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Full Length Research Paper

Essential and potentially toxic trace elements in selected antimalarial plants: A pilot study in Kilembe copper mine catchment, Kasese District, Uganda

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Majority of people in rural areas of Uganda and other malaria-endemic parts of the world use medicinal plants to treat the disease. This study documented medicinal plants used to treat malaria around Kilembe copper mines and assessed the presence of essential and potentially toxic elements. Household surveys and key informant interviews were carried out while anti-malarial plants were sampled, prepared and concentrations of Fe, Mn, Zn, Co, Cu and Ni determined by atomic absorption spectrometry. It was established that *Vernonia amygdalina* (40%), *Ocimum suave* (35%), *Justicia betonica* (32%) and *Aloe felox* (20%) were the most used plants to treat malaria. Leaves were the most commonly used plant part (83%) while decoctions were reported by 51% of respondents. Concentration of trace elements (mg/kg) in the four plant species ranged from 50.4-422 (Mn), 16.7-202 (Fe), and 19.6-198 (Zn) and from 1.6-44.1, 0-7, and 0.1-31.5 for Cu, Co and Ni, respectively. Fe, Cu and Ni exceeded the recommended thresholds in almost all Kilembe mine samples as well as controls while Mn, Zn and Co exceeded thresholds in more than 25% of the samples. Remediation of Kilembe catchment soils as well as public sensitisation on the safety of medicinal plants is recommended.

Key words: Malaria, medicinal plants, trace elements, Kilembe mine.

INTRODUCTION

The increasing environmental pollution especially soil contamination with heavy metals such as Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Arsenic (As), and Nickel (Ni), has led to their consumption by human beings through food chains (Kulhari et al., 2013). Heavy metals can be emitted into the environment by natural causes associated with weathering of rocks and minerals, erosion and flooding or anthropogenic causes associated with industrialization, mining, waste disposal, urban effluent discharge, vehicle exhaust fumes, fertilizer and sewage sludge application in agriculture land all of which influence the uptake, accumulation and

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concentration of trace elements in plants (Zhao et al., 2007).

Environmental pollution by heavy metals is very prominent in areas of mining and old mine sites and pollution reduces with increasing distance away from mining sites (Peplow, 1999). Peplow (1999) reported that hard rock mines operate from 5 to 15 years until the minerals are depleted, but metal contamination that occurs as a consequence of hard rock mining persist for hundreds of years after the cessation of mining operations.

Kilembe copper mine was opened in 1959 and operated until 1979 when mining and mineral processing ceased due to civil unrest and fall in copper prices on the world market. Up to 15 Mt. of rock waste was generated during the processing of the copper-cobaltiferous pyrite ores (Owor et al., 2007). Several studies have drawn attention to the serious environmental consequences of copper mining pollution of Kilembe area and the surrounding environment (Mwesigye et al., 2016; Mwesigye and Tumwebaze, 2017). Mwesigye et al. (2019) reported wide scale soil, water and food contamination with trace metals which exposed local residents to trace metal toxicity.

Malaria remains one of the most important global health issue accounting for more than 1 million deaths per year (Greenwood et al., 2005). Medicinal plants are traditionally used to treat diseases ranging from common colds to malaria, arthritis and ulcers among others (Radulescu et al., 2013). In Uganda, medicinal plants have contributed significantly to current malaria therapy (Katuura et al., 2007a; Stangeland, 2011). According to WHO (2013), 80% of the people living in malaria-endemic parts of Africa depend on medicinal herbs to treat the disease. The affordability of most traditional medicines makes them more attractive at a time of soaring health-care costs and nearly universal austerity (WHO, 2013). In Uganda, over 90% of the rural population rely on herbal medicine for their health care because it is easily accessible and effective (Orem and Zikusooka, 2010). Trace elements in medicinal plants can have negative health effects on consumers if they exceeded recommended thresholds (Szentmihályi et al., 2006). This study was conducted to assess the levels of trace elements and safety of commonly used medicinal plants around Kilembe mine catchment.

MATERIALS AND METHODS

Study area

The study area covered Kilembe Mine (0°12' N 30°0E), located in Western Uganda, 10 km west of Kasese town on the slopes of Rwenzori mountain range (Figure 1). The valley area is bisected by River Nyamwamba and is occupied by copper mining infrastructure such as the Old Mine complex, the waste tailings dams, and Kilembe mine housing estates. This study was undertaken to document plants used locally in treatment of malaria by communities around Kilembe copper mines in Kasese and to assess the presence of essential and potential toxic elements in the most commonly used plants, namely: *Vernonia amygdalina* Delile *(Asteraceae) (NS01), Ocimum sauve* L (Lamiaceae) (NS10), *Aloe ferox* (Aloaceae) (NS04), and *Justicia betonica* L (Acanthaceae) (NS08).

Field survey

A survey was conducted targeting traditional health practitioners (THPs), the elderly and mothers who are the custodians of indigenous knowledge in management of various diseases (Katuura et al., 2007b). Qualitative data was obtained using questionnaires. A total of 103 consenting adults were interviewed, and comprised THPs (n=21), herbal medicine collectors/vendors (n=10), Traditional Birth Attendants (TBAs) (n=5), and the elderly residents (n=67).

Medicinal plants sampling

About 200 g of fresh leaves of medicinal plants at different maturity stages were randomly collected from mature disease free medicinal plants growing within the same plots within a radius of 50 m between January and April 2019. The medicinal plant species targeted included *V. amygdalina*, *O. suave*, *A. ferox*, and *J. betonica*, growing within the contaminated soils (Mwesigye et al., 2016) close to the defunct ore processing site and mine tailing dumps. The four plant species were purposively selected because they are the commonly used in the treatment of malaria. Where more than one plant of the same species was found in one place or within 20 m range, leaves were randomly picked from each plant and mixed up to form a homogenized sample.

Five composite control samples representing each of the four plant species under study were randomly collected from 4 to 7 different and well established medicinal plants growing upstream of Kilembe mine area, approximately 3 km from the Kilembe copper mine site. All samples were collected using a stainless-steel knife and first rinsed with tap water followed by distilled and deionized water. Samples were packed in polythene bags before transportation to Makerere University laboratory for further preparation and analysis.

Sample preparation and analysis

In the laboratory, the samples were oven dried at 70°C for 48 h to remove all moisture. The cut pieces were carefully ground in a ceramic mortar, sieved through nylon mesh (2 mm) and the resultant powder packed and sealed in plastic ziplock bags. At the Department of Chemistry, Makerere University, the powdered samples were further ground using a Phillips blender (Model: HR 2058) and sieved through 0.053-micron sieve. Approximately, 0.5 g from each sample was then weighed into Pyrex conical flask. Up to 30 ml of aquaregia solution was added to the samples in the flasks and heated slowly at a low temperature of 100°C using a hotplate for about 50 min until digestion was completed. The resultant solution was filtered using Whatman filter paper (40 mm × 100 mm) to remove residues. Trace elements in the medicinal plants were analysed using Atomic Absorption Spectrophotometer (Agilent 200AA Series).

Quality control

For quality control, all samples were prepared and analyzed in duplicates. Blanks were used during digestion and analytical

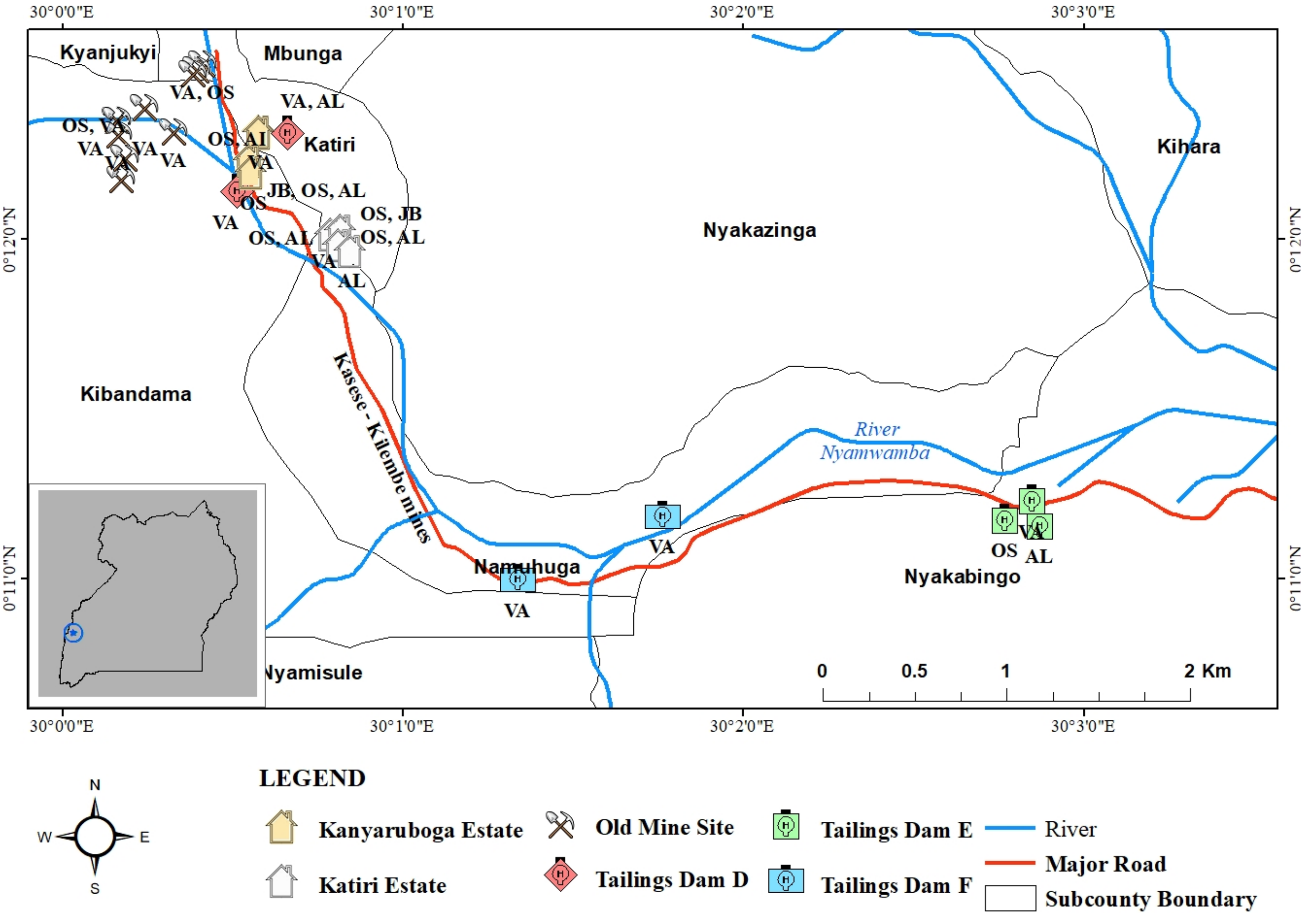

Figure 1. Sketch map showing location of Kilembe mine in Kasese district Uganda, and the sampling points within the study area. VA-*V. amgydalina*; OS-*O. sauve*; AL- *A. ferox*, JB-*J. betonica* samples).
Source: Author

processes. The reagents used for sample preparations were of analytical grade (AR) or trace analytical grade (TAG).

Statistical analysis

Survey data was cleaned and entered into SPSS Version16 to generate descriptive statistics. Data for elemental concentrations in the leaf samples of different medicinal plants were analyzed using Pearson's correlation to determine whether there was any linear association between the elements. A two-sample t-test was conducted using Mintab version 18 to assess if there were significant differences in elemental concentrations of medicinal plants growing in contaminated and control sites. All statistical tests were conducted at 5% significance level.

RESULTS AND DISCUSSION

Socio-demographic factors associated with use of medicinal plants

Majority of medicinal plant users were females (65%), aged between 35 and 55 years (48%), mostly with only the seven-year primary level education (38%). Majority of the respondents were peasant farmers (57%) with low income levels and depended on subsistence farming for their livelihood.

Thirty seven plants species distributed among 27 families were mentioned by respondents to be used traditionally in treatment of malaria. Out of these, 29 were scientifically identified to species level. Most of the plants were from the family of Fabaceae, Asteraceae and Myrsinaceae, respectively. Results of the study revealed that *V. amygdalina* was the most frequently used medicinal plant to treat malaria (40%), followed by *O. suave* (35%), *J. betonica* (32%) and *A. felox* (20%), respectively. Earlier studies (Katuura et al., 2007; Stangeland et al., 2011) also reported *V. amydalina, J. betonica* and *A. ferox* as the most commonly used plants to treat malaria in Mbarara district in Western Uganda. Twenty four plants reported in this study were found to be documented to treat malaria in other parts of Uganda as

Table 1. Elemental concentrations in selected medicinal plant expressed in mg/kg, DW.

Plant species		Elements		
		Mn	Fe	Zn
Vernonia amygdalina (n=12)	Range	129-271	53.1-511.5	19.60-38.60
	Mean±SD	168±44.7	218±121	29.6±5.55
Control (n=5)	Control Range	04.6-228	78.1-190.6	28.4-82.1
	Control Mean±SD	149±78.9	127±42.5	45±22.7
Ocimum suave (n=10)	Range	50.4-144	86-137	24.3-56
	Mean±SD	106±34	501±504	41±13.1
Control (n=5)	Control Range	04.2-76.2	129-483	26.4-52.4
	Control Mean±SD	55.3±14.4	268±154	42±10.1
Aloe ferox (n=17)	Range	109-422	2.7-228	34.7-198
	Mean±SD	245±117	110.6±66.9	82.9±56.8
Control (n=5)	Control Range	92-641	58.8-118	26.2-63.6
	Control Mean±SD	279±228	85±27	51±15
Justicia betonica (n=14)	Range	129-181	151-202	24-37
	Mean±SD	155±37	156.65±7.71	30.4±9.2
Control (n=5)	Control Range	4.2-78.9	95.4-483.0	36-50
	Control Mean±SD	63.7±14	232.8±180.7	44.8±5.5
Permissible levels in medicinal plants by WHO/FAO (1998)		200 mg/kg[a]	20 mg/kg[a]	50 mg/kg[b]

DW: Dry weight.
Source: Author

well as in different countries in Africa and across Asia and Latin America (Bhat and Surolia, 2001; Willcox and Bodeker, 2004). The use of the same plant species in malaria treatment across different traditions strongly suggests that these species may be highly effective in treating malaria (Orwa and Pharm, 2002). According to various studies (Challand and Willcox, 2009; Kumar et al., 2017; Anywar et al., 2020), extracts of *A. ferox* and *V. amygdalina* have good anti plasmodial activity against chloroquine sensitive strain of *Plasmodium falciparum* (MRC-2) *in vivo* and are effective in treating malaria in adult patients.

Concentrations of essential elements of Fe, Zn, Mn in four selected medicinal plants

Table 1 shows the concentration of Fe, Zn and Mn found in the leaves of *V. amygdaline*, O. *suave*, *J. betonica* and *A. ferox* on dry weight basis.

Iron (Fe) had the highest concentrations across all the four plant species sampled, followed by Mn and Zn. However, there was no correlation among the three elements, suggesting no common uptake and transportation mechanism. *O. suave* had the highest mean concentrations of Fe followed by *V. amygdalina, J. betonica* and *A. forex* at 501±504, 218±1201, 157±7.7 and 111±67, respectively. Iron concentrations in *V. amygdalina* were significantly higher than the control samples (P=0.04), whereas Mn concentrations in *O. suave* were significantly higher than in the control sample (P= 0.03). Iron concentration in *J. betonica* was higher in the control than in the treatment, suggesting that area mineralogy and geological could be key in influencing trace element in soils, besides past mining activities. Iron concentrations in all the four different species exceeded the WHO/FAO permissible levels in medicinal plants of 20 mg/kg (WHO/FAO, 1998). The concentrations of Fe (111- 501 mg/kg), Zn (29.6-83 mg/kg) and Mn (105.9-245.3 mg/kg) observed in this study were higher than those reported for medicinal plants from Khetri copper mines in India of Fe (228-461 mg kg^{-1}), Zn (24.9-49.9 mg kg^{-1}) and Mn (37.0-57.1 mg kg^{-1}) (Maharia et al., 2010). This can be attributed to the differences in the geochemical soils characteristics, geographic region and the ability of different plant species to selectively accumulate these elements (Korfali et al., 2013). Iron has several key functions in human body including oxygen supply, energy production, immunity and its deficiency may result in anaemia. However, excessive iron intake is associated with symptoms of vomiting and nausea, dizziness, diarrhoea, liver damage and joint pain. Continuous intake of excess Fe may damage the mucosal cells which may result in hematemesis (Obi et al., 2006).

The observed mean concentrations for Mn were 245±117, 168±45, 155±37 and 106±34 for *A. forex*, *V. amygdalina*, *J. betonica* and O. *suave*, respectively. Mean concentration of Mn in *A. ferox* was higher in the controls. For Zn, *A. ferox* had the highest mean concentration at 83±57; followed by *O. suave, J. betonica* and *V. amygdalina* at 41±13, 30.40±9.2 and 29.6±5.6, respectively. Results of this study showed higher ranges of Mn compared to the selected medicinal herbs of Kenya which ranged from 3.2 to 17.3 mg/kg, and were considered safe (Maobe et al., 2012). High Mn concentrations were also observed in the medicinal plants of Egypt (Khan et al., 2008) and selected medicinal plants commonly used in Tanzania which ranged from 44.6 to 339 ppm and 12.07 to 317.23 ppm, respectively (Nkuba et al., 2017). High Mn concentrations in medicinal plants could be due to high Mn concentrations in the soils in which the plants are growing since the main route of uptake in plants is via roots (Kabata-Pendias, 2011). Manganese is an essential trace element known to be less toxic than any other metals and acts as co-factor for many enzymes. However, continuous exposure to concentrations of more than 5 mg/kg can cause severe health problems including neurological disorders (Kulhari et al., 2013).

From the elemental concentrations observed in different medicinal plants, it can be noted that the four medicinal plants sampled have different metal uptake and bioaccumulation potentials, due to differences in physiology. The tailings from Kilembe cooper mine have been reported to have high concentration of Fe, Zn and Mn (Mwesigye et al., 2016) and this implies that plants growing in soils contaminated by tailing are likely to have these minerals in high concentration following root uptake from the soil solution (Gajalakshmi et al., 2012).

Concentration of potentially toxic trace elements of Cu, Co, and Ni in four selected medicinal plant samples

The concentrations of Cu, Co and Ni in the four medicinal plants sampled, on dry weight basis are presented in Table 2.

Copper was the most accumulated trace element across the four sampled medicinal plants, followed by Ni and Co. The concentration of Cu in *J. betonica* and *O. suave* were higher in control samples than Kilembe catchment soils, suggesting that area geology and mineralogy could be a key factor in soil elemental concentrations (Owor et al., 2007). There was no correlation among the elements suggesting lack of a common uptake and transportation mechanism. *O. suave* had the highest observed mean concentration of Cu (mg kg^{-1}) at 19.38±8.85, followed by *V. amygdalina* (16.36±6.35) and *A. ferox* (12.11±14.42), while *J. betonica* had the lowest (4.6±4.24). Further still, *V. amygdalina* had the highest observed mean concentration of Ni at 7.82±8.05, while *O. suave* had a mean concentration of 5.2 ±4.9. *J. betonica* and *A. ferox* had 6.60±1.56 and 6.02±3.24 mg/kg, respectively. Cobalt showed the lowest mean concentrations observed in the four medical plant species sampled. The highest was 5.2±2.4 mg/kg for *A. ferox* followed by 3.8±2.2 mg/kg for *O. suave* and 3.±2.6 mg/kg for *V. amygdalina*. *J. betonica* had the lowest Co concentrations observed at 2.5 mg/kg. Cobalt concentrations in *J. betonica* (n=14) were significantly higher in the control samples (p= 0.03). Copper concentration in *V. amygdalina, A. ferox* and *O. suave* were all above the 10 mg/kg WHO threshold in medicinal plants implying that these medicinal plants could potentially negatively affect consumers. Only *J. betonica* had Cu concentration of 4.6±4.24, which was below the 10 mg/kg threshold (WHO, 1998). In related studies, Maharia et al. (2010) found high accumulation of Cu in the leaves of all medicinal plants growing in contaminated soils around Khetri copper mine in India. However, the Cu concentration levels observed in the present study which ranged from 4.60 to19.38 mg/kg, were significantly lower than the levels recorded in medicinal plants from Khetri, which ranged from 31.6 and 76.5 mg kg$_{-1}$. As Kabata-Pendias (2011) noted, the common route for trace elements in plants is through root uptake and therefore the differences in plant trace element uptake and accumulation could be associated with soil elemental concentrations.

Copper is essential to the human body in very minute amount, since it forms a component of many enzyme systems, such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood (Nkuba et al., 2017). However, high Cu accumulation levels in these medicinal plants are not only a danger to plants but pose negative human health effects due to copper toxicity. The Cu ion is said to aid production of reactive oxygen species, hence causing oxidative damage in human system (Maharia et al., 2010). Copper toxicity is also associated with Wilson's disease, liver damage and jaundice, which may lead to death in absence of treatment (Murray et al., 2000; Soetan et al., 2010).

Nickel concentration in all the four plant species sampled exceeded the WHO/FAO (1998) permissible limit of 1.5 mg/kg and in *A. ferox,* concentration was slightly higher in the control samples (P=0.055). The concentration of Ni in all the four medicinal plants was higher in controls than in the treatments. This could possibly suggest that Ni concentrations are not only

Table 2. Trace elemental concentrations in four selected medicinal plants in mg/kg, DW.

Plant species		**Elements**		
		Cu	**Co**	**Ni**
Vernonia amygdalina (n=12)	Range	1.6-24.7	0 -7.	0.1-31.5
	Mean±SD	16.36±6.35	3.0±2.571	7.82±8.1
Control (n=5)	Control Range	11.90-19.1	0-4.7	5.7-23.6
	Control Mean±SD	16.54±3.04	2±1.7	15.7±8.2
Ocimum suave (n=10)	Range	9-33.2	1.-6.1	0.4-12.9
	Mean±SD	19.4±8.9	3.8±2.2	5.2±4.91
Control (n=5)	Control Range	17.6-27.1	2.3-21.3	1.2-11.3
	Control Mean±SD	23.1±3.51	7.3±7.9	6.28±3.58
Aloe ferox (n=17)	Range	2.2-44.1	0-6.9	2.5-1.8
	Mean±SD	12.1±14.4	5.2±2.4	6.1±3.2
Control (n=5)	Control Range	5.4-9.8	2.6-5.7	6.9-20.5
	Control Mean±SD	7.7±2.1	4.4±1.2	13.7±6.35
Justicia betonica (n=14)	Range	1.6-7.6	2.5-2.5	5.5-7.7
	Mean±SD	4.6±4.24	2.5±0.1	6.6±1.56
Control (n=5)	Control Range	6.5-27	2.8-5.1	6. -19.8
	Control Mean±SD	16.4±8.9	3.96±0.9	10.6±5.6
Permissible levels in medicinal plants by WHO/FAO (1998)		10 mg/kg[a]	0.14-0.48	1.5 mg/kg[a]

DW: Dry weight.
Source: Author

higher in contaminated soils of Kilembe as reported by Mwesigye et al. (2016) but also higher in the surrounding hills of Kilembe where the controls were sampled. Nickel concentration observed in *O. sauve* was lower than that of the same plant from Khetri soils from India (Maharia et al., 2010). High Ni concentrations were also reported in different herbal remedies used in Nigeria (Obi et al., 2006). Nickel is a known carcinogen reported to adversely affect lungs and nasal cavities (Nkuba et al., 2017). It has also been reported to be toxic to human reproductive system (Obi et al., 2006). According to Annan et al. (2010), Ni is also associated with dose-related decreases in bone marrow cellularity and in granulocyte macrophage and pluripotent stem cell proliferative responses. The results of the present study reveal high accumulated levels of Ni above the WHO/FAO (1998) permissible levels of 1.5 mg/kg in medicinal plants, which suggest possible health risks for consumers.

Cobalt was recorded to have the lowest mean concentration levels in all the four plant species sampled. The Co concentrations observed in the present study ranged from 0 to 7 mg/kg. This was lower than the range of Co concentrations reported in the selected common medicinal herbs of Haripur, Pakistan where the minimum Co concentration was 3.41 and the maximum was 11.26 mg/kg (Jabeen et al., 2010). However, Co concentration observed in this study are much higher than those observed from medicinal plants collected from the contaminated soil of Khetri copper mine in India where the highest Co mean concentration was 1.80±0.12 mg/kg as compared to 5.2±2.4mg/kg observed in the current study. Even in the controls, the Co concentrations in the present study are higher (7.25±7.92 mg/kg) than those from Haridwar in India which was equally far away from the copper mine where the highest concentration level was 1.09±0.1 mg/kg (Maharia et al., 2010). The wide variations in metal concentrations in the analysed medicinal plants could possibly be attributed to differences in the plant metal uptake and translocation capabilities. In human body, Co is required as constituent of vitamin B12 in minute amounts (Soetan et a.l, 2010). However, human toxicity symptoms associated with excessive Co intake include goitre, hypothyroidism and heart failure (Murray et al., 2000). The high concentration of Cu, Co, and Ni observed in Kilembe mine medicinal plants can be attributed to high concentrations of these elements in the soils around Kilembe copper mine which were reported to be above the world average crust as a result of erosion and contamination from the mine tailings (Mwesigye et al., 2016). Since controls also exhibited high elemental Co concentrations, geological sources of Co in soils are also highly likely.

Conclusion

The study established that there is wide scale use of traditional herbal medicine within Kilembe mine area and surroundings. However, it also confirmed the presence and varying concentrations of essential and potentially toxic trace elements in the four selected medicinal plants. Iron, Ni and Cu concentrations exceeded thresholds in all samples of different medicinal plant species collected while Zn, Mn and Co were also significantly higher and exceeding thresholds in over 20% of the medicinal plant species sampled. The high concentrations of essential and potentially toxic elements in medicinal plants could pause health risks to the users of anti-malarial herbal medicines from Kilembe mine area. Also, contrary to the common belief among community members that traditional herbal medicine is safe and free of side effects, the results of this study indicate that herbal medicines may contain potentially toxic elements when grown in soils that are highly contaminated with trace elements from anthropogenic or geological sources. The high concentration of trace elements in Kilembe medicinal plants calls for urgent efforts to remediate the polluted soils. Equally important is the need to create awareness among the communities of the potential risks associated with use of herb medicines collected from Kilembe catchment soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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A comparison of selected heavy metals in soils mixed with domestic and industrial sludges and assessment of effects of the sludge pollutants on oxidative stress markers of the African kale (*Brassica oleracea* var acephala) grown using sewage sludge manure

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Sewage sludge is used as fertiliser and contains nutrients required for plant growth. It also contains contaminants that can leach into crops. The effects of sewage sludge on kale plants were investigated. Mixture ratios of 50:50 and 20:80 for soil and sludge were prepared. The soil-sludge blends were analysed for selected metal residues. Kale seedlings of 10-13 cm were planted on the different soil-sludge combinations. Leaf lengths were measured 21 days post transplanting for four weeks. After sixty days, the leaves were analysed for metal residue levels and antioxidant enzyme activities. The results showed higher metal concentrations in soil blended with industrial sludge than in soil mixed with domestic sludge. The highest growth of plants was observed after 28 days in plants grown on 50% soil-industrial sludge mixtures. Superoxide dismutase and glutathione peroxidase activities were higher in plants grown on soil applied with sewage sludge when compared to enzyme activities in plants grown on sludge-free soil. The high levels of metals and enhanced antioxidant enzyme activity observed were attributed to the contaminants in the sewage sludge. Preassessment of sewage sludge to be used as soil manure is recommended to safeguard the health of plants and, indirectly, humans who consume the crops.

Key words: Sewage sludge, metals, pollutants, plants.

INTRODUCTION

The semi-solid sewage sludge, a by-product of sewage treatment of industrial or municipal wastewater, is a sink of various compounds, some of which are toxic to living organisms. It is the final product of wastewater treatment processes. It is composed of multiple components of raw wastewater and by-products of anthropogenic activities such as heavy metals, pesticides and pharmaceutical residues that find their way to a sewerage system

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catchment area (Agoro et al., 2020). Domestic and industrial effluents are components of municipal sewage. Sewage sludge contains high levels of valuable organic substances and nutrients such as nitrogen and phosphorus. These components of sewage sludge make it a good soil improver. It improves the soil's physical, chemical, and biological properties, including aggregate stability, bulk density, water movement, and retention (Rashid et al., 2018). The utilization of sewage sludge in agriculture increases the content of nutrients in the soil while decreasing the need for chemical fertilizers required ensuring high crop yields (Lamastra et al., 2018; Iticescu et al., 2018). The utilisation of solid sewage sludge in urban settlements where agriculture occurs has become popular because of its fertiliser properties and the meagre costs of this bio-solid matter. Kacprzak et al. (2017) noted that sewage sludge is exploited for agricultural purposes as a strategy for managing waste. The exploitation of sewage sludge in farming as a way of solving problems associated with sewage sludge disposal is also highlighted by Ekane et al. (2021).

Sewage sludge also contains matter that is nonbeneficial to the soil. Contents of sewage sludge include inorganic compounds such as heavy metals and pathogens like viruses and bacteria (Rashidi et al., 2018). Literature has shown that pollutants from sludge can potentially leach into crops (Olowoyo and Mugivhisa, 2019). A transfer of heavy metals from dumpsite soil to plants grown on this soil was recorded by Obasi et al. (2017) and Fei-Baffoe et al. (2021) also reported an accumulation of heavy metals in plants grown on soil treated with sewage sludge. Nunes et al. (2021) observed a collection of heavy metals and other pollutants on vegetables grown on soil treated with sludge. Sludge applied to agricultural soil which contains pollutants like polycyclic aromatic hydrocarbons, pesticides and surfactants pose risk to the environment and human health (Yang et al., 2022). As such contamination of vegetables by heavy metals leaching from sewage sludge is of great concern considering the world over; people are encouraged to consume a lot of vegetables to improve health as vegetables are associated with essential vitamins, minerals, antioxidants, and fibre. Concern about the use of sewage sludge as manure mainly stems from its pollutant load. Vegetables contaminated with heavy metals are a significant threat to human health (Souri et al., 2019). The level of pollutants in sewage sludge depends on the source of the wastewater and the efficiency of the waste treatment plant.

In Bulawayo, Zimbabwe, sewage sludge is used by peri-urban small-scale farmers as soil improvers. Unfortunately, there is no prior toxicological testing of sewage sludge targeted for use as manure. There are no studies that show the extent of contamination of sewage sludge in this southern region of the country. This study, therefore, was carried out to evaluate the levels of selected metal pollutants in amended sludge soils and in vegetables grown using sewage sludge manure. The effects of the contaminants in the amended sludge soil on the growth and some enzymes of the widely consumed kale plants were assessed.

METHODS

Sampling of soil -sewage sludge manure and exposure of plants

Air-dried domestic and industrial sewage sludge from a wastewater treatment plant's drying bed was mixed with topsoil collected to a depth of 20 cm from a field free from any soil amendments. The sludge-soil mixtures were prepared in the ratios of 50:50 and 80:20 for domestic and industrial sewage sludge. The control soil preparation was sludge free.

Conductivity and pH were measured on the different soil-sludge mixtures and the control soil. Kale plant seedlings of 10-13 cm were planted on the soil-sludge treatments, with some grown on the control soil. Leaf length measurements were taken 21 days post-planting, and the leaf lengths were recorded weekly for four weeks. The plant leaves were harvested 60 days post-planting. Heavy metal levels were determined in the soil-sludge samples, and the African kale plant leaves using an Atomic Absorption Spectrophotometer. The plant leaves were homogenised and centrifuged to produce fractions used for enzymatic and non-enzymatic determinations.

Protein determination

Protein concentration was determined following Lowry et al. (1951) and using bovine serum albumin (BSA) as a standard. In a reaction tube, 5 ml of an alkaline solution (2.5 ml of 0.5% copper sulphate in 1% potassium sodium tartrate) was added, followed by 125 ml of 2% sodium carbonate in 0.1 M sodium hydroxide. Lastly, the test solution at a volume of 0.5 ml was added. The contents of the reaction tube were mixed thoroughly and allowed to stand at room temperature for 10 minutes, after which 0.5 ml of 1 M Folin-Ciocalteau reagent was added, mixed rapidly, and the reaction mixture was left to stand for an additional 30 minutes at room temperature. Absorbance was measured against an appropriate blank at 750 nm.

Superoxide dismutase activity

Superoxide dismutase activity in plant homogenates was determined following the method of Sun et al. (1988). Superoxide anion radicals were generated by reacting xanthine and xanthine oxidase. The free radicals reacted with nitroblue tetrazolium chloride to form a red formazan dye measured at 560 nm. The reactant blend was composed of 0.5 ml plant sample or copper-zinc, superoxide dismutase standard (0-300 ng/tube), and 2.45 ml SOD Assay Reagent (SODAR). The SODAR contained 0.3 mM xanthine, 0.6 mM ethylenediaminetetraacetic acid, 150 µM, 400 mM sodium carbonate, 0.1 w/v bovine serum albumin and 150 µM nitroblue tetrazolium in the ratio 4:2:1.2:2:0.6 respectively. The reaction mixture was placed in a water bath at 25°C for 20 min after adding 50 µl xanthine oxidase. After the incubation, the reaction mixture was terminated by adding 1 ml of 0.8 mM copper chloride. The working range for the Cu, Zn-SOD standard curve was 0-300 mg/ml. One enzyme unit of superoxide dismutase is defined as the amount which inhibits the nitroblue tetrazolium reaction by 50 %.

Table 1. Mean pH and conductivity of soil and soil-sludge treatments.

Soil treatments	Control	50% DS-Soil	80% DS-Soil	50% IS-Soil	80% IS-soil	WHO limit
pH	6.81±0.08^a	6.35±0.05^a	6.11±0.06^a	5.57±0.06^a	5.02±0.03^a	6.5-8.5
Conductivity µS/cm	500±16.80^a	521±20.25^a	605±26.35^b	607±18.55^b	630±32.20^b	700

DS = Domestic sludge and IS = Industrial sludge. Values represent the average of triplicate samples expressed as mean ± SD. Different letters between each group indicate significant differences (p<0.05).
Source: Authors

Glutathione peroxidase activity

Glutathione peroxidase activity was determined in plant samples following Flohé and Günzler (1984). The following reagents were added in a quartz cuvette: 1.5 mL of 0.05 M potassium phosphate buffer (pH 7.0), 300 µL of 0.01 M glutathione, 150 µL of 0.02 M sodium azide, 300 µL of 0.002 M reduced form of nicotinamide adenine dinucleotide phosphate in 0.1% w/v sodium bicarbonate, 300 µL of glutathione reductase (10 U/mL), 300 µL of plant sample (1 mg/mL) and lastly 150 µL of 0.0015 M hydrogen peroxide. The blank contained 300 µL of phosphate buffer in place of the plant sample. Absorbance was measured at 340 nm for 5 min.

Malondialdehyde levels

Malondialdehyde (MDA) levels were determined in leaf tissue following Draper and Hadley (1988). Fresh leaf tissues were homogenized in 0.1% (w/v) trichloroacetic acid solution in 1:3 ratios. The homogenates were centrifuged at 12,000 x g at 4°C. Aliquots of the supernatants (1 mL) were added to 0.5% thiobarbituric acid made in 20% trichloroacetic acid, and the mixtures were heated at 95°C for 30 min. After rapid cooling on ice, the mixtures were centrifuged at 10,000 × g for 10 min. The absorbance of each sample was measured at 532 nm.

Statistical analysis

The results were reported as mean ± SD. Two-way analysis of variance (ANOVA) in Tukey's multiple comparison tests indicated statistical significance at $p < 0.05$ using the GraphPad Prism 8 software for the different soil-sludge samples compared with control samples.

RESULTS

pH and conductivity analysis of soil samples

A decrease in pH with an increase in sludge concentration was observed. The control site had the highest pH value of 6.81 (Table 1). The soils mixed with industrial sludge had lower pH values than those mixed with domestic sludge. The soil with 80% sludge had the lowest pH of 5.02 (Table 1). Conductivity increased with the increase in sludge levels in the soil (Table 1). Electrical conductivity was higher in all sludge-soil mixtures when compared to conductivity in the control sludge-free soil. Conductivity recorded for the 80% domestic sludge-soil mixture was 605 µS/cm, while in the 80% industrial sludge-soil mixture; it was 630 µS/cm.

Heavy metals concentration of different soil treatments

The concentration of heavy metals in soils blended with sludge significantly increased with an increase in domestic and industrial sludge than in control soils (p<0.05). The concentrations of cadmium, copper, and zinc were higher in industrial sludge compared to the content in domestic sludge (Table 2). The highest concentrations of all the analysed metals were observed in soil with 80% industrial sludge (Table 2).

Heavy metals concentrations in plant tissue

Heavy metals were observed in all kale plants grown on control and sludge-treated soils. The concentrations of heavy metals were higher in plants grown on soil mixed with sewage sludge than in plants grown on control soils. Comparing heavy metals in plants grown on soil combined with different sewage sludges showed higher levels of metals in plants grown on soil blended with industrial sludge than on soil mixed with domestic sludge. The highest levels of all analysed metals were in soil mixed with 80% industrial sludge (Table 3).

Growth of African kale leaves

The results showed time-dependent increases in lengths of leaves of kale plants grown on different sludge–soil mixtures, with the highest growth observed in the fourth week of leaf measurements. The most increased leaf lengths were observed in kale plants grown on soil mixed with 50% industrial sewage sludge for all time intervals (Figure 1).

Superoxide dismutase (SOD) activity

Superoxide dismutase in all kale plants grown on different sludge-soil mixtures was significantly enhanced when compared to the enzyme activity in kale plants grown on sludge-free soil (p<0.05) (Figure 2). For domestic and industrial sludges, superoxide dismutase activity was higher in kale plants grown on 80% sludge than plants grown on control sludge-free soil (Figure 2).

Table 2. Metal residues in soil- sludge mixtures.

Metal	Metal	residue	level	(mg/kg)		
	Control	**50% DS-Soil**	**80% DS-Soil**	**50% IS-Soil**	**80% IS-soil**	**WHO limit (mg/kg)**
Cadmium	2.95±0.3^{a}	4.55± 0.3^{b}	6.25± 0.4^{b}	6.25± 0.6^{b}	10.80 ± 0.5^{c}	3.0
Copper	10.40± 0.9^{a}	25.08± 1.1^{b}	82.10± 10.0^{c}	102.40±11.2^{d}	237.50± 15.9^{e}	100
Zinc	18.00± 0.8^{a}	255.10 ± 50.0^{b}	397.50± 40.2^{c}	602.30± 20.2^{d}	965.30± 25.1^{e}	300

DS = Domestic sludge and IS = Industrial sludge. Values represent the average of triplicate samples expressed as mean ± SD. Different letters between each group indicate significant differences ($p<0.05$).
Source: Authors

Table 3. Metal residues in plant tissue.

Metal	Metal	residue	level (mg/kg)			
	Control	**50% DS-Soil**	**80% DS-Soil**	**50% IS-Soil**	**80% IS-soil**	**WHO limit mg/kg)**
Cadmium	1.10 ± 0.07^{a}	1.55 ± 0.06^{a}	2.10 ± 0.04^{a}	2.95 ± 0.6^{a}	4.80 ± 0.9^{b}	0.2
Copper	7.90 ± 0.9^{a}	19.48 ± 1.1^{b}	29.70 ± 3.0^{c}	49.45± 3.2^{d}	95.50± 2.9^{e}	10
Zinc	7.50 ± 0.8^{a}	62.50 ± 5.0^{b}	120.50 ± 4.2^{c}	150.30± 5.2^{d}	245.30± 8.1^{e}	5

DS = Domestic sludge and IS = Industrial sludge. Values represent the average of samples expressed as mean ± SD. Different letters between each group indicate significant differences ($p<0.05$).
Source: Authors

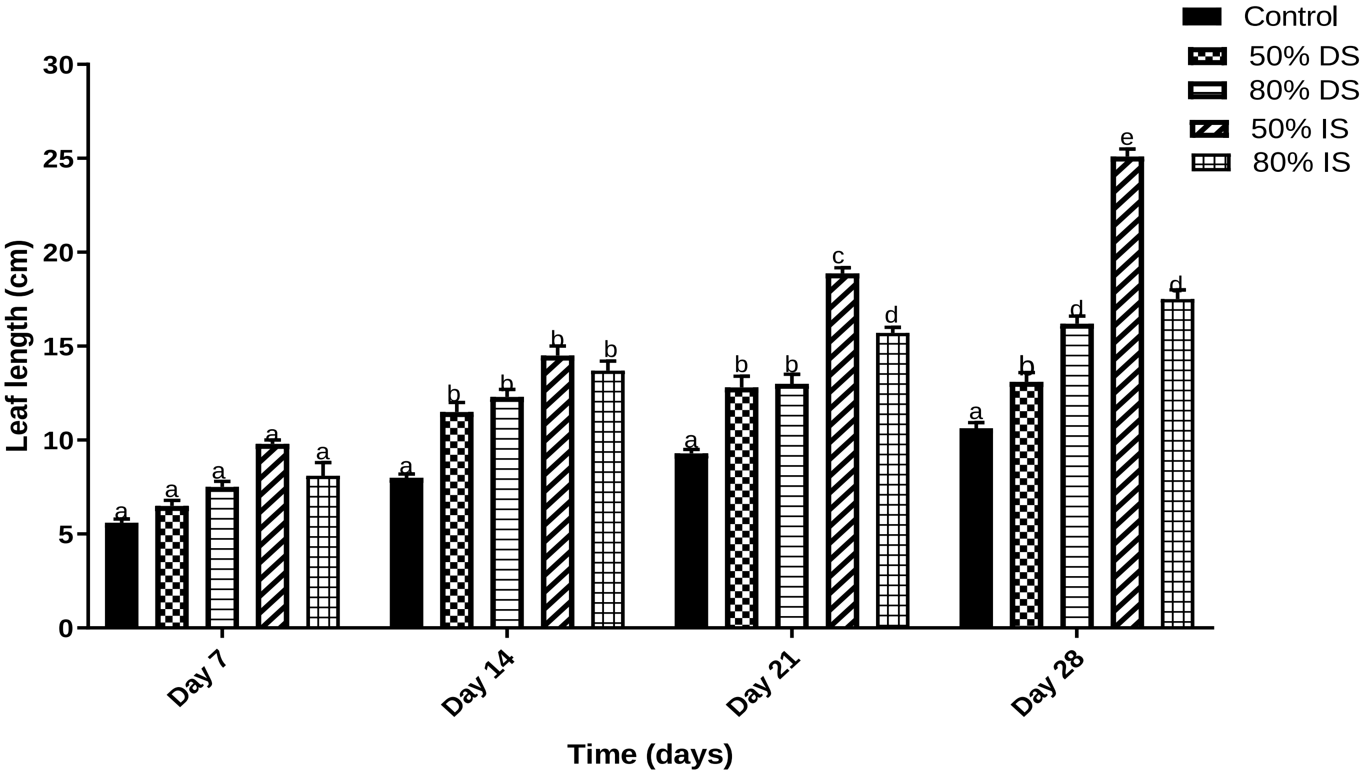

Figure 1. Length of kale plants leaves of plants grown on different sludge-soil mixtures. Plants were grown over 28 days. Leaf measurements were started 21 days post-planting, and the measurements were taken at seven days intervals. The 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% Industrial sewage sludge + 20% soil. Bars with different letters indicate significant differences at $p<0.05$. Values represent the average of triplicate exposures.
Source: Authors

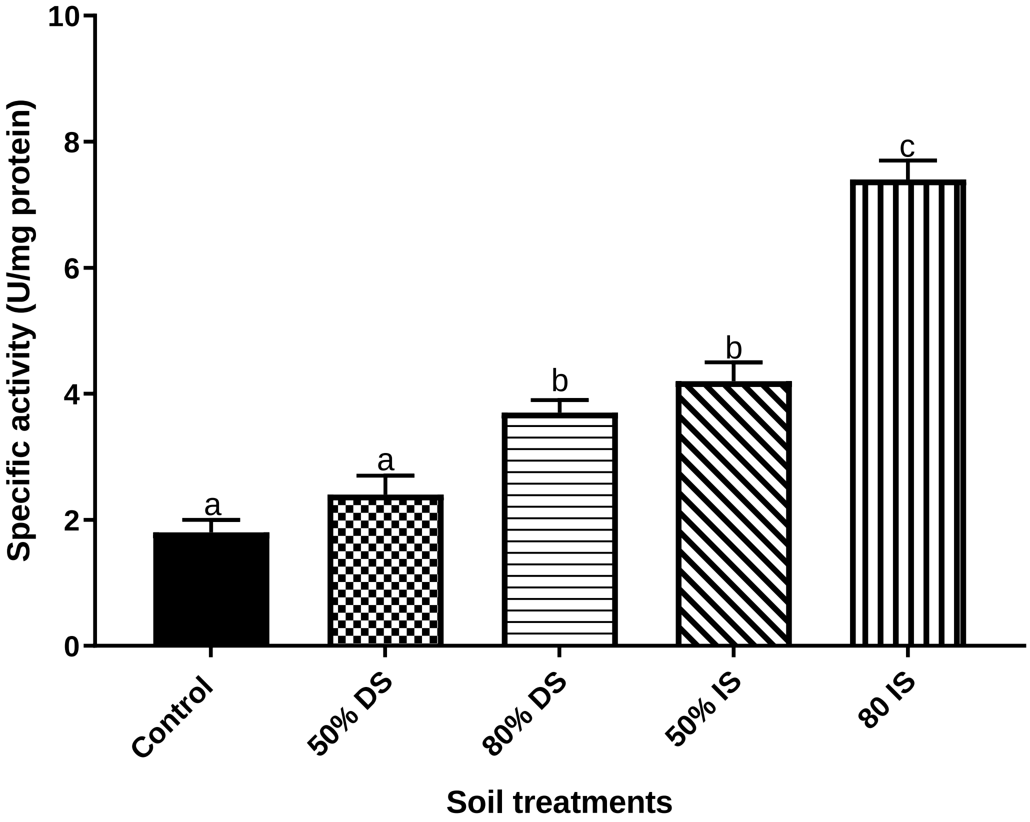

Figure 2. Effect of the different soil-sludge mixture on superoxide dismutase activity in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% Industrial sewage sludge + 20% soil. Bars with different letters indicate significant differences at p<0.05. Values represent the average of triplicate exposures, expressed as mean ± SD.
Source: Authors

The SOD activity in kale plants grown on 80% domestic sludge–soil mixture was enhanced twofold. The same enzyme activity for the kale plants grown on 80% industrial sludge-soil was increased four times than the SOD activity in plants grown on control soils (Figure 2).

Glutathione peroxidase (GPx) activity

Significantly higher glutathione peroxidase activities were observed in kale plants grown on different sludge–soil blends than the activities of GPx in kale plants grown on sludge-free soils (p<0.05). The highest activation of GPx activity was observed in kale plants grown on soil blended with 80% industrial sludge (Figure 3).

Malondialdehyde (MDA) levels

All the kale plants grown on different sludge-soil mixtures showed higher malondialdehyde (MDA) levels than MDA levels in kale plants grown on the control, sludge-free soils. The levels of MDA in kale plants grown on soils blended with sludge were; 2, 7, 8, and 12 times more than MDA levels in plants grown on control soils for 50% DS, 80% DS, 50% IS, and 80% IS respectively (Figure 4).

DISCUSSION

Sewage sludge is a semi-solid, mud-like residuum left behind after wastewater treatment. The high nitrogen, phosphorus, and potassium content present in sewage sludges make them ideal substitutes for fertilisers. They enhance the quality of soil and improve the growth of crops.

Although sewage sludge possesses fertiliser properties, it contains high levels of pollutants, including heavy metals and organic materials such as aromatic hydrocarbons. Literature has shown that these pollutants, particularly non-biodegradable ones like heavy metals, may accumulate in crops, posing health risks to humans who consume the vegetables (Krishna et al., 2021; Salim

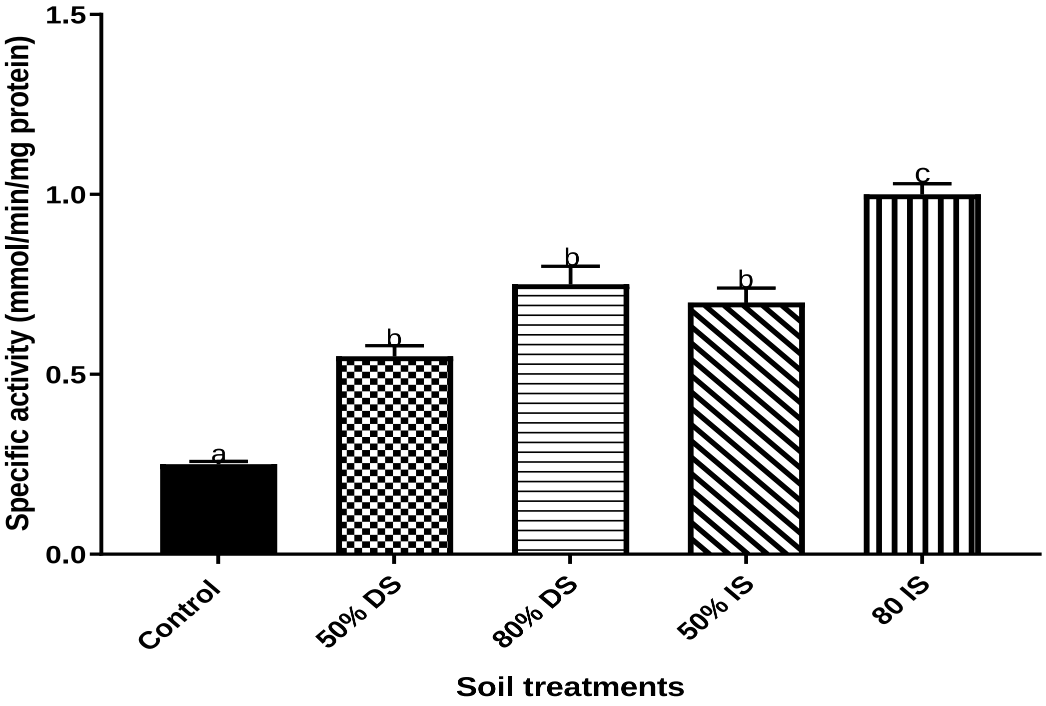

Figure 3. Effect of different soil-sludge mixtures on glutathione peroxidase activity in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% Industrial sewage sludge + 20% soil. Bars with different letters indicate significant differences at p<0.05. Values represent the average of triplicate exposures, expressed as mean ± SD.
Source: Authors

et al., 2021). It is, therefore, necessary to assess the toxin levels of sludge for soil amendments to ensure that it does not have excess pollutant load, which could accumulate in plant crops. In Zimbabwe, the use of sewage sludge is popular for small-scale farmers on the outskirts of cities. However, there is no information or enough studies in the country on the toxins present in both domestic and industrial sludges.

Conductivity and pH data recorded are shown in Table 1. The pH of the control, sludge-free soil, was 6.81, and it falls in the permissible pH range of 6.5-8.5 set by WHO (Tomno et al., 2020). All four sludge samples had pH ranging from 5.02 – to 6.35, indicating an acidic nature (Table 1). The acidic pH observed was attributed to the components of the sludge. The results differ from the results recorded by Jaffar et al. (2017), who generally observed slightly alkaline pH values for the soil samples they worked with. Soil electric conductivity measures levels of ions of water-soluble salts in soils and reflect the soil's salinity. In the present study, it was observed that EC levels in sludge-soil mixtures ranging from 521 µS/cm to 630 µS/cm, and the levels fall within the allowable EC range set by the World Health Organisation (Tomno et al., 2020). In contrast to the results, Suanon et al. (2016) reported a low mean EC value of 4.7 µS/cm in the sludge they used for their study. Another study by Zoghlami et al. (2016) revealed a mean EC value of 1702 µS/cm in the sewage sludge targeted for agricultural use they characterised; the high electric conductivity value observed was attributed to the high content of heavy metals in the sludge.

The soil and soil-sludge samples were analysed for the following metals; cadmium, copper, and zinc. After growing the plants for sixty days, the plants were analysed for the three metals, cadmium, copper, and zinc. All analysed metals were present in all the soil samples. The observed cadmium levels in soil-sludge samples were in the range of 4.55-10.8 mg/kg and were above the WHO permissible levels in soil (Tomno et al. 2020). The results were much higher than the levels reported by Tomno et al. (2020). They reported concentrations of cadmium in soil samples in the range of 0.00534 to 0.0072 mg/kg (Tomno et al. 2020). The concentrations of cadmium in soils reported in this present study were, however, much higher than those reported in Eastern Cape Province, South Africa in a study carried out by (Agoro et al. 2020) who reported cadmium levels in sludge ranging from 0.13 to 0.5 mg/kg. Aloud et al. (2022) also reported cadmium levels in the soil much higher than the limit set by WHO. In their study which assessed the accumulation of heavy metals in soil and plants from an industrial area in Saudi Arabia, they

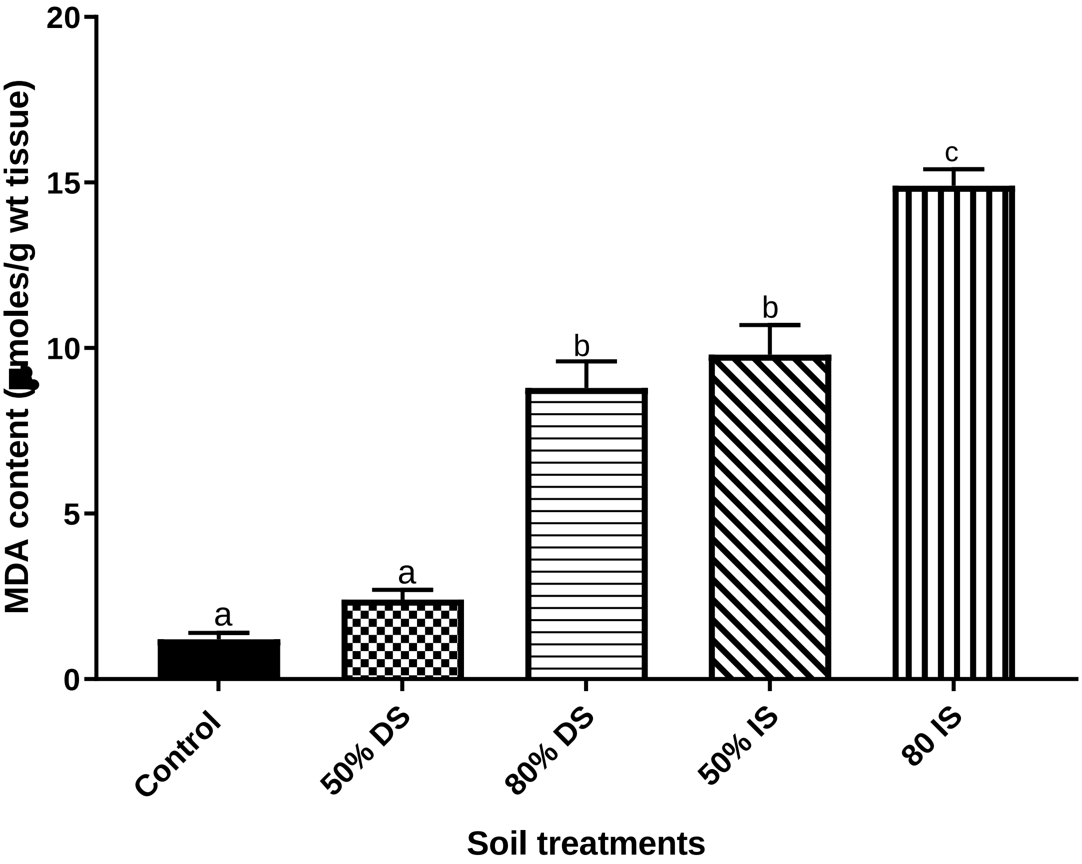

Figure 4. Effect of different soil-sludge mixtures on malondialdehyde (MDA) in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% Industrial sewage sludge + 20% soil. Bars with nonidentical alphabetical symbols show significant differences at $p<0.05$. Values represent the average of triplicate exposures expressed as mean ± SD.
Source: Authors

noted cadmium levels of up to 22.0 mg/kg (Aloud et al., 2022). In *Cyperus laevigatus* plants, a mean cadmium concentration of 2.4 mg/kg was observed (Aloud et al., 2022). In kale plants, the cadmium concentrations observed in the present study ranged from 1.55 to 4.8 mg/kg in plants grown on sludge-soil blends and they were all above the 0.02 mg/kg permissible limits as set by WHO (Kinuthia et al. 2020) in plants. Our results were higher than the concentrations reported by Salim et al. (2020) who recorded 0.05 to 2.4 mg/kg in Mung beans. In another study, Ramanos et al. (2021) recorded cadmium levels ranging from 0.32 to 0.66 in wheat grown on sludge amended soil. The copper levels the authors observed in soil-sludge samples ranged from 25.08 mg/kg to 237.50 mg/kg and they were very high when compared to the levels of copper in soil-sludge samples analysed by Fei-Baffoe et al. (2021) who reported a mean copper concentration of 53.10 mg/kg.

Turkmen et al. (2016) also observed an accumulation of copper of up to 85.6 mg/kg in edible plants collected from the Giresun basin. The copper concentrations reported in kale plants grown on soil blended with industrial sludge in the current study of 49.5 mg/kg and 95.5 mg/kg for 50% IS and 80% IS respectively were all above the 5 mg/kg permissible limit set by WHO (Tomno et al. 2020). In contrast to our results, Reis et al. (2020) in their study observed copper concentrations in leaves of maize plants of up to 4.58 mg/kg which were much lower than those recorded in our study.

Zinc concentrations reported in sludge-soil mixtures in the current study were within the permissible limit set by US EPA (Agoro et al., 2020). The findings, however, were higher than those reported by Romanos et al. (2021) who recorded zinc levels in the range of 8.93 to 18.14 mg/kg in soil amended with sewage sludge. Zinc is an important trace element required in various metabolic processes and it becomes toxic at high concentrations. In the present study, zinc concentrations observed in kale plants grown using sludge fertilizer were in the range of 62.50 to 245.30 mg/kg and were much higher than the

WHO's recommended limit of zinc in plants of 5 mg/kg (Tomno et al. 2020). The zinc levels in plants grown on the 80% domestic sludge-soil, and 80% industrial sludge-soil mixtures were 24 and 49 times more than the permissible limit set by WHO (Tomno et al., 2020). Similar observations were made by Krishna et al. (2021) whose data revealed a zinc mean concentration of 105.14 mg/kg in spinach grown on sludge. Turkmen et al. (2016) also recorded high zinc concentrations in the range of 10.1 – 110.0 mg/kg in plants obtained from the Giresun basin and the levels were above the acceptable limit set by WHO (Tomno et al., 2020). Our results showed that increasing the sludge content resulted in increases in the uptake of metals by the kale plants. The results also indicated that decreases in pH levels of the sludge soil mixtures resulted in increases in the uptake of metals by the plants. The highest uptake of metals was recorded in plants grown on soil blended with 80% industrial sludge and the 80% IS had the lowest mean pH. The acidic conditions in the soil-sludge treatments probably enhance the mobility of toxic metals in soil and increase leaching into the plants. The results are supported by Štofejová et al. (2021) who reported decreased availability of metals in plants as pH levels rise above 7.

There were increases in leaf lengths of all the plants grown on different soil treatments over the 28 days when leaf measurements were taken (Figure 1). Time-dependent growth of the kale plants indicated by increases in leaf lengths of the kale plants was observed. Growth was recorded in all plants grown on sludge-soil mixtures and plants grown on control sludge-free soils; however, more pronounced growth was observed in kale plants grown on sludge-soil mixtures.

The higher growth rate observed in kale plants grown on sludge-soil mixtures than in plants grown on control sludge-free soil was probably due to sewage sludge supplemented on the soil. The nutrients and other organic matter in the sludge likely contributed to the enrichment of the soil and provision of nutrients to the plants resulting in enhanced plant growth. The findings are supported by the work of Fei-Baffoe et al. (2021), who also reported increased yields of cabbage and lettuce in plants that were grown using sludge as fertiliser. Abdul Khaliq et al. (2017) also reported a study which is in line with the results. They observed higher yields of beans and radish grown on soil with sludge compared to plants grown on sludge-free soil (Abdul Khaliq et al., 2017). Positive effects of sewage sludge soils on maize and triticale plants were recorded by Tomócsik et al. (2016) as higher quantities of the crops were harvested from plants grown on sludge amended soils compared to the amounts of crops harvested from plants grown on the control soils. The enhanced yield of crops was linked to the sludge added to the soil, which provided nutrients that positively impacted the soil structure, improving soil aeration and enhancing the activities of living organisms within the soil structure. Wang et al. (2016) highlighted that nutrients in sewage sludge are responsible for growth improvement observed in crops grown on soil enriched with sewage sludge. In the current study, the highest growth was recorded in plants grown on soil mixed 50:50 with industrial sludge for all time intervals. Increasing the industrial sludge content above the 50% ratio probably had adverse effects on the growth of the kale plants, possibly because of the very high content of toxin particles associated with sludge.

The effect of sludge on the oxidative defence system was investigated. The kale plants exposed to domestic and industrial sludges were analysed for the antioxidant enzymes superoxide dismutase and glutathione peroxidase. Plants synthesise numerous antioxidant molecules and enzymes as protection against xenobiotic-induced oxidative stress.

Superoxide dismutase activities in all kale plants grown on sludge–soil mixtures were higher than the enzyme activities in plants grown on sludge-free soils. The highest enhancement of superoxide dismutase activity was in plants grown on the 80% industrial sludge–soil mixture, with an increase of more than 4-fold, compared to enzyme activity in plants grown on control soils. The enhancement of superoxide dismutase was probably caused by the pollutant levels, including heavy metal residues observed in sludge–soil mixtures. The results are in accord with the findings of Hakeem et al. (2022) who observed increases in SOD activities of the garden peas, *Pisum sativum*, grown on sludge. However, in a different study Sasi et al. (2019) revealed lower SOD activities in the common bean (*Phaseolus vulgaris*) grown on soil enriched with sewage sludge compared to enzyme activities in plants grown on controls, findings that are in contrast to the results observed in the current study. Glutathione peroxidase activities, just like the SOD activities, were elevated in kale plants grown on sludge-soil mixtures compared to enzyme activities in plants grown on control soils in the current study. The highest glutathione peroxidase activity was observed in kale plants grown in soil mixed with 80% industrial sludge. The activation of activities of SOD and GPx were probably protective mechanisms in plants to safeguard their well-being. The dose-dependent increase in SOD and GPx activities in sludge-soil mixtures supports the fact that the enhancement of the antioxidant defense system, namely SOD and GPx, was to counteract the oxidative effects of pollutants in the sludge as increases in sludge levels resulted in increased uptake of metal residues in kale plants ultimately causing enhancement of antioxidant enzyme activities in the kale plants. Increased SOD and GPx protect the plant cells from oxidative stress caused by sludge pollutants, including the high heavy metal load. In essence SOD and GPx are the first protective molecules of living organisms both animals and plants that encounter xenobiotically induced reactive oxygen species and transform them into less toxic molecules (Ighodaro and Akinloye, 2018). Other researchers have also reported similar findings. Yap et al. (2021) observed elevated antioxidant SOD in plants exposed to the metal copper. Benhamdi et al. (2021) also reported results with similar trends to our findings. They recorded activations in the activities of SOD and GPx in plants exposed to zinc (Benhamdi et al. 2021).

Levels of malondialdehyde were also determined in kale plants grown on different soil sludge blends. The malondialdehyde (MDA) levels observed in the current study were significantly enhanced in kale plants grown on soil enriched with sludge compared with malondialdehyde concentrations in plants grown on sludge-free soil.

Malondialdehyde is a marker for lipid peroxidation. Reactive oxygen species generated from detoxification of pollutants causes lipid peroxidation of polyunsaturated fatty acids in membranes of plants producing MDA which is generally used as a sensitive marker for membrane damage (Morales and Munné-Bosch, 2019). High levels of MDA are associated with lipid peroxidation resulting from pollutant-induced oxidative stress. The sewage-sludge manure that was used to enrich the soil contained pollutants such as heavy metals, which caused oxidative stress indicated by elevated MDA levels observed in plants grown on the soil blended with sludge compared to MDA levels in plants grown on control soils. The findings of Benhamdi et al. (2021) are in agreement with the results. They recorded enhanced MDA concentrations and antioxidant enzymes SOD and GPx in plants exposed to the heavy metal zinc (Benhamdi et al. 2021). Likewise, Georgiadou et al. (2018) reported high levels of MDA concentration in *Ocimum basilicum* plants exposed to copper and zinc. Similar data were reported by Hussain et al. (2021) who observed increases in the MDA content of carrot plants grown with industrial effluent. The enhanced MDA levels in plants reflect the failure of the plant's antioxidant protection system to effectively counteract pollutant-induced oxidative stress. The chemical pollutants in sludge undoubtedly caused the generation of free radicals in plants resulting in lipid peroxidation in plant cells. The results clearly show that there was leaching of pollutants from the soil-sludge mixtures to the plants. As such, sludge designated for agricultural use should be screened for pollutants to protect crops and indirectly the health of humans who consume the crops.

Conclusion

The use of sewage sludge, without a doubt, improves the physical and chemical properties of the soil, thereby facilitating enhanced crop yield. The main drawback to the use of sewage sludge as manure is the presence of toxic pollutants such as heavy metals in sludge material. The soil amended with sludge contained high loads of metal residues that exceeded the WHO permitted limits. Soil mixed with industrial sludge had a higher concentration of heavy metals than soil amended with domestic sludge. Heavy metals were found in all plants grown on soil mixed with sludge. The acidic conditions of the sludge-soil mixture probably increased the bioavailability of the heavy metal residues enabling the leaching of the metal residues in the plant leaves. The seemingly healthy kale plants contained heavy metals at concentrations that exceeded WHO permissible limits. There is a need for the pre-assessment of pollutant levels in sludge targeted for use as compost to protect plants from toxic matter in sludge and also indirectly protect the health of people who consume the crops.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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