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ORIGINAL ARTICLE

Growth response of vetiver grass (*Chrysopogon zizanioides* (L.) Roberty) to chemical amendments in assisted phytoremediation of contaminated mined soil

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Abstract
The application of chemical amendment to improve metal availability is a key strategy in phytoremediation and an important determinant for successful removal of heavy metals from soil, although empirical data on their effects on plants used in phytoremediation are scanty. In this study, field-based assisted phytoremediation with ethylene-diamine-tetra-acetic acid (EDTA), nitrogen-potassium-phosphorus fertilizer (NPK) and combination of EDTA and NPK modelled after the completely randomized block design was used to determine the effects of chemical amendments on some morphological and physiological growth parameters of vetiver grass (*Chrysopogon zizanioides* (L.) Roberty) as well as the relative effects of chemical amendment and free heavy metal ions contamination. Results showed that the soil amendments (EDTA, NPK, EDTA + NPK) enhanced plant height and diameter, and reduced the toxicity of free metal ions. On the other hand, heavy metals reduced plant chlorophyll-a and -b, and plant root, and correlated with lipid peroxidation. Notably, EDTA contributed the least to enhancing plant height, diameter, and root length although it interacted positively with NPK to enhance the above-mentioned parameters. In general, the results of this study confirm the effectiveness of chemical amendments (EDTA and NPK in this case) in reducing the toxicity of free heavy metal ions in plant during phytoremediation.

Keywords *Chrysopogon zizanioides* (L.) Roberty · Plant growth · Phytoremediation · NPK · EDTA

Introduction

Phytoremediation has become a fascination to most environmentalists in the wake of global industrialization and its resultant effects on the environment (Miller et al. 2008a, b; Farid et al. 2013; Suman et al. 2018; Shehata et al. 2019; Yan et al. 2020). Several research aimed at improving the effectiveness, efficiency and the market value of this biotechnology have been carried out over the last few decades (Zeremski-Škorić et al. 2010; Saifullah et al. 2015; Li et al. 2017; Anning and Akoto 2018; Bian et al. 2018). A sizable number of these studies have focused on improving the bioavailability of heavy metals in soil (Yu et al. 2019). Chelants (Epelde et al. 2008; Miller et al. 2008b; Glinska et al. 2014), acidifiers (Palma and Mecozzi 2007; Anning and Akoto 2018), phytohormones (López et al. 2005) and other amendments (Kamari et al. 2010) capable of enhancing metal bioavailability have been extensively investigated with significant and interesting findings of great implications for phytoremediation. Additionally, the effects of plant species, source of contamination, metal type and the time of application on the efficacy of chemical amendments are also well documented (Nowack et al. 2006; Shahid et al. 2014; Anning and Akoto 2018). Significant improvement in heavy metal availability observed after soil amendment (Wu et al. 2004; López et al. 2005; Liphadzi and Kirkham 2006a; Ebrahimi 2013) has made this process an integral part of phytoremediation of heavy metals from soil.

Successful phytoremediation, however, requires more than enhanced metal availability in soil. As noted by Garbisu and Alkorta (2001), successful phyto-extraction is also

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strongly dependent on plant biomass and efficient transfer of metals from plant root to shoot, thereby eliminating the need for replanting after every phytoremediation cycle, and reducing the overall cost of the process. Given their interdependency (Garbisu and Alkorta 2001; López et al. 2005; Shahid et al. 2014), a balance between heavy metal availability, plant growth and uptake is vital to achieving efficient phytoremediation. Hence, chemical amendments used to improve metal availability should not hinder the growth of plants.

Recent studies, however, have shown that most chemical amendments, especially the artificial ones, directly or indirectly exert significant effects on plant morphological and physiological properties (Saifullah et al. 2010; Anning and Akoto 2018). Ethylene-diamine-tetra-acetic acid (EDTA), for example, contains 10% nitrogen which can mineralize and increase nitrogen concentration in the soil above their threshold limits and affect plant growth (Oviedo and Rodríguez 2003). On the other hand, EDTA has also been associated with detrimental effects on plant morphological (Liphadzi and Kirkham 2005; Saifullah et al. 2010; Sulaivani and Mezori 2015) and physiological (Collins et al. 2002; Yu et al. 2019) parameters. This effect poses a challenge for phytoremediation given that plant growth is fundamental to the success of this biotechnology. There is, therefore, the need to carefully assess and evaluate the toxicity of chelating agents and their metal complexes in soil to inform the choice of appropriate phytoremediation options. Soil physical, chemical and biological characteristics (Blight 2011), seasonal variations (Kidd et al. 2015) and concentration of free metal ions in soil solution (Oviedo and Rodríguez 2003; Liu et al. 2007) have been shown to exert detrimental effects on plant growth. Thus, it is important to examine the effects of the various amendments in relations with these plant growth-limiting factors. Yet, to date, the effects of even the most commonly used amendment (i.e., EDTA; Liphadzi and Kirkham 2005; López et al. 2005; Ebrahimi 2013; Mirza et al. 2014) on plants are mixed and limited at best. The relative effects of chemical amendments and other plant growth-limiting factors are also not clear. This knowledge will help determine the actual effects of chemical amendments on plant growth and inform decisions regarding appropriate phytoremediation.

In this study, field-based chemically assisted phytoremediation was used to determine the effects of some chemical amendments (EDTA, NPK, EDTA + NPK) on some morphological (height, diameter, root length) and physiological parameters (MDA, chlorophyll *a* and chlorophyll *b*) of the vetiver grass (*Chrysopogon zizanioides* (L.) Roberty). The study addressed the following questions: (1) does contamination of soil by heavy metals affect plant growth? (2) how do EDTA and NPK amendments modulate the effects of heavy metal-contaminated soil on plant growth? (3) what is the relative importance of metal concentration and treatment on plant growth? It was hypothesized that plant growth-limiting factors like free metal ion concentration in soil would affect plant morphological and physiological parameters more than the chemical amendments.

Materials and methods

Study area

The study was conducted in the southeastern side of the mining lease of Mensin Gold Bibiani limited, located in the Bibiani-Anhwiaso-Bekwai Municipality of the Western North Region of Ghana (Fig. 1). The Bibiani-Anhwiaso-Bekwai Municipality is among the areas covered by the north-western part of the moist semi-deciduous forest of Ghana and is characterized by a bi-modal rainfall pattern making farming a lucrative activity in the municipality. The study site forms part of an old tailing storage facility for processed sulphide ore from the main pit and underground working, but the tails were evacuated and reprocessed in the early part of the year 2000. A recent study by Akoto and Anning (2021) showed that the area is still enriched with heavy metals, and hence appropriate for this study.

Study plant

Chrysopogon zizanioides (L.) Roberty, also known as vetiver grass or Khus, is a perennial grass of the genus *Chrysopogon* and the Poaceae family. The grass is native to Asia but widely introduced and cultivated in tropical and subtropical regions of the world. It is a high biomass-producing and robust plant with massively fine deep-root system and tolerant of wide pH ranges (3.0–9.5; Danh et al. 2009). The ability of vetiver to tolerate other harsh conditions (low nutrient concentration and high levels of heavy metals and other contaminants) on tailings dam is also well documented (Fonseca et al. 2006; Arochas et al. 2010). According to Shahid et al. (2014), it is vital for candidate plants for EDTA-assisted phytoremediation to have sets of characteristics required to reduce leaching of EDTA-mobilized heavy metals, effectively take up the contaminants and thrive. Vetiver appears to exhibit these suites of characteristics, hence its selection for this research. All plant samples used for this study were obtained from the CSIR-Plant Genetic Resources Research Institute, Ghana.

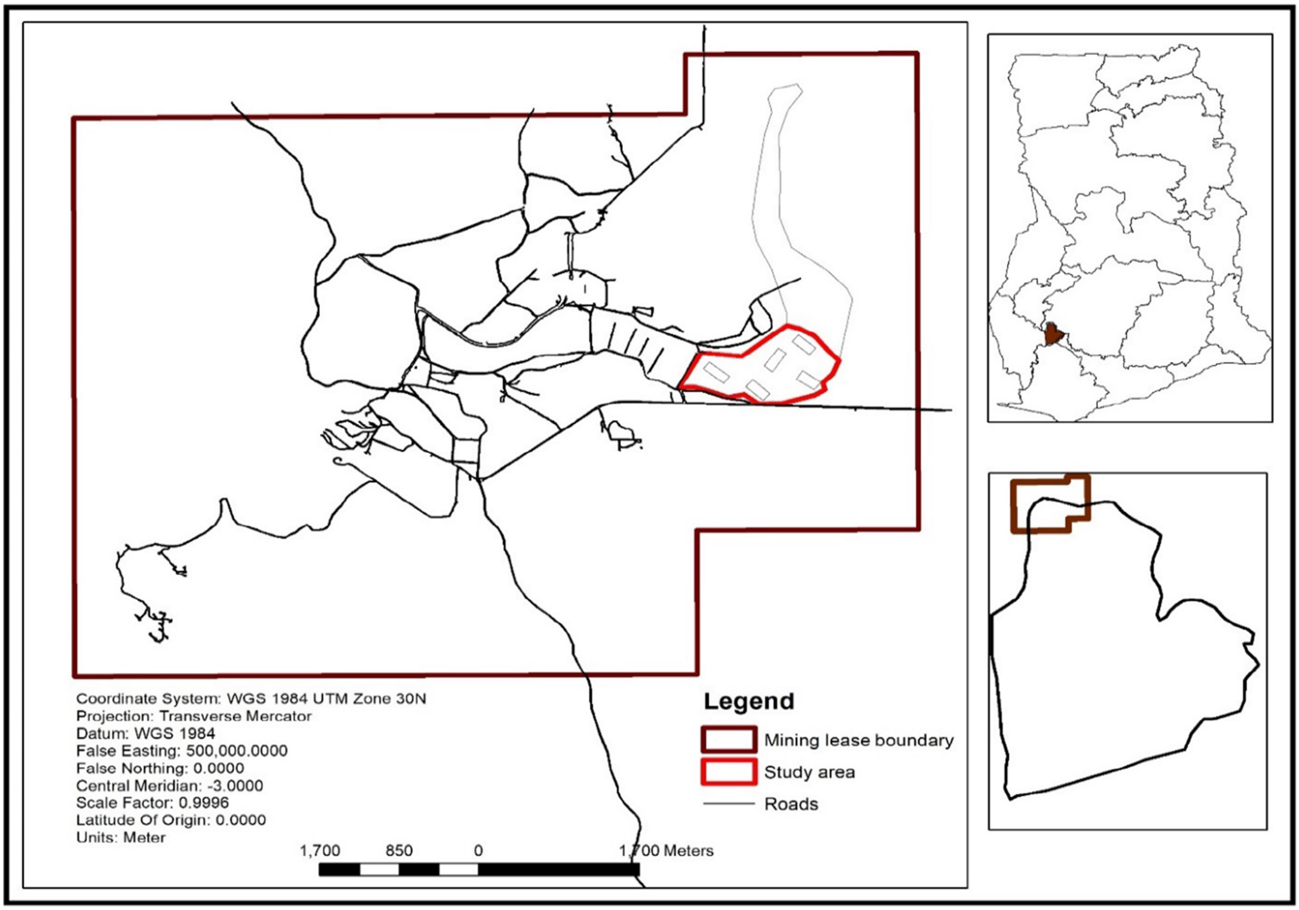

Fig. 1 Mining lease of Mensin Gold Bibiani Limited. Area edged "mars red" is the study area and areas edged green are the study blocks

Study design

Field experiment

Completely randomized block design was adopted for the field experiment conducted from the 1st of April 2019 to 31st March 2020. Here, the study area was divided into four blocks/replicates of dimensions 45 m × 12 m. Three composite soil samples were obtained from each block at 0–60 cm depth to determine the baseline levels of heavy metals (Pb, As, Cd, Fe, Cu). Each block was then subdivided into four plots (each measuring 2 m × 2 m), with one of four treatments (Control, EDTA, NPK fertilizer, and NPK fertilizer + EDTA) assigned to each plot. Also a plot of 2 m × 2 m was demarcated on an uncontaminated site (reference site), sampled and analyzed for baseline heavy metal concentration.

Application of treatments

According to Nowack et al. (2006), a metal–chelant concentration (mole) ratio of at least one (1) is needed to solubilize all targets metals. Based on this, 50 g/L of EDTA, representing metal-EDTA mole ratio of 1:2 was used in this study. In addition to the total concentration of all target metals, major cations (Ca, Mg, K, and Na) which could compete with the metals for EDTA were taken into consideration in determining the metal–EDTA concentration used for this study. Studies have shown that application of NPK (15-15-15) fertilizer at a dose of 1000 kg/ha produces the highest result in height, wet and dry weight of fruits and other plant parts (Hariyadi et al. 2019). Thus, 20 g of NPK fertilizer per plant was used in this study.

Transplanting young plants

Vetiver tilers of approximately similar heights (20 cm), root length (7 cm), weight, and age (12 months) were pruned and transplanted early in the morning on the study site as well as the reference site. Plants were planted at a row distance of 30 cm and plant distance of 30 cm resulting in a density of 89,000 plants per hectare (Ghosh et al. 2018). Plants were then tagged with unique identification codes for easy and accurate identification, and allowed a period of two weeks (18th March to 1st of April 2019) to acclimatize to their new environment.

Sample analysis

Study parameters were monitored periodically using chemical and physical methods.

Physical monitoring of plant parameters

Plant morphological parameters for this study (height, diameter and root lengths) were monitored on monthly and quarterly basis. Plant height and root length were monitored with tape measure and the diameter with a calliper.

Chemical analysis of plant and soil samples

Heavy meals (Fe, Cu, Cd, As, Pb) in soil collected at 0–60 cm depth and plant samples were determined before, during and after the field study. In addition, physiological (MDA, chlorophyll-a and b) content of plants was analyzed following the methods. Heavy metals were analyzed using atomic spectrometry technique (atomic absorption spectrometry, AAS) according to the protocol adopted by Idera et al. (2015) with slight modifications. Composite sample was weighed (5 g) into a conical flask. Concentrated sulphuric acid (20 ml) was added and the mixture allowed to stand for 45 min at room temperature. Five milligram of nitric acid was then added to the mixture, heated and allowed to cool at room temperature before perchloric acid (5 ml) was added and further heated gradually until the mixture was clear. The mixture was then filtered with Whatman No. 41 filter paper and diluted with double distilled water. Analysis of heavy metals was performed in triplicates after calibrating the AAS (atomic absorption spectrophotometer, Agilent 240AA) with standard solution of the element (Ultra Scientific, at concentration of 1000 µg/mg) to be determined. Chlorophyll was extracted according to Li (2000) with slight modifications. Here, the extraction was made from a 100 mg-fresh sample in 5 mL acetone (100%). The tubes containing the extracts were wrapped with Parafilm and placed in a −25 °C freezer for 3–6 h. The samples were removed from the freezer and filtered through a 0.45 µm pore size PTFE syringe cartridge filter attached to a disposable plastic syringe and immediately placed in a darkened environment. Each filtered sample was then vortexed and approximately 500 µL of extract transferred to the HPLC (Agilent 1260 instrument equipped with diode-array detection) vial for analysis. The following parameters were used;

Column: Supelcosil LC318 C18 column (25 cm × 4.6 mm × 5 µm for computer modelling work and 10 cm × 4.6 mm × 5 µm for pigment isolations).

Flow rate: 1.0 mL/min.

Mobile phase A: 70:30 (v/v) methanol, 28 mM aqueous TBAA, pH 6.5; Mobile phase B:

Methanol.

The percentage of mobile phase was linear from 5 to 100% in 20 min.

Microplate reader (BioTek Instrument, Synergy H1, USA) was used to quantify malondialdehyde (MDA) levels in the plant samples using protocols described by Sari et al. (2012) with slight modifications. Here, extraction was made from a 100 mg-fresh sample in 5 mL acetone (100%) and the tubes immediately covered with Parafilm and placed in a −25 °C freezer for at least 1 h. A 0.3 mL of thiobarbituric acid solution was pipetted into a 1.5 mL centrifuge tube before 0.1 mL of the extract was added. The reaction lasted for 30 min at 95 °C before the centrifuge tubes were placed in an ice bath to cool down to room temperature. The reaction solution was then centrifuged at 10000r for 10 min. The supernatant solution was then filtered through a 0.45 µm nylon membrane filter. A 200 µL aliquot of the extract was pipetted onto the 96-well plate and the absorbance measured at 532 nm and 600 nm. MDA content was calculated using the below formula;

$$\mathrm{MDA} = [\Delta \mathrm{A} \times V\mathrm{total} \div (\varepsilon \times d) \times 109] \div V\mathrm{sample} = 51.6 \times \Delta A$$

Vtotal: Total volume of reaction system;

ε: Malondialdehyde molar extinction coefficient; 155 × 103 L/mol/cm;

d: 96-well plant light path; 0.5 cm;

Vsample: The volume of extracts.

Data analysis

The Bartlett and multivariate normality tests (MVN package) were performed to determine the homogeneity of variances and the normality of the data set, respectively. Results showed that the data set in general met the assumptions for using parametric tests. Hence, the differences in the heavy metal levels between the mine site and the reference site were evaluated using a t test. ANOVA was used to determine the significance of the effect of the treatments and heavy metal concentration on plant study morphological (height, diameter, root length) and physiological parameters (chlorophyll-a and b, MDA) of the study plant. Also, regression analysis was employed to quantify and compare the effects of metal concentration, treatments and time on the studied parameters. The relative effects of treatment and metal concentration on the study plant morphological and physiological parameters were quantified by decomposing the total variance explained (R^2) in a multiple linear regression by averaging the sequential sum of squares over all orderings of the explanatory variables. The results were then normalized to sum to 100%. This analysis was implemented using the 'relaimpo' version 3.6.3 package in the R statistical software (R Core Team, 2020), which also contains functions for estimating 95% bootstrap confidence intervals using 1000 replications from the original data (see Anning and Mccarthy 2013).

Table 1 Mean concentrations of heavy metals from the two studied sites with their corresponding threshold limits [[a]FEPA (1997), [b]FEPA (1991) and [c]UNEP (2015)]

Metal	Mean metal concentration (mg/Kg)		*P* value	Threshold Limits (mg/Kg)
	Study site (*n* = 15)	Reference site (*n* = 15)		
Fe	6031.02 (535.2)	382.95 (11.7)	< 0.001	400[a]
Cu	39.54 (3.4)	10.20 (0.4)	< 0.001	10.10[b]
As	18.6 (0.8)	3.46 (0.4)	< 0.001	5[c]
Cd	3.22 (0.2)	0.73 (0.1)	< 0.001	0.8[b]
Pb	13.82 (0.8)	1.00 (0.1)	< 0.001	1.6[a]

Values are means of 15 replicate samples with standard deviation in parenthesis

Results

Heavy metal contamination of the mine soil and accumulation by vetiver grass

Mean concentrations of Fe, Cu, As, Cd and Pb in the mine soil far exceeded that of the reference site as well as their respective threshold limits (Table 1). Notably, metal concentrations in the reference site were within the threshold limits for all the studied metals. In general, Fe with mean concentrations of 6031.02 and 382.95 was the most abundant heavy metal (mg/Kg) in the mined and reference sites respectively, followed by Cu (39.54, 10.20), As (18.6, 3.46) and Pb (13.82, 1.00). Cd was the least abundant metal, and differed between the mined site (3.22 mg/Kg) and reference site (0.73 mg/Kg).

Heavy metals in the study soil accumulated in the study plants at significantly high concentration (Fig. 2). Heavy metals in the study plant increased significantly from throughout the study period however, the rate of increase depended on the time and the treatment type. For Cu, Cd and As, accumulation was higher at the first two quarter compared to the last two. On the other hand, accumulation of Fe and Pd were relatively higher at the third and fourth quarters. However, it is clear that heavy metal level in the study plant also varied significantly with treatment. Fe and Cd accumulation by plant in the treated soil were higher than Fe and Cd in their counterpart in the untreated

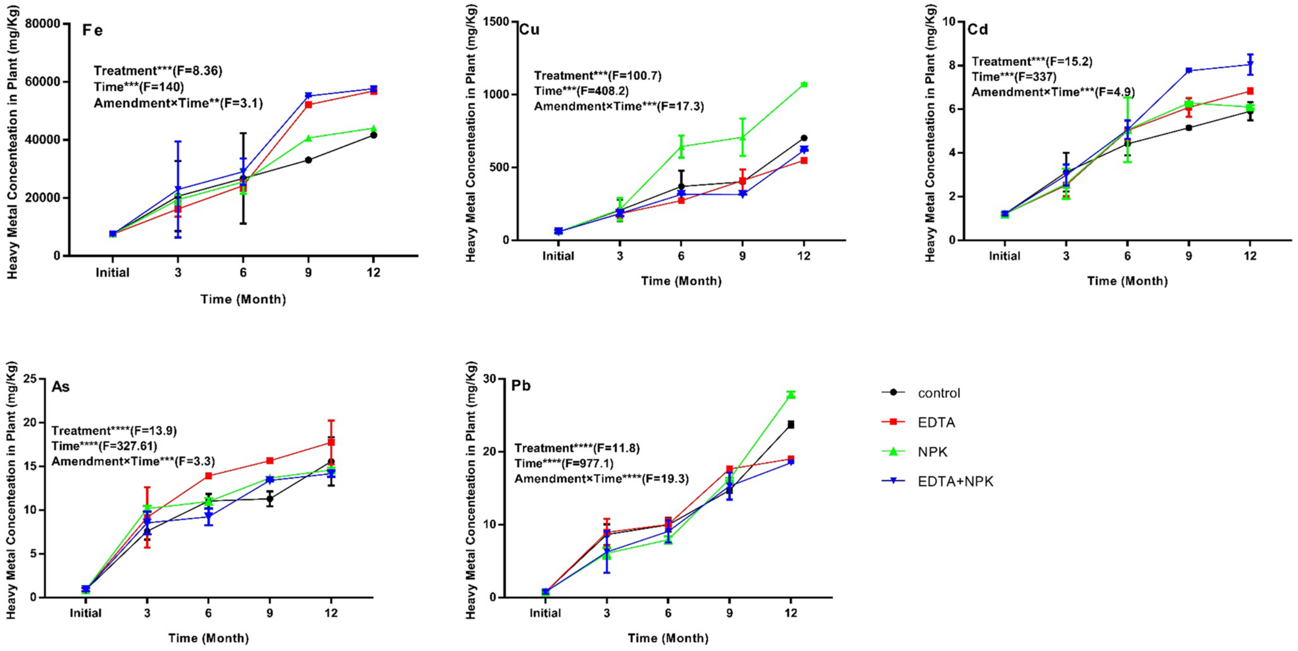

Fig. 2 Heavy metal accumulation by vetiver grass over time compared for treated and untreated soil. $p < 0.05$ denotes significance of effect

soil. Treatment with EDTA + NPK exerted the strongest influence on accumulation of Fe and Cd on the other hand, treatment exerted mixed effects on accumulation of Cu, As and Pb with NPK, EDTA and NPK exerted the strongest effects on Cu, As and Pb respectively.

Heavy metal accumulation and its effects on vetiver growth

Growth parameters of vetiver grass were influenced by heavy metals concentration in the soil (Fig. 3). Height of vetiver on the reference site (uncontaminated soil) consistently increased during the study period and was clearly higher (219 cm) than those of the contaminated soil (25 cm). Height growth on the contaminated site increased initially but reduced slightly in the second half of the study period. Like the trend observed with plant height, vetiver from the reference site exhibited significantly ($p<0.05$) greater diameter growth compared to those from the contaminated soil.

Regression analysis indicated significant relationships ($p < 0.05$) between metal type and various plant morphological characteristics (Table 2). Fe, Cu, As, and Pb were positively correlated with height, but negatively with the diameter of the study plant. Cd negatively influenced height and diameter of plant. However, regression analysis showed that the study metals typically influenced plant diameter (Fe, Cu, Pb) more relative to height (Cd, Pb). Like the morphological parameters, different heavy metals exerted varied effects on plant physiological parameters (Table 3). Fe, As and Cd had positive effects on chlorophyll-a whereas Cu and Pb had a negative effect. On the other hand, all the study metals negatively influenced chlorophyll-b. MDA levels in the plants significantly increased with decreasing metal (Fe, Cu, As and Cd) concentration in soil (and increasing metals concentration in plants) but did not respond to Pb in the plant ($p > 0.05$). Cd and Pb were the dominant metals affecting the plant physiological parameters.

Table 2 Regression analysis between heavy metal concentrations and plant morphological parameters

Metal (mg/Kg)	Regression equations	
	Height (cm)	Diameter (cm)
Fe	$Y = 0.00036x + 23.43$ $(R^2 = 0.07)$	**$Y = -0.00026x + 4.5$** **$(R^2 = 0.50^{***})$**
Cu	$Y = 0.11x + 22.3$ $(R^2 = 0.18)$	**$Y = -0.04x + 4.5$** **$(R^2 = 0.41^{***})$**
As	$Y = 0.004x + 24.9$ $(R^2 = 0.001)$	$Y = -0.006x + 3.4$ $(R^2 = 0.02)$
Cd	**$Y = -1.0x + 27.9$** **$(R^2 = 0.36^{*})$**	$Y = -0.3x + 4.3$ $(R^2 = 0.05)$
Pb	**$Y = 0.81x + 14.8$** **$(R^2 = 0.37^{*})$**	**$Y = -0.1x + 5.3$** **$(R^2 = 0.22^{*})$**

Note: Bold face represents significant relationships (*P*-value > 0.05), Y = plant morphological parameter, X = metal concentrations. For each metal, asterisk (***) denotes significant effect ($p<0.05$) on morphological parameter

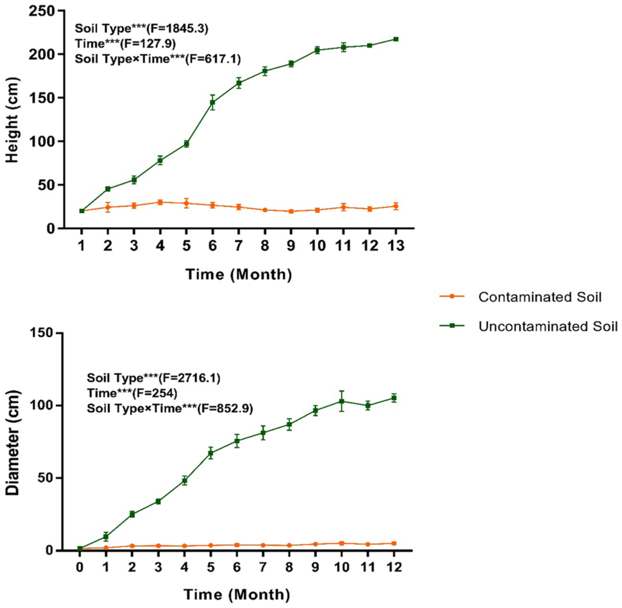

Fig. 3 Growth parameters of vetiver grass in the study and reference site. Asterisk (***) denotes significance of effect ($p<0.05$) while F values denotes the magnitude of the effect

Effects of EDTA and NPK treatments on plant growth

The treatments significantly affected plant height and diameter growth (Fig. 4). Height of vetiver in treated soil were significantly higher than their counterparts in the untreated soil. NPK exerted the strongest positive effects on plant height while EDTA had the least effects. Similarly, vetiver in the treated soil were significantly wider than those in the untreated soil. Generally, increase in plant diameter across treatments was higher in the first half of the study. Vetiver in the NPK-treated soil recorded the largest diameter growth while those in the EDTA-treated soil had the least. Like the trends observed for plant height and diameter, the treatments exerted significant but mixed effects on root length. EDTA + NPK initially exerted detrimental effect on root length but this effect diminished with time. Vetiver in EDTA-treated soil recorded the least increase in root length throughout the study.

Levels of chlorophylls-a and chlorophyll-b in the study plant generally followed a similar pattern over time and in response to the treatments (Fig. 5). NPK and EDTA + NPK significantly improved chlorophyll-a and chlorophyll-b contents more than the control. However, the improvement in

Table 3 Regression analysis between heavy metal concentrations and plant physiological parameters

Metals (mg/Kg)	Regression equations		
	Chlorophyll-a	Chlorophyll-b	Malondialdehyde
Fe	$Y = 0.002x + 98.1$ ($R^2 = 0.03$)	$Y = -0.0007x + 44.1$ ($R^2 = 0.05$)	**$Y = 0.0007x + 2.6\ (R^2 = 0.47{*}{*}{*})$**
Cu	$Y = -0.23x + 111.3$ ($R^2 = 0.01$)	**$Y = -0.3x + 48.6\ (R^2 = 0.25{*})$**	**$Y = 0.1x + 3.3\ (R^2 = 0.23{*})$**
As	$Y = 0.01x + 3.4$ ($R^2 = 0.02$)	$Y = -0.04x + 41.9$($R^2 = 0.01$)	**$Y = 0.004x + 6.5\ (R^2 = 0.10{*})$**
Cd	**$Y = 0.14x + 105.2\ (R^2 = 0.16{*})$**	**$Y = -1.52x + 45.6(R^2 = 0.20{*})$**	**$Y = 2.4x - 1.5\ (R^2 = 0.38{*})$**
Pb	**$Y = -4.9x + 166.6\ (R^2 = 0.22{*})$**	**$Y = -2.4x + 70.7\ (R^2 = 0.57{*}{*}{*})$**	$Y = 0.7x + 4.6$ ($R^2 = 0.01$)

Note: Bold face represents significant relationships (*P*-value > 0.05), Y = plant physiological parameter, X = metal concentrations. For each metal, asterisk (***) denotes significant effect ($p < 0.05$) on morphological parameter

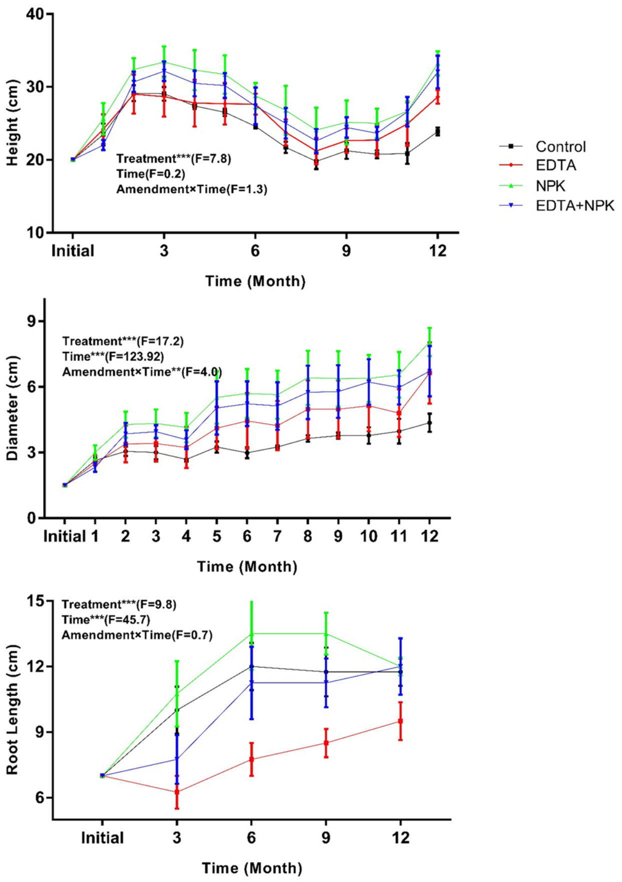

Fig. 4 Variations in the effect of soil amendments (EDTA, NPK and EDTA + NPK) on plant growth over time. Error bars represent the standard errors of the means. For each treatment, asterisk (***) denotes significance of effect ($p < 0.05$) while F values denote the magnitude of the effect

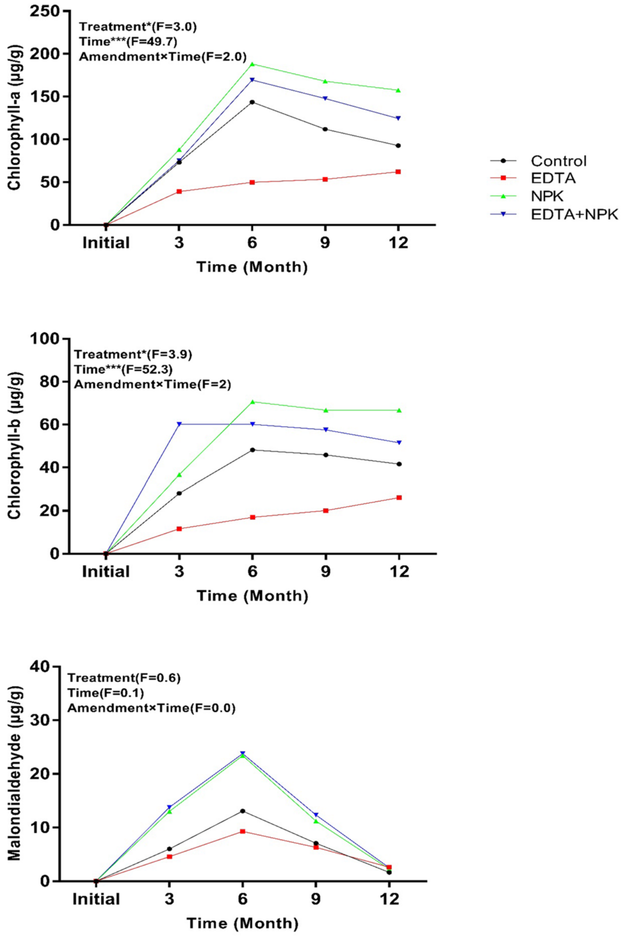

Fig. 5 Effects of soil amendments (EDTA, NPK and EDTA + NPK) on plant physiological parameters over time. Error bars represent the standard errors of the means. For each treatment, asterisk (***) denotes significance of effect ($p < 0.05$) while F values denotes the magnitude of the effect

these parameters was clearly limited to the first two quarters of the study period. On the other hand, vetiver in soil treated with EDTA recorded the least chlorophyll-a and chlorophyll-b contents. On the contrary, MDA content in the study plant was not significantly affected by the treatment, time or their interactions ($p > 0.05$). This notwithstanding, the plants in the NPK and EDTA + NPK were stressed in the first six months of the study though this effect dwindled in the last two quarters.

Relative effects of heavy metal concentration and treatment on plant morphological and physiological parameters

Heavy metal concentrations and treatments accounted for more than 50% of variations in plant height (53.8%), diameter (54.33%) and root length (56.47%; Fig. 6). For plant height and diameter, treatments proved to be the dominant factor, contributing 75% and 78% of their variations, respectively, while heavy metal concentrations accounted for 98% of variations in root length. Treatments and metal concentrations, however, contributed less than 20% of the variations in the chlorophylls a and b as well as MDA levels in the plant. Regardless, metal concentration was more dominant than treatments in affecting MDA levels‾accounting for 90% of the observed r^2 value.

Discussion

Heavy metal contamination status of the study soil and its effects on plant growth

Wastes from mine development and production per their nature are rich with inorganic contaminants like heavy metals (Blight 2011; Anning and Akoto 2018) which when

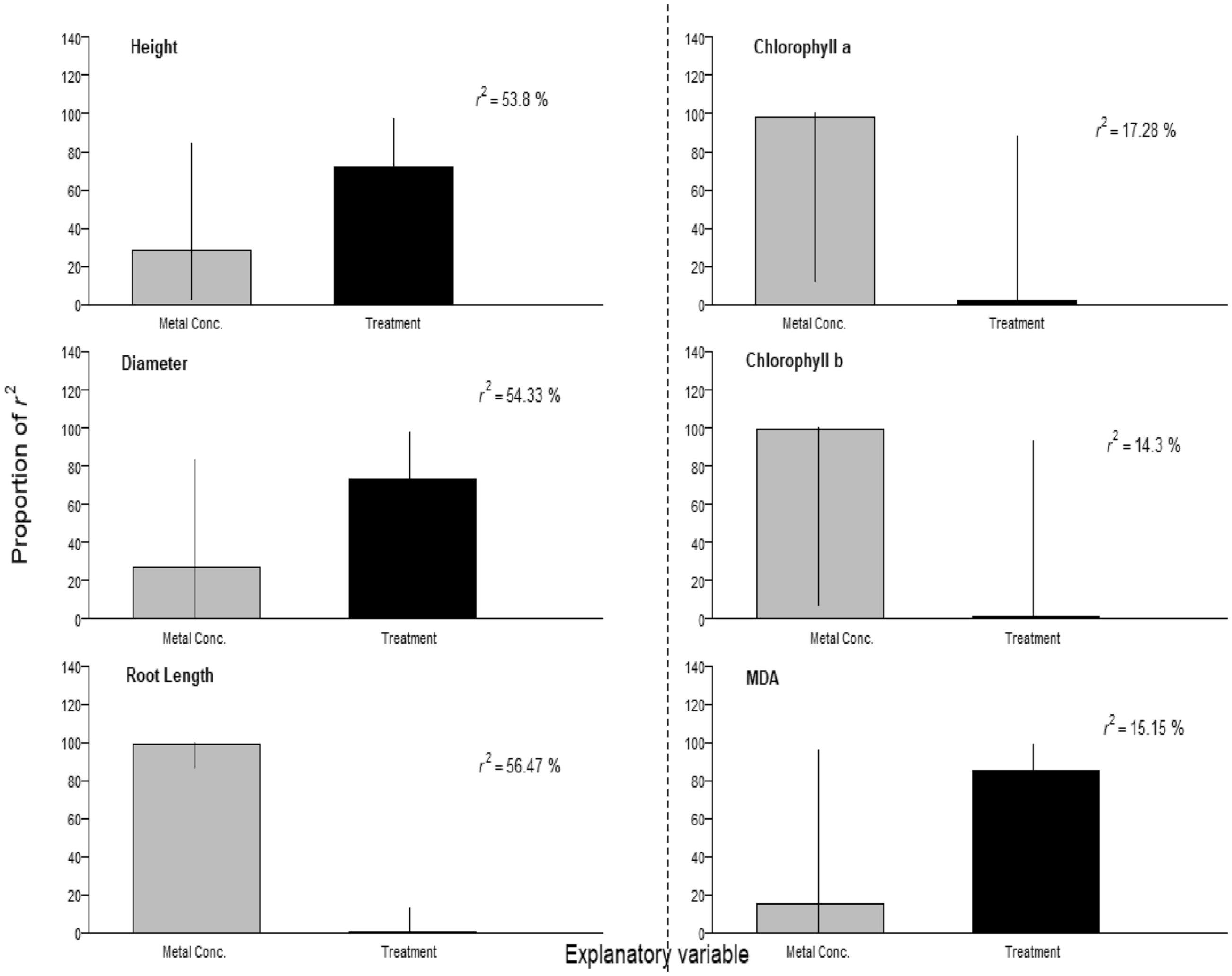

Fig. 6 Relative effects of heavy metal concentration and treatment on plant morphological and physiological parameter

exposed to the environment can alter the biogeochemical composition of soil (Kinneberg et al. 1998; Akoto and Anning 2021). In this study, the significantly high concentration of the study metals in soil from the mine site compared to the reference site as well as the FEPA (1991; 1997) and the UNEP (2015) threshold limits shows that the site is enriched with heavy metals from the mine tailings.

The enrichment of the soil with heavy metals two decades after the tailings were mined out is indicative of the persistent nature of these metals, underscoring the need to ensure that tailings storage facilities (TSF) are lined with high-density polyethylene plastic to prevent leaching of potentially toxic elements into soil. Metals in soil pose a threat to soil microbial community (Lwin et al. 2018) and plants because they are capable of inhibiting essential plant enzymatic processes (Muradoglu et al. 2015; Arsenov et al. 2019) and can hinder plant's ability to perform some of its key ecosystem functions (Lwin et al. 2018) when accumulated in plants. As clearly shown in the results of this study, plants have the capacity of accumulating heavy metals in soil at significantly high concentration with significant implication for the health of the ecosystem. Thus, removal of heavy metals from environmental media is essential for the health of ecosystems. However, the primary requirement for metal accumulation by plant is the solubility of metal in the soil solution, which is difficult to satisfy (Oviedo and Rodríguez 2003; Shahid et al. 2014), hence the need for amendments (natural or artificial) to boost nutrient content of soil and generally enhance the uptake of metals during phytoremediation.

Detoxification of heavy metals in plants through chelation, sequestration and compartmentalization on heavy metals in inactive compartments like the vacuoles is very desirable suit of characteristics for traditional phytoremediation plants (Thakur et al. 2016; Yan et al. 2020). These mechanisms provide effective protection against the detrimental effects of heavy metals by removing them from sensitive sites (Sheora et al. 2011). However, as plants accumulate more metals above the threshold limits, the above-mentioned strategies become inadequate and increased accumulation of metal ions in the cytoplasm triggers the production of reactive oxygen species (ROS), hence oxidation stress in plants with its resultant effect on growth performance (Ruley et al. 2004; Singh et al. 2011). These effects include damage to plant cells, inhibition of photosynthetic activities, damage to DNA, stunted growth and reduction in root length (Huang et al. 2012; DalCorso et al. 2019). The significantly wide difference in height and diameter of vetiver from the heavy metal-contaminated sites and the reference site is ample evidence of the potential detrimental effects of heavy metals on plant morphological parameters. Pb is considered one of the systematic toxicants which causes considerable damage to exposed plants even in low concentrations (Xiong 1997), and has been implicated with toxic effects like reduced root length growth (Wu et al. 2011; Yang et al. 2020). In this study, plant height decreased with decreasing Pb concentration in soil and increasing concentration in plants. This suggests that Pb accumulation in plants poses significant effect on plant growth. Cd, on the other hand, exerted adverse effect on plant height whether accumulated in plant or sequestered in soil, giving empirical indication of its toxic effects on plants (Hindarti and Larasati 2019).

The most generalized effects of heavy metals in plant are their attack on the photosynthetic apparatus (Shakya et al. 2008; Wu et al. 2011). Reduction in plant photosynthetic pigments including chlorophyll- a and -b and other accessory pigments on exposure to heavy metals has been reported both in laboratory (Krupa et al. 1996; Kastori et al. 1998) and field (Chettri et al. 1998; Shakya et al. 2008; Yilmaz et al. 2009; Arsenov et al. 2019) studies. As with the morphological parameters, Cd exerted significant effect on the study physiological parameters even at low concentration in plant (high concentration in soil). Cd in plant is associated with interruptions to uptake of metal nutrients like Fe, Zn, Cu, Mn (Zhang et al. 2002; Wu and Zhang 2002), inducing lipid peroxidation and chlorophyll breakdown (Malecka et al. 2001; MacFarlane 2003; Manios et al. 2003) as observed in this study. An interesting finding of this study is that chlorophyll-a content increased with Pb concentration in plants as also recorded by Küpper et al. (1996); Yilmaz et al. (2009) and Yang et al. (2020). Mg de-chelation is considered a major cause of chlorophyll breakdown in plants (Küpper et al. 1996; Yang et al. 2020). However, Küpper et al. (1996) reported the following order of metal complex formation with chlorophyll—$Hg^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Pb^{2+}$— indicating that Pb has the greatest tendency to bond with the center Mg of the chlorophyll molecule, consistent with our observed patterns. Alternatively, Yilmaz et al. (2009) and Yang et al. (2020) recorded increasing trends in chlorophyll-a at higher concentration of Pb (greater or equal to 300 mg/kg). The mean Pb concentration of the studied soil, though higher than the recommended threshold, was relatively low (13.82 mg/kg) compared to the above-mentioned concentration.

Another significant finding of this study is the differential responses of chlorophyll-a and chlorophyll-b to heavy metal contamination. With the exception of Pb, increase in heavy metal concentration in the plant (decrease in soil) resulted in reduction in chlorophyll-a and an increase in chlorophyll-b, a result similar to that of Chettri et al. (1998) and Shakya et al. (2008). According to Chettri et al. (1998), the apparent increase in chlorophyll-b may be an indirect effect of metal stress. Metal stress induces the oxidation of methyl group on ring II of chlorophyll-a to aldehyde, hence the formation of chlorophyll-b (Bidwell 1979). The main indicator for oxidative stress in plant is MDA. Results of this study show that with the exception of Pb, all the studied metals are capable of causing oxidative stress in plants (Houri et al. 2020). This suggests that oxidative stress is common to most heavy metals, hence the need to take precautionary measures to reduce the effect of metals on plants during phytoremediation.

Effects of chemical amendments on plant growth

Attempts to enhance plant uptake of metals have led to the use of supplementary interventions, mostly in the form of chemical amendments. However, some studies have associated these interventions to significant reduction in plant growth (Collins et al. 2002; Liphadzi and Kirkham 2006a, b; Anning and Akoto 2018). In this study, soil amendments (EDTA, NPK, EDTA + NPK) enhanced plant height, diameter, and to an extent, root length growth more than the control, a finding similar to that of Glinska et al. (2014). Heavy metal detoxification at intracellular level in some traditional phytoremediation plants is achieved through various mechanisms such as chelation of heavy metal ions with organic ligands like organic acids, amino acids, phytochelatins (PCs), metallothioneins (MTs), and cell wall proteins (Hall 2002; Sharma and Dietz 2006; Gupta et al. 2013) and compartmentalization of the chelated heavy metal in inactive compartments (Dalvi and Bhalerao 2013). The introduction of the artificial amendments may have enhanced this mechanism, thus reducing the toxicity of metal ions on plant growth (Sorvari and Sillanpää 1996; Glinska et al. 2014; Liu et al. 2007). Alternatively, the amendments may have introduced or made available plant nutrients otherwise unavailable to plants.

According to Lestan et al. (2008), metal–EDTA complex or EDTA alone cannot pass across the plasma membrane due to its large size (Du et al. 2011). Thus, enhanced metal uptake by plants might be due to physical damages caused by free or complexed EDTA to plant root (Luo et al. 2006; Chaney et al. 2010; Zaier et al. 2010). While physical damages to plant root were not investigated in this study, the significantly lower root length growth recorded for plants in the EDTA-treated soil suggests detrimental effect of EDTA on plant root. Apparently, this effect was alleviated with the addition of NPK, suggesting that EDTA can be used in combination with EDTA to reduce the effects of EDTA on plant root.

Reduction in plant photosynthetic pigments has been attributed to the toxic effect of heavy metals (Chettri et al. 1998; Shakya et al. 2008; Yilmaz et al. 2009; Arsenov et al. 2019). However, it is clear from this study that chemical amendments, especially EDTA significantly influenced plant chlorophyll content as also documented by previous investigators (e.g., Collins et al. 2002; Saifullah et al. 2010). EDTA toxicity to plant chlorophyll is mostly linked to de-chelation of Mg in the chlorophyll structure (Kotaka and Krueger 1969). This finding provides further evidence that EDTA is taken up by plants, and can be recovered after phytoremediation. This recovery can reduce the cost of phytoremediation as well as the environment risk of EDTA.

It is worth noting that though treatments exerted significant effect on chlorophylls a and b, their contribution to these parameters was small compared to that of time (season). This is evident by the fact that treatment effects on plant chlorophyll-a and b and MDA level were more apparent in the first six month of the study. This suggests that the treatments cause significant changes in soil physicochemical and biological parameters in the first six month after application of the amendments as observed by Akoto et al. (2021).

Relative importance of heavy metal concentration and treatment for vetiver plant growth

Heavy metal concentration and treatment exerted varied effects on the studied plant morphological and physiological parameters. Treatment proved to be the dominant factor affecting plant height, diameter while heavy metal concentration accounted for a greater percentage in the variations in root length, as well as chlorophylls a and b contents. Notably, treatment enhanced height and diameter more than the control. The results of this study suggest that treatment and metal concentration were not the dominant regressors for the study physiological parameters as the recorded R^2 was significantly lower than 50%. This suggests that other factors (for example time and age of plants used) might have greater influence on the studied plant physiological parameters. This notwithstanding, it is clear from the percentage contributions of the various treatments to the total variation in R^2 for all the morphological parameters, that EDTA was the least dominant treatment although it interacted positively with NPK to enhance plant growth, once again providing evidence of the effectiveness of NPK as a partner for EDTA.

Conclusion

Heavy metal stress can have significant adverse effect on plant morphological and physiological parameters as evidenced from the strong negative effects of the studied metals on chlorophyll a, chlorophyll b and root length.

Although all the studied metals affected the morphological and physiological parameters of the studied plant species, Pb and Cd were by far the dominant factors influencing the transformation of chlorophyll a to chlorophyll b. The application of soil amendments (EDTA, NPK, EDTA + NPK) enhanced plant height and diameter, reduced the toxicity of free metal ions, evidenced by their insignificant effect on MDA. Likewise, treatment with NPK and EDTA + NPK significantly enhanced chlorophyll-a and chlorophyll-b. However, EDTA failed to enhance chlorophyll-a and b content more than the control. Also, it is worth noting that EDTA contributed the least in enhancing plant height, diameter and root length, although EDTA + NPK application significantly improved height and diameter more than the stand alone EDTA application.

Plant morphological and physiological parameters are important factors of plant growth, hence the success of phytoremediation. Thus, the significant improvement in the study plant growth parameters after the application coupled with improved metal mobility after soil amendment with chemical amendment suggests significant improvement in phyto-extraction of metals. This has significant implication for the economics of phytoremediation as heavy metals taken up by plants plant be recovered for re-use in industries.

This notwithstanding, the significant improvement in metal mobility after soil amendment of the chemical amendment may cause phytotoxicity in plants. EDTA for instance have 10% nitrogen in its molecular content which when mineralized may result in algae bloom in aquatic system or result in excessive nitrogen in soil and its concomitant effect on plant growth.

Study limitation

Though the environmental impact of chemical amendment is also an important consideration factor in their selection to aid phytoremediation, this study only looked at the effects of the study amendment on plant growth parameter.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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