Trace element composition of two wild vegetables in response to soil-applied micronutrients

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Wild vegetables are an important commodity in the subsistence farming sector. They are considered to be rich in micronutrients and can therefore be used to overcome inadequate nutrition. However, research on micronutrients in wild vegetables remains limited and sporadic. In this study, we evaluated the responses of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – to micronutrients added to the soil in comparison with a reference crop, Swiss chard (*Beta vulgaris* var. *cicla*). Swiss chard concentrated significantly ($p<0.01$) higher amounts of Cu, Zn and Mn in the leaves than did the wild vegetables. Variations in micronutrients among the vegetables were greater for Zn (72–363 mg/kg) and Mn (97.9–285.9 mg/kg) than for Cu (8.8–14 mg/kg). *C. olitorius* had the least capacity to concentrate Mn and Zn in the leaves. However, *C. olitorius* concentrated significantly more Fe (327 mg/kg) in the leaves than did *A. cruentus* (223 mg/kg) or *B. vulgaris* (295 mg/kg). The mean per cent S concentration in the leaves ranged from 0.26% in *C. olitorius* to 0.34% in *A. cruentus* and *B. vulgaris*. We conclude that the different vegetables had different abilities to concentrate Cu and Zn in the order *B. vulgaris* > *A. cruentus* > *C. olitorius*. These results seem to contradict the belief that wild vegetables have an inherent ability to concentrate mineral micronutrients in their tissues.

Introduction

A review of micronutrients in diets in South Africa and other developing countries by Steyn and Herselman[1] revealed widespread shortages. They concluded that the poor micronutrient status among the poor is caused by insufficient and undiversified diets and compounded by soils of low micronutrient status. There are three main ways in which micronutrients can be added to foods: (1) food fortification or supplementation, (2) genetic bio-fortification and (3) agronomic biofortification.[2] Food fortification is widespread and efficient for those who can afford fortified foods, but is not applicable to most rural populations in Africa, which produce most of their staple crops, vegetables and meat at their farms and therefore do not generally purchase micronutrient fortified foods. Biofortification through conventional breeding shows promise, but research is currently limited to a small range of staple crops.[3] Genetic biofortification is still mostly at the experimental stage, and even when it becomes viable, it might be some time before the genotypes that result are widely available and affordable to subsistence farmers. Agronomic biofortification, which is the increase in plant tissue micronutrient content through soil or foliar application of fertilisers, also involves the purchase of these mineral fertilisers and is only viable in commercial agriculture. Another strategy, which is the long-term goal of our present study, is to use locally available wild, semi-domesticated and domesticated vegetable species that have the ability to concentrate the mineral micronutrients Zn, Cu and Fe, even from depleted soils. The possibility of exploiting the variability in shoot mineral content among different plant species in combating micronutrient under-nutrition has been discussed in detail by Broadley et al.[4,5]

Our targeting of wild vegetables is based on the knowledge that they are widely available and relatively easily accessible to poor subsistence farmers. These wild vegetables (also known in South Africa as African leafy vegetables, indigenous vegetables, *morogo* and *imfino*) are variously reported to be superior to conventionally cultivated vegetables such as Swiss chard (*Beta vulgaris* var. *cicla*) and cabbage (*Brassica oleracea* var. *capitata*)[6,7] in micronutrients, including beta-carotene and minerals such as Zn, Fe and Cu. These vegetables are important to subsistence farmers, especially the poor, some of whom rely on wild vegetables as a major form of relish used to complement and accompany staple meals such as *phutu, pap, ugali* and *sadza*.[8]

A recent (2012) report on the importance of African leafy vegetables reaffirmed the potential of these vegetables in the eradication of micronutrient under-nutrition.[7] The report particularly highlighted the prevalence of vitamin A and Fe deficiencies in rural parts of South Africa. Although Zn deficiency was reported as not being well documented in developing countries[9], including South Africa[7], the World Health Organization has identified Zn as one of the most serious deficiencies in the past decade.[10] The consumption of fruits and micronutrient-rich vegetables has been reported to be low, leading to monotonous and micronutrient-poor diets in several provinces of South Africa.[7,11] It is against such a background that promotional and research efforts for wild vegetables need to be taken seriously.

Wild vegetables are widely reported to have superior nutritional properties with respect to micronutrients compared to conventional vegetables such as cabbage (*Brassica oleracea* var. *capitata*) and *Beta vulgaris* var. *cicla*.[12-16] There is wide variability in the nutritional composition reported for these vegetables in different studies. The claim that uncultivated indigenous vegetables could have superior micronutrient levels to cultivated conventional vegetables suggests that there is the possibility of increasing their micronutrient content if micronutrients are added to the soil.[17] For most conventional crops, research has established critical points for deficiency or excess (toxicity).[18] Such information is important in crop production if correct amounts of fertilisers are to be applied. The toxicity risks associated with applying excessive amounts of micronutrients to the soil were extensively reviewed by White et al.[18] The levels of nutrients applied in crop production also determine the ability of a food crop to supply nutrients to humans. Deficiencies of these micronutrients in the soil also lead to reduced crop yields, thereby presenting a double problem: reduced amounts of food of poor nutritional quality.[17] Some studies that have reported the superior nutrient composition of wild vegetables in South Africa[12-16] were not controlled experiments and involved collection from the wild or purchasing from the market and conducting tests. The aim of the present study was to compare, under well-defined conditions, the accumulation of Zn, Cu and Fe in the leaves of two leafy wild vegetable species commonly consumed in the rural areas of South Africa – wild okra (*Corchorus olitorius)* and pigweed (*Amaranthus cruentus* var. Arusha) – and a reference vegetable crop that is also widely eaten – Swiss chard (*Beta vulgaris* var. *cicla*).

Materials and methods

The experiment was conducted on potted plants in a greenhouse at the University of Zululand (28°51'S; 31°51'E). The growing medium used was soil collected from the university farm. The soil has been classified as Glenrosa soil form.[19] Prior to filling the pots, the soil was sieved through a 13-mm diamond mesh wire mounted on a wooden frame, to homogenise it and to remove large stones, clods, sticks and grass.

Treatments

There were two factors in the study – vegetable species and micronutrient fertiliser. Vegetable species were of three types: *Corchorus olitorius*, *Amaranthus cruentus* var. Arusha and *Beta vulgaris* var. *cicla*. Micronutrient fertiliser had four levels: 0 kg/ha (control), 5 kg/ha, 10 kg/ha and 15 kg/ha each of Cu, Fe and Zn mixed and applied in the form of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$ (Table 1). The combination of the two factors resulted in a 3x4 factorial experiment with 12 treatments arranged in a randomised complete block design with three replications. The micronutrient, basal fertiliser and nitrogen top dressing rates were calculated per plant based on a standardised plant density of 100 000 plants per hectare for the three vegetable species. Because basal fertiliser was not a treatment in this study, a general basal fertiliser of 5 g per plant of NPK 2:3:2 (14) was added pre-plant to all the pots resulting in 0.2 g N, 0.3 g P and 0.2 g K applied per plant. Lime ammonium nitrate (LAN, 28% N) top dressing fertiliser was also applied 10 days after transplanting to all pots at a rate of 3 g per plant resulting in 0.84 g of N applied per plant. Each pot contained 2.4 kg of air-dried soil. The micronutrient treatments were added 15 days after transplanting.

Plant management

Seedlings of each of the three test species were germinated and grown for 38 days in the commercial growth medium Hygromix® (Hygrotech Sustainable Solutions, Pretoria, South Africa) in polystyrene trays. Seedlings of uniform size were selected and transplanted into moist soil in which basal fertiliser had been applied pre-plant as described above. One seedling was planted per pot and there were four pots for each treatment. Thereafter, plants were watered in a way that avoided leaching of the nutrients from the soil. There were no plant mortalities such that at harvesting time each treatment had four plants. Samples of the youngest fully expanded leaves (blade and petiole) were taken 26 days after the application of micronutrients for chemical analysis. Samples were harvested from all the four plants per treatment so as to collect enough samples for analysis. Leaf samples were washed in distilled water soon after harvesting. Excess water was allowed to drain off for 4 h before the samples were placed in new brown paper bags and dried in the oven at 60 °C until they had attained uniform mass.

Chemical analyses of youngest fully expanded leaves

Dried plant samples were milled in a Retsch ZM200® mill (Retsch GmbH, Haan, Germany) to pass through a 0.5-mm sieve. They were then submitted for analysis to the Fertiliser Advisory Services of the South African Sugar Research Institute, Mount Edgecombe, KwaZulu-Natal.

Measurement of leaf nutrients

All the analysed elements were determined according to methods described by Wood et al.[20] For Zn, Cu, Fe, Mn and S, a 1-g dried leaf sample was digested in 15 mL nitric acid followed by 5 mL perchloric acid. After digestion, the resultant mixture was filtered and then made up to 50 mL using water. The elements were then determined using atomic absorption spectrophotometry. S was determined colorimetrically. For N, P, K, Ca and Mg, a 0.25-g dried leaf sample was digested in 2 mL selenised sulphuric acid for 1.5 h in a Kjeldatherm block digester at a temperature of 370 °C. After digestion, the samples were diluted by adding water to a volume of 1 L and K, Ca and Mg were determined by atomic absorption spectrophotometry; N and P were determined colorimetrically.

Soil analyses

Information on physico-chemical attributes of the soil (Table 2) was obtained prior to fertiliser application. Soil pH was determined in 0.01 M $CaCl_2$. Exchangeable acidity was determined by titration after extraction with 1 M KCl. The macronutrients P, K, Ca, Mg and Ca and micronutrients Fe, Cu, Zn and Mn were determined using the method described by Van der Merwe et al.[21] after extraction by the Ambic–2 extraction method using EDTA-di-ammonium solution. Truog-extractable P was determined colorimetrically after extraction with 0.02 N sulphuric acid. Si was determined as described by Miles et al.[22] after overnight extraction with 0.01 M $CaCl_2$.

Data analysis

Analysis of variance was performed on the data using the Genstat 12 statistical package to test for significant treatment effects. Statistical significance was evaluated at $p<0.05$. Where the *F*-tests were significant, the treatment means were separated using the least significant difference test.[23,24]

Results and discussion

The effects of the micronutrient mixture on leaf nutrient concentration varied according to plant species and application rate. *B. vulgaris* concentrated Cu, Zn and Mn in the leaves significantly ($p<0.01$) more than did A. *cruentus* and *C*. *olitorius* (Table 3). The variations among the vegetables in these three micronutrients were greater for Zn (72–363 mg/kg) and Mn (98–286 mg/kg) than they were for Cu (9–14 mg/kg). In a previous study, in which Swiss chard was grown in several different types of soil in South Africa,[1] wide variations in micronutrient concentrations (2.72–152.12 mg/kg for Cu and 11.9–623.8 mg/kg for Zn) were reported. In that same study, Swiss chard samples from fruit and vegetable markets and shops had Cu levels of 9.4–111.7 mg/kg and Zn levels of 34.0–816.0 mg/kg.[1] The large discrepancies between our study and that of Steyn and Herselman's[1]

Table 1: Combinations of the sulphates of Cu, Fe and Zn to achieve the desired micronutrient treatments used in the experiment

Amount of each of elemental Cu, Fe and Zn applied (kg/ha)	Amount of the sulphate of each of Cu, Fe and Zn in grams applied per plant to achieve desired rate of element in kg/ha based on a standardised plant population of 100 000 plants/ha		
	$ZnSO_4.7H_2O$	$CuSO_4.5H_2O$	$FeSO_4.7H_2O$
0	0	0	0
5	0.22	0.25	0.20
10	0.44	0.50	0.40
15	0.66	0.75	0.60

in leaf Cu and Zn concentration ranges of Swiss chard are difficult to explain. Similar variations in Zn concentrations in plant tissues used for food were also reported in other studies.[25] In the present study, *C. olitorius* had the least capacity to concentrate Mn and Zn in the leaf, which suggested that this vegetable is a less satisfactory candidate for agronomic biofortification of these micronutrients. However, *C. olitorius* leaves concentrated significantly more Fe (327 mg/kg) than did *A. cruentus* (223 mg/kg) or *B. vulgaris* (295 mg/kg).

Table 2: Soil physico-chemical analysis results of the soil used in the study

Soil attribute	Units	Value
pH (0.01 M $CaCl_2$)	–	4.58
Phosphorus	mg/L	10.1
Potassium	mg/L	169.9
Calcium	mg/L	1432.3
Magnesium	mg/L	525.4
Sodium	mg/L	104.3
Exchangeable acidity	cmol/L	0.19
Total cations	cmol/L	12.55
Acid saturation	%	1.51
Exchangeable sodium	%	3.6
Calcium/magnesium ratio	–	1.65
Zinc	mg/mL	2.4
Copper	mg/mL	2.7
Manganese	mg/mL	6.3
Iron	mg/mL	395
Silicon	mg/mL	28.54
Clay estimate	%	23
Organic matter estimate	%	4.3
Volume weight	g/mL	1.23

Table 3: The main effect of vegetable plant species on leaf concentrations of Cu, Fe, Zn and Mn

	Concentration (mg/kg)			
Vegetable	**Cu**	**Fe**	**Zn**	**Mn**
Corchorus olitorius	9.2[b]	327[a]	72[c]	97.9[c]
Amaranthus cruentus var. Arusha	8.8[b]	223[b]	235[b]	199.8[b]
Beta vulgaris var. *cicla*	14.3[a]	295[a]	363[a]	285.8[a]
Significance	**	*	**	**
Least significant difference	2.4	70	63	28.9

**significant at* $p<0.05$*; **significant at* $p<0.01$

Means followed by different letters in the same column are significantly different at $p<0.05$ *according to the least significant difference test.*

Leaf Cu, Zn and S concentrations increased with increasing application rate, whereas Fe concentrations did not show a defined pattern (Table 4). The application of micronutrient fertilisers did not affect the concentration of macronutrients Ca, K, Mg, P and N (not shown), but there were significant ($p<0.05$) differences in the concentrations of Ca, Mg, P and S among the plant species tested (Table 5). The general trend of *B. vulgaris* > *A. cruentus* > *C. olitorius* in terms of leaf micronutrient content was also observed for the macroelements Mg and P (Table 5). However, the trend was reversed for Ca concentration, with *C. olitorius* leaves having three times the Ca content of *B. vulgaris*. However, a regression analysis of this negative relationship showed that it was not significant. The concentration of shoot Ca and Mg and their variations across different plant families have been investigated in comprehensive studies.[4,5] These studies revealed that the order Caryophyllales, to which *Amaranthus* and Swiss chard belong, has a tendency towards high shoot Mg levels, resulting in lower Ca:Mg ratios, as observed in *B. vulgaris* in the present study.

Table 4: The main effect of soil-applied micronutrients on leaf Cu, Fe, Zn and Mn concentrations of *Beta vulgaris* var. *cicla, Corchorus olitorius* and *Amaranthus cruentus* var. Arusha

	Concentration (mg/kg)			
Micronutrient mixture amount† (kg/ha)	**Cu**	**Fe**	**Zn**	**Mn**
0	4.69[c]	280	60[c]	0.23[c]
5	9.27[b]	274	218[b]	0.32[b]
10	14.75[a]	312	277[ab]	0.34[a]
15	14.49[a]	258	338[a]	0.34[a]
Significance	**	ns	**	**
Least significant difference	2.80	–	72	0.02

*ns, not significant; *significant at* $p<0.05$*; **significant at* $p<0.01$

Means followed by different letters in the same column are significantly different at $p<0.05$ *according to the least significant difference test.*

†Micronutrient mixture consisted of the following treatments: 0 kg/ha (control), 5 kg/ha, 10 kg/ha and 15 kg/ha each of elemental Cu, Fe and Zn mixed together and applied to the soil as $CuSO_4{\cdot}5H_2O$*;* $FeSO_4{\cdot}7H_2O$ *and* $ZnSO_4{\cdot}7H_2O$*.*

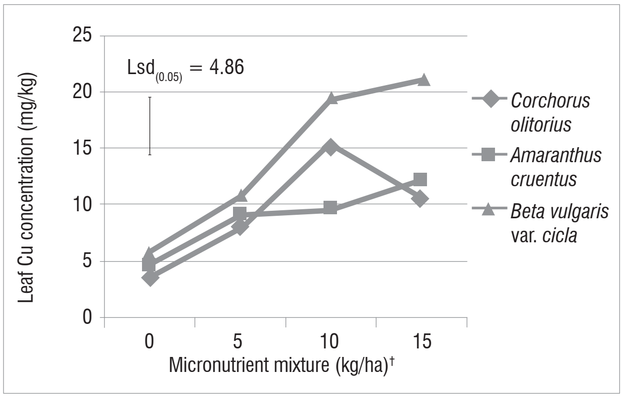
Table 5: Macronutrient composition of three vegetable species in response to micronutrient fertiliser added to soil

	Concentration (mg/kg)					
Vegetable	**Ca**	**K**	**Mg**	**P**	**N**	**S**
Corchorus olitorius	1.77[a]	3.15	0.30[b]	0.35[c]	3.86	0.27[b]
Amaranthus cruentus var. Arusha	1.37[ab]	3.33	1.01[a]	0.51[b]	3.80	0.34[a]
Beta vulgaris var. *cicla*	0.55[b]	3.50	1.04[a]	0.61[a]	3.84	0.34[a]
Significance	*	ns	**	**	ns	**
Least significant difference	0.83	–	0.08	0.09	–	0.05

*ns, not significant; *significant at* $p<0.05$*; **significant at* $p<0.01$

Means followed by different letters in the same column are significantly different at $p<0.05$ *according to the least significant difference test.*

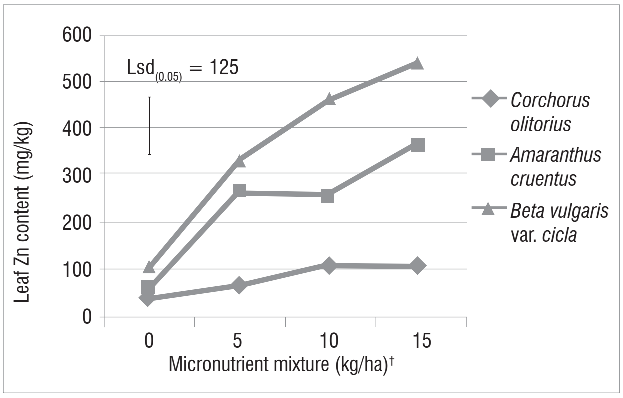
There were significant ($p<0.05$) interactions (Figure 1 and 2) among plant species and fertiliser rate in terms of leaf Zn and Cu. In all three vegetables, the leaf Cu concentration increased with Cu addition up to 10 kg/ha but declined at 15 kg/ha (Figure 1). The decline in leaf Cu concentration at 15 kg/ha Cu was more marked in *C. olitorius* than in Swiss chard and *A. cruentus*. The reason for the decline in Cu concentration in *C. olitorius* at high application rates in the current study is not known but could be a result of toxicity or simply the inherent inability of *C. olitorius* as a species to accumulate the microelement, as alluded to by several researchers.[4,5,18,25,26] Swiss chard concentrated more Zn and Cu at all fertiliser application rates (Figures 1 and 2), which contradicted our hypothesis that wild vegetables have a greater inherent ability to accumulate micronutrients from the soil than the widely cultivated exotic vegetables. Nonetheless, the wild vegetables also responded positively to the incremental application of the two micronutrients, which supported our postulate that the addition of micronutrients would result in increased micronutrient concentrations in wild vegetables leaves. Thus, when the vegetables were grown in soil with augmented micronutrient content, they concentrated increased quantities from the soil.



†Micronutrient mixture consisted of increasing quantities of each of elemental Cu, Fe and Zn mixed together and applied to the soil as $CuSO_4.5H_2O$; $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$.

LSD, least significant difference

Figure 1: Concentration (mg/kg) of Cu in the leaves of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – and a conventional vegetable, Swiss chard (*Beta vulgaris* var. *cicla*) after application of 0 kg/ha, 5 kg/ha, 10 kg/ha or 15 kg/ha micronutrient mixture.



†Micronutrient mixture consisted of increasing quantities of each of elemental Cu, Fe and Zn mixed together and applied to the soil as $CuSO_4.5H_2O$; $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$.

LSD, least significant difference

Figure 2: Concentration (mg/kg) of Zn in the leaves of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – and a conventional vegetable, Swiss chard (*Beta vulgaris* var. *cicla*) after application of 0 kg/ha, 5 kg/ha, 10 kg/ha or 15 kg/ha micronutrient mixture.

The concentrations of micronutrients tested in this study could be considered high, or even toxic to crops. We did not evaluate toxicity in this study, but observed no visible toxicity symptoms. The concentrations of micronutrients obtained from the leaf analysis results after applying micronutrient fertiliser were much higher than those reported for wild vegetables collected from the wild.[12-16] However, not all micronutrients present in crops are available for uptake by humans. Some are complexed in unavailable forms by various biomolecules.[2,25] It is therefore necessary to investigate if the increase in leaf micronutrient level has a positive effect on bioavailability of the micronutrients in wild vegetables. Micronutrients are generally applied in smaller quantities than macronutrients,[27] but mostly in commercial agriculture. There is a danger of accumulation of micronutrients to hazardous levels in soils associated with high application rates of micronutrient fertilisers such that some countries have statutory maximum limits of micronutrients to be applied to soils to prevent accumulation to toxic levels.[18] The nutrient content of most soils in the subsistence farming sector is generally not well known, yet there is a belief among agriculturalists that micronutrients occurring naturally in the soil are adequate for crop production. In contrast, in commercial agriculture, in which the nutrient status of soils is well known, farmers periodically apply micronutrients, either as foliar sprays or chelates to soils.[28]

Conclusions

The different vegetable species investigated demonstrated different abilities to take up Cu and Zn, in the order Swiss chard > *A. cruentus* > *C. olitorius*, and they responded to soil-applied micronutrients by taking up more from the soil, as more was supplied, but up to a certain point. The trend *B. vulgaris* > *A. cruentus* > *C. olitorius* was observed for the macroelements Mg and P but it was reversed for Ca concentration, with *C. olitorius* leaves containing three times the Ca content of *B. vulgaris.* Our results contradict the current claim that wild vegetables have superior micronutrient content to exotic vegetable species.

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Authors' contributions

All authors contributed to the research and write-up. S.M. was responsible for the design and conduct of the experiments, and the data collection and analysis. W.P.d.C. and M.M. were responsible for overall supervision of the project.

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