

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 (%) \pm SE									
	Co	Cr	F	I	Se	Si	Sn	V	Composite	Composite - F
Control	96 \pm 0.9 ^{bc}	96 \pm 1.2 ^a	96 \pm 1.6 ^c	96 \pm 1.6 ^a	96 \pm 1.2 ^a	96 \pm 1.2 ^a	96 \pm 1.2 ^a	96 \pm 1.2 ^a	96 \pm 1.2 ^b	96 \pm 1.2 ^a
Treatment 1	99 \pm 1.3 ^c	96 \pm 0.3 ^a	99 \pm 1.3 ^c	93 \pm 3.3 ^a	96 \pm 4.0 ^a	93 \pm 1.8 ^a	96 \pm 1.9 ^a	96 \pm 2.3 ^a	95 \pm 1.3 ^b	97 \pm 1.3 ^a
Treatment 2	95 \pm 0.7 ^b	99 \pm 0.9 ^a	88 \pm 6.1 ^b	95 \pm 3.5 ^a	93 \pm 2.7 ^a	88 \pm 2.3 ^a	96 \pm 0.9 ^a	99 \pm 0.7 ^a	92 \pm 2.3 ^b	96 \pm 0.0 ^a
Treatment 3	91 \pm 0.7 ^a	88 \pm 4.1 ^a	0 \pm 0.0 ^a	97 \pm 1.3 ^a	93 \pm 1.5 ^a	99 \pm 0.7 ^a	91 \pm 5.5 ^a	95 \pm 2.4 ^a	0 \pm 0.0 ^a	97 \pm 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 (%) \pm SE									
	Co	Cr	F	I	Se	Si	Sn	V	Composite	Composite - F
Control	96 \pm 4.0 ^a	96 \pm 4.0 ^a	96 \pm 4.0 ^c	96 \pm 4.0 ^a	96 \pm 4.0 ^a	96 \pm 4.0 ^a	96 \pm 4.0 ^b	96 \pm 4.0 ^b	96 \pm 4.0 ^c	96 \pm 4.0 ^a
Treatment 1	84 \pm 2.3 ^a	88 \pm 4.0 ^a	82 \pm 2.0 ^b	84 \pm 8.0 ^a	92 \pm 0.0 ^a	88 \pm 2.3 ^a	77 \pm 5.3 ^a	83 \pm 1.1 ^a	86 \pm 6.0 ^c	88 \pm 2.3 ^a
Treatment 2	78 \pm 6.0 ^a	90 \pm 6.0 ^a	84 \pm 4.0 ^b	73 \pm 3.5 ^a	72 \pm 8.0 ^a	76 \pm 8.0 ^a	73 \pm 1.3 ^a	88 \pm 0.0 ^{ab}	69 \pm 5.3 ^b	87 \pm 1.3 ^a
Treatment 3	84 \pm 4.0 ^a	88 \pm 4.0 ^a	0 \pm 0.0 ^a	84 \pm 4.0 ^a	74 \pm 2.0 ^a	87 \pm 5.8 ^a	82 \pm 2.0 ^{ab}	82 \pm 2.0 ^a	0 \pm 0.0 ^a	85 \pm 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) \pm SE									
	Co	Cr	F	I	Se	Si	Sn	V	Composite	Composite - F
Control	16.6 \pm 1.0 ^b	16.6 \pm 1.0 ^c	16.6 \pm 1.0 ^c	16.6 \pm 1.0 ^a	16.6 \pm 1.0 ^b	16.6 \pm 1.0 ^a	16.6 \pm 1.0 ^b	16.6 \pm 1.0 ^{ab}	16.6 \pm 1.0 ^c	16.6 \pm 1.0 ^b
Treatment 1	20.4 \pm 0.9 ^b	13.1 \pm 0.9 ^{bc}	16.2 \pm 1.1 ^c	13.4 \pm 1.0 ^a	21.1 \pm 1.2 ^b	16.8 \pm 1.0 ^a	13.6 \pm 1.0 ^b	22.2 \pm 5.2 ^b	13.7 \pm 0.9 ^c	28.5 \pm 1.6 ^c
Treatment 2	10.6 \pm 0.9 ^a	11.2 \pm 0.7 ^b	6.1 \pm 0.6 ^b	17.1 \pm 1.2 ^a	18.7 \pm 1.4 ^b	16.4 \pm 1.3 ^a	8.0 \pm 0.5 ^a	20.0 \pm 1.3 ^b	4.6 \pm 0.3 ^b	19.5 \pm 0.9 ^b
Treatment 3	16.9 \pm 1.2 ^b	3.6 \pm 0.3 ^a	0 \pm 0.0 ^a	17.0 \pm 1.0 ^a	8.0 \pm 0.6 ^a	17.3 \pm 1.2 ^a	7.3 \pm 0.5 ^a	14.4 \pm 0.9 ^a	0 \pm 0.0 ^a	8.2 \pm 0.5 ^a
<i>H</i> ₃	42.181	120.176	184.738		68.727		68.832	9.991	200.003	111.647
<i>P</i>	< 0.001	< 0.001	< 0.001	NS	< 0.001	NS	< 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) \pm SE									
	Co	Cr	F	I	Se	Si	Sn	V	Composite	Composite - F
Control	4.7 \pm 0.6 ^a	4.7 \pm 0.6 ^b	4.7 \pm 0.6 ^b	4.7 \pm 0.6 ^b	4.7 \pm 0.6 ^c	4.7 \pm 0.6 ^c	4.7 \pm 0.6 ^a	4.7 \pm 0.6 ^c	4.7 \pm 0.6 ^c	4.7 \pm 0.6 ^b
Treatment 1	4.8 \pm 0.6 ^a	4.6 \pm 0.6 ^b	4.1 \pm 0.7 ^b	3.5 \pm 0.3 ^b	3.3 \pm 0.4 ^{bc}	4.8 \pm 0.6 ^c	4.0 \pm 0.6 ^a	3.3 \pm 0.6 ^b	3.1 \pm 0.3 ^{bc}	4.4 \pm 0.5 ^b
Treatment 2	4.0 \pm 0.4 ^a	4.4 \pm 0.4 ^b	2.6 \pm 0.3 ^b	2.3 \pm 0.6 ^a	2.0 \pm 0.2 ^{ab}	2.4 \pm 0.5 ^a	3.7 \pm 0.6 ^a	3.2 \pm 0.3 ^b	2.0 \pm 0.2 ^b	3.3 \pm 0.4 ^a
Treatment 3	4.6 \pm 0.5 ^a	1.9 \pm 0.1 ^a	0 \pm 0.0 ^a	3.2 \pm 0.4 ^b	1.7 \pm 0.2 ^a	3.8 \pm 0.6 ^b	3.5 \pm 0.6 ^a	1.9 \pm 0.1 ^a	0.0 \pm 0.0 ^a	2.7 \pm 0.3 ^a
<i>F</i> ₃	NS	50.213	121.983	10.853	33.604	35.809	NS	32.217	127.38	20.76
<i>P</i>	NS	< 0.001	< 0.001	0.013	< 0.001	< 0.001	NS	< 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.

3.6 References

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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 (Euroola *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 (Euroola *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.



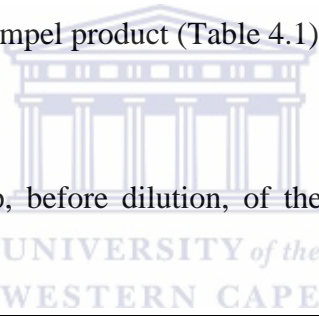
4.3 Materials and Methods

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 °C during the day and 10 °C 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.

Element	Chemical form	Concentration (g/kg)	
		Treatment 1	Treatment 2
Co	CoSO ₄ .7H ₂ O	0.0002	0.002
Cr	CrO ₃	0.001	0.01
F	NH ₄ F	0.004	0.04
I	KIO ₃	0.004	0.04
Se	Na ₂ SeO ₄	0.002	0.02
Si	*	0.04	0.4
Sn	SnCl ₂ .2H ₂ O	0.0002	0.002
V	NH ₄ VO 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 70° 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 Li₂SO₄.H₂O were added to 420 ml of 100 vol. H₂O₂. Selenium was not added to the mixture as selenium concentrations had to be determined. The solution was mixed well and 350 ml of conc. H₂SO₄ 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 Unicam 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, Spearman's 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) \pm standard deviation			Significance
		Control	Treatment 1	Treatment 2	
Cabbage	Roots	4.38 \pm 2.70 ^{ab}	10.20 \pm 6.67 ^b	2.54 \pm 1.70 ^a	$H_{(2,15)} = 7.819, P = 0.020$
	Shoots	39.66 \pm 9.72 ^a	48.84 \pm 24.31 ^b	14.01 \pm 9.59 ^a	$H_{(2,15)} = 7.980, P = 0.018$
Spinach	Roots	10.87 \pm 6.83	6.91 \pm 4.98	5.00 \pm 3.73	NS
	Shoots	40.21 \pm 14.92 ^b	20.75 \pm 17.48 ^{ab}	6.04 \pm 3.38 ^a	$H_{(2,15)} = 9.260, P = 0.010$
Lettuce	Roots	5.68 \pm 3.07	4.91 \pm 1.17	5.75 \pm 0.89	NS
	Shoots	25.97 \pm 12.50 ^b	21.05 \pm 10.13 ^{ab}	11.46 \pm 2.06 ^a	$H_{(2,15)} = 7.980, P = 0.018$
Turnip	Roots	6.00 \pm 2.86	7.33 \pm 9.41	3.37 \pm 1.92	NS
	Shoots	34.32 \pm 14.71	29.06 \pm 18.03	16.60 \pm 9.26	NS
		Mean dry mass (g) \pm standard deviation			
Cabbage	Roots	0.98 \pm 0.44 ^{ab}	2.50 \pm 1.64 ^b	0.50 \pm 0.38 ^a	$H_{(2,15)} = 7.819, P = 0.020$
	Shoots	3.61 \pm 1.48	4.81 \pm 2.23	2.60 \pm 1.42	NS
Spinach	Roots	2.61 \pm 2.51	2.13 \pm 1.60	1.48 \pm 1.06	NS
	Shoots	3.39 \pm 1.48	2.70 \pm 1.84	1.93 \pm 0.62	NS
Lettuce	Roots	0.50 \pm 0.26	0.60 \pm 0.15	0.81 \pm 0.28	NS
	Shoots	3.21 \pm 1.07	3.60 \pm 0.84	2.07 \pm 0.79	NS
Turnip	Roots	1.21 \pm 0.68	1.14 \pm 1.11	0.57 \pm 0.34	NS
	Shoots	4.15 \pm 1.31	3.71 \pm 1.80	3.38 \pm 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.

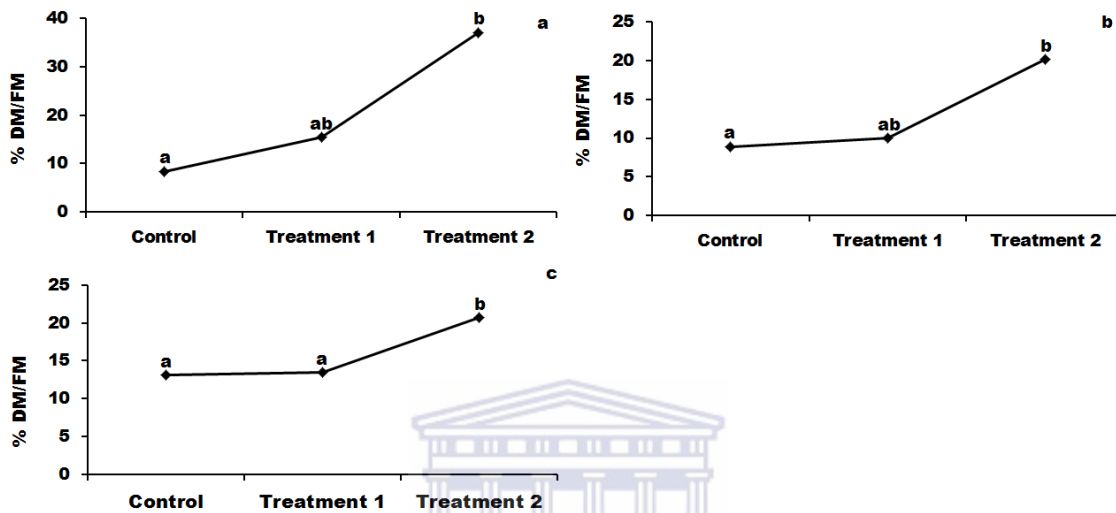


Figure 4.1: Relationship between the dry (DM) and fresh (FM) masses of spinach (a), cabbage (b), and turnip (c) plants. Comparisons between the different treatments were made within organs (roots or shoots), and not between roots and shoots. Significant differences ($P < 0.05$) within the roots and shoots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

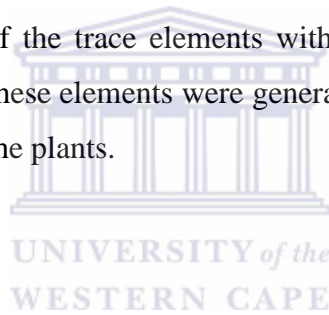


Table 4.4: Accumulation of trace elements in the roots and shoots of four biofortified vegetable crop species. Concentrations with the same letters are not significantly different ($P \geq 0.05$) from one another.

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

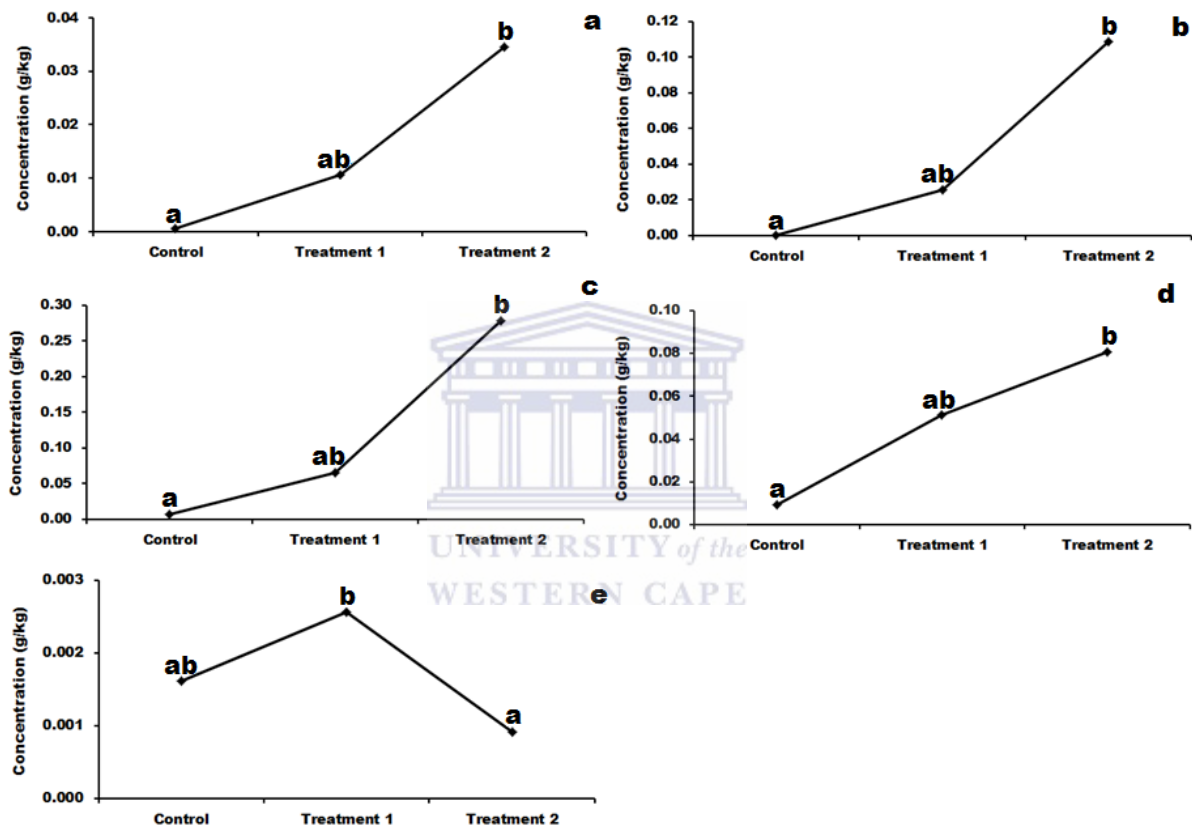


Figure 4.2: Cobalt (a), chromium (b), vanadium (c), selenium (d) and silicon (e) concentrations in the edible portions of spinach plants. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

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).

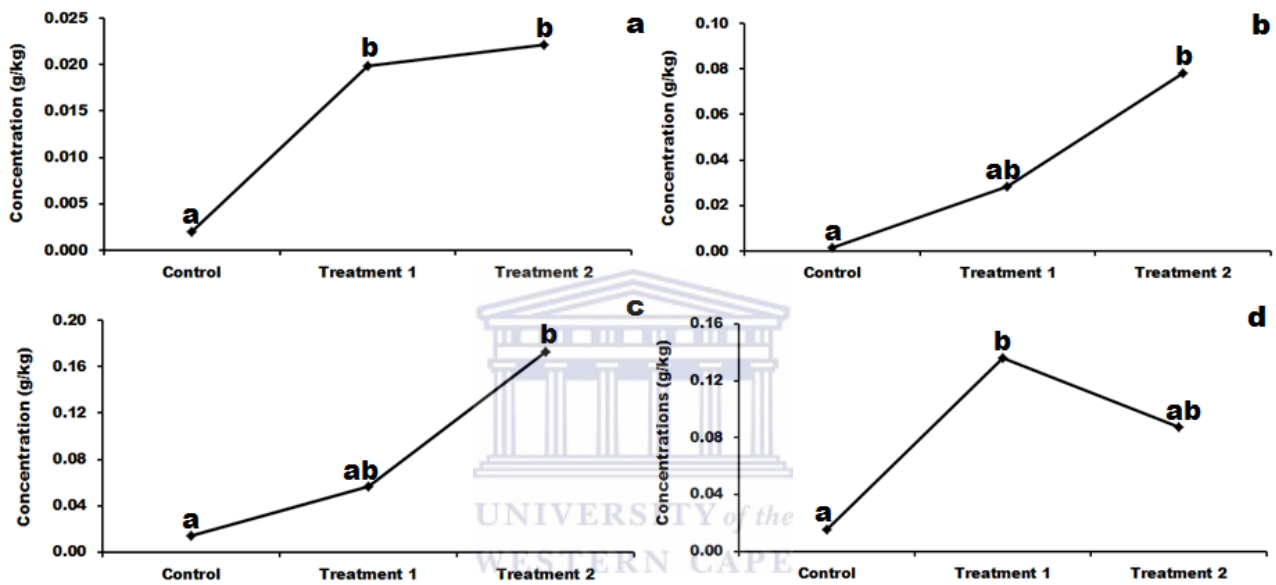


Figure 4.3: Cobalt (a), chromium (b), vanadium (d) and selenium (d) concentrations in the edible portions of cabbage plants. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

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).

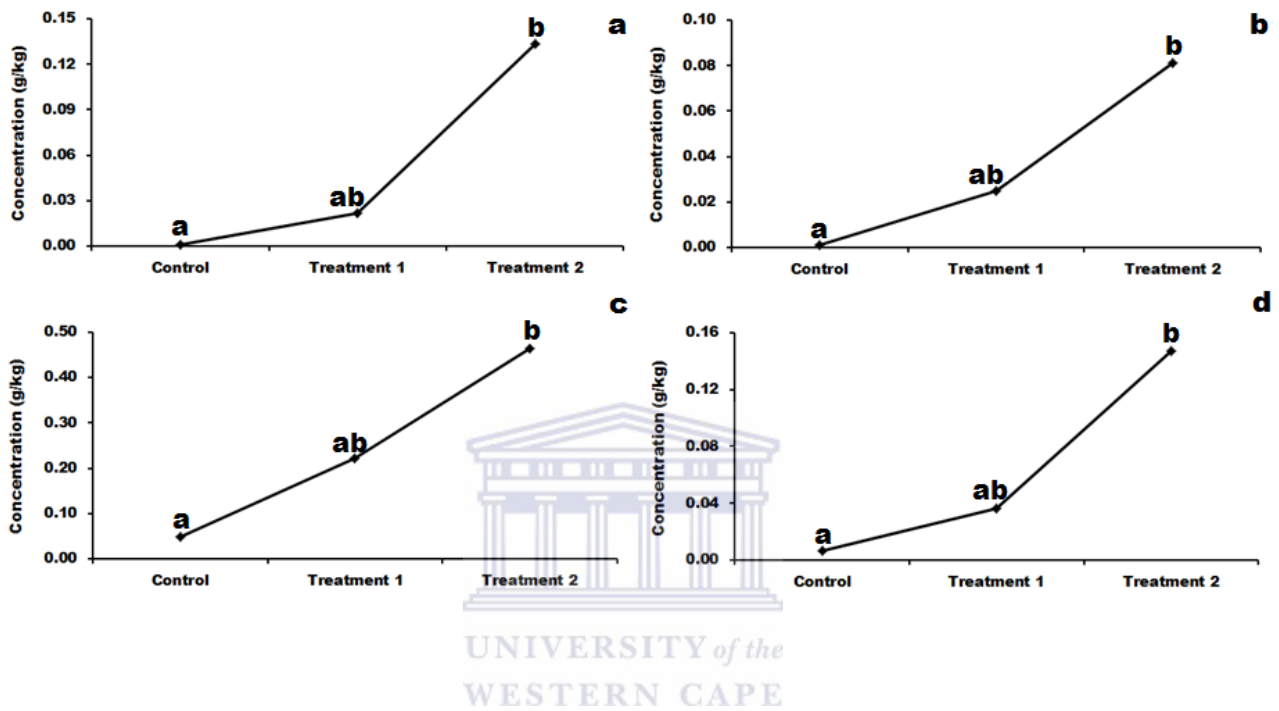


Figure 4.4: Cobalt (a), chromium (b), vanadium (c) and selenium (d) concentrations in the edible portions of turnip plants. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

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).

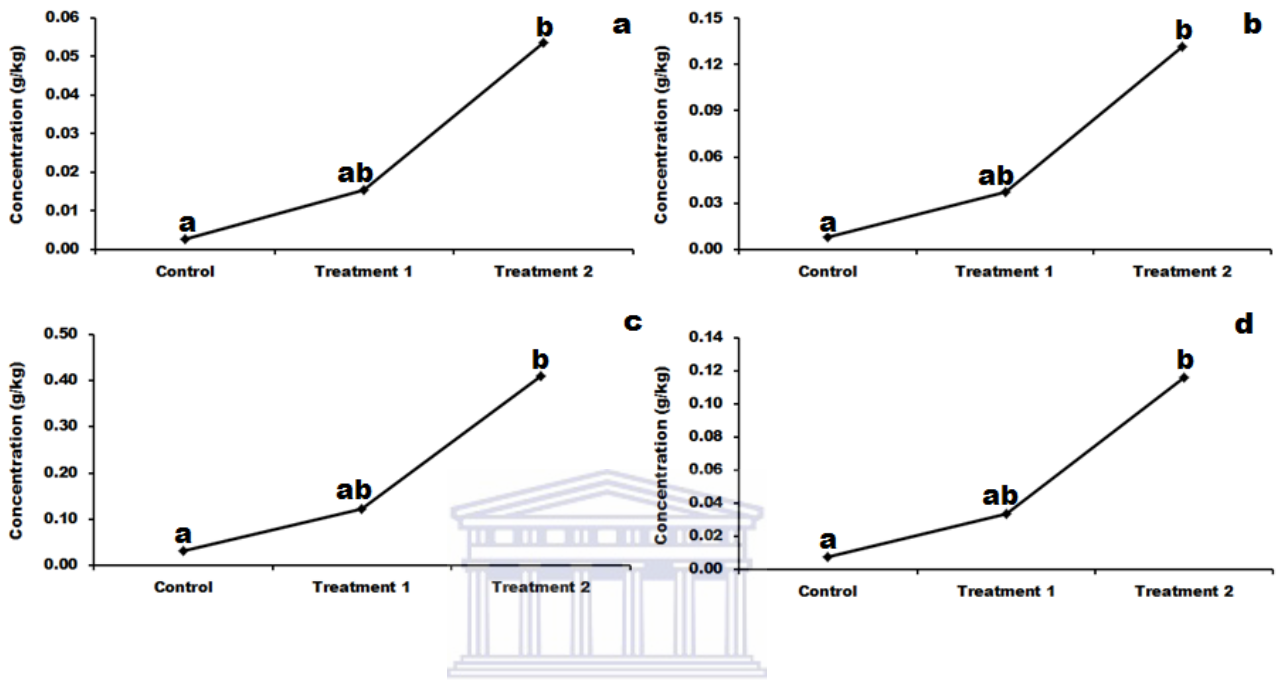


Figure 4.5: Cobalt (a), chromium (b), vanadium (c), and selenium (d) concentrations in the edible portions of lettuce plants. Mean concentrations that are not significantly different ($P \geq 0.05$) between the different treatments for each element, is shown by the same letters.

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 ($R^2 = 0.9087$, $\rho = 0.549$, $P = 0.034$) and lettuce ($R^2 = 0.6344$, $\rho = 0.577$, $P = 0.024$) as well as iodine concentrations in lettuce ($R^2 = 0.6077$, $\rho = 0.520$, $P = 0.047$) 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) \pm standard deviation								
		N	P	Mn	Mg	K	Fe	Ca	Cu	Zn
Spinach	Control	4.35 \pm 1.91	0.38 \pm 0.10	0.08 \pm 0.04	0.46 \pm 0.15	9.57 \pm 3.52	0.23 \pm 0.09	5.61 \pm 0.77	0.003 \pm 0.001	0.01 \pm 0.00
	Treatment 1	3.05 \pm 1.27	0.42 \pm 0.16	0.06 \pm 0.03	0.54 \pm 0.43	7.59 \pm 5.28	0.19 \pm 0.06	5.68 \pm 0.33	0.003 \pm 0.002	0.01 \pm 0.00
	Treatment 2	3.59 \pm 2.69	0.4 \pm 0.14	0.04 \pm 0.01	0.49 \pm 0.18	5.77 \pm 4.37	0.20 \pm 0.04	5.89 \pm 0.82	0.003 \pm 0.001	0.01 \pm 0.00
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cabbage	Control	5.98 \pm 1.63	0.48 \pm 0.11	0.04 \pm 0.00	0.53 \pm 0.14	11.28 \pm 2.50	0.21 \pm 0.02	5.07 \pm 0.83	0.003 \pm 0.002 ^{ab}	0.01 \pm 0.00
	Treatment 1	4.91 \pm 1.29	0.45 \pm 0.12	0.03 \pm 0.00	0.31 \pm 0.10	8.76 \pm 1.87	0.19 \pm 0.06	4.78 \pm 0.50	0.003 \pm 0.001 ^a	0.01 \pm 0.00
	Treatment 2	8.62 \pm 3.23	0.68 \pm 0.37	0.07 \pm 0.02	1.01 \pm 0.59	11.93 \pm 8.53	0.27 \pm 0.24	15.28 \pm 18.99	0.006 \pm 0.002 ^b	0.05 \pm 0.07
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	8.796 0.012	NS
Turnip	Control	14.22 \pm 7.68	0.73 \pm 0.41	0.05 \pm 0.01	1.41 \pm 1.01	28.58 \pm 17.58	0.26 \pm 0.06	6.9 \pm 4.31	0.005 \pm 0.003	0.02 \pm 0.01
	Treatment 1	8.90 \pm 4.54	0.74 \pm 0.40	0.04 \pm 0.02	1.34 \pm 0.81	23.17 \pm 12.57	0.19 \pm 0.04	6.61 \pm 2.03	0.004 \pm 0.002	0.01 \pm 0.01
	Treatment 2	17.66 \pm 2.67	0.89 \pm 0.06	0.05 \pm 0.02	1.69 \pm 0.39	32.13 \pm 4.74	0.16 \pm 0.09	9.25 \pm 3.65	0.005 \pm 0.002	0.02 \pm 0.00
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lettuce	Control	11.25 \pm 2.77 ^{ab}	0.79 \pm 0.39 ^b	0.08 \pm 0.01 ^b	5.18 \pm 1.87	24.6 \pm 7.35	0.26 \pm 0.06	10.73 \pm 4.17	0.018 \pm 0.009	0.05 \pm 0.01 ^b
	Treatment 1	14.04 \pm 3.37 ^b	0.78 \pm 0.18 ^b	0.05 \pm 0.01 ^a	5.90 \pm 3.22	23.7 \pm 3.32	0.21 \pm 0.02	7.24 \pm 0.90	0.015 \pm 0.006	0.03 \pm 0.00 ^a
	Treatment 2	7.73 \pm 4.09 ^a	0.39 \pm 0.11 ^a	0.07 \pm 0.01 ^{ab}	6.72 \pm 3.11	15.64 \pm 4.59	0.19 \pm 0.02	6.87 \pm 1.59	0.015 \pm 0.005	0.04 \pm 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) \pm standard deviation								
		N	P	Mn	Mg	K	Fe	Ca	Cu	Zn
Spinach	Control	22.70 \pm 3.54	1.87 \pm 0.58	0.13 \pm 0.01	3.63 \pm 0.83	104.98 \pm 8.79 ^b	0.24 \pm 0.05	6.86 \pm 1.57	0.005 \pm 0.003	0.02 \pm 0.00 ^b
	Treatment 1	18.49 \pm 2.92	1.63 \pm 0.38	0.12 \pm 0.00	3.52 \pm 1.13	83.63 \pm 22.76 ^{ab}	0.20 \pm 0.02	7.62 \pm 0.81	0.004 \pm 0.002	0.02 \pm 0.00 ^a
	Treatment 2	15.89 \pm 7.17	1.49 \pm 0.65	0.12 \pm 0.02	5.43 \pm 2.68	61.06 \pm 28.09 ^a	0.16 \pm 0.04	9.35 \pm 1.74	0.004 \pm 0.001	0.02 \pm 0.00 ^{ab}
	$H_{(2, 15)}$ P	NS	NS	NS	NS	6.980 0.031	NS	NS	NS	6.020 0.049
Cabbage	Control	21.61 \pm 3.82	1.01 \pm 0.16 ^b	0.09 \pm 0.01 ^b	3.24 \pm 0.77	108.26 \pm 6.71 ^b	0.13 \pm 0.04	12.69 \pm 1.48 ^b	0.003 \pm 0.001	0.02 \pm 0.00
	Treatment 1	22.29 \pm 3.58	1.01 \pm 0.08 ^b	0.09 \pm 0.00 ^b	3.25 \pm 0.43	79.48 \pm 7.06 ^{ab}	0.13 \pm 0.07	12.24 \pm 0.40 ^{ab}	0.003 \pm 0.001	0.02 \pm 0.00
	Treatment 2	15.59 \pm 2.53	0.61 \pm 0.06 ^a	0.06 \pm 0.01 ^a	2.43 \pm 0.27	44.89 \pm 2.82 ^a	0.08 \pm 0.03	9.95 \pm 1.43 ^a	0.003 \pm 0.001	0.01 \pm 0.00
	$H_{(2, 15)}$ P	NS	9.397 0.009	8.660 0.013	NS	12.500 0.002	NS	6.860 0.032	NS	NS
Turnip	Control	22.56 \pm 3.33	1.22 \pm 0.16 ^b	0.07 \pm 0.01	2.66 \pm 0.33	89.99 \pm 5.02 ^b	0.12 \pm 0.05 ^b	10.85 \pm 0.78	0.004 \pm 0.002	0.02 \pm 0.01 ^b
	Treatment 1	20.42 \pm 4.81	0.97 \pm 0.33 ^{ab}	0.06 \pm 0.01	2.34 \pm 0.69	69.55 \pm 17.36 ^{ab}	0.09 \pm 0.07 ^{ab}	9.84 \pm 1.74	0.003 \pm 0.001	0.01 \pm 0.00 ^a
	Treatment 2	16.32 \pm 3.23	0.67 \pm 0.14 ^a	0.07 \pm 0.01	2.27 \pm 0.29	46.08 \pm 5.78 ^a	0.05 \pm 0.02 ^a	9.86 \pm 1.28	0.002 \pm 0.001	0.02 \pm 0.00 ^{ab}
	$H_{(2, 15)}$ P	NS	7.220 0.027	NS	NS	10.140 0.006	7.280 0.026	NS	NS	6.260 0.044
Lettuce	Control	10.66 \pm 5.00	0.75 \pm 0.23	0.11 \pm 0.05	2.38 \pm 0.80	66.07 \pm 24.17	0.21 \pm 0.07 ^b	9.15 \pm 2.65	0.004 \pm 0.003	0.05 \pm 0.02
	Treatment 1	11.43 \pm 3.30	0.27 \pm 0.13	0.07 \pm 0.01	1.76 \pm 0.16	44.04 \pm 9.36	0.14 \pm 0.03 ^{ab}	7.33 \pm 1.75	0.003 \pm 0.001	0.03 \pm 0.01
	Treatment 2	13.35 \pm 5.59	0.42 \pm 0.31	0.06 \pm 0.01	1.47 \pm 0.32	40.21 \pm 10.20	0.11 \pm 0.02 ^a	7.21 \pm 1.89	0.003 \pm 0.001	0.04 \pm 0.00
	$H_{(2, 15)}$ P	NS	NS	NS	NS	NS	6.260 0.044	NS	NS	NS

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 world's population (Bouis and Islam 2011, FAO *et al.* 2012). It is estimated that 60 – 80 % of the world's 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 (Euroola *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

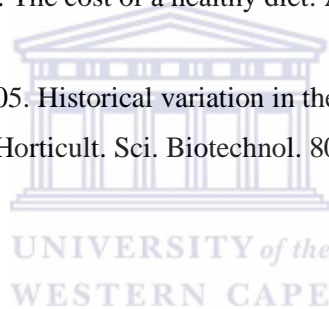
Due to the low micronutrient and trace element concentrations found in the vegetable based foods collected in the current study (Chapter 2), it is suggested that further research into these deficiencies are done, not only as market basket surveys, but also farm specific sampling in order to determine 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 earth's 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 ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) 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. Spearman's 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 & Fargášová 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.

Acknowledgements

Mosilo Machere is thanked for her contribution in the growth experiments. The National Research Foundation provided the funding to do this study. The opinions expressed and conclusions arrived at, are however those of the authors.

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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.



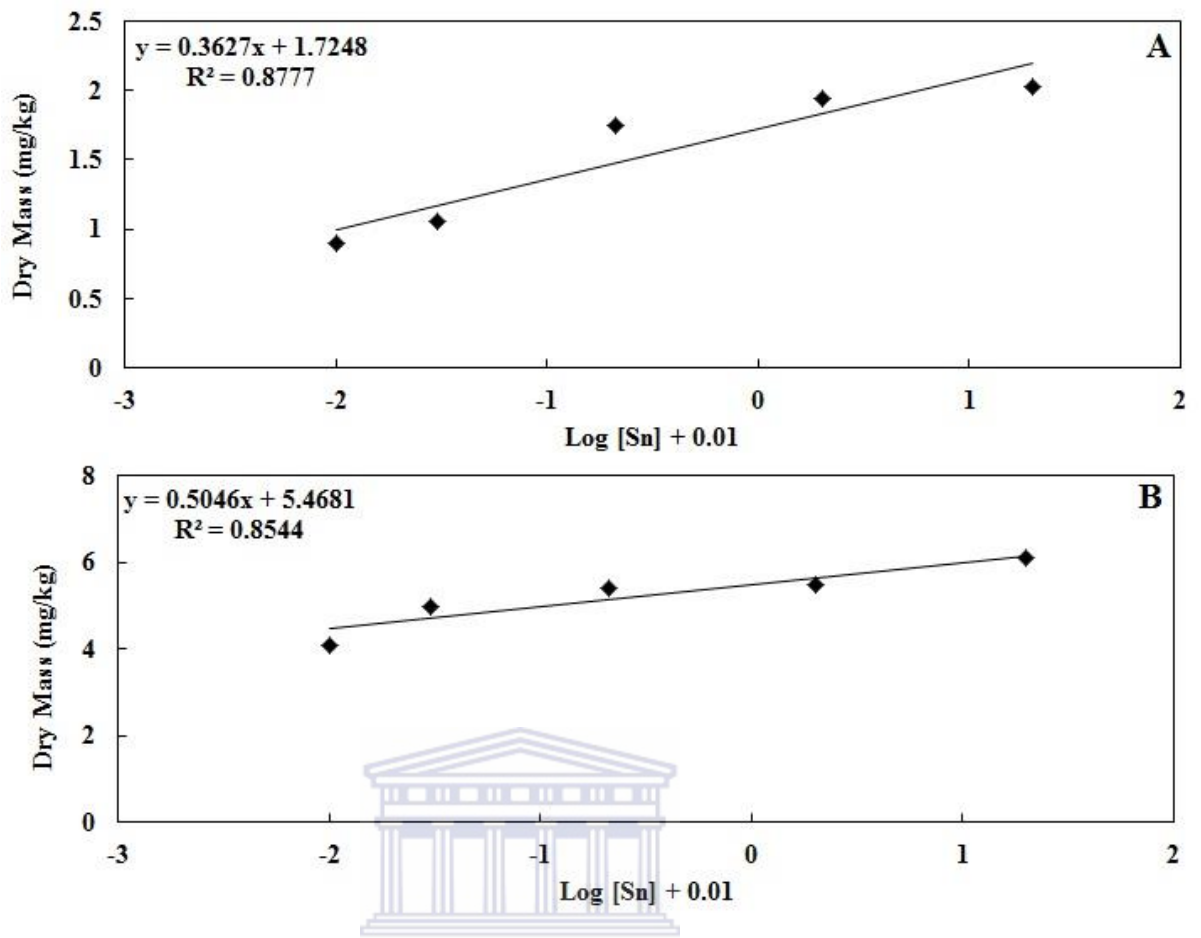


Figure 1



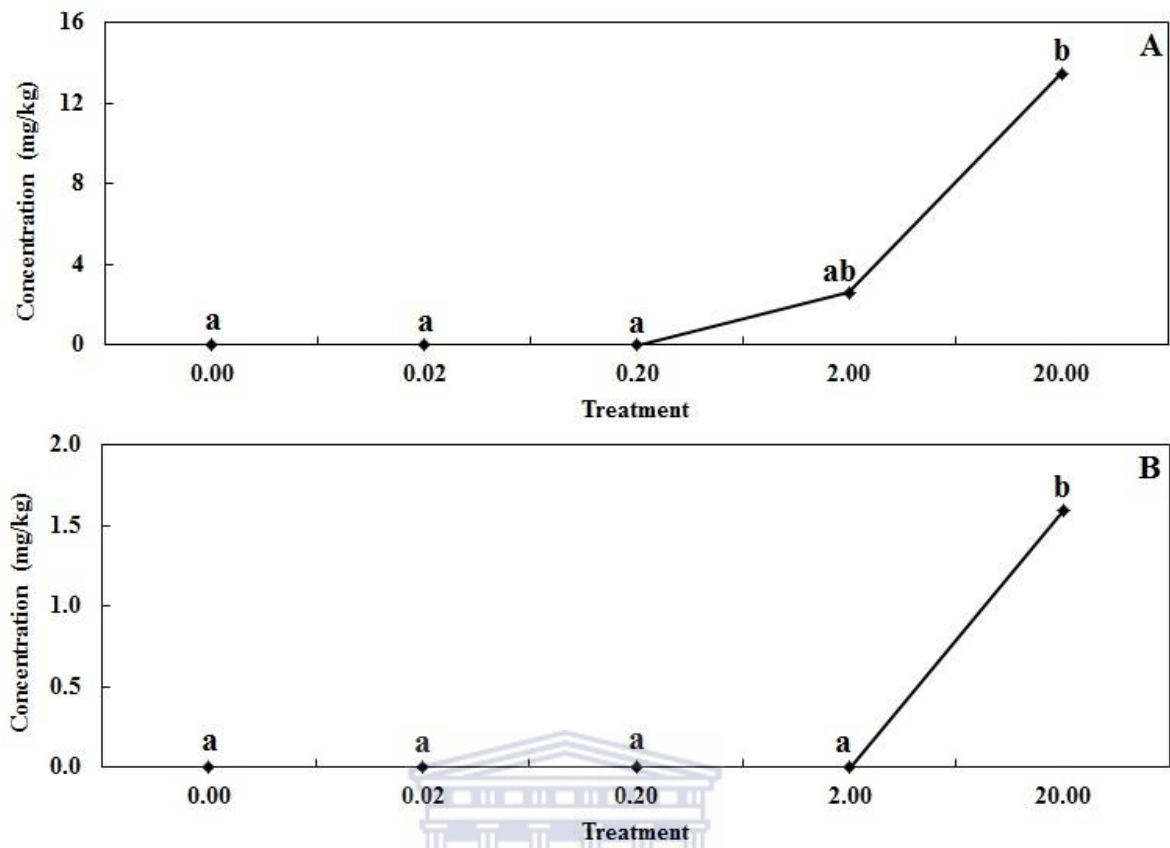


Figure 2

