Crisp Great Lakes) and cress (*Lepidium sativum* L.var. Garden Cress Common) were germinated under controlled conditions in 90 mm petri dishes on filter paper (n = 25 seeds/petri dish). Temperatures were kept at a constant 20 ºC during the day and 10 ºC during the night over a 14 hour day and 10 hour night. These crops were germinated using four treatments which were replicated three times. Table 3.1 provides an overview of the concentrations of the focus trace elements that were used in treatments one, two and three. The stock nutrient solution (Plant Food- Starke Ayres) was used as the control treatment (Table 3.2). The composite treatments were made using the concentrations given in treatments one to three.

Table 3.1: Trace element concentrations (g/kg) added to the nutrient solution and the chemical form in which they were added to the solution.

		Concentration (g/kg)		
Element	**Chemical form**	**Treatment 1**	**Treatment 2**	**Treatment 3**
Co	$CoSo_4.7H_2O$	0.0002	0.002	0.02
Cr	CrO_3	0.001	0.01	0.1
F	NH_4F	0.004	0.04	0.4
I	KIO_3	0.004	0.04	0.4
Se	Na_2SeO_4	0.002	0.02	0.2
Si	*	0.004	0.04	0.4
Sn	$SnCl_2.2H_2O$	0.0002	0.002	0.02
V	NH_4VO_3	0.0002	0.002	0.02

***1000 mg/L Spectrosol standard solution for atomic absorption spectroscopy

Table 3.2: Elemental make-up, before dilution, of the nutrient solution (control treatment) applied to the vegetable crops.

Concentration (g/kg)									
N	**P**	**K**	**Mg**	**Fe**	**B**	**Zn**	**Cu**	**Mn**	**Mo**
146	43	274	29	1.8	0.24	0.05	0.02	0.24	0.01

3.3.2 Calculation of growth indices and phytotoxicity

Cress was allowed to grow for six days (five days after sowing) and lettuce for 15 days (14 days after sowing), after which they were harvested. The total amount of seed germination (%) and the rate at which germination occurred (number of seeds germinating per day) were determined during the growing period while root lengths were measured at the end of the growing period. Seeds with a radicle length of 1 mm were considered to have germinated. The seeds which did not germinate were given a root length value of zero. The percentages of relative seed germination (RSG) and relative root growth (RRG) were calculated in order to determine the germination index (GI) (Miaomaio *et al.* 2009, Jakubus 2012).

RSG (%) = (Number of seeds germinated in treatments / Number of seeds germinated in control) x 100

RRG (%) = (Mean root length in treatments / Mean root length in control) X 100

GI (%) = (RSG x RRG) / 100

3.3.3 Statistical analyses

The Statistical Package for the Social Sciences (SPSS Inc., Chicago IL) was used to test the data for normality using a Shapiro-Willks test. Percentage seed germination data was transformed back to numbers after which the data was log + 0.01 transformed. An analysis of variance was done to determine whether there were statistically significant differences ($P \leq 0.05$) in seed germination between the different treatments for each crop species. After differences were found, a LSD Post Hoc test was analysed to determine between which treatments these differences occurred. Root length data was subject to a Kruskal-Wallis analysis to determine whether there were significant differences between the different treatments.

3.4 Results and Discussion

3.4.1 Germination

In this study the majority of the trace elements applied as single nutrient additions to the seeds of both cress and lettuce plants had no negative effects on seed germination ($P \geq 0.05$). However, cress seed germination (Table 3.3) was found to be significantly reduced by the additions of the higher concentrations of cobalt and fluoride as single nutrient additions, while lettuce seed germination (Table 3.4) was significantly reduced by the additions of the higher concentrations of fluoride, tin and vanadium as single nutrient additions. When the composite treatment of all eight trace elements were applied to cress (Table 3.3) and lettuce (Table 3.4) seeds, it was found that seed germination was significantly reduced by the treatments containing the higher concentrations of the trace elements (treatment two and tree).

These significant inhibitory effects on germination by the addition of the composite treatment was believed to be as a result of the fluoride in the treatments based on what was found when fluoride was applied to both cress and lettuce seeds as a single nutrient addition. In both instances, fluoride, applied as a single nutrient addition, and within the composite treatment, affected lettuce germination more severely than cress. The inhibitory effects of fluoride applied to the lettuce seeds occurred at lower concentrations while cress was only affected at the higher concentrations.

These inhibitory properties of fluoride on seed germination have been well documented. The results obtained in this study corresponds to the findings of Sabal *et al.* (2006), Gupta *et al.* (2009), Bhargava and Bhardway (2010), Chakrabari *et al.* (2012), Saini *et al.* (2012), Gadi *et al.* (2012) and Singh *et al.* (2013) who found that seed germination significantly decreased with increasing fluoride applied to the seeds of several plant species. To determine whether fluoride was the major inhibitory factor influencing germination, a composite treatment without the addition of fluoride was applied to cress (Table 3.3) and lettuce (Table 3.4) seeds.

Without the addition of fluoride in the treatments, no significant decreases in germination ($P \geq 0.05$) were found between the different treatments in both cress and lettuce seeds. Thus, confirming that the significant inhibition of seeds observed by the application of the

composite treatments was as a result of the fluoride in the treatment. These significant inhibitory effects of fluoride on seed germination are thought to be as a result of the inhibitory properties of fluoride on carbohydrate metabolism (Weinstein 1977), specifically the reduction of amylase activity (Gadi *et al.* 2012) in germinating seedlings.

Table 3.3: Percentage cress seed germination after the addition of eight trace elements as single nutrient additions and as composite treatments. Percentages with the same letters are not significantly different from one another ($P \geq 0.05$). Comparisons were made between treatments for each element and not between elements.

	Germination (%) ± SE									
	Co	**Cr**	**F**	**I**	**Se**	**Si**	**Sn**	**V**	**Composite**	**Composite - F**
Control	96 ± 0.9^{bc}	96 ± 1.2^{a}	96 ± 1.6^{c}	96 ± 1.6^{a}	96 ± 1.2^{a}	96 ± 1.2^{a}	96 ± 1.2^{a}	96 ± 1.2^{a}	96 ± 1.2^{b}	96 ± 1.2^{a}
Treatment 1	99 ± 1.3^{c}	96 ± 0.3^{a}	99 ± 1.3^{c}	93 ± 3.3^{a}	96 ± 4.0^{a}	93 ± 1.8^{a}	96 ± 1.9^{a}	96 ± 2.3^{a}	95 ± 1.3^{b}	97 ± 1.3^{a}
Treatment 2	95 ± 0.7^{b}	99 ± 0.9^{a}	88 ± 6.1^{b}	95 ± 3.5^{a}	93 ± 2.7^{a}	88 ± 2.3^{a}	96 ± 0.9^{a}	99 ± 0.7^{a}	92 ± 2.3^{b}	96 ± 0.0^{a}
Treatment 3	91 ± 0.7^{a}	88 ± 4.1^{a}	0 ± 0.0^{a}	97 ± 1.3^{a}	93 ± 1.5^{a}	99 ± 0.7^{a}	91 ± 5.5^{a}	95 ± 2.4^{a}	0 ± 0.0^{a}	97 ± 1.3^{a}
F_3	***10.955***	NS	***12204.87***	NS	NS	NS	NS	NS	***64196.24***	NS
P	***0.003***		***< 0.001***						***< 0.001***	

ns = not significant ($P \geq 0.05$)

Table 3.4: Percentage lettuce seed germination after the addition of eight trace elements as single nutrient additions and as composite treatments. Percentages with the same letters are not significantly different from one another ($P \geq 0.05$). Comparisons were made between treatments for each element and not between elements.

	Germination (%) ± SE									
	Co	**Cr**	**F**	**I**	**Se**	**Si**	**Sn**	**V**	**Composite**	**Composite - F**
Control	96 ± 4.0^{a}	96 ± 4.0^{a}	96 ± 4.0^{c}	96 ± 4.0^{a}	96 ± 4.0^{a}	96 ± 4.0^{a}	96 ± 4.0^{b}	96 ± 4.0^{b}	96 ± 4.0^{c}	96 ± 4.0^{a}
Treatment 1	84 ± 2.3^{a}	88 ± 4.0^{a}	82 ± 2.0^{b}	84 ± 8.0^{a}	92 ± 0.0^{a}	88 ± 2.3^{a}	77 ± 5.3^{a}	83 ± 1.1^{a}	86 ± 6.0^{c}	88 ± 2.3^{a}
Treatment 2	78 ± 6.0^{a}	90 ± 6.0^{a}	84 ± 4.0^{b}	73 ± 3.5^{a}	72 ± 8.0^{a}	76 ± 8.0^{a}	73 ± 1.3^{a}	88 ± 0.0^{ab}	69 ± 5.3^{b}	87 ± 1.3^{a}
Treatment 3	84 ± 4.0^{a}	88 ± 4.0^{a}	0 ± 0.0^{a}	84 ± 4.0^{a}	74 ± 2.0^{a}	87 ± 5.8^{a}	82 ± 2.0^{ab}	82 ± 2.0^{a}	0 ± 0.0^{a}	85 ± 2.7^{a}
F_3	NS	NS	***21220.235***	NS	NS	NS	***5.622***	***8.477***	***5229.192***	NS
P			***< 0.001***				***0.035***	***0.033***	***< 0.001***	

ns = not significant ($P \geq 0.05$)

3.4.2 Root Growth

Root length was found to be a more sensitive indicator of the effects of several of the focus trace elements applied to the seeds as single nutrient additions. In both cress (Table 3.5) and lettuce (Table 3.6) root growth was significantly reduced by several of the focus trace elements. However, the treatments containing iodine and silicon applied to cress seeds, as well as cobalt and tin applied to lettuce seeds, as single nutrient additions, did not affect root growth ($P \geq 0.05$). Both cress and lettuce root length were however significantly reduced by the additions of chromium, fluoride, selenium and vanadium, applied to the seeds as single nutrient additions, at the highest concentrations (treatments two and/or tree). This significant decrease in root length corresponds to the findings of several other researchers, who found that the addition of several of these trace elements, as single nutrient additions, decreased root growth of various plant species as the concentrations of the trace elements in the treatments applied to the plants increased (Stevens *et al.* 1998, Wang and Liu 1999, Singh *et al.* 2006, Gupta *et al.* 2009, Jun *et al.* 2009, Hema and Subramani 2013).

It thus came as no surprise that the addition of the composite treatment of all eight elements also significantly reduced both cress (Table 3.5) and lettuce (Table 3.6) root growth. Unlike germination which was affected primarily by the addition of fluoride to the treatment, root length was reduced by several elements. Thus, even without the addition of fluoride in the composite treatments, root length remained significantly reduced by the addition of these treatments applied to the seeds at the highest concentrations (treatments two and tree).

Table 3.5: Mean root length (mm) of cress seeds after the addition of eight trace elements as single nutrient additions and as composite treatments. Means with the same letters are not significantly different from one another ($P \geq 0.05$). Comparisons were made between treatments for each element and not between elements.

	Mean root length (mm) ± SE									
	Co	**Cr**	**F**	**I**	**Se**	**Si**	**Sn**	**V**	**Composite**	**Composite - F**
Control	16.6 ± 1.0^{b}	16.6 ± 1.0^{c}	16.6 ± 1.0^{c}	16.6 ± 1.0^{a}	16.6 ± 1.0^{b}	16.6 ± 1.0^{a}	16.6 ± 1.0^{b}	16.6 ± 1.0^{ab}	16.6 ± 1.0^{c}	16.6 ± 1.0^{b}
Treatment 1	20.4 ± 0.9^{b}	13.1 ± 0.9^{bc}	16.2 ± 1.1^{c}	13.4 ± 1.0^{a}	21.1 ± 1.2^{b}	16.8 ± 1.0^{a}	13.6 ± 1.0^{b}	22.2 ± 5.2^{b}	13.7 ± 0.9^{c}	28.5 ± 1.6^{c}
Treatment 2	10.6 ± 0.9^{a}	11.2 ± 0.7^{b}	6.1 ± 0.6^{b}	17.1 ± 1.2^{a}	18.7 ± 1.4^{b}	16.4 ± 1.3^{a}	8.0 ± 0.5^{a}	20.0 ± 1.3^{b}	4.6 ± 0.3^{b}	19.5 ± 0.9^{b}
Treatment 3	16.9 ± 1.2^{b}	3.6 ± 0.3^{a}	0 ± 0.0^{a}	17.0 ± 1.0^{a}	8.0 ± 0.6^{a}	17.3 ± 1.2^{a}	7.3 ± 0.5^{a}	14.4 ± 0.9^{a}	0 ± 0.0^{a}	8.2 ± 0.5^{a}
H_3	***42.181***	***120.176***	***184.738***	NS	***68.727***	NS	***68.832***	***9.991***	***200.003***	***111.647***
P	***< 0.001***	***< 0.001***	***< 0.001***		***< 0.001***		***< 0.001***	***0.019***	***< 0.001***	***< 0.001***

ns = not significant ($P \geq 0.05$)

Table 3.6: Mean root length (mm) of lettuce seeds after the addition of eight trace elements as single nutrient additions and as composite treatments. Means with the same letters are not significantly different from one another ($P \geq 0.05$). Comparisons were made between treatments for each element and not between elements.

	Mean root length (mm) ± SE									
	Co	**Cr**	**F**	**I**	**Se**	**Si**	**Sn**	**V**	**Composite**	**Composite - F**
Control	4.7 ± 0.6^{a}	4.7 ± 0.6^{b}	4.7 ± 0.6^{b}	4.7 ± 0.6^{b}	4.7 ± 0.6^{c}	4.7 ± 0.6^{c}	4.7 ± 0.6^{a}	4.7 ± 0.6^{c}	4.7 ± 0.6^{c}	4.7 ± 0.6^{b}
Treatment 1	4.8 ± 0.6^{a}	4.6 ± 0.6^{b}	4.1 ± 0.7^{b}	3.5 ± 0.3^{b}	3.3 ± 0.4^{bc}	4.8 ± 0.6^{c}	4.0 ± 0.6^{a}	3.3 ± 0.6^{b}	3.1 ± 0.3^{bc}	4.4 ± 0.5^{b}
Treatment 2	4.0 ± 0.4^{a}	4.4 ± 0.4^{b}	2.6 ± 0.3^{b}	2.3 ± 0.6^{a}	2.0 ± 0.2^{ab}	2.4 ± 0.5^{a}	3.7 ± 0.6^{a}	3.2 ± 0.3^{b}	2.0 ± 0.2^{b}	3.3 ± 0.4^{a}
Treatment 3	4.6 ± 0.5^{a}	1.9 ± 0.1^{a}	0 ± 0.0^{a}	3.2 ± 0.4^{b}	1.7 ± 0.2^{a}	3.8 ± 0.6^{b}	3.5 ± 0.6^{a}	1.9 ± 0.1^{a}	0.0 ± 0.0^{a}	2.7 ± 0.3^{a}
F_3	NS	***50.213***	***121.983***	***10.853***	***33.604***	***35.809***	NS	***32.217***	***127.38***	***20.76***
P		***< 0.001***	***< 0.001***	***0.013***	***< 0.001***	***< 0.001***		***< 0.001***	***< 0.001***	***< 0.001***

ns = not significant ($P \geq 0.05$)

3.4.3 Phytotoxicity

The germination index (GI) was used to assess phytotoxicity. A percentage equal to or greater than 80 % indicated that the treatment exerted no phytotoxic effect on the seeds (Araujo and Monteiro 2005, Paradelo *et al.* 2012). In this study all eight trace elements, as well as the composite treatments were found to exert phytotoxic effects on cress and/or lettuce seeds at one or more of the concentrations applied to the seeds (Table 3.7).

Table 3.7: Germination index (%) indicating phytotoxicity of each of the elements as well as the composite treatments on cress and lettuce seeds. Values below 80% indicates that the elements are exerting phytotoxic effects on the seeds.

	Germination Index					
	Treatment 1		**Treatment 2**		**Treatment 3**	
	Cress	**Lettuce**	**Cress**	**Lettuce**	**Cress**	**Lettuce**
Cobalt	126	90	**64**	**69**	97	86
Chromium	80	90	**70**	89	**20**	**38**
Fluoride	101	**75**	**34**	**49**	**0**	**0**
Iodine	79	**65**	102	**38**	104	**59**
Selenium	82	**68**	**47**	**32**	**43**	**28**
Silicon	98	94	91	**41**	107	**74**
Tin	134	**69**	121	**60**	82	**64**
Vanadium	127	**61**	116	**66**	**48**	**36**
Composite	82	**59**	**27**	**32**	**0**	**0**
Composite - F	174	87	117	**63**	**51**	**51**

The germination index obtained for iodine in cress seeds was found to be marginally below 80 % at the lowest iodine treatment. However, because no differences in seed germination and root growth were found in these seeds, it was not considered that iodine had no phytotoxic effects on the cress seeds. None of the trace elements supplied as single nutrient additions, and as the composite treatments at the lowest concentrations (treatment 1) were found to exert phytotoxicity on cress seeds. Lettuce seeds however, experienced phytotoxicity when fluoride, iodine, selenium, tin, vanadium and the composite treatment

of all eight trace elements was supplied at the lowest concentrations (treatment 1). When fluoride was excluded from the composite treatment, the phytotoxicity exerted by the treatments was only observed in the seeds that received the higher trace element concentrations (treatments two and/or three) in both cress and lettuce seeds.

3.5 Conclusion

Under these experimental conditions, the addition of the trace elements to both cress and lettuce seeds affected seed germination and root growth. Root length was found to be a better indicator of the phytotoxic effects of the trace elements applied to the seeds than seed germination. Kapustka (1997) reported that seed germination bioassays have many deficiencies, and are less sensitive to toxic substances than plant growth bioassays. According to the same author, this is because of two important properties of seeds. The first is that many chemicals cannot penetrate the seed coat and are thus not absorbed by the seeds, and the second is that most of the nutritional requirements of seed embryos are obtained from internally stored nutrient stocks.

In this study however, fluoride was found to be the major inhibitor of seed germination as a single nutrient treatment as well as in the composite treatment. This was confirmed when germination increased when fluoride was left out of the composite treatment. Root growth was affected by several of the trace elements as well as the composite treatments.

As single nutrient additions, several of the elements were found to exert phytotoxicity on both cress and lettuce seeds. Lettuce seeds, compared to cress seeds, were found to be more prone to the phytotoxic effects of the single nutrient additions, as well as the composite treatments, even at the lowest concentrations. However, when fluoride was left out of the composite treatment, phytotoxicity due to the addition of the composite treatments only occurred at the higher concentrations. The addition of fluoride as a single nutrient and in the composite treatments thus affected germination, root growth and overall phytotoxicity.

The addition of trace element and micronutrient enriched fertilizers (without the addition of fluoride) at low concentrations (treatment one and/or two) under these experimental conditions were thus found to be a possible option to enrich agricultural soils and thus also the human diet with these essential trace elements. However, due to the different responses observed by the different seeds, it is suggested that similar studies are done on a wider variety of seeds and this should also be done under field conditions to determine whether the results obtained in this study remain true under field conditions.

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CHAPTER 4

The use of a trace element enriched fertilizer to increase the concentrations of essential trace elements in the edible portion of vegetable crops.

4.1 Abstract

There is substantial evidence indicating the loss of essential nutrients from agricultural soils and crops. This contributed to nearly two billion people suffering from 'the hidden hunger', a term used to describe deficiencies in essential micro- and trace nutrients. Agronomic biofortification has been proposed as a means to enrich agricultural soils, and the fresh produce grown on these soils, with the nutrients lacking from human diets. However, the focus has only been placed on a few essential elements supplied as single nutrient additions to certain staple crops. This study investigates the effects of eight trace elements, supplied as a composite treatment, on the growth, water content and nutrient uptake of four crop species. Lettuce, turnip, spinach and cabbage were grown in a random block experiment under controlled conditions. Fresh and dry mass, water content as well as mineral nutrient content was determined for all four crops. Fresh mass, as well as water content were significantly reduced in certain of the crops that received the highest trace element concentrations however, dry mass, which is the more accurate indicator for biomass production, was not as severely affected. The addition of the trace elements at the high concentrations also affected the uptake of several essential plant nutrients, but the concentrations of the elements affected generally remained higher than the concentrations needed for adequate growth of agricultural crops. Several of the trace elements supplied to the plants were also found to be retained in the roots of the vegetable crops however, as the concentrations supplied to the plants increased, so did the concentrations found in the edible portions of the crops.

Agronomic biofortification of vegetable crops with simultaneous additions of multiple trace elements, under these experimental conditions, were thus found to be a viable option

to increase the concentrations of essential mineral nutrients in the edible portions of vegetable crops. However, further research on a wider variety of crops as well as research under field conditions is needed to determine whether these findings remain relevant.

Key words: hidden hunger, trace elements, mineral nutrients

4.2 Introduction

Few people recognise the connection between agricultural soils and human health. However, soils play a crucial role in agriculture as it provides agricultural crops with the nutrients they need to grow, which in turn, provides humans with the nutrients they need to sustain themselves. Agriculture thus, plays an important role in the acquisition of mineral nutrients by humans and animals (FAO and WHO 2001). Over the last few decades however, emphasis has been placed on the expansion of agricultural land and increasing crop yields at lower costs, rather than the nutritional quality of the crops. There is substantial evidence indicating loss of essential nutrients from the soil as well as from agricultural crops, and since most of these nutrients are only exceptionally added to commercial fertilizers, it is possible that by continual cropping they can become depleted from agricultural soils, and are therefore not supplied in sufficient quantities to humans that eat the crops (Johnsson 2005, Legard 2005, Tan *et al.* 2005, White and Broadley 2005, Garvin *et al.* 2006, Marler and Wallin 2006, Fan *et al.* 2008, Sands *et al.* 2009, Zhao *et al.* 2009).

This micronutrient and trace elements deficient diet has eventually led to an international dilemma in which nearly two billion people are believed to suffer from the "hidden hunger", a term used to describe deficiencies in micronutrients and trace elements (Bouis and Islam 2011, FAO *et al.* 2012). Although various traditional strategies to mitigate and overcome these deficiencies exist (Table 1.7, Chapter 1), the prevalence of micronutrient deficiencies as well as under nutrition remains staggeringly high, especially in the developing and poor countries (FAO and WHO 2001, Winkler 2011, FAO *et al.* 2012). Recently, agronomic biofortification has been proposed as a possible alternative to the traditional approaches to enhance the nutrient content in human diets.

Due to the availability and global use of macronutrient fertilizers, the addition of essential trace elements to these fertilizers could be a more realistic and feasible method to overcome deficiencies in essential trace nutrients (Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005, White and Broadley 2005, Cakmak 2008, Broadley *et al.* 2010). There have been many successful implementations of agronomic biofortification strategies. Finland was the first country to biofortify food crops with selenium enriched fertilizers to increase selenium concentrations in the population diet to the recommended

levels (Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005). In the UK, it was demonstrated that selenium concentrations in wheat grains can be increased nearly ten-fold by the addition of selenium enriched fertilizers to the soil (Broadley *et al.* 2010), and in the Xingjaing province of China, enriching irrigation water with iodine increased iodine concentrations of crops and by doing so, decreased infant mortalities related to iodine deficiencies (DeLong *et al.* 1997).

All of these successful implementations of agronomic biofortification have however only been implemented as single nutrient additions to the agricultural crops. Due to the significant global nutrient depletion from agricultural soils, concurrent shortages of several trace elements are likely to occur (Table 1.3, Chapter 1). This study thus aims to determine the effects of the addition of eight trace elements (Co, Cr, F, I, Se, Si, Sn, and V) as a composite treatment, on the growth and water content of four crop species, lettuce, turnip, spinach and cabbage, and the subsequent effects or lack there-of that the addition of these eight elements have on the uptake of other essential plant nutrients.

4.3 Materials and Methods

4.3.1 Plant materials and growing conditions

A randomized block experiment was carried out in which four crop species were grown using three treatments, and these were replicated five times. Lettuce (*Lactuca sativa* L. var. Eish), turnip (*Brassica rapa* var. Early purple top globe), spinach (*Beta vulgaris* var. Fordhook Giant) and cabbage (*Brassica oleracea* var. Giant Drumhead) were grown in 15 cm pots in sand culture under controlled conditions. Temperature was kept at a constant 20 ºC during the day and 10 ºC during the night over a 14 hour day and 10 hour night.

For the first week after sowing, the crops only received deionized water, after which they received a nutrient solution (Chemicult – Kompel). During the second and third week, the nutrient solution was only supplied at half strength, and thereafter at full strength. After week seven, the plants were supplied a new nutrient solution (Plant food - Stark Ayres) due to the discontinuation of the Kompel product (Table 4.1).

Table 4.1: Elemental make-up, before dilution, of the nutrient solutions applied to the vegetable crops.

	Nutrient concentration (g/kg)												
	N	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Mn**	**B**	**Zn**	**Cu**	**Mn**	**Mo**
A	65	27	130	70	22	75	1.5	0.24	0.24	0.05	0.02	0.01	/
B	146	43	274	/	29	/	1.8	/	0.24	0.05	0.02	0.24	0.01

A) Chemicult – Kompel **B)** Plant Food – Stark Ayres

From week eight the different treatments were applied together with the full strength nutrient solution (Plant food - Stark Ayres). Treatments were now supplied twice a week. The control treatment consisted of the full strength stock nutrient solution, while the first and second treatments consisted of the nutrient solution with the addition of the trace elements at different concentrations (Table 4.2).

Table 4.2: Trace element concentrations (g/kg) added to the nutrient solution (Plant food - Stark Ayres) and the chemical form in which they were added to the solution.

		Concentration (g/kg)	
Element	**Chemical form**	**Treatment 1**	**Treatment 2**
Co	$CoSO_4.7H_2O$	0.0002	0.002
Cr	CrO_3	0.001	0.01
F	NH_4F	0.004	0.04
I	KIO_3	0.004	0.04
Se	Na_2SeO_4	0.002	0.02
Si	*	0.04	0.4
Sn	$SnCl_2.2H_2O$	0.0002	0.002
V	NH_4VO_3	0.0002	0.002

*1000 mg/L Spectrosol standard solution for atomic absorption spectroscopy

4.3.2 Nutrient Determination

Crops were harvested after 12 weeks and separated into roots and shoots after which the fresh mass of the shoots were determined while root mass was determined after washing the crops with deionized water and blotting it dry. The samples were then oven dried at 70º C for 72 hours. After oven drying, dry mass was determined. The dried samples were then milled, and stored for the determination of mineral nutrients.

4.3.2.1 Sample digestion and elemental analyses

A sulphuric - peroxide digestion method was used (Moore and Chapman 1986). 14 g of $Li_2SO_4.H_2O$ were added to 420 ml of 100 vol. H_2O_2. Selenium was not added to the mixture as selenium concentrations had to be determined. The solution was mixed well and 350 ml of conc. H_2SO_4 was carefully added to it. The mixture was cooled down by placing it on ice during the addition of the acid. After every session of use, the digestion mixture was stored in a fridge at approximately 4 °C. A 0.4 g sample of dry ground material was weighed into cigarette paper and placed in a digestion tube. A 5 ml sample of the digestion mixture was then added. The mixture was digested in a heating block in a fume cupboard at 150 °C until the initial reaction was complete, thereafter gradually increased to 380 °C,

until an almost colourless solution was obtained. Glass funnels were used to cover the opening of the digestion tube to minimize the loss of fumes. After digestion, the solution was transferred into a 100 ml volumetric flask after filtering and diluted to volume with deionised water. Four blank solutions where only the cigarette paper was digested were also made (Moore and Chapman 1986).

Analyses of Co, Cr, Na, Sn, V, K, Fe, Mn, Zn, Cu, and Mg were done using a Unican M-series Pye Solar Atomic Absorption Spectrophotometer (Unicam Unlimited, Cambridge, UK). Se and Si concentrations were determined using Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES). N concentrations were determined using a Büchi nitrogen distillation (K-350) unit (Labotec, Büchi, Switzerland) and P, I and F concentrations were determined using a Spectroquant ® Pharo 300-M unit (Merc (Pty.) Ltd.).

4.3.3 Statistical analyses

The Statistical Package for the Social Sciences version 21(SPSS Inc., Chicago IL) was used to test the data for normality using a Shapiro-Wilks test after which a Kruskal-Wallis (*H*) analysis was done to determine whether there were statistically significant differences ($P \leq 0.05$) in the growth, trace element as well as essential plant nutrient concentrations between the different treatments and also between the root and shoots. After differences were found, a Post Hoc test was done to determine between which treatments these differences occurred. Where there were no differences found in the trace element concentrations using the Kruskal-Wallis analysis, Spearmans Rho (ρ) correlations were done to determine whether there were any correlations between the uptake of the trace elements and the concentrations at which it was supplied to the vegetable crops.

4.4 Results and Discussion

4.4.1 Biomass production and water content in the roots and shoots

Although there seemed to be an apparent decrease in both fresh and dry mass of all four vegetable species as the concentrations of the focus trace elements in the treatments increased, no differences ($P \geq 0.05$) were found in fresh and/or dry mass of more than one of the crop species (Table 4.3). However, where significant differences were found in both fresh and dry mass, a significant reduction in mass was observed in the vegetable crops that received the treatments containing the highest trace element concentrations. Similar reductions in biomass was also observed by various other researchers who supplied crops with excess concentrations of selenium (Molnarova and Fargasova 2009), vanadium (Wang and Liu 1999), chromium (Singh *et al.* 2006), fluoride (Gupta *et al.* 2009) and cobalt (Li *et al.* 2009) as single nutrient additions.

Table 4.3: Fresh and dry mass (g) of vegetable crop species. Masses with the same letters are not significantly different ($P \geq 0.05$) from one another.

		Mean fresh mass (g) ± standard deviation			Significance
		Control	**Treatment 1**	**Treatment 2**	
Cabbage	Roots	4.38 ± 2.70 ^{ab}	10.20 ± 6.67 ^{b}	2.54 ± 1.70 ^{a}	$H_{(2,15)} = 7.819$, $P = 0.020$
	Shoots	39.66 ± 9.72 ^{a}	48.84 ± 24.31 ^{b}	14.01 ± 9.59 ^{a}	$H_{(2,15)} = 7.980$, $P = 0.018$
Spinach	Roots	10.87 ± 6.83	6.91 ± 4.98	5.00 ± 3.73	NS
	Shoots	40.21 ± 14.92 ^{b}	20.75 ± 17.48 ^{ab}	6.04 ± 3.38 ^{a}	$H_{(2,15)} = 9.260$, $P = 0.010$
Lettuce	Roots	5.68 ± 3.07	4.91 ± 1.17	5.75 ± 0.89	NS
	Shoots	25.97 ± 12.50 ^{b}	21.05 ± 10.13 ^{ab}	11.46 ± 2.06 ^{a}	$H_{(2,15)} = 7.980$, $P = 0.018$
Turnip	Roots	6.00 ± 2.86	7.33 ± 9.41	3.37 ± 1.92	NS
	Shoots	34.32 ± 14.71	29.06 ± 18.03	16.60 ± 9.26	NS
		Mean dry mass (g) ± standard deviation			
Cabbage	Roots	0.98 ± 0.44 ^{ab}	2.50 ± 1.64 ^{b}	0.50 ± 0.38 ^{a}	$H_{(2,15)} = 7.819$, $P = 0.020$
	Shoots	3.61 ± 1.48	4.81 ± 2.23	2.60 ± 1.42	NS
Spinach	Roots	2.61 ± 2.51	2.13 ± 1.60	1.48 ± 1.06	NS
	Shoots	3.39 ± 1.48	2.70 ± 1.84	1.93 ± 0.62	NS
Lettuce	Roots	0.50 ± 0.26	0.60 ± 0.15	0.81 ± 0.28	NS
	Shoots	3.21 ± 1.07	3.60 ± 0.84	2.07 ± 0.79	NS
Turnip	Roots	1.21 ± 0.68	1.14 ± 1.11	0.57 ± 0.34	NS
	Shoots	4.15 ± 1.31	3.71 ± 1.80	3.38 ± 1.67	NS

NS = Not significant ($P \geq 0.05$)

When the relationship between dry (DM) and fresh (FM) mass was determined (Fig 4.1), it was found that as the trace element concentrations in the treatments increased, the mass fraction of the roots of the vegetable crops remained relatively constant between the different treatments ($P \geq 0.05$). However, there was a significant increase observed in the mass fraction of the shoots of spinach ($H_{(2, 15)} = 12.500$, $P = 0.002$), cabbage ($H_{(2,15)} = 10.500$, $P = 0.005$) and turnip ($H_{(2,15)} = 9.380$, $P = 0.009$) plants.

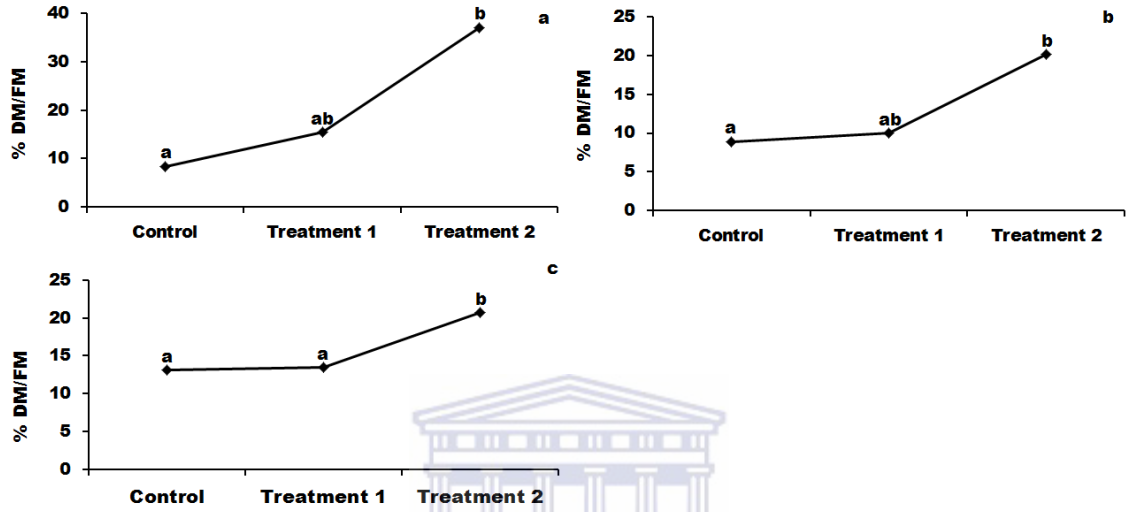

Figure 4.1: Relationship between the dry (DM) and fresh (FM) masses of spinach (a), cabbage (b), and turnip (c) plants. Comparisons between the different treatments were made within organs (roots or shoots), and not between roots and shoots. Significant differences ($P < 0.05$) within the roots and shoots respectively of each crop species are shown by different letters.

The increase in the mass fraction indicates a reduction in the water content in the shoots of the vegetable crops that received the treatments containing the highest trace element concentrations. This indicates that the high trace element concentrations affects the translocation of water from the roots to the shoots of the vegetable crops, or, the excess concentrations of the trace elements within the plants affects the rate of transpiration, by increasing the amount of water lost through the leaves. These could be the reasons for some of the visual toxicity symptoms, including loss of leaf turgor pressure, severe wilting and the initial stages of chlorosis, observed in the crops that received the highest trace element concentrations. These symptoms are characteristic of plants that received excess concentrations of cobalt, chromium and selenium (Sharma *et al.* 1995, Zayed *et al.* 1998, Molnarova and Fargasova 2009).

4.4.2 Concentrations of the focus trace elements in the edible portions of the vegetable crops

Iodine and fluoride were detected in both roots and shoots of all four crop species however, there was no prevalence of accumulation ($P \geq 0.05$) in either the roots or shoots in any of the plant species. In this study, iodine was supplied to the plants as iodate. Iodate has however been found to be less available for the uptake by plant roots, and also not as readily transported within plans, as iodide (Zhu *et al.* 2003). Tin could not be detected in the roots or shoots of any of the plant species. The literature on the uptake and accumulation of tin in plants is limited thus, further experiments on the uptake of tin by spinach plants is included (appendix 1). From these results, it is possible to see that tin was only taken up at detectible concentrations when it was supplied to the plants at relatively high concentrations (2 and 20 mg/L), after which the majority of the element is accumulated in the roots of the plants. However, where significant differences were found in the area of accumulation of the trace elements within the plants in the current study (Table 4.4), it was found that these elements were generally retained in significantly higher concentrations in the roots of the plants.

Table 4.4: Accumulation of trace elements in the roots and shoots of four biofortified vegetable crop species. Concentrations with the same letters are not significantly different ($P \geq 0.05$) from one another.

			Concentration (g/kg) ± Standard Deviation		Significance
			Roots	**Shoots**	
Co	Cabbage	Treatment 1	0.0093 ± 0.0020	0.0079 ± 0.0011	NS
		Treatment 2	0.0524 ± 0.0312 **b**	0.0088 ± 0.0025 **a**	$H_{(1, 10)} = 6.818$, $P = 0.009$
	Turnip	Treatment 1	0.0072 ± 0.0077	0.0039 ± 0.0030	NS
		Treatment 2	0.0430 ± 0.0208 **b**	0.0091 ± 0.0029 **a**	$H_{(1, 10)} = 6.818$, $P = 0.009$
Cr	Cabbage	Treatment 1	0.0280 ± 0.0111 **b**	0.0113 ± 0.0049 **a**	$H_{(1, 10)} = 5.806$, $P = 0.016$
		Treatment 2	0.0687 ± 0.0308 **b**	0.0313 ± 0.0138 **a**	$H_{(1, 10)} = 4.811$, $P = 0.028$
	Turnip	Treatment 1	0.0454 ± 0.0407	0.0100 ± 0.0077	NS
		Treatment 2	0.0929 ± 0.0438 **b**	0.0329 ± 0.0147 **a**	$H_{(1, 10)} = 6.818$, $P = 0.009$
V	Cabbage	Treatment 1	0.0625 ± 0.0078 **b**	0.0227 ± 0.0063 **a**	$H_{(1, 10)} = 6.818$, $P = 0.009$
		Treatment 2	0.0866 ± 0.0274	0.0693 ± 0.0213	NS
	Lettuce	Treatment 1	0.1209 ± 0.0499 **b**	0.0500 ± 0.0199 **a**	$H_{(1, 10)} = 5.771$, $P = 0.016$
		Treatment 2	0.1413 ± 0.0534	0.1664 ± 0.0729	NS
Se	Spinach	Treatment 1	0.0044 ± 0.0056 **a**	0.0207 ± 0.0132 **b**	$H_{(1, 10)} = 6.818$, $P = 0.009$
		Treatment 2	0.0064 ± 0.0029	0.0325 ± 0.0138	NS
	Cabbage	Treatment 1	0.0093 ± 0.0031 **a**	0.0548 ± 0.0131 **b**	$H_{(1, 10)} = 6.818$, $P = 0.009$
		Treatment 2	0.0275 ± 0.0327	0.0350 ± 0.0025	NS
	Lettuce	Treatment 1	0.0238 ± 0.0081 **b**	0.0137 ± 0.0070 **a**	$H_{(1, 10)} = 3.938$, $P = 0.047$
		Treatment 2	0.0408 ± 0.0188	0.0472 ± 0.0128	NS
Si	Spinach	Treatment 1	0.0004 ± 0.0003 **a**	0.0010 ± 0.0004 **b**	$H_{(1, 10)} = 5.345$, $P = 0.021$
		Treatment 2	0.0004 ± 0.0006	0.0003 ± 0.0004	NS
	Turnip	Treatment 1	0.0006 ± 0.0001	0.0006 ± 0.0001	NS
		Treatment 2	0.0007 ± 0.0001 **b**	0.0006 ± 0.0001 **a**	$H_{(1, 10)} = 5.714$, $P = 0.017$

NS = Not significant ($P \geq 0.05$)

One of the key factors that needs to be achieved through biofortification is to increase the concentrations of essential mineral nutrients in the edible portions of crops (White and Broadley 2005a, Winkler 2011). Here it is however clear that the prevalence in many of the crop species is to retain the trace elements in their roots. Apart from turnip which is a tuber, all three other vegetables grown in this study are leafy vegetables, which mean that most of the additional trace elements supplied to the plants were retained in the non-edible portions of the crops.

However, when the concentrations of the focus trace elements were assessed in only the edible portions of the vegetable crops, it was found that cobalt, chromium, vanadium, selenium and silicon were taken up and incorporated into the edible portions of the crops at varying concentrations. This was however not uniformly found throughout all four crop species, and the elements found in the edible portions of the crops also were not the same in all four crop species.

Within the spinach plants, it was found that as the concentrations of cobalt ($H_{(2,\ 15)}$ = 11.010, P = 0.004), chromium ($H_{(2,\ 15)}$ = 12.727, P = 0.002), vanadium ($H_{(2,\ 15)}$ = 12.020, P = 0.002), selenium ($H_{(2,\ 15)}$ = 7.740, P = 0.021) and silicon ($H_{(2,\ 15)}$ = 6.575, P = 0.037) increased in the treatments, so did the concentrations found in the edible portions of the spinach plants (Fig. 4.2).

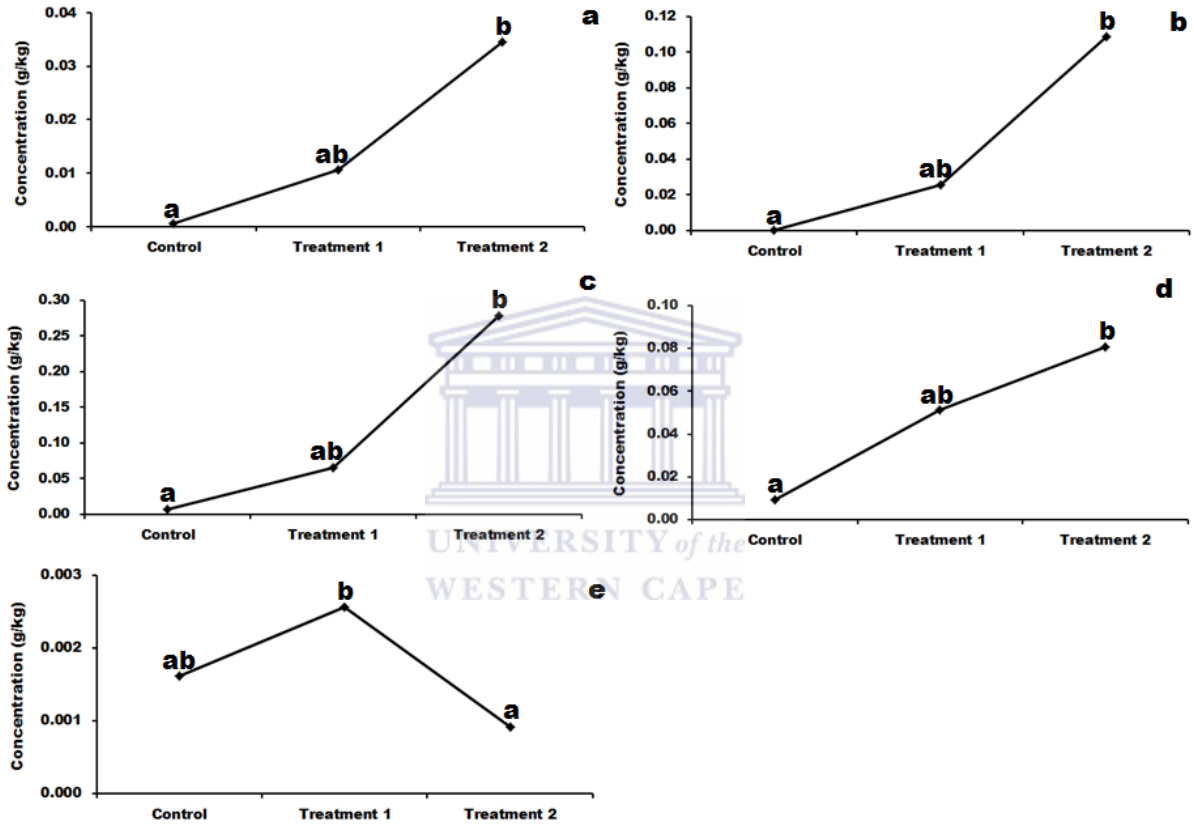

Figure 4.2: Cobalt (a), chromium (b), vanadium (c), selenium (d) and silicon (e) concentrations in the edible portions of spinach plants. Mean concentrations that are not significantly different (P ≥ 0.05) between the different treatments for each element, is shown by the same letters.

The edible portions of cabbage plants were found to have cobalt ($H_{(2, 15)}$ = 9.420, P = 0.009), chromium ($H_{(2, 15)}$ = 12.500, P = 0.002), vanadium ($H_{(2, 15)}$ = 12.020, P = 0.002) and selenium ($H_{(2, 15)}$ = 11.580, P = 0.003) concentrations that were significantly higher in the crops that received treatments one and/or two, compared to those crops that received the control treatment (Fig. 4.3).

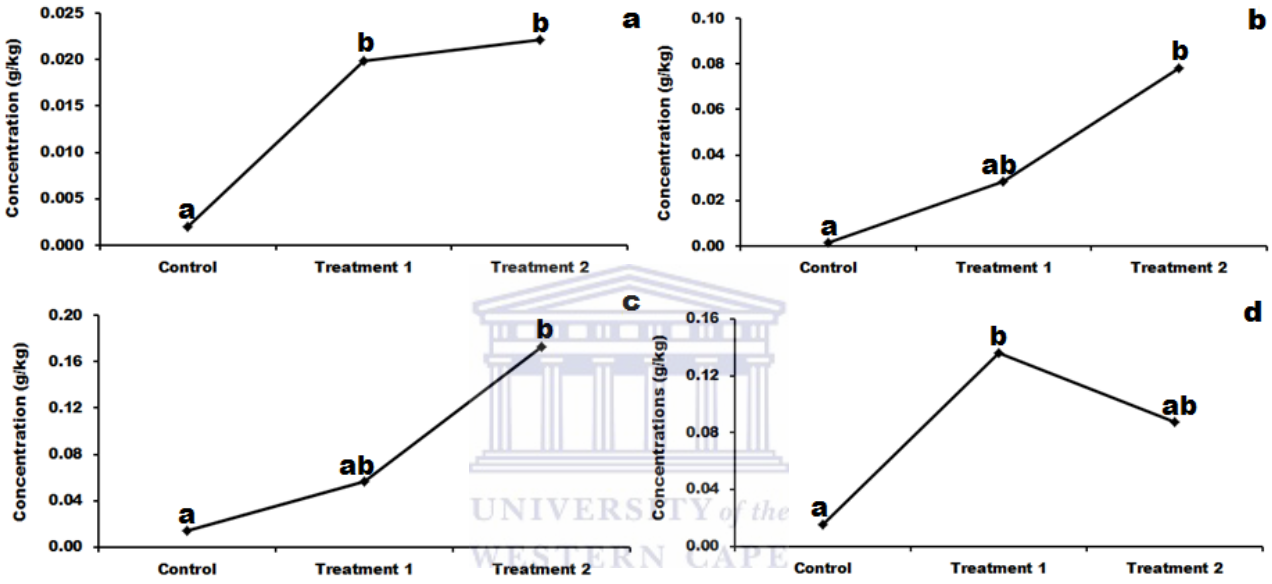

Figure 4.3: Cobalt (a), chromium (b), vanadium (d) and selenium (d) concentrations in the edible portions of cabbage plants. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

Turnip plants that received the treatment containing the highest concentrations of the focus trace elements, had cobalt ($H_{(2,15)}$= 11.080, P = 0.004), chromium ($H_{(2,15)}$ = 10.334, P = 0.006), vanadium ($H_{(2,15)}$ = 8.000, P = 0.018) and selenium ($H_{(2,15)}$ = 10.287, P = 0.006) concentrations that were significantly higher in the edible portions than the concentrations found in the plants that received the control treatment and/or treatment one (Fig. 4.4).

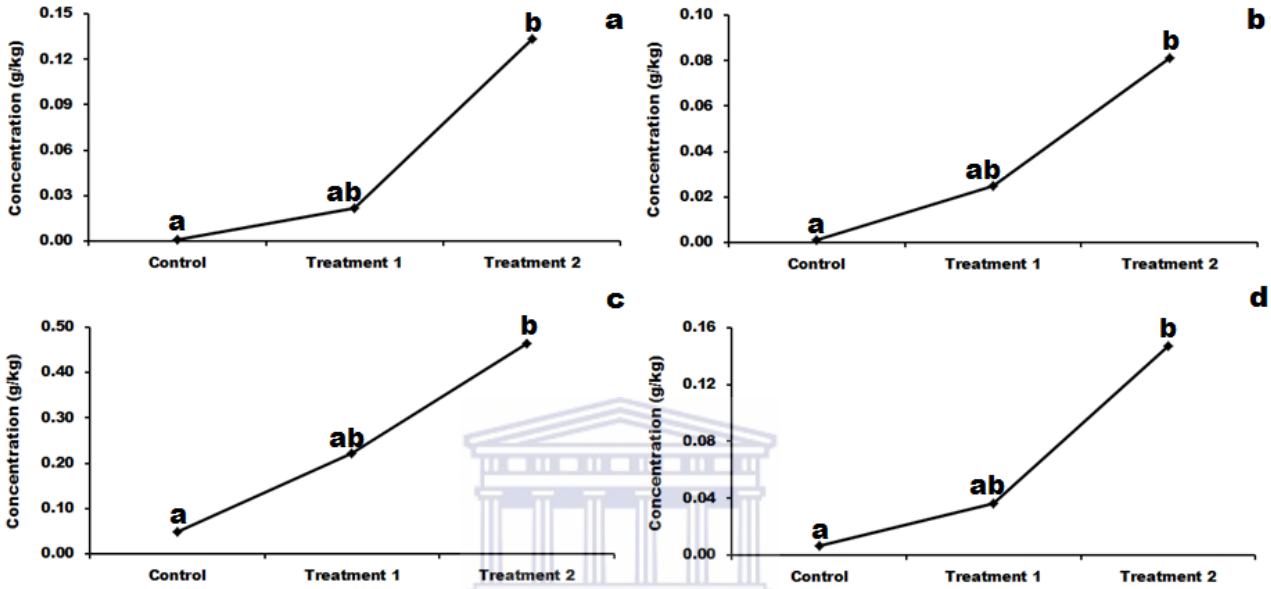

Figure 4.4: Cobalt (a), chromium (b), vanadium (c) and selenium (d) concentrations in the edible portions of turnip plants. Mean concentrations that are not significantly different (P ≥ 0.05) between the different treatments for each element, is shown by the same letters.

The concentrations of cobalt ($H_{(2,15)}$ = 12.020, P = 0.002), chromium ($H_{(2,15)}$= 12.500, P = 0.002), vanadium ($H_{(2,15)}$= 12.020, P = 0.002), and selenium ($H_{(2,15)}$= 9.423, P = 0.009) found in the edible portions of lettuce plants were found to increase in the plant as the concentrations of these elements in the treatments increased (Fig. 4.5).

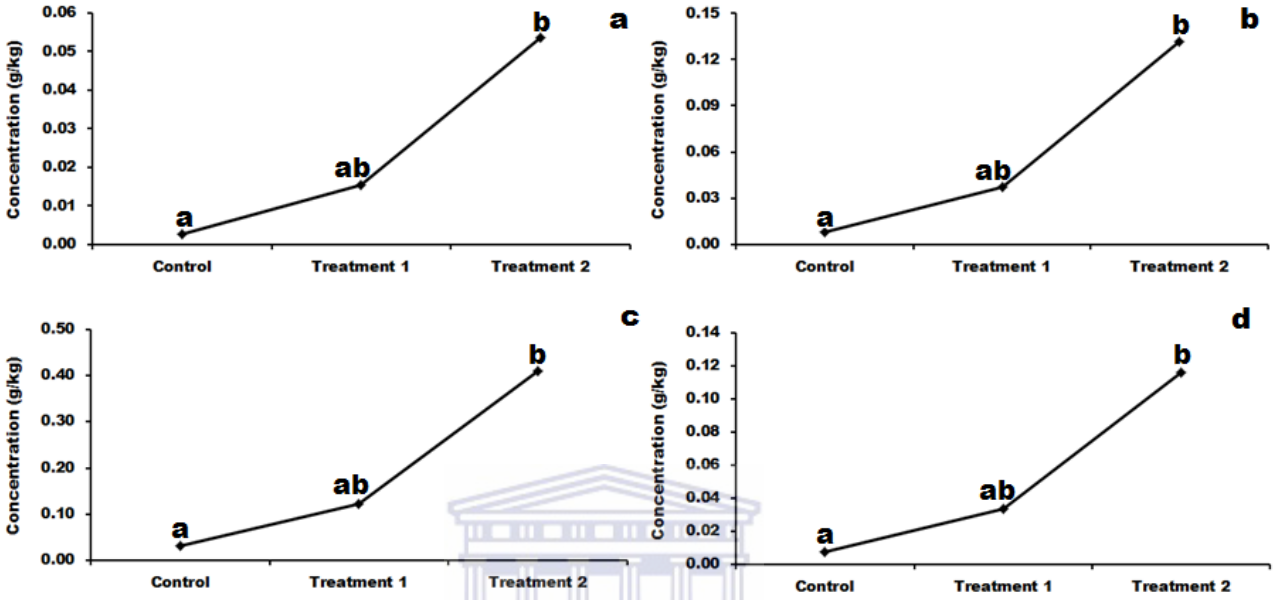

Figure 4.5: Cobalt (a), chromium (b), vanadium (c), and selenium (d) concentrations in the edible portions of lettuce plants. Mean concentrations that are not significantly different (P ≥ 0.05) between the different treatments for each element, is shown by the same letters.

Although fluoride and iodine was not found to be incorporated into the edible portions of the vegetable crops at statistically significantly higher concentrations than the concentrations found in the plants that received the control treatments, strong positive correlations were found between the concentrations of fluoride in spinach (R^2 = 0.9087, ρ = 0.549, P = 0.034) and lettuce (R^2 = 0.6344, ρ = 0.577, P = 0.024) as well as iodine concentrations in lettuce (R^2 = 0.6077, ρ = 0.520, P = 0.047) supplied to the crops and the concentrations found in the edible portions of the crops. Based on these results, it is clear that if these elements are supplied in sufficient quantities to the vegetable crops, increases in the concentrations of these trace elements in the edible portions of the crops can be

achieved, even though the majority of the elements are retained in the non-edible portions of certain of the vegetable crops.

4.4.3 Uptake of essential plant nutrients

The addition of the trace elements to the vegetable crops affected the uptake and incorporation of several of the essential plant nutrients. However, this was not uniformly found for all of the essential plant nutrients in each of the crop species. Certain of the essential plant nutrient concentrations were affected only in the roots of the plants (Table 4.5), while others were only affected in the shoots of the plants (Table 4.6) however, certain of the elements concentrations were found to be affected in both the roots and shoots of certain of the vegetable crop species. In general, as the concentrations of the trace elements in the treatments supplied to the crops increased, there was a decrease in the concentrations of the essential plant nutrients in the roots and/or shoots of the vegetable crops.

However, although the concentrations of the essential plant nutrients decreased, their concentrations in the shoots of the crops remained higher than the concentrations required for normal growth of agricultural crops (Larcher 2003, Epstein and Bloom 2005). Phosphate concentrations in the shoots of the crops were marginally below the required concentrations, but this was also observed in the plants that received the control treatments. According to Epstein and Bloom (2005), the uptake of phosphate by plant roots is greatly reduced at soil pH greater than 6. In this study, the soil pH was kept between 6.4 and 6.6 to increase the uptake of the trace elements supplied to the crops.

Table 4.5: Concentration (g/kg) of essential plant nutrients found in the roots of four crop species that were biofortified with eight trace elements essential to humans but not the crops. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

		mean concentration (g/kg) ± standard deviation								
		N	**P**	**Mn**	**Mg**	**K**	**Fe**	**Ca**	**Cu**	**Zn**
Spinach	**Control**	4.35 ± 1.91	0.38 ± 0.10	0.08 ± 0.04	0.46 ± 0.15	9.57 ± 3.52	0.23 ± 0.09	5.61 ± 0.77	0.003 ± 0.001	0.01 ± 0.00
	Treatment 1	3.05 ± 1.27	0.42 ± 0.16	0.06 ± 0.03	0.54 ± 0.43	7.59 ± 5.28	0.19 ± 0.06	5.68 ± 0.33	0.003 ± 0.002	0.01 ± 0.00
	Treatment 2	3.59 ± 2.69	0.4 ± 0.14	0.04 ± 0.01	0.49 ± 0.18	5.77 ± 4.37	0.20 ± 0.04	5.89 ± 0.82	0.003 ± 0.001	0.01 ± 0.00
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cabbage	**Control**	5.98 ± 1.63	0.48 ± 0.11	0.04 ± 0.00	0.53 ± 0.14	11.28 ± 2.50	0.21 ± 0.02	5.07 ± 0.83	0.003 ± 0.002 **ab**	0.01 ± 0.00
	Treatment 1	4.91 ± 1.29	0.45 ± 0.12	0.03 ± 0.00	0.31 ± 0.10	8.76 ± 1.87	0.19 ± 0.06	4.78 ± 0.50	0.003 ± 0.001 **a**	0.01 ± 0.00
	Treatment 2	8.62 ± 3.23	0.68 ± 0.37	0.07 ± 0.02	1.01 ± 0.59	11.93 ± 8.53	0.27 ± 0.24	15.28 ± 18.99	0.006 ± 0.002 **b**	0.05 ± 0.07
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	*8.796* 0.012	NS
Turnip	**Control**	14.22 ± 7.68	0.73 ± 0.41	0.05 ± 0.01	1.41 ± 1.01	28.58 ± 17.58	0.26 ± 0.06	6.9 ± 4.31	0.005 ± 0.003	0.02 ± 0.01
	Treatment 1	8.90 ± 4.54	0.74 ± 0.40	0.04 ± 0.02	1.34 ± 0.81	23.17 ± 12.57	0.19 ± 0.04	6.61 ± 2.03	0.004 ± 0.002	0.01 ± 0.01
	Treatment 2	17.66 ± 2.67	0.89 ± 0.06	0.05 ± 0.02	1.69 ± 0.39	32.13 ± 4.74	0.16 ± 0.09	9.25 ± 3.65	0.005 ± 0.002	0.02 ± 0.00
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lettuce	**Control**	11.25 ± 2.77 **ab**	0.79 ± 0.39 **b**	0.08 ± 0.01 **b**	5.18 ± 1.87	24.6 ± 7.35	0.26 ± 0.06	10.73 ± 4.17	0.018 ± 0.009	0.05 ± 0.01 **b**
	Treatment 1	14.04 ± 3.37 **b**	0.78 ± 0.18 **b**	0.05 ± 0.01 **a**	5.90 ± 3.22	23.7 ± 3.32	0.21 ± 0.02	7.24 ± 0.90	0.015 ± 0.006	0.03 ± 0.00 **a**
	Treatment 2	7.73 ± 4.09 **a**	0.39 ± 0.11 **a**	0.07 ± 0.01 **ab**	6.72 ± 3.11	15.64 ± 4.59	0.19 ± 0.02	6.87 ± 1.59	0.015 ± 0.005	0.04 ± 0.01 **ab**
	$H_{(2, 15)}$ P	*7.220* *0.027*	*8.180* *0.017*	*8.000* *0.018*	NS	NS	NS	NS	NS	*6.020* *0.049*

NS = Not significant ($P \geq 0.05$)

Table 4.6: Concentration (g/kg) of essential plant nutrients found in the shoots of four crop species that were biofortified with eight trace elements essential to humans but not the crops. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

		mean concentration (g/kg) ± standard deviation								
		N	**P**	**Mn**	**Mg**	**K**	**Fe**	**Ca**	**Cu**	**Zn**
Spinach	**Control**	22.70 ± 3.54	1.87 ± 0.58	0.13 ± 0.01	3.63 ± 0.83	104.98 ± 8.79 **b**	0.24 ± 0.05	6.86 ± 1.57	0.005 ± 0.003	0.02 ± 0.00 **b**
	Treatment 1	18.49 ± 2.92	1.63 ± 0.38	0.12 ± 0.00	3.52 ± 1.13	83.63 ± 22.76 **ab**	0.20 ± 0.02	7.62 ± 0.81	0.004 ± 0.002	0.02 ± 0.00 **a**
	Treatment 2	15.89 ± 7.17	1.49 ± 0.65	0.12 ± 0.02	5.43 ± 2.68	61.06 ± 28.09 **a**	0.16 ± 0.04	9.35 ± 1.74	0.004 ± 0.001	0.02 ± 0.00 **ab**
	$H_{(2, 15)}$	NS	NS	NS	NS	*6.980*	NS	NS	NS	*6.020*
	P					*0.031*				*0.049*
Cabbage	**Control**	21.61 ± 3.82	1.01 ± 0.16 **b**	0.09 ± 0.01 **b**	3.24 ± 0.77	108.26 ± 6.71 **b**	0.13 ± 0.04	12.69 ± 1.48 **b**	0.003 ± 0.001	0.02 ± 0.00
	Treatment 1	22.29 ± 3.58	1.01 ± 0.08 **b**	0.09 ± 0.00 **b**	3.25 ± 0.43	79.48 ± 7.06 **ab**	0.13 ± 0.07	12.24 ± 0.40 **ab**	0.003 ± 0.001	0.02 ± 0.00
	Treatment 2	15.59 ± 2.53	0.61 ± 0.06 **a**	0.06 ± 0.01 **a**	2.43 ± 0.27	44.89 ± 2.82 **a**	0.08 ± 0.03	9.95 ± 1.43 **a**	0.003 ± 0.001	0.01 ± 0.00
	$H_{(2, 15)}$	NS	*9.397*	*8.660*	NS	*12.500*	NS	*6.860*	NS	NS
	P		*0.009*	*0.013*		*0.002*		*0.032*		
Turnip	**Control**	22.56 ± 3.33	1.22 ± 0.16 **b**	0.07 ± 0.01	2.66 ± 0.33	89.99 ± 5.02 **b**	0.12 ± 0.05 **b**	10.85 ± 0.78	0.004 ± 0.002	0.02 ± 0.01 **b**
	Treatment 1	20.42 ± 4.81	0.97 ± 0.33 **ab**	0.06 ± 0.01	2.34 ± 0.69	69.55 ± 17.36 **ab**	0.09 ± 0.07 **ab**	9.84 ± 1.74	0.003 ± 0.001	0.01 ± 0.00 **a**
	Treatment 2	16.32 ± 3.23	0.67 ± 0.14 **a**	0.07 ± 0.01	2.27 ± 0.29	46.08 ± 5.78 **a**	0.05 ± 0.02 **a**	9.86 ± 1.28	0.002 ± 0.001	0.02 ± 0.00 **ab**
	$H_{(2, 15)}$	NS	*7.220*	NS	NS	*10.140*	*7.280*	NS	NS	*6.260*
	P		*0.027*			*0.006*	*0.026*			*0.044*
Lettuce	**Control**	10.66 ± 5.00	0.75 ± 0.23	0.11 ± 0.05	2.38 ± 0.80	66.07 ± 24.17	0.21 ± 0.07 **b**	9.15 ± 2.65	0.004 ± 0.003	0.05 ± 0.02
	Treatment 1	11.43 ± 3.30	0.27 ± 0.13	0.07 ± 0.01	1.76 ± 0.16	44.04 ± 9.36	0.14 ± 0.03 **ab**	7.33 ± 1.75	0.003 ± 0.001	0.03 ± 0.01
	Treatment 2	13.35 ± 5.59	0.42 ± 0.31	0.06 ± 0.01	1.47 ± 0.32	40.21 ± 10.20	0.11 ± 0.02 **a**	7.21 ± 1.89	0.003 ± 0.001	0.04 ± 0.00
	$H_{(2, 15)}$	NS	NS	NS	NS	NS	*6.260*	NS	NS	NS
	P						*0.044*			

NS = Not significant ($P \geq 0.05$)

4.5 Conclusion

Each of the vegetable crops responded differently to the addition of the trace elements. This was seen in all of the parameters that were assessed. One of the prerequisites for biofortification is increasing the essential nutrient concentrations in the edible portions of crops. However, this should not happen at the expense of other essential parameters such as biomass production, yield and plant mineral nutrition. In this study, fresh mass of several of the crop species were greatly reduced by the addition of the trace elements. However, dry mass, a more accurate parameter to assess biomass production, was not significantly affected. The uptake of essential plant nutrients were found to be reduced by the addition of the trace elements. However the concentrations of the elements affected were still above the concentrations required for normal growth of agricultural crops. Finally, although the majority of the trace elements supplied to the leafy vegetables were retained at higher concentrations in the non-edible portions of the crops, the concentrations found in the shoots of these crops significantly increased as the concentrations of the trace elements supplied to the plants increased.

Agronomic biofortification of vegetable crops with simultaneous additions of multiple trace elements, under these experimental conditions, were thus found to be a viable option to increase the concentrations of essential mineral nutrients in the edible portions of vegetable crops. However, due to the different responses observed by each of the vegetable crop species, it is suggested that similar studies are done on a wider variety of crop species. Once this is done, further research under field conditions are required in order to determine whether the results observed under experimental conditions remain relevant and true under field conditions.

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CHAPTER 5

A new paradigm for agriculture – conclusions and recommendations from the current study

5.1. Conclusion

Micronutrient and trace element malnutrition is a global problem that is affecting more than one-third of the worlds population (Bouis and Islam 2011, FAO *et al.* 2012). It is estimated that 60 – 80 % of the worlds population is iron deficient, 30 % is zinc deficient, 30 % is iodine deficient and 15 % is selenium deficient (White and Broadley 2005). These are however, only those elements with well documented deficiencies. Many more people suffer from other, less-well documented micronutrient and trace element deficiencies but, because their symptoms are less obvious, they are often overlooked (Bruulsema *et al.* 2012). Millions of people die yearly, either directly or indirectly, as a result of micronutrient and trace element malnutrition. The majority of these deaths are however, as a result of preventable diseases which are able to manifest as a result of the compromised immune systems of the people suffering from these deficiencies due to poor nutrition (Calder and Jackson 2000, Calder and Kew 2000, Bouis and Welch 2010).

In the current study, it was found that the situation described above could easily become a reality in South Africa. The nutritional quality, based on the concentrations of the focus trace elements, of several of the vegetable based food items collected in the Western Cape Province of South Africa, is a cause for great concern. Up to 53 % of the items collected from different geographical locations had concentrations below the detection limit of certain of the focus trace elements while others only contained low concentrations that contributed less than one percent per food portion to the daily recommended intake of these elements (Chapter 2). Due to poverty, the majority of South Africans living in the poorer and rural communities have a low dietary diversity (Labadarios *et al.* 2005), based on staples and cereals as they cannot afford a diet rich in fresh produce (Temple and Steyn 2009, Labadarios *et al.* 2011a). Maize meal is one of the more commonly consumed staples among the South Africans living in rural and poor communities in South Africa. However, from the current study, it was indicated that maize meal, in most instances, had the lowest, or one of the lowest trace element concentration compared to the other produce

collected. Maize meal had no contribution to the daily recommended intake of selenium and ranged from zero to approximately 6 % for the other trace elements. This monotonous diet of many of the poorer communities of South Africa, based on cereals and staples, is the primary reason why the majority of the people living in these areas are at risk of, or are already suffering from multiple micronutrient and trace element deficiencies (Darmon and Drewnowski 2008, Temple and Steyn 2011, Labadarios *et al.* 2011a,b).

The World Health Organisation (WHO) defines human health as "a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity" (Bruulsema *et al.* 2012). From this definition, one realises that human health promotion extends beyond the medical field. The awarding of the 1970 Nobel Peace Prize to Dr. Norman Borlaug, for his work on the green revolution, indicates the significant link between agriculture and human health (Bruulsema *et al.* 2012). Good nutrition can prevent infectious diseases and deaths as a result of preventable illnesses, and good nutrition is a variable that can be controlled through proper management strategies and the correct infrastructure. Modern agriculture and fertilizer use has generally been successful in increasing crop production, specifically cereals and staples, to provide enough calories to most of the poor communities in developing countries. However, as indicated in the current study, cereals and staples, such as maize meal, are weak sources of several of the essential trace elements. Unfortunately, the role of good quality and nutritious foods in preventing illnesses and diseases has been replaced by pharmaceutical solutions. Many of these pharmaceutical solutions are however, too costly for the people living in the poorer communities in developing countries to afford. Ironically, it is these communities that are more prone to develop diseases and illnesses as a result of micronutrient and trace element malnutrition (Niedzweicki and Rath 2005, FAO *et al.* 2012).

The use of home and community gardens to supply people living in poor and rural communities with a wider variety of vegetable crops is a good strategy to try and change these individuals' diets to the healthier options. However, as shown in chapter 2, the majority of vegetable crops contain insufficient amounts of several trace elements. In order to find long term, sustainable and cost effective solutions to the global problem of micronutrient and trace element deficiencies, and all the problems associated with them, interdisciplinary approaches between plant and soil scientists, farmers and the medical community is needed. To reach the millennium development goals target of halving the

proportion of undernourished people by 2015, it is clear that we cannot rely exclusively on food aid and fortification programmes anymore. New agricultural approaches are needed to work with the other approaches if the millennium developments goal is to be reached in the near future. Thus, agriculture must now focus on a new paradigm, a second green revolution, that will not only focus on increasing the production of certain agricultural crops (mostly cereals and staples), to feed a human population estimated to reach 10 billion by 2050, but also to deliver foods that are of better quality and are more nutrient-dense (Bouis *et al.* 2012). These crops will not only need to stave off hunger as the first green revolution did, but also help promote human health from a nutritional point of view. One such a strategy is agronomic biofortification (Chapter 4). The global production and increased use of macronutrient fertilizers to increase agricultural production, makes these fertilizers the ideal means of supplying agricultural crops with the trace elements that are also essential for human growth and development. While the current role of fertilizers in supporting human health, by providing enough food is large, the opportunity to expand it even more is also substantial.

From the literature, it is possible to see that the addition of the elements that have well documented deficiencies (I, Se, Zn and Fe) to agricultural crops through modified fertilizers, can significantly reduce the prevalence of the deficiencies caused by these elements (Eurola *et al.* 2005, Hartikainen 2005, Johnsson 2005, Legard 2005, Broadley *et al.* 2010). From the current study, it is clear that the addition of other trace elements and micronutrients, with less-well documented deficiencies, if supplied in sufficient amounts, can result in increased concentrations of these elements in the edible portions of the vegetable crops (Chapter 4). By producing foods whose edible portions are more dense in bioavailable nutrients, plant and soil scientists can help farmers provide a means for the medical community to provide natural alternatives to pharmaceutical solutions to reduce the prevalence of disease and illnesses related to micronutrient and trace element malnutrition. Agronomic biofortification can thus be used as an additional tool to help millions of people to overcome micronutrient and trace element malnutrition, not only of those elements with well documented deficiencies, but all essential trace elements.

There is nothing more important than supplying all people with all the nutrients required for a healthy and productive life. The sustainable means to this end must thus come from agriculture as there can be no human health without food. However, to achieve this goal,

various other barriers preventing people from having a healthy diet, the most significant of which is poverty, needs to be addressed. More nutrient-dense crops can provide a cheaper, means for more people to achieve nutrient efficiency, even when a diverse diet cannot be afforded. For this to be realised, informed government policies, as well as large investments in agricultural research are needed in the developing countries, in order to make these healthier options available to all.

5.2. Recommendations

Due to the low micronutrient and trace element concentrations found in the vegetable based foods collected in the current study (Chapter 2), it is suggested that further research into these deficiencies are done, not only as market basket surveys, but also farm specific sampling in order to determine where these deficiencies are more prevalent. This will allow decision makers to take the necessary steps to overcome these deficiencies at the source. Also, based on the miniscule contribution many of the food sources have to the DRI of these elements, it is suggested that people should be tested in order to determine whether they are suffering from micronutrient and trace element deficiencies. This is important as many of these deficiencies might not manifest as physical impairments, but could significantly affect cognitive abilities.

Although agronomic biofortification was shown to be a possible means to enrich crops with essential trace elements, the phytotoxicity exerted by the additional trace elements on seeds germination and root growth of seeds grown under controlled conditions is worrying (Chapter 3). It is this suggested that these experiments are carried out under field conditions to determine whether these phytotoxic effects remain true under field conditions. Also, a wider variety of seeds should be used as there were significant differences found between the seeds used in the current study. Although the crops that were biofortified with the trace elements did not show any major deficiency or toxicity symptoms, the reduction in water content as well as the reduction in uptake of essential plant nutrients could become a problem (Chapter 4). Because this study only tested the effects of a composite treatment, it is impossible to determine which of the elements could result in these symptoms. It is thus suggested that further research be done, where plants are biofortified with these elements as single nutrient additions, to determine which are the trace elements that causes in the symptoms described in chapter 4. This study (Chapter 4) should also be conducted on a wider variety of crops as results from the current study show large variations between the different crop species. This should also be done under field conditions to determine whether the results found in the current study remain true under these conditions.

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Appendix 1

The effects of tin additions to spinach plants

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Abstract

Agricultural crops form one of the major sinks for both essential and toxic elements released in the terrestrial environment and are often also important sources of toxic elements to the human diet. The increase in bioavailable tin in the environment could result in bioaccumulation of tin in agricultural crops, and thus, have adverse consequences on human lives. This study aims to investigate the effects of tin additions on the uptake of Sn, Mn, Fe, Cu, Zn, K, Ca, Mg and Na by spinach plants, as well as on the growth of the spinach plants. Spinach plants were grown in sand culture and received tin at concentrations of 0.02, 0.2, 2 and 20 mg/L along with a nutrient solution. Tin additions resulted in no visual toxicity symptoms, and might be beneficial to biomass production. Uptake at detectible concentrations only occurred at the highest concentrations (2 and 20 mg/L), after which it was mostly retained in the roots of the plants. Further field trials are needed to ensure that these experimental results remain true under field conditions.

Key words: beneficial; heavy metals; root allocation; stannous chloride

Introduction

Tin is a naturally occurring element in the earths' crust, and thus, is also a component of many soils. Normal concentrations in unpolluted soils range from >1 mg/kg to 200 mg/kg (WHO 2005) however, due to the increase in anthropogenic activities that uses, and releases tin products into the environment, the amount of bioavailable tin in the environment has significantly increased (Laughlin & Linden 1983, Weber 1985, Snoeij *et al.* 1986). In certain countries in Europe and North America, tin concentrations in sewage sludge ranged from 40 – 700 mg/kg dry weight, while manure and poultry wastes contained 3.7 – 7.4 mg/kg and 2.0 – 4.1 mg/kg respectively, before it is added to agricultural soils (Senesi *et al.* 1999). It is thus important to understand the uptake and allocation of tin by agricultural crops as tin accumulation in these crops is possible (Weber 1985). Agricultural crops form one of the major sinks for both essential and toxic mineral elements, released in the terrestrial environment. They are often also important sources of toxic elements to the human diet. The ingestion of relatively high concentrations of tin is known to cause toxicity in various mammalian species. Toxicity symptoms range from fatigue, headaches, diarrhoea, vomiting, muscular weakness and paralyses, anaemia, excessive damage to the liver and kidneys, and a reduction in various levels of neurotransmitters in the brain (Gerren *et al.* 1976, Graham *et al.* 1976, WHO 1980, Snoeij *et al.* 1987, WHO 2005). The aims of the current study was thus to assess the effects of different concentrations of tin on the uptake and allocation of Sn, Mn, Fe, Cu, Zn, K, Ca, Mg and Na as well as the growth of spinach plants, grown in sand culture.

Materials and Methods

Growing conditions and sample preparation

Spinach (*Spinacea oleracea* L.) was grown in a random block design, in sand culture under controlled conditions. The plants were watered with tap water daily until the seedlings were established, after which the plants received a nutrient solution (Chemicult – Kompel), with the addition of tin as stannous chloride ($SnCl_2.2H_2O$) at concentrations of 0.02, 0.2, 2 and 20 mg/L. The full strength nutrient solution was used as the control treatment.

The plants were harvested after nine weeks and separated into roots and shoots. Fresh mass of the shoots were determined after which both roots and shoots were oven dried at 50 °C to a constant mass. After oven drying, the dry mass of both roots and shoots were determined, after which the dried material was milled and stored for nutrient determination. The milled samples were digested using a sulphuric-peroxide digestion method (Moore & Chapman 1986) and the digested samples were thereafter filtered and diluted to 100 ml with deionised water.

Elemental Analyses

After digestion, Sn, Mn, Fe, Cu, Zn, K, Ca, Mg and Na concentrations in the roots and shoots of the spinach plants were determined using a Unicam Solaar M-series Atomic Absorption Spectrophotometer (AAS). Tin concentrations were determined in furnace mode while the other nutrient concentrations were determined in flame mode.

Statistical analyses

The Statistical Package for the Social Sciences version 21 (SPSS Inc., Chicago IL) was used to perform a Kruskal-Wallis analyses to determine whether there were statistically significant differences ($p \leq 0.05$) in tin concentrations as well as essential plant nutrient concentrations between the different treatments and also between the root and shoots of the spinach plants. Spearmans Rho (ρ) was used to determine the relationship between the tin additions and dry mass of the roots and shoots of the spinach plants.

Results and Discussion

The addition of tin to spinach plants resulted in no visual toxicity symptoms, and did not affect the uptake of the essential plant nutrients ($P \geq 0.05$). Romney *et al.* (1975) however, noted that the addition of tin to bush bean plants resulted in an increase in manganese and zinc concentrations and a decrease in iron concentrations while Cohen (1940), indicated that at high tin concentrations (> 1 – 100 mg/L), various toxicity symptoms occurred in pea and corn plants. A strong positive correlation was however found between the tin

concentrations supplied to the plants and the increase in dry mass of both roots ($n = 16$, $\rho = 0.702$, $p = 0.002$) and shoots ($n = 20$, $\rho = 0.503$, $p = 0.024$) of the spinach plants (Fig. 1).

…Figure 1…

This increase in the dry mass suggests that tin might have some beneficial effects on biomass production, and an increase in biomass production is known to be a prerequisite for increasing yields (Molnárová & Fargásová 2009). Cohen (1940) showed similar results, and found that the addition of tin to pea and corn plants at low concentrations (0.2 and 1 mg/L) increased root growth as well as the height of the corn plants.

In this study, tin could not be detected in the roots and shoots of the plants that received the treatments containing less than 0.2 mg/L tin, however, at the concentrations where tin was taken up at detectable concentrations by the plants, concentrations in the roots ($H_{(4,18)} = 16.260$, $p = 0.003$) and shoots ($H_{(4,18)} = 16.941$, $p = 0.003$) were significantly higher than that found in the control treatment (Fig. 2).

…Figure 2…

Within these plants, it was found that tin was allocated in significantly higher concentrations in the roots of the plants (Table 1), suggesting apoplastic localization, resulting in the poor transport of tin from the roots to the aerial parts of the plants. This corresponds with the literature suggesting that tin, even when applied at relatively high concentrations, is not readily available to plants, and when it is taken up, it is accumulated in the roots of the plants (Cohen 1940, Romney *et al.* 1975). However, these apoplastic regions could become saturated which results in the transfer of nutrients to the above ground parts of the plants (Prasad 2004).

… Table 1…

It is shown that different crop species respond differently to increased tin concentrations, and thus research on a wider variety of crop species is needed. However, because the current study was conducted under controlled conditions, it is suggested that further field trials are needed to ensure that these results remain true under field conditions.

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Table 1: Allocation (mg/kg) of tin in the roots and shoots of spinach plants

	Treatment	
	2 mg/L	**20 mg/L**
Roots	1.55	8.39
Shoots	Not detected	0.44
$H_{(1,8)}$	***6.054***	***5.398***
p	***0.014***	***0.02***

Figure 1: The effects of the addition of tin on the dry mass (g/kg) of spinach roots (A) and shoots (B)

Figure 2: Tin concentration (mg/kg) in the roots (A) and shoots (B) of spinach plants. Significant differences ($p < 0.05$), in the roots and shoots respectively, are shown by different letters.

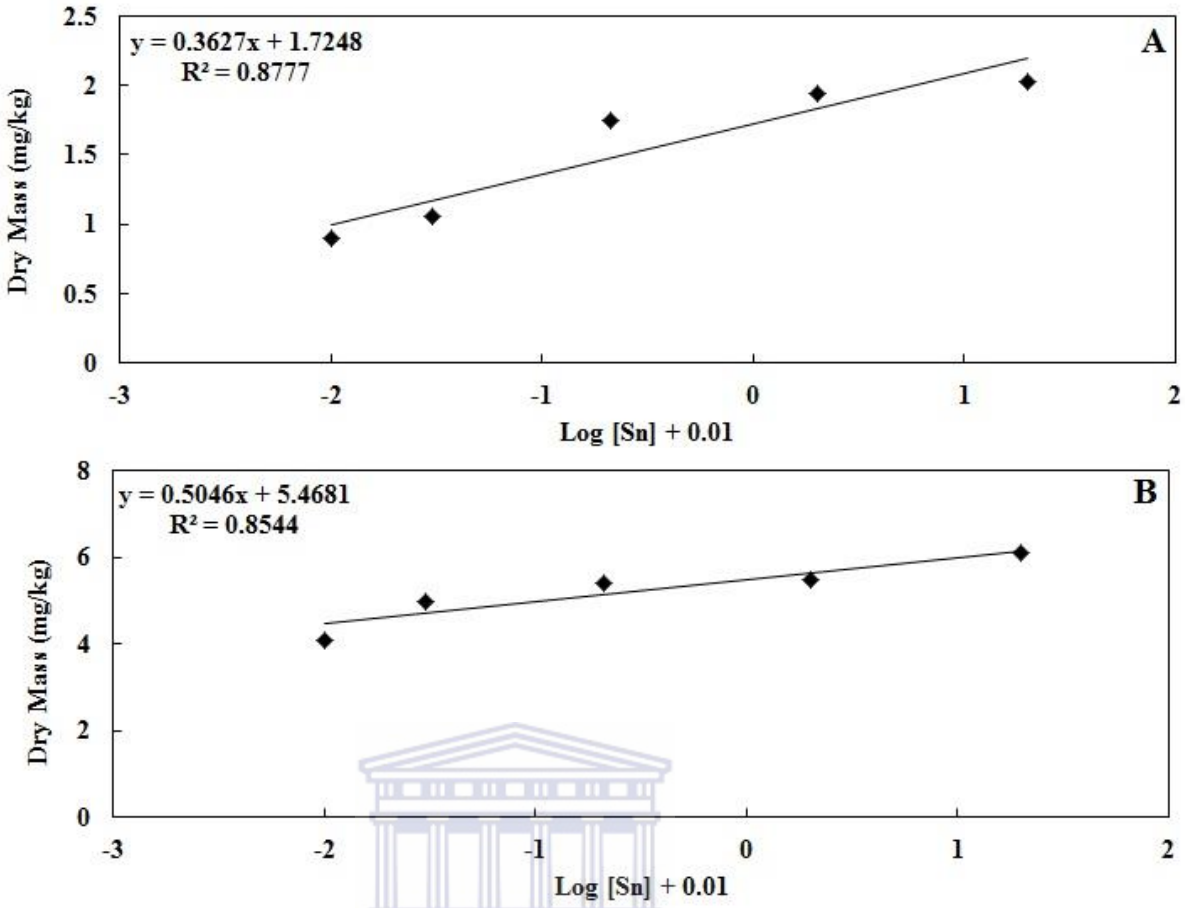

Figure 1

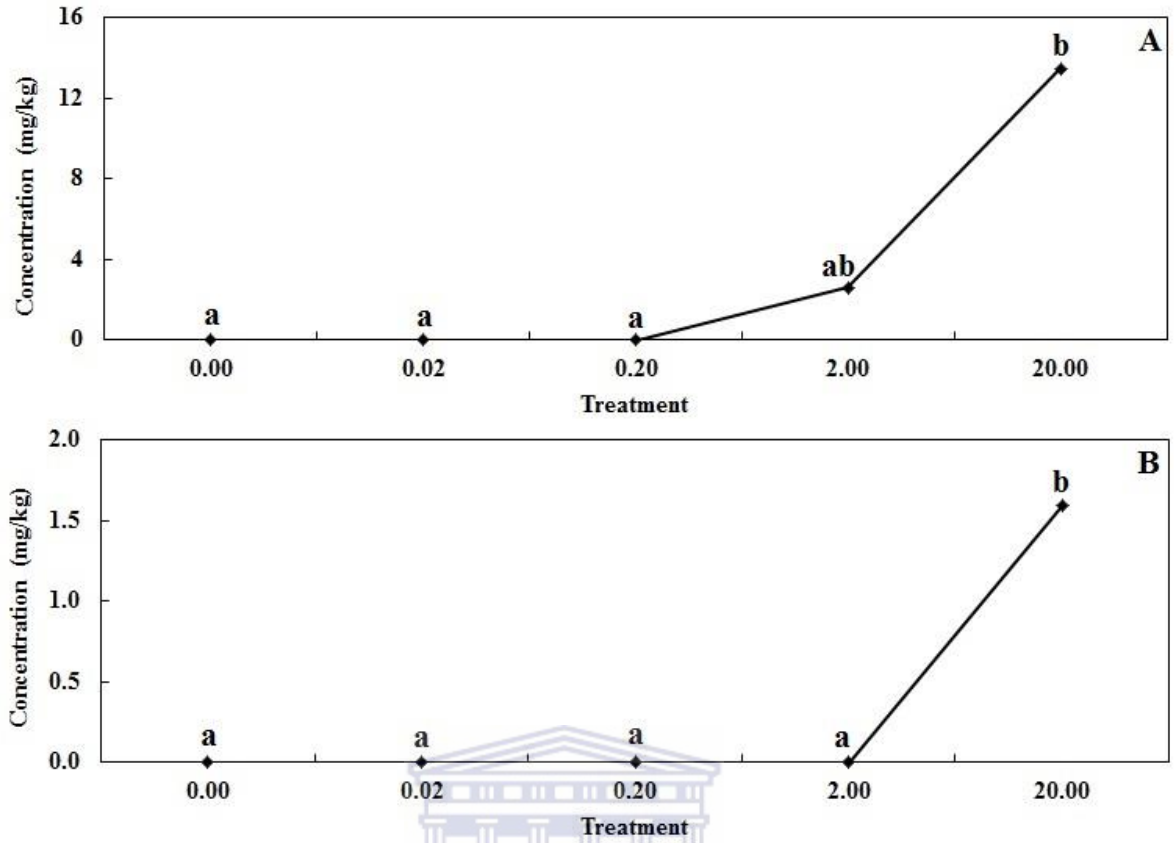

Figure 2