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An assessment of the uptake of selected heavy metals, antioxidant response and lipid peroxidation in *Spinacia Oleracea* vegetables, cultivated on soil from a coal mining area in Matabeleland North Region of Zimbabwe

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ABSTRACT

Coal mining in Matabeleland North region plays an important role in the production of energy in Zimbabwe. Coal mining, however, results in the release of pollutants such as heavy metals into the surrounding soil environments, putting communities involved in vegetable gardening at risk. This study aimed to quantify the levels of the heavy metals, cadmium (Cd), copper (Cu) and zinc (Zn) in soil from a coal mining area in Matabeleland North region, and in spinach (*Spinacea oleracea*) grown in these soils, as well as assess superoxide dismutase (SOD) and catalase (CAT) antioxidant enzyme activities in the cultivated spinach. Malondialdehyde (MDA) levels were also measured as an index of lipid peroxidation. Soil was collected from 3 sites and the pH and electrical conductivity were measured. Spinach seeds were planted in the soils in polythene bags and left to germinate and grow outdoors for 30 days, with daily watering. Spinach leaves were harvested. Heavy metals were quantified, SOD and CAT activities assessed and MDA levels measured. Soil pH from Sites 1, 2 and 3 ranged between 6.2 and 6.9, while conductivity was in the 1.50-1.59 µS range. Compared to the heavy metal levels in the reference soil, Cd levels from Sites 2 and 3 were significantly ($p< 0.05$) higher. Copper and Zn levels from each of the sites were significantly higher than in the reference soil. In spinach, Cd levels ranged between 1.20-2.23 mg/kg. Both Cu and Zn levels were significantly ($p< 0.05$) higher than in spinach grown on reference soil, ranging between 38-47 mg/kg and 64-89 mg/kg respectively. There was a significant increase in SOD and CAT enzyme activities in spinach grown on Sites 1-3 soils, compared to enzyme activities of plants grown on reference soil. Malondialdehyde levels were significantly higher in plants grown on Site 1 and 2 soils compared to plants grown on reference soil. The findings suggest that spinach bioaccumulates pollutants from soil in the coal mining area.

Keywords: Heavy metals, coal mining, *Spinacea oleracea*, superoxide dismutase, catalase, malondialdehyde.

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1. INTRODUCTION

Coal is a combustible rock rich in carbon and forms a crucial component of the energy matrix that fuels the whole globe (Masto *et al*., 2015). While coal mining and its utilisations in energy production play an important role in the development of any country, there are environmental quandaries associated with it (Harmanescu *et al*., 2011). In Zimbabwe, the biggest coal producing mines are in the Matabeleland North region of the country, however, years of coal mining has left the surrounding town whelmed in coal dust (Masara, 2017). Coal dust discharged from coal mining activities is rich in a variety of harmful trace elements, particularly heavy metals (Harun et al., 2014).

Heavy metals are defined as metallic elements which have a density five times that of water (Fergusson, 1990). While some metals, for instance copper, are

deemed essential micronutrients for growth and metabolism in plants, at concentrations above the normal threshold they become detrimental (Ercal *et al*., 2001; Stern, 2010). Heavy metals tend to accumulate in surrounding soil environments, due to their slow movement processes in soil. (Shahid *et al*., 2016). With most human food obtained from the earth, cultivation on such soils must be minimised. Studies have found that metals, such as cadmium, have high mobility in the soil-plant system and can thus be easily absorbed by the plant. This subsequently affects plant physiological processes and the metals enter the food cycle, ultimately affecting the final human consumer (Shahid et al., 2016).

A vegetable that forms a conventional part of the human diet is the *Spinacia oleracea*, commonly known as spinach, whose leaves have high nutritional value (Khan *et al*., 2009). Due to its relatively high growth and absorption rates, *S. oleracea* has also been employed in studies assessing growth and toxicity responses to contaminants such as heavy metals (Alia et al., 2015). The uptake of such contaminants induces production of reactive oxygen species (ROS) which results in oxidative stress in the plant cells. Oxidative stress causes damage to different biomolecules including induction of lipid peroxidation, compromising membrane fluidity and function (Repetto *et al*., 2012). To ensure optimal protection against oxidative stress, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are engaged (Oliva *et al*., 2012; Valavanidis *et al*., 2006). The induced and/ or inhibited enzyme activities thus serve as important biomarkers for demonstrating oxidative stress status due to pollutant uptake (Pandey *et al*., 2003; Li *et al*., 2011;Tkachenko *et al*., 2014).

According to a report by the Center for Natural Resource Governance on the situation of coal mining in Matabeleland South region, vegetation in the mining and surrounding areas is heavily intoxicated with chemical substances, and local inhabitants who grow and harvest vegetables for consumption are at risk of harmful consequences (CNRG, 2016). Due to the paucity of information on the exact nature and amount of chemical substances taken up by cultivated vegetables, the aim of the present study was to firstly, assess the levels of heavy metals, namely, cadmium (Cd), copper (Cu) and zinc (Zn) in soil from selected sites around the coal mining area. Secondly, the study sought to cultivate spinach in these soils and assess uptake of the named metals, measure SOD and CAT antioxidant response as well as malodialdehyde (MDA) levels, as an indicator of lipid peroxidation status.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals, substrates and enzymes were purchased from Sigma Aldrich Chemical Company, Germany. All other laboratory reagents were of analytical grade.

2.2 Soil sampling

Soil samples were collected from three sites demonstrating agricultural activity, within the coal mining area in Matabeleland North province of Zimbabwe. The sites identified were: Site 1, a water treatment plant; Site 2, an old coal mine; and Site 3, two kilometres away from a coal power station (Figure 1). The reference soil was collected at a community along Deka River, away from the coal mining vicinity (Figure 1). A wooden shovel was used to collect the soil, at a depth of approximately 3 cm. From each site, three samples were collected in polythene bags and pooled into one compound sample of approximately 20kg. The bags were transported at 20 °C to the laboratory for pH and heavy metal analyses, as well as spinach cultivation.

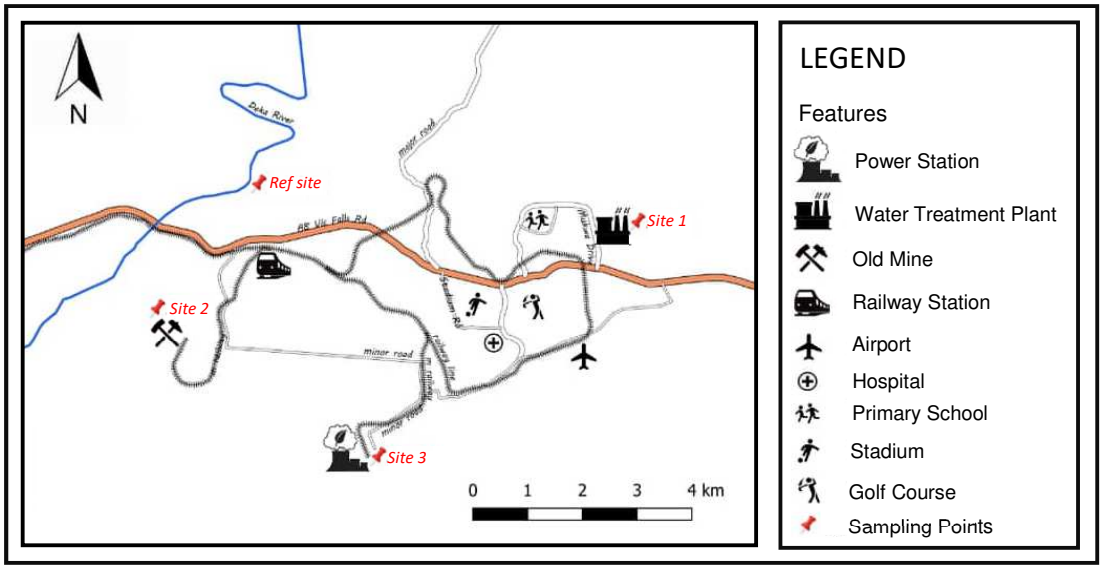

Figure 1. Sampling points in Matabeleland North, Zimbabwe

2.3 Soil pH and conductivity analysis

As described by Houba *et al.*, (2000), soil samples were first air dried and sieved through a No 10 sieve (2 mm mesh) to remove the coarser soil fraction. To prepare the soil slurry, 10 g of the sieved soil was placed in a beaker and 50 ml of 0.1 M calcium chloride solution was added. The slurry was mixed thoroughly using a shaker for 1 hour and both pH and conductivity were measured using the Eutech Cyberscan Instruments water proof series meters (oxygen: DO300 Series, pH: 300 Series, conductivity: 410 Series).

2.4 Spinach (*S. oleracea*) cultivation

Spinach seeds were purchased from a local supplier in Bulawayo, Zimbabwe. In six replicates, 500 g of soil from each sampling site was weighed and placed in plastic polythene bags. The spinach seeds were then planted and allowed to germinate and grow outdoors. The plants were allowed to grow for 30 days and were watered daily with municipal tap water, prior to harvesting for heavy metal analysis and homogenisation of plant tissue.

2.5 Heavy metal analysis of soil and plant tissue

Prior to analysis, soil and plant tissue were digested. Digestion of soil samples involved oven-drying the soils at 100°C for 12 hours. The samples were then pulverised using a pestle and mortar. In triplicates, 1 g of soil was placed in a 100 ml beaker, followed by 1 ml dH_2O, 12 ml 32% hydrochloric acid (HCl) and 4 ml 69% nitric acid (HNO_3). The mixture was allowed to reflux for 20 min, after which 5 ml of dH_2O was run down the sides of each beaker and further heated for 10 min, prior to cooling. For the digestion of plant tissue, 2 g of spinach leaves and 5 ml of concentrated HNO_3 were placed in a beaker and left to stand overnight. The mixture was then heated on a hot plate at 80°C for 1 hour until the production of red NO_2 fumes ceased. The temperature was

increased to 120 °C, and the mixture further heated for 1 hour. A volume of 1 ml of hydrogen peroxide was added, and the mixture heated for another hour.

All digests were allowed to cool and were subsequently filtered using the Whatman No 42 filter, into 50 or 100 ml volumetric flasks. The contents were diluted to the mark with dH_2O. Levels of copper, cadmium and zinc were confirmed using the GBC XplorAA Dual atomic absorption spectrophotometer (Victoria, Australia). Metal determination was carried out in triplicate.

The transfer coefficient, i.e. the index of soil to plant transfer of heavy metals was expressed as the ratio of the metal concentration in the plants to metal concentrations in the soil.

2.6 Homogenisation of plant tissue for post mitochondrial fraction (PMF)

Approximately 200 mg of leaf tissue was weighed and homogenized in 1.2 ml of 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The samples were centrifuged at 15,000 × g for 20 min at 4 ℃. The supernatant (PMF) was removed and kept, while the pellet was resuspended in 0.8 ml of the same buffer, and centrifuged for another 15 min at the same conditions. The supernatants were combined and stored at -80 ℃ prior to protein determination as well as SOD and CAT enzyme assays.

2.7 Protein determination

Protein determination was carried out following a modified method of Lowry et al., (1951) using bovine serum albumin (BSA) as a standard. Five ml of the alkaline solution [2.5 ml of 0.5% copper sulphate ($CuSO4.5H_2O$) in 1% potassium sodium tartrate; 125 ml of 2% sodium carbonate (Na_2CO_3) in 0.1M sodium hydroxide (NaOH)] was added to 0.5 ml of the PMF solution. This was mixed thoroughly and allowed to stand at room temperature for 10 min, after which 1M Folin-Ciocalteau reagent (0.5 ml) was added, mixed rapidly and the reaction mixture left to stand for an additional 30 min at room temperature. Absorbance was measured against an appropriate blank at 750 nm. The total protein concentration in the plant leaf samples was obtained from the standard curve.

2.8 Assessment of superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured according to the method of Sun et al., (1988). Xanthine and xanthine oxidase were used to generate superoxide anion radicals which react with nitroblue tetrazolium (NBT), forming a red formazan dye. In each test tube, the reaction mixture contained 0.5 ml of sample (PMF) [1 mg/ml] or standard Cu, ZnSOD (0–600 ng/tube) and 2.45 ml SOD Assay Reagent (SODAR). The SODAR contained 0.3 mM xanthine, 0.6 mM ethylenediaminetetraacetic acid (EDTA), 150 µM (NBT), 400 mM sodium carbonate and 0.1% w/v BSA in the ratio 4:2:2:1.2:0.6 respectively. Test tubes were placed in a water bath at 25°C. The reaction was started with 50 µl of 167 U/L xanthine oxidase and allowed for 30 min. The absorbance was measured at 560 nm and one enzyme unit superoxide dismutase is defined as the amount which inhibits the NBT reaction by 50%. Specific activity was defined as units/mg protein.

2.9 Assessment of catalase (CAT) activity

The activity of catalase was assayed according to the method of Claiborne (1985). A decrease in absorbance was determined at 240 nm for 1 min following the decomposition of hydrogen peroxide (H_2O_2). The reaction mixture (3 ml) contained 2950µl of 19 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 50 µl of sample (PMF) [1 mg/ml] at 25℃. The activity was calculated using the extinction coefficient (46 mM^{-1} cm^{-1}) for H_2O_2.

2.10 Determination of lipid peroxidation

Membrane lipid peroxidation in fresh leaf tissue was estimated by measuring the malondialdehyde (MDA) concentrations, as described by Heath and Packer (1968). Fresh leaf tissues were homogenized in 0.1% (w/v) trichloroacetic acid (TCA) solution in 1:3 ratio. After centrifugation (15 min, 12 000 x g at 4℃), an aliquot of the supernatant was added to 0.5% thiobarbituric acid (TBA) made in 20% TCA and heated at 95°C for 30 min. After rapid cooling on ice, the mixture was centrifuged at 10 000 x g for 10 min and absorbance recorded at 532 nm.

2.11 Statistical analysis

Data was reported as means ± SD and statistical differences were analysed using the two-way analysis of variance (ANOVA) with multiple comparisons (Tukey's multiple comparisons test). Graph plots and statistical analysis were done using GraphPad Prism 6. Significance of results was ascertained at $p< 0.05$.

3 RESULTS

The pH of soils from Sites 1, 2 and 3 were in the 6.2-6.9 range, and did not differ significantly from the reference soil, while conductivity ranged between 1.50- 1.59 µS (Table 1).

Regarding the levels of Cd in the soil, Site 1 had 6.93± 0.19 mg/kg and did not differ significantly from in reference soil (3.58± 0.19 mg/kg) [Table 1]. From Site 2 and 3 soils, Cd levels were 10.22± 0.11 mg/kg and 10.25± 0.23 mg/kg respectively, and were significantly ($p<0.05$) higher than levels in the reference soil (Table 1). Levels of Cu in soils from each of the Sites were significantly ($p<0.05$) higher than those in the reference soil (Table 1). Comparing Site 1, 2 and 3 soils, Cu levels were significantly different, with Site 3 soil having the highest amount (79.00± 2.30 mg/kg) [Table 1]. Compared to the reference soil, Zn levels in the soils from each of the sites were significantly ($p<0.05$) higher (Table 1). Levels of Zn in Site 1 and Site 3 soils were 332.50± 5.75 mg/kg and 327.50± 9.46 mg/kg respectively and did not differ significantly from each other, while Zn levels in Site 2 soil were significantly ($p<0.05$) lower (298.12± 1.86 mg/kg) [Table 1].

Regarding the levels of heavy metals in spinach, Cd levels ranged between 1.20-2.23 mg/kg in plants grown in soils from Sites 1- 3 and did not differ significantly from spinach cultivated in reference soil (Table 1). Both Cu and Zn levels in spinach grown in Site 1, 2 and 3 soils were significantly higher than those observed in spinach grown in reference soil (Table 1). Spinach grown in Site 1 soil had 38.07± 3.10 mg/kg of Cu, which did not differ significantly from spinach cultivated in Site 2 soil, having 41.87± 3.37 mg/kg (Table 1). Spinach cultivated in Site 3 soil had 46.55± 2.32 mg/kg of Cu and did not differ significantly from the levels observed in spinach from Site 2 soil (Table 1). Zn levels of spinach grown in Site 1, 2 and 3 soils were 64.08±3.82 mg/kg, 82.07± 4.57 mg/kg and 89.50± 5.77 mg/kg respectively, with spinach from Site 3 soil having the highest concentration (Table 1).

Soil-plant transfer coefficients of Cd and Zn were all below 0.5, whereas the Cu transfer coefficient for plants cultivated in Site 1 and 2 soils was 0.67± 0.05, and from Site 3, 0.59± 0.02 (Table 1).

Table 1. Analysis of soil pH and conductivity; soil and spinach heavy metal content; and metal transfer coefficient.

		Site 1	Site 2	Site 3	Reference
Soil	**pH**	6.90±0.00	6.26±0.05	6.76±0.03	6.12±0.05
	Conductivity (µS)	1.50±0.02	1.53±0.10	1.54±0.07	1.59±0.07
	Cd (mg/kg)	6.93±0.19	10.22* ±0.11	10.25*±0.23	3.58 ±0.19
	Cu (mg/kg)	56.70^{a*}±0.45	62.35^{b*}±0.24	79.00^{c*}±2.30	24.22±0.40
	Zn (mg/kg)	332.50^{a*}±5.73	298.12^{b*}±1.86	327.50^{a*}±9.44	226.25± 5.73
Spinach	**Cd (mg/kg)**	2.23±0.63	1.20±0.26	1.23±0.26	0.48±0.03
	Cu (mg/kg)	38.07^{a*}±3.10	41.87^{ab*}±3.37	46.55^{bc*}±2.32	10.73±1.30
	Zn (mg/kg)	64.08^{a*}±3.82	82.07^{b*}±4.57	89.50^{c*}±5.77	25.83±3.82
Transfer coefficient	**Cd**	0.32±0.03	0.11±0.02	0.12±0.06	0.13±0.01
	Cu	0.67±0.05	0.67±0.05	0.59±0.02	0.45±0.05
	Zn	0.18±0.02	0.18±0.02	0.24±0.05	0.11±0.02

Values are expressed as mean ± SD. Asterisks indicate statistically significant differences ($p^*< 0.05$) compared to the reference. Different letters within the same row indicate significant differences ($p< 0.05$) of heavy metal levels between soils or spinach grown on soils from Sites 1, 2 and 3.

The activities of SOD and CAT, as well as MDA levels in spinach grown in the different soils are shown in Figure 2. The SOD activity in spinach grown on Site 1, 2 and 3 soils (i.e. SS1, SS2 and SS3 respectively) was significantly ($p< 0.05$) higher than that of spinach cultivated in reference soil (SR) [Figure 2A]. Enzyme activity ranged between 1.7-1.9 U/ mg protein, with SS3 being significantly ($p< 0.05$) higher than SS2 and SS1 (Figure 2A). A similar trend was observed with CAT activity (Figure 2B).

The average CAT activity in spinach grown in Site 1 and 2 soils (SS1 and SS2) was 0.052 U/ mg protein, and for spinach grown in Site 3 soil (SS3), ~ 0.059 U/ mg protein (Figure 2B). Regarding MDA levels, spinach cultivated in soils from Site 1 and 2 (SS1 and SS2) had MDA levels of 6 µmoles/ g wt tissue and 8 µmoles/ g wt tissue respectively, and both were significantly ($p< 0.05$) higher than in spinach cultivated in reference soil (SR) and Site 3 soil (SS3) [Figure 2C].

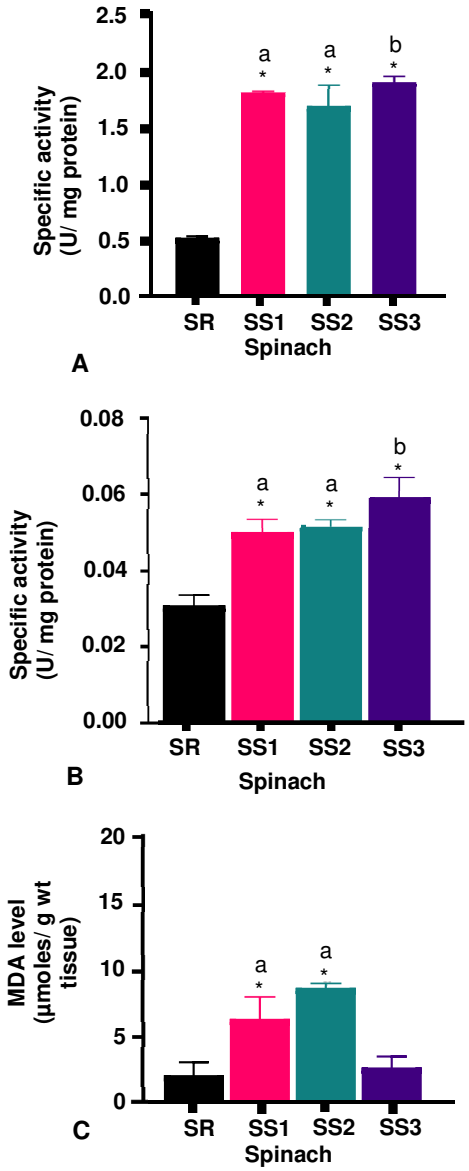

Figure 2. Specific enzyme activities of (A) SOD, (B) CAT, and MDA levels (C) of Spinach (*S. oleracea*) cultivated on different soils. SR: spinach cultivated on Reference soil, SS1: spinach cultivated on soil from Site 1, SS2: spinach cultivated on soil from Site 2, SS3: spinach cultivated on soil from Site 3. Spinach was cultivated on soils for 30 days and watered daily, prior to harvesting leaves. Values are expressed as mean ± SD. Asterisks indicate statistically significant differences ($p^*< 0.05$) compared to the reference. Different letters indicate significant differences ($p< 0.05$) of specific activity or MDA levels between spinach grown on soils from Sites 1, 2 and 3

4.0 DISCUSSION

Coal mining and its utilisation in energy production generates by-products such as coal ash, that are deposited in lowland areas, contaminating nearby agricultural soils (Mondal *et al.*, 2010). Contamination results in changes in soil physicochemical parameters as well as subsequent uptake of toxic elements by cultivated vegetables (Zocche *et al.*, 2017).

Soil pH is one of the important factors that influences the amount of soluble nutrients and metals available to the plant (Fayad *et al.*, 2013). In the present study, soil pH ranged between 6.2-6.9, falling in the 5.5-6.8 range, i.e. desired pH for good vegetation (Goswami and Sarma 2008). This possibly contributed to the growth of the spinach in the present study, as well as the vegetation that was observed at each of the sampling sites. The soil pH values of the present study were, however, slightly higher than those found by Rahman *et al.*, (2017). They observed an average pH value of 5.7 in the soil from the Barapukia coal mine area (Rahman *et al.*, 2017). A measure of soluble salts present in the soil, i.e. electrical conductivity, gave values ranging between 1.5 and 1.9 µS. According to Goswami and Sarma (2008), conductivity less than 0.5 µS is safe for plant growth, whilst higher values can be toxic. In the present study, conductivity was above 0.5 µS, suggesting the presence of a large amount of ionic substances and soluble salts, perhaps due to high evaporation of soil water resulting in the transport and accumulation of salt in the top soil (Ma et al., 2019).

According to the World Health Organisation (WHO), the permissible limits for heavy metals in soil for Cd, Cu and Zn are 0.8 mg/kg, 36 mg/kg and 50 mg/kg respectively

(WHO, 1996). In the present study, Cd levels across Sites 1-3 exceeded WHO limits by at least 12 fold, while Cu levels were on average 2 fold higher and Zn levels 6 fold higher. The high levels could be attributed to the activities associated with mining, such as coal mining, washing, storage and transport, which result in the release of products known to contain various species of metals such as Cd, Cu and Zn (Huang et al., 2018; Ghose 2007). Interestingly, the metal levels found in this study were significantly higher than those obtained by Liu and colleagues (2019) from a national study with data obtained from 50 coal mines in China. The average Cd, Cu and Zn levels they found were 0.5 mg/kg, 33 mg/kg and 79 mg/kg respectively.

The soils from Site 2 (an old mine) and Site 3 (power station) were typically expected to have high heavy metal concentrations, due to the activities in those surrounding areas. Burning coal produces airborne products such as fly ash and bottom ash which contain large quantities of heavy metals which settle or wash out on land (Li *et al.*, 2017). The soil from the reference site, despite being away from the coal mining vicinity, had Cd and Zn levels which were slightly higher that the stated WHO permissible limits. This may have been due to several factors such as the transport of coal combustion products by air and/ or water, or perhaps anthropogenic activities such as farming, where heavy metal containing pesticides may have been used by community members in that area.

Regarding heavy metal levels in plants, WHO stipulates that plants should contain no more than 0.02 mg/kg of Cd, 10 mg/kg of Cu and 0.6 mg/kg of Zn (WHO, 1996). In this study, spinach cultivated on each of the soils, accumulated heavy metals at levels above WHOs permissible limits, despite the relatively low soil-plant transfer coefficients observed. This implies that the heavy metals had high mobility and bioavailability to the spinach. The accumulation of heavy metals in plants grown on heavy metal contaminated soil also tends to be inevitable, owing to the co-uptake and translocation of necessary nutrients such as nitrogen and phosphorus (Gupta *et al.*, 2014). Regarding Cd uptake, the findings in the present study are in agreement with those of Cobb and co-workers (2009) who cultivated vegetables on soil mixed with mine waste, and found significantly high Cd levels in the leaves of leafy vegetables. The accumulation of Cd mainly in the leaves of leafy vegetables was found to be due to non-immobilisation in the roots, accumulating more in the above ground parts (Leita *et al.*, 1996; John *et al.*, 1972). Contrary to the Cobb and colleagues (2009) findings, however, spinach leaf Zn levels in the present study were significantly high. This suggests there may not have been any zinc-resistance mechanisms that restricted root to shoot translocation, as postulated in the Cobb study (Cobb *et al.*, 2009). The presence of these metals in spinach leaves certainly implies that their consumption would pose a high risk to human health.

The enhanced SOD and CAT enzyme activities in spinach grown on Site 1-3 soils, when compared to the plants grown on reference soils, also give an indication of the uptake of pollutants from soil. These pollutants could have included other heavy metals associated with coal mining e.g. arsenic, selenium; organic components such as polyaromatic hydrocarbons; as well as inorganics like silicates (Ghose, 2007). Due to resource and time constraints, however, an analysis of all the possible pollutants present could not be done in the present study. An increase in the enzyme activities is a result of a rapid adaptive response to pollutant exposure. The enzymes scavenge ROS species by dismutation of superoxide anion radicals by SOD, forming hydrogen peroxide, which is then broken down by CAT to water (Bhagat *et al.*, 2016). Our findings are in agreement with those from several studies. After 30 days of germination, spinach seedlings exposed to heavy metal (lead) polluted soil,

showed enhanced SOD activity, when compared to seedlings grown on unpolluted soil (Wang *et al.*, 2010). *Atriplex hortensis* (mountain spinach) grown in soil polluted with heavy metals had increased CAT activity while maize seedlings cultivated in soil with Zn also showed increased SOD activity in comparison to plants grown on unpolluted soil (Sai Kachout *et al.*, 2009; Cui and Zhao, 2011). *Brassica napus* exposed to soil with Cu alone or in combination with other metals had high SOD activity, but low CAT activity (Li et al., 2018). In *Brasssica juncea* cultivated on cadmium (Irfan *et al.*, 2014) and zinc (Prasad *et al.*, 1999) contaminated soil, SOD and CAT activities increased when compared to the plants grown on control soils. The inconsistencies regarding CAT activity, may be due to the differences in plant growth conditions.

In the present study, the antioxidant enzymes may have successfully played a protective role in preventing lipid peroxidation in spinach grown on Site 3 soil, as the MDA levels were low, similar to the levels in spinach grown on the reference soil. The higher MDA levels observed in spinach grown on soils from Sites 1 and 2 may be due to the nature of the pollutants present, which may have overwhelmed the overall protective antioxidant system.

5.0 Conclusion

The present study showed that although the levels of cadmium, copper and zinc in soil from the Hwange coal mining area differed from site to site, they were nonetheless higher than the WHO permissible limits. The spinach cultivated on these soils also bioaccumulated the metals at high levels. Increased superoxide oxidase and catalase enzyme activities in spinach, also indicated uptake of heavy metals and possibly other mine soil related pollutants. The elevated MDA levels in spinach grown on soils from Site1 and Site 2 suggest increased toxicity to the plant and an overwhelmed antioxidant protective system. High levels of heavy metals in vegetables pose a serious health hazard to human consumers, thus measures need to be taken by relevant Environmentalists to remediate the soils in the coal mining area and educate the public on the risks associated with cultivating in those areas.

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