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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

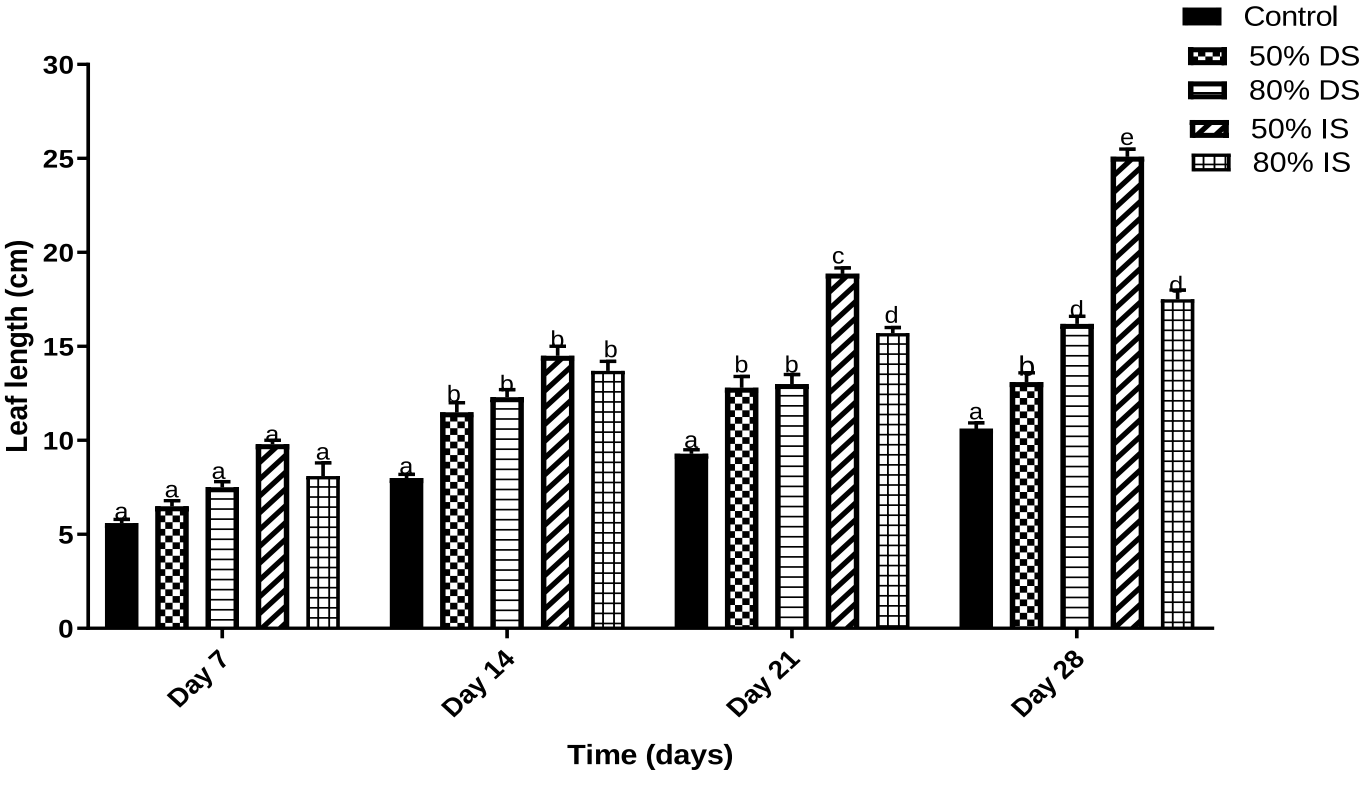

Figure 1. Length of kale plants leaves of plants grown on different sludge-soil mixtures. Plants were grown over 28 days. Leaf measurements were started 21 days post-planting, and the measurements were taken at seven days intervals. The 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic 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

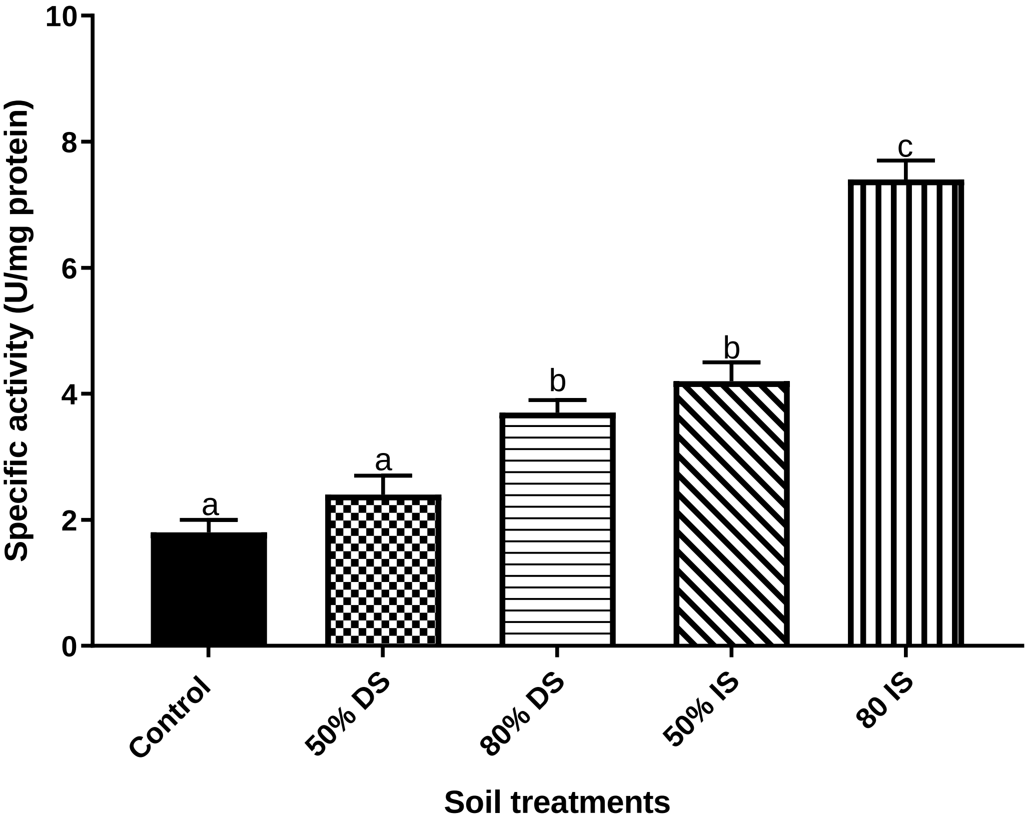

Figure 2. Effect of the different soil-sludge mixture on superoxide dismutase activity in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% 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

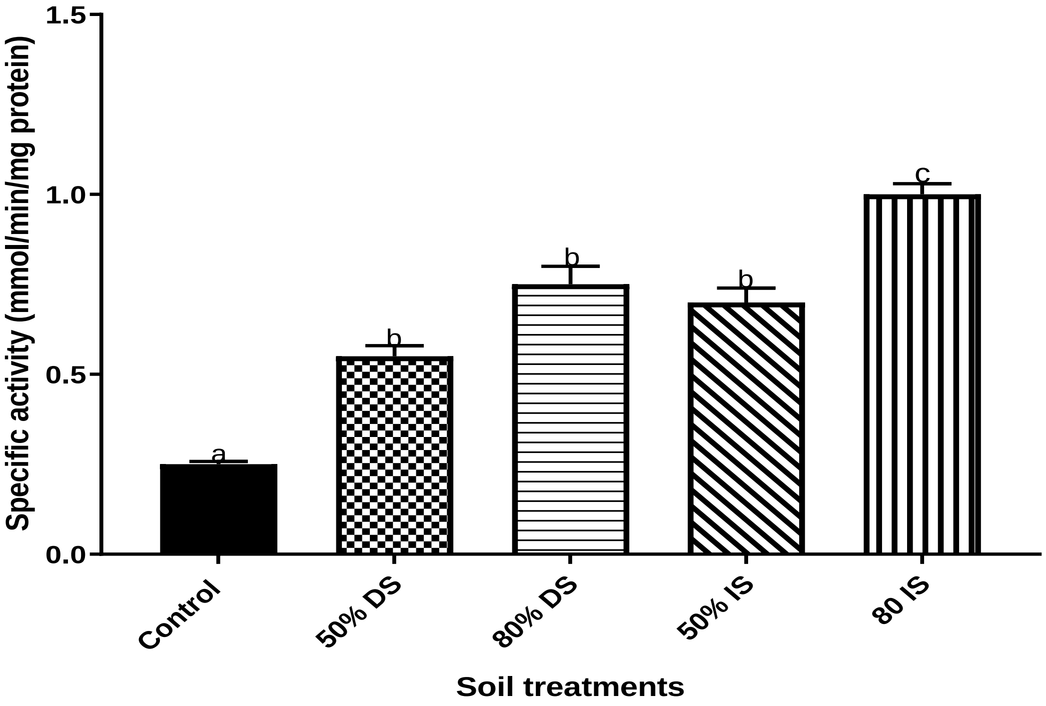

Figure 3. Effect of different soil-sludge mixtures on glutathione peroxidase activity in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% 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

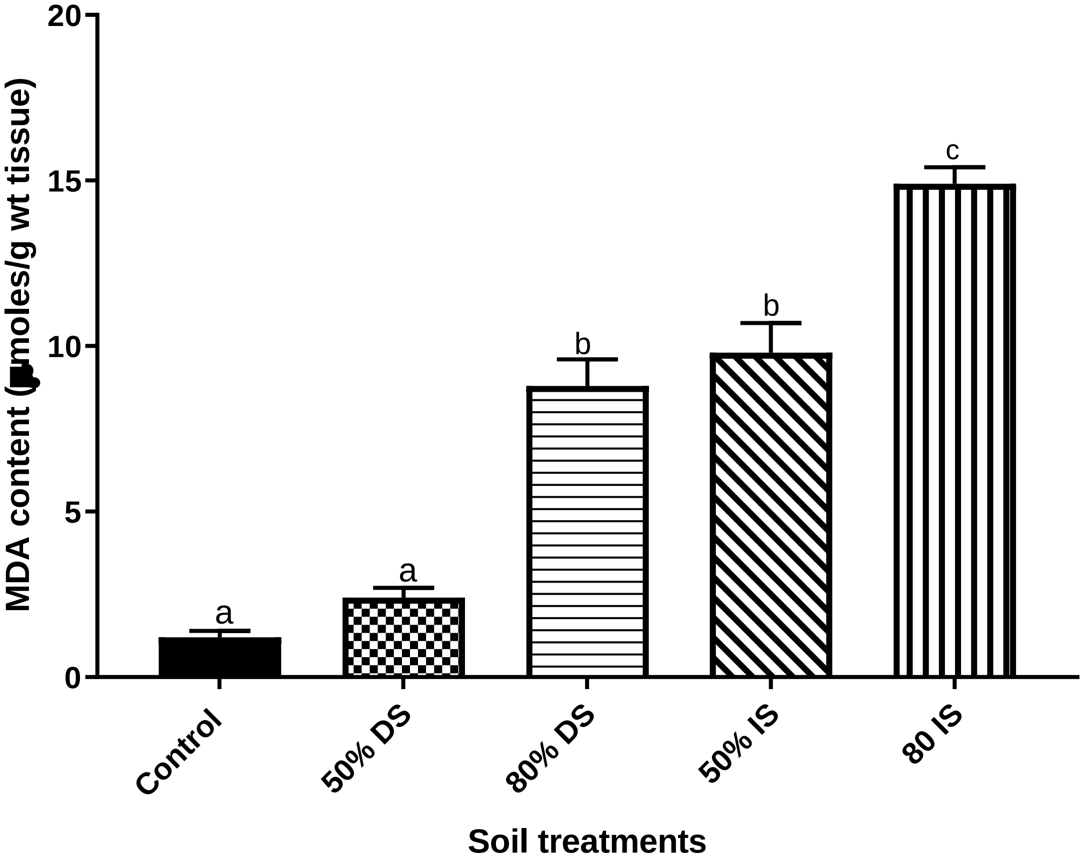

Figure 4. Effect of different soil-sludge mixtures on malondialdehyde (MDA) in leaves of kale plants allowed to grow for 60 days. 50% DS = 50% Domestic sewage sludge + 50% soil, 80% DS = 80% Domestic sewage sludge + 20% soil, 50% IS = 50% Industrial sewage sludge + 50% soil and 80% IS = 80% Industrial 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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