

Evaluation of sphagneticola trilobata and amaranthus hypochondriacus on the phytoremediation of soils polluted by heavy metals

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Abstract

Soil pollution by heavy metals is a major environmental problem. Heavy metals can seep through the soil layers to pollute groundwater or surface water through runoff and enter the food chain causing major health problems for humans and biodiversity. This study was conducted to assess the phytoremediation potential of two plant species, the sphagneticola trilobata, and, amaranthus hypochondriacus, in soils polluted with lead (Pb), nickel (Ni), and zinc (Zn) using the atomic absorption spectrophotometer (AAS) method. The results showed that the two plant species absorbed heavy metals differently. The sphagneticola trilobata absorbed Pb the least with an average concentration, ranked highest to lowest, of 2.334 mg/kg, 1.409 mg/kg, and 1.399 mg/kg for the leaves, roots, and stems, respectively, over 4 months. However, Zn accumulated the most in the same plant with concentration values, ranked highest to lowest, of 2.576 mg/kg, 2.398 mg/kg, and 2.061 mg/kg for the roots, leaves, and stems, respectively, over 4 months. Overall, amaranthus hypochondriacus was the only species that absorbed Ni, with low average concentrations compared to Pb and Zn. The average concentration values for amaranthus hypochondriacus, ranked lowest to highest, were: Ni (1.600, 1.493, 1.358 mg/kg), Pb (2.018, 1.965, 2.285 mg/kg) and Zn (6.426, 4.767, 5.820 mg/kg) for leaves, stems and roots respectively for all metals. Therefore, a bioaccumulation factor (BCF) < 1 for all plants and a translocation factor (TF) > 1 indicates the sphagneticola trilobata is a phytoextractor of Pb and Zn, and the amaranthus hypochondriacus can be used as a phytoextractor of Pb, Ni, and Zn in soil.

Keywords

Phytoremediation, Heavy metals, Amaranthus hypochondriacus, Sphagneticola trilobata.

1.Introduction

Soil pollution by heavy metals has been a major environmental problem of the last three decades. Although heavy metals exist naturally in the soil, anthropogenic activities have increased their concentration to levels that are toxic to humans, fauna, and flora health [1]. Activities that produce heavy metals include the excessive use of fertilizers and pesticides in agriculture, fossil fuel combustion, traffic, mining, smelting, sewage sludge reuse, wastewater irrigation, metal products in industries, sewage sludge and municipal waste disposal [2, 3]. Soils contaminated with toxic amounts of heavy metals could potentially cause health problems by ending up in groundwater [4, 5] or spreading to the surface by runoff [6].

Heavy metals can also enter and poison the food chain [7].

The United Nations Environment Programme (UNEP) proved soil pollution by heavy metals had become the second-highest reported environmental crime in Kenya UNEP, [8]. The state agency recorded 23 cases of soil pollution, compared to 97 and 11 for air and water contamination cases, respectively [9]. According to UNEP, exposure to heavy metals causes health problems, such as intellectual disability, deafness, and kidney failure [8]. Zinc (Zn) and lead (Pb) caused 38.47% and 29.80% of cancer cases, 45.54% and 34.73% of stroke cases and 46.02% and 28.12% of cardiovascular disease cases, respectively, in Jua kali, Kiambu county, and Kahoya areas in Eldoret county [10]. Therefore, with an increasing presence of toxic

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quantities of Pb, Nickel (Ni), and Zn and their negative health implications, it is necessary and urgent to find a remedy.

Conventional soil remediations using physical and chemical techniques are too expensive and limited due to their complexity of implementation and their undesirable effects, such as destruction of the soil structure [11]. However, phytoremediation appears to be an effective, green and environmentally friendly method for cleaning up soil polluted by heavy metals. Phytoremediation is a technique using only plants, where plants are used to extract, remove or decrease the bioavailability of pollutants in the soil [12]. Some plants can absorb or reduce the toxicity of organic pollutants or heavy metals and radioelements present in the soil [13]. Naturally, they accumulate, transform, degrade, stabilize or volatilize them [14].

There are four methods often used as bioremediation processes. In phytostabilization, plants reduce the mobility and bioavailability of contaminants in the soil, or rhizosphere, by chemical immobilization (precipitation, stabilization, absorption or trapping) preventing lateral or deep movement via erosion or leaching [15]. Phytoextraction, also known as phytoaccumulation, occurs when plants remove contaminants, such as bioavailable trace metals and metalloids and certain types of organic contaminants, from the soil and accumulate them in their aerial parts, which can then be harvested [16].

Phytodegradation occurs when plants absorb and degrade organic pollutants in their tissues or secrete organic pollutants in their tissues or secrete enzymes related to degradation in the rhizosphere [17]. In phytovolatilization, organic pollutants and some inorganic compounds are extracted from the soil by plants, transported in their vascular system and then released into the atmosphere by transpiration. This is an attractive technology because the pollutants are completely volatilized (in the form of gases), so there is no need to harvest and treat the plants used [18].

Previous studies have proven that some species of plants, such as *sphagneticola trilobata* [19] and *amaranthus hypochondriacus* [20] can remove metals from the soil and air. Therefore, the objectives of this paper are:

1. To investigate the phytoremediation potential of *amaranthus hypochondriacus* and *sphagneticola trilobata*, to remove Pb, Ni, and Zn from the soil.
2. To determine the bioconcentration and translocation factors of Pb, Ni, and Zn in

sphagneticola trilobata and *amaranthus hypochondriacus*.

2.Literature review

Plants have always been considered, in most cultures around the world, as a source of food and energy and for their therapeutic abilities [21, 22]. For about two to three decades, researchers have developed a particular interest in a plant's capacity to store, degrade or mineralize certain polluting substances in the air, water, and soil [23].

2.1Sphagneticola trilobata

Sphagneticola trilobata, also known as *wedelia*, is a flowering herb in the plant family Asteraceae. Rachmadiarti et al. [24] revealed that *syzigium oleina* and *wedelia trilobata*, grown in diponegoro, J.A. suprpto and H.R. muhamad streets in Surabaya, absorbed Pb contained in the polluted air. They found the different levels of Pb absorption in each plant had affected chlorophyll. The researchers concluded the ability of plants to absorb metallic Pb was influenced by the leaf surface. *Wedelia trilobata* had larger leaves that were able to absorb 0.288 ppm of Pb compare to 0.186 ppm for *syzigium oleina*.

Kaewtubtim et al. [25] conducted a phytoremediation study of cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), Ni, Pb and Zn. Several plants growing in the mangroves of Pattani Bay were used, including the *wedelia*. The results showed the *wedelia* absorbed 53 mg/kg of Zn, 42 mg/kg of Ni, and 27.8 mg/kg of Pb. In addition, all the translocation factors (TF) and bioconcentration factors (BCF) were greater than 1. Therefore, the study concluded that *wedelia* met the criteria of a hyperaccumulator for Zn, Ni and Pb.

Qian et al. [26] conducted a study on plants grown in a hydroponic system for 10 days to evaluate the ability of *wedelia* to remove heavy metals, such as baron (B), Cd, Cr, Cu, Pb, Mn, mercury (Hg), Ni and selenium (Se) in wastewater. In addition, the system was constantly fed with nutrients, such as ferric ethylenediaminetetraacetic acid, molybdic acid, calcium, potassium dihydrogen phosphate, potassium nitrate, magnesium sulfate, boric acid and a calcium chelating agent. The concentrations found in the plant were: (880, 360, 900, 620,767, 804, 324, 715, 184 and 158) mg/kg corresponding respectively to B, Mn, Cd, Cu, Pb, Cr, Hg, Ni, Arsenic (As) and Se. *Wedelia* can be used as an hyperaccumulator of these heavy metals.

Patel et al. [27] conducted studies on different plants, including the wedelia trilobata, which were exposed to different concentrations of metals, such as mercuric chloride (Hg_2Cl_2) and stannous chloride (SnCl_2), to observe their bioaccumulation efficiency. The results of this study showed that wedelia trilobata survived only for 13 days for the 0.20 mg/ml concentration of (HgCl_2) and 15 days for the 0.6 mg/ml concentration of (SnCl_2).

Lin et al. [28] investigated the effect of the phosphate-solubilizing bacterium (PSB) on soil polluted with Cu to enhance the phytoremediation capacity of wedelia trilobata. Results proved that there was a strong significance between PSB and the growth of wedelia trilobata. On another hand, the results showed that the efficiency of Cu removal by wedelia trilobata increased with increasing PSB concentration, and both Cu TFs (i.e. leaf: root and stem: root) were significantly upregulated by PSB.

2.2 Amaranthus hypochondriacus

Huang et al. [29] conducted a phytoextraction experiment within a period of two months in the greenhouse with several Cd hyperaccumulator plants, including amaranthus hypochondriacus. The plants were planted in two soils with different pH (5.93 and 7.43). The results showed most of the accumulator plants grew better in the acidic pH soil, increasing the biomass between 19.59 to 39.63% higher than in the alkaline pH soil. Also, most plants achieved a leaf Cd BCF greater than 10 in the acidic pH soil, compared to a BCF less than 4 in the alkaline pH soil.

Wu et al. [20] worked on the phytoremediation capacity of amaranthus hypochondriacus for thallium (Tl) contaminated soil. The experiment focused on the potential culture of amaranthus hypochondriacus for 100 days. In the first step, the researchers noticed that despite the presence of Tl in the soil, the whole plant was growing in the polluted soil. Amaranthus hypochondriacus also grew as the Tl content of the soil decreased, indicating a good capacity of amaranthus hypochondriacus to extract Tl. Finally, the BCF was greater than 1 for all levels of Tl studied, and the BCF increased with increasing Tl content in the soil. A similar trend in the increase of the translocation factor (TF) was observed under conditions of high Tl pollution. The researchers, therefore, proved the amaranthus hypochondriacus had a good potential to remediate thallium-contaminated soils.

Wang et al. [30] used a chemical to permanently bind positive ions to form a chelating agent to improve the phytoextraction capacity of amaranthus hypochondriacus for cadmium-polluted soil. The results showed that the chelating agents enhanced a large amount of extracted Cd.

Li et al. [31] conducted container experiments to evaluate the efficacy of phytoextraction of Cd by the amaranthus hypochondriacus. This study examined the effect of fertilization, repeated harvesting and growing time on the efficiency of soil Cd removal. The results showed that fertilization with nitrogen phosphorus and potassium (NPK) increased the dry growth time of the biomass by a factor of 4.2, which translates into a significant increase in Cd accumulation. Repeated harvesting had a significant effect on Cd accumulation. Plant growth time had been shown to have a significant impact on the amount of Cd extracted by amaranthus hypochondriacus. This study finally indicated the amaranthus hypochondriacus has a high potential for phytoremediation in cadmium-contaminated soils.

Most of the recent studies reviewed above show that amaranthus hypochondriacus has been used as a hyperaccumulator for Cd and Ti removal in soil. The sphagneticola trilobata has also been used as hyperaccumulators of Zn, B, Cd, Cr, Cu, Pb, Mn, Hg and Ni in the air and mangrove sediments. However, none of these studies showed the potential of sphagneticola trilobata and amaranthus hypochondriacus to remediate soils polluted by Pb, Ni, and Zn.

3. Methods

3.1 Sampling area

Two soil samples were obtained from the Juja area in Kenya. The first soil sample was taken from Jomo Kenyatta University of Agriculture and Technology (JKUAT) farm site B ($1^\circ 05'20''\text{S}$ - $37^\circ 00'40.85''\text{E}$). The second sample was taken from the county government of Kiambu, Juja sewage ($1^\circ 05'47''\text{S}$ - $37^\circ 01'22''\text{E}$). The last sample was taken from Apex steel in Nairobi ($1^\circ 18'08.515''\text{S}$ - $36^\circ 50'45.52''\text{E}$). First, each sampling area was divided into four sub-areas of 5 square meters, from which the soil samples were taken using an auger and trowel at 15 points in each sampling unit to a depth of 15 cm. Next, the samples were placed in a bucket previously cleaned with water and labelled. Finally, all samples were transported to JKUAT ($1^\circ 05'35''\text{S}$ - $37^\circ 00'84''\text{E}$), to a greenhouse where the experiments were conducted.

The heavy metals chosen for this phytoremediation study were those with tolerance limits exceeding those established by the World Health Organization (WHO) heavy metals guidelines (*Table 1*).

Table 1 FAO/WHO metals guidelines in vegetables

Heavy metals	Upper limit of concentration (mg/kg)
Copper	73.3
Zinc	100
Lead	0.3
Nickel	67.9

Source: [32]

Physical and chemical characteristics of the three soil samples collected, are presented in *Table 2*.

Table 2 Physical and chemical characteristics of soils

Soil properties	Soil samples		
	S1	S2	S3
pH	5.20	7.20	7.12
Organic Matter (%)	1.21	5.71	3.55
Electrical Conductivity (ms/cm)	2.53	0.0096	0.008
Nickel (mg/kg)	NA	162	NA
Zinc (mg/kg)	0.55	11.60	106.6
Lead (mg/kg)	36.62	NA	NA
Copper (mg/kg)	0.29	1.80	
Soil Texture	Loam	Sandy clay loam	Sandy Loam
Soil Structure	Granular	Granular	Single grain

Juja Sewage (S1), Apex (S2), JKUAT Farm (S3)

3.2 Experimental design

Four seeds of *sphagneticola trilobata* were planted in each of the three plastic pots, with an inner diameter of 20 cm and a depth of 15 cm (*Figure 1*). Four seeds of *amaranthus hypochondriacus* were also planted in each of the three plastic pots, with an inner diameter of 15 cm and a depth of 20 cm (*Figure 2*). Each pot had 2.5 kg of soil polluted by different heavy metals. The plants were watered with tap water three days a week with 0.5 liters of water per pot.

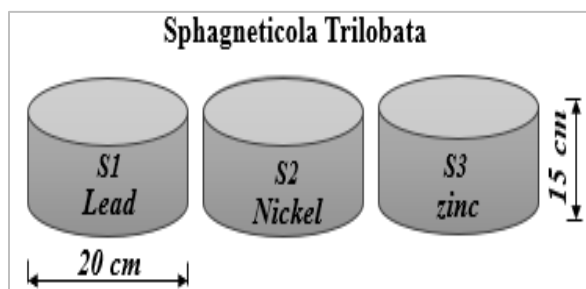


Figure 1 *Sphagneticola trilobata* set-up

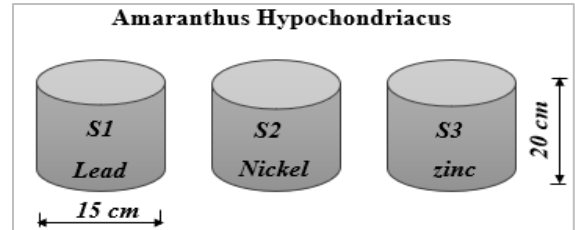


Figure 2 *Amaranthus hypochondriacus* set-up

3.3 Data collection and plant sample preparation

During the experiment, the evaluation of the heavy metal concentrations in the plants was carried out over four successive months, from 20th February to 20th June 2021. For each month, in each pot, a plant was collected for testing.

Heavy metal concentrations were determined in the soil and the aerial and root biomass of plants. *Sphagneticola trilobata* and *amaranthus hypochondriacus* samples were thoroughly washed with tap water and then washed twice with distilled water. The samples were dried in filter paper before being weighed for the digestion process. Half a gram of dried *sphagneticola trilobata* and 0.5 g of *amaranthus hypochondriacus* samples of the root, stem, and leaves were taken separately and placed in an oven at 50 °C for 24 hours to dry. The plant parts were grounded into powder and weighed. Then, 50 ml of 0.5 M HNO₃ and 10 ml of distilled water were added to the powder samples. The mixture was boiled to the boiling point, cooled, and filtered with Whatman No. 42 to perform the AAS using equipment AA-7000 Shimadzu.

3.4 Phytoremediation mechanism

The mechanism of phytoremediation makes it possible to assess how plants remove heavy metals and the quantity of heavy metal absorbed by the plant from the soil, air, or water. However, the determination of this mechanism requires knowledge of two essential factors. First, BCF is a calculated value that indicates the ability of plants to remove trace metals from the soil [33]. The second element, the TF, shows the ability of a plant to transfer heavy metals from its roots to its above-ground biomass [34]. The TF shows the efficiency of the plant to translocate heavy metals from its roots to the above-ground biomass. The formulas used to calculate BCF and TF are those proposed by 35. Asdeo and Loonker [35] and Boonyapookana et al. [33].

$$BCF = \frac{\text{(Metal in tissue of whole plant (mg/kg))}}{\text{(Metal in soil (mg/kg))}}$$

TF = (Metal concentration in shoot (mg/kg)) / (Metal concentration in root (mg/kg)) The correlation analysis was performed using the Minitab software, version 19. Means and standard deviations of heavy metals were calculated using Microsoft Excel 2016, and data were expressed as mg/kg.

The mechanism used by the sphagneticola trilobata and amaranthus hypochondriacus to remove Pb, Ni, and Zn from the soil was found using the outlined steps. First, the soil polluted with Pb, Ni and Zn was collected. Then, sphagneticola trilobata and amaranthus hypochondriacus seeds were planted in the soil and watered frequently. The plants were allowed to grow and accumulate the heavy metals via their roots until the heavy metals were translocated to the shoots. Next, the sphagneticola trilobata and amaranthus hypochondriacus were harvested and analyzed in the laboratory. The concentrations of Pb, Ni and Zn obtained from each part of the plants were used to calculate the BCF and TF. Finally, based on the BCF and TF values, the type of mechanism was determined (Figure 3).

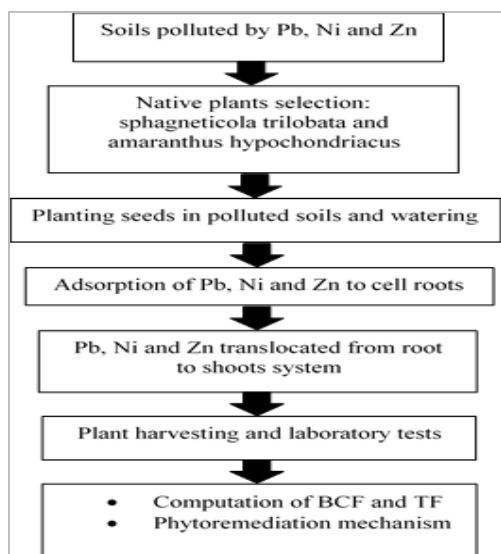


Figure 3 Block diagram for the phytoremediation mechanism

4. Results

4.1 Accumulation of lead, nickel, and zinc in sphagneticola trilobata and amaranthus hypochondriacus

In this study, we investigated the phytoremediation potential of the sphagneticola trilobata and amaranthus hypochondriacus planted in three different soils polluted with Pb, Ni and Zn. The concentration values of Pb, Ni, and Zi absorbed by

the leaves, stems, and roots of the sphagneticola trilobata and amaranthus hypochondriacus for the four months of the study conducted are shown below in Table 3. The heavy metal concentrations in the whole plant of the sphagneticola trilobata were highest in the last month, ranging from 6.735 mg/kg, 0 mg/kg and 8.923 mg/kg for Pb, Ni, and Zn respectively. The test results also showed that the amaranthus hypochondriacus absorbed heavy metals throughout the study with heavy metal concentrations of 8.182 mg/kg, 6.301 mg/kg, and 20.375 mg/kg for Pb, Ni, and Zn respectively, during the last month.

Figure 4 and Figure 5 show the uptake of heavy metals by the sphagneticola trilobata and amaranthus hypochondriacus respectively. The trends of the curves are almost the same for both plants. Zn was absorbed the most, followed by Pb and Ni. However, only the amaranthus hypochondriacus was able to absorb Ni. The results also show that there is a gradual accumulation of heavy metals from the first to the fourth month.

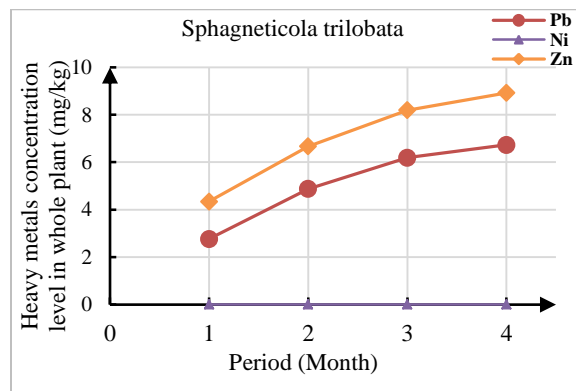


Figure 4 Uptake of lead, nickel, and zinc in all plants of sphagneticola trilobata within the 4 months

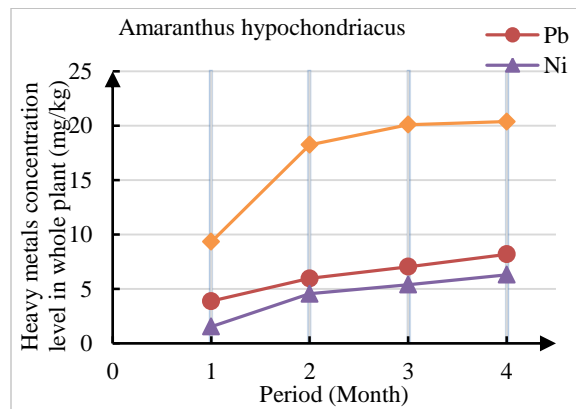


Figure 5 Uptake of lead, nickel, and zinc in all plants of amaranthus hypochondriacus within the 4 months

Table 3 Heavy metal concentrations in plants

Plant Species	Heavy Metal (mg/kg)	Plant Part	Period				Mean	SD
			1 st month	2 nd month	3 rd month	4 th month		
Sphagneticola Trilobata	Lead (Pb)	Leaves	1.870	2.240	2.530	2.697	2.334	0.363
		Stems	0.331	1.285	1.864	2.115	1.399	0.792
		Roots	0.563	1.352	1.798	1.923	1.409	0.615
		A. Plant	2.764	4.877	6.192	6.735	5.142	1.636
	Nickel (Ni)	Leaves	Nd	Nd	Nd	Nd	0.000	0
		Stems	Nd	Nd	Nd	Nd	0.000	0
		Roots	Nd	Nd	Nd	Nd	0.000	0
		A. Plant	0	0	0	0	0	0
	Zinc (Zn)	Leaves	1.425	2.377	2.816	2.972	2.398	0.514
		Stems	0.988	1.746	2.571	2.938	2.061	0.551
		Roots	1.933	2.548	2.809	3.013	2.576	0.784
		A. Plant	4.346	6.671	8.196	8.923	7.034	1.873
Amaranthus Hypochondriacus	Lead (Pb)	Leaves	1.436	1.867	2.097	2.670	2.018	0.586
		Stems	1.228	1.894	2.234	2.503	1.965	0.791
		Roots	1.218	2.206	2.705	3.009	2.285	0.725
		A. Plant	3.882	5.967	7.036	8.182	6.267	1.693
	Nickel (Ni)	Leaves	0.889	1.483	1.725	2.304	1.600	0.363
		Stems	0.344	1.595	1.981	2.050	1.493	0.792
		Roots	0.308	1.485	1.690	1.947	1.358	0.615
		A. Plant	1.541	4.563	5.396	6.301	4.450	1.912
	Zinc (Zn)	Leaves	3.483	7.249	7.442	7.530	6.426	1.966
		Stems	3.026	4.989	5.490	5.563	4.767	1.188
		Roots	2.831	5.999	7.163	7.282	5.82	2.070
		A. Plant	9.340	18.237	20.095	20.375	17.012	4.82

Nd: Below detectable limit, A: all

4.1.1 Correlation

Figures 6 to 10 show the results of the correlation analysis between time and heavy metal concentration levels in plant parts (leaves, stems and roots) of sphagneticola trilobata and amaranthus hypochondriacus, established using bivariate statistics. The results of the calculations showed the correlation coefficients between the concentration of heavy metals in plant parts and time were significantly and positively correlated for sphagneticola trilobata and amaranthus hypochondriacus. The correlation coefficients (r)

between time and heavy metal concentrations in the plant parts were 0.987, 0.967 and 0.950 for Pb and 0.943, 0.989 and 0.964 for Zn for the the leaves, stem and roots respectively in the sphagneticola trilobata and 0.987, 0.976 and 0.967 for Pb, 0.810, 0.881 and 0.904 for Zn and 0.988, 0.898, and 0.912 for Ni for the leaves, stem and roots respectively in the amaranthus hypochondriacus. Therefore, both plants have a strong correlation coefficient, which could mean that the plants accumulate Pb, Ni and Zn gradually over time.

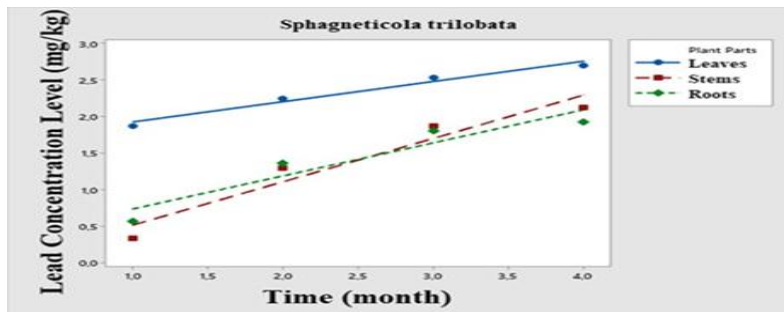


Figure 6 Correlation between concentration of Pb in plant parts and time for sphagneticola trilobata

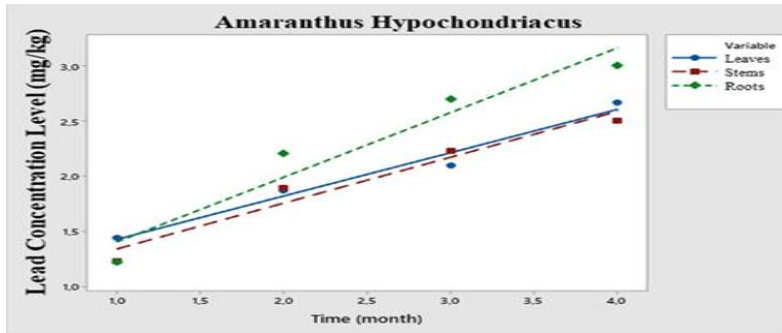


Figure 7 Correlation between concentration of Pb in plant parts and time for amaranthus hypochondriacus

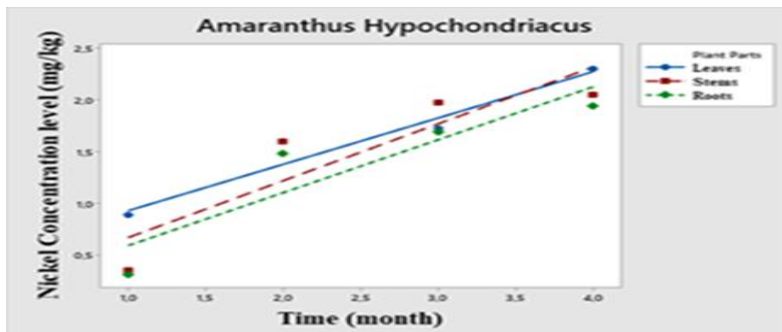


Figure 8 Correlation between concentration of Ni in plant parts and time for amaranthus hypochondriacus

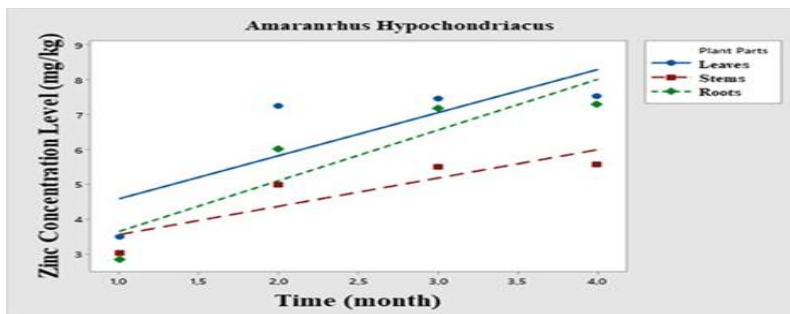


Figure 9 Correlation between concentration of Zn in plant parts and time for amaranthus hypochondriacus

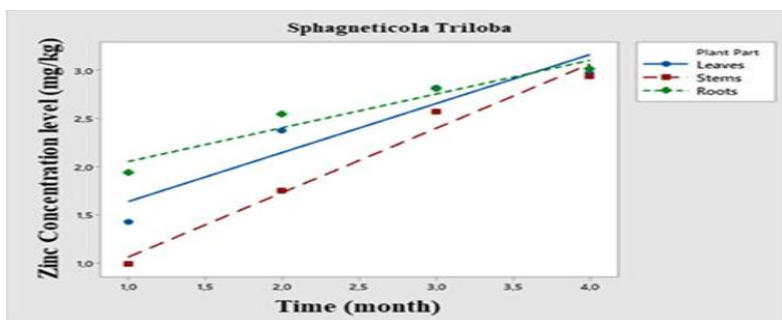


Figure 10 Correlation between concentration of Zn in plant parts and time for sphagneticola trilobata

4.2 Phytoremediation mechanism

The BCF, calculated for both plants and all their parts exposed to Pb, Ni and Zn, showed all had a mean with a BCF < 1 (Table 4). On the other hand, the TF

of all heavy metals (Pb, Ni and Zn) in both species had calculated values where TF > 1, except for Ni in sphagneticola trilobata, which was below detection limits (Table 4).

Table 4 Bioconcentration factor and translocation factor

Plant species	Heavy Metals (mg/kg)	Bioconcentration factor				Translocation factor				Mean	
		1 st month	2 nd month	3 rd month	4 th month	1 st month	2 nd month	3 rd month	4 th month	BCF	TF
Sphagneticola	Lead	0.08	0.13	0.17	0.18	3.91	2.61	2.44	2.50	0.14	2.87
Trilobata	Nickel	0	0	0	0	0	0	0	0	0.00	0.00
	Zinc	0.04	0.06	0.08	0.08	1.25	1.62	1.92	1.96	0.07	1.69
Amaranthus	Lead	0.11	0.16	0.19	0.22	2.19	1.70	1.60	1.72	0.17	1.80
Hypochondriacus	Nickel	0.01	0.03	0.03	0.04	4.00	2.07	2.19	2.24	0.03	2.63

5. Discussion

Plants for the last two to three decades have been studied for their ability to degrade and sequester organic pollutants in the soil via different constituent parts. The sphagneticola trilobata and amaranthus hypochondriacus have been used to accumulate a variety of toxic substances in the soil [36, 37]. For this reason, their proven phytoremediation potential has gained importance in the restoration of polluted soil.

In this study, the evaluation of sphagneticola trilobata and amaranthus hypochondriacus in the phytoremediation of Pb, Ni and Zn was conducted. It can be observed in *Table 3* that the sphagneticola trilobata absorbed Pb and Zn but not Ni. Also, according to *Figure 4*, the concentration of Pb and Zn increased gradually. Similarly to previous studies, plants absorb heavy metals until they show signs of phytotoxicity or die [38].

The concentrations of Pb and Zn in sphagneticola trilobata, sampled regularly over four months, are summarized in *Table 3*. Overall, Zn concentrations in sphagneticola trilobata were much higher than those of Pb. These results are consistent with findings reported by a previous study [25]. The high concentration of Zn in the sphagneticola trilobata could be attributed to the fact that Zn is an essential nutrient for plants [39] and major component of the fertilizer used in the JKUAT farm soils.

The results of Ni, presented in *Table 3* for the sphagneticola trilobata, show the concentration of Ni was below the detection limit. The absence of Ni in the sphagneticola trilobata contrasts with previous research, where two studies found sphagneticola trilobata to be a Ni accumulator [28, 29]. One possible reason of this contrast is the previous studies investigated the phytoremediation of sphagneticola trilobata in a zone permanently saturated, where the roots remained in contact with the humidity [25].

Similar findings have also been found in the research of Qian et al. [26], who observed positive effects of

sphagneticola trilobata in the absorption of Ni when the phytoremediation was done in a very hydrous environment. Thus, sphagneticola trilobata was shown it can be used as a Ni accumulator.

As shown in *Table 3*, the amaranthus hypochondriacus absorbed Pb, Ni and Zn. The present research also revealed different parts of the plant have different capacity for the removal and accumulation of Pb, Ni and Zn, which confirmed and coincided with the results obtained by another study [20]. *Figure 5* also shows that Pb, Ni and Zn were absorbed gradually from the first to the fourth month of study. These results are in line with the previous studies. Wei et al. supported that heavy metal concentrations in soil increased over time with increasing years of cultivation [2]. The amaranthus hypochondriacus was found to accumulate Pb, Ni and Zn from the soil. However, previous research has only studied amaranthus hypochondriacus in the phytoremediation of Cd and Ti [29, 20].

According to results found in this study, it can be observed that the amaranthus hypochondriacus had higher Pb, Ni, and Zn levels than the sphagneticola trilobata. This difference in uptake could be explained by the fact that plants do not accumulate heavy metals in the same way, depending on their cellular and molecular constitution [23].

The low concentrations of Pb and Zn in the sphagneticola trilobata could be explained by the fact that its seeds germinate or grow with difficulty or hardly at all. Studies conducted in the laboratory found that low light significantly inhibited seed germination. Leaf extracts also reduce seed germination and inhibit seedling growth, and significant relationships were found between low light and leaf extracts on seed germination [40]. Thus, even in a greenhouse, where this study took place, seeds may need a lot of light to grow easily.

The study also looked at the linear correlation between time and the amount of heavy metal accumulated in the plant species. As time increased,

the amount of Pb accumulated in shoots and roots increased for both plants. The correlation coefficient for all plant parts of the *amaranthus hypochondriacus* were 0.987, 0.976 and 0.967 for the leaves, stem and roots respectively, while the correlation coefficients for all plant parts of the *sphagneticola trilobata* were 0.987, 0.967 and 0.950 for the leaves, stem and roots respectively. Both plants had correlation coefficients greater than 0.8 indicating a strong correlation between time and Pb accumulated in all plant parts [41]. The *amaranthus hypochondriacus* also showed a strong correlation between time and the amount of Ni absorbed, with correlation coefficients of 0.988, 0.898 and 0.912 for the leaves, stems and roots respectively. For the *amaranthus hypochondriacus*, there is a strong correlation between time and Zn absorbed by the leaves, stem and roots with correlation coefficients of 0.810, 0.881 and 0.904 respectively. The correlation between time and Zn absorbed by the leaves, stem and roots was also strong for the *sphagneticola trilobata*, with correlations values of 0.943, 0.989 and 0.964 for the leaves, stem and roots respectively. Therefore, Zn uptake in all plant parts increases with time in both plant species as both have a strong correlation coefficients [41].

Bioconcentration and translocation factors of *sphagneticola trilobata* and *amaranthus hypochondriacus* (Table 4) revealed all the mean BCF values were less than 1 and all TF values were higher than 1. The TF of the *sphagneticola trilobata* was 2.87, 0, and 1.68 for Pb, Ni, and Zn respectively. *Amaranthus hypochondriacus* showed an average TF value of 1.80 for Pb, 2.63 for Ni, and 1.99 for Zn. Values where the BCF < 1 and TF > 1 for Pb, Ni, and Zn suggest that the mechanism involved is phytoextraction [42]. Phytoextraction is a process whereby plants are used to transport and concentrate heavy metals from the soil into the harvestable parts of roots and shoots [43]. The *sphagneticola trilobata* and *amaranthus hypochondriacus* had a TF greater than 1. These values suggest both plants were able to absorb Pb, Ni and Zn from the soil and move them to the stems and leaves and the aerial part absorbed more heavy metals than in the roots [44].

Therefore, *sphagneticola trilobata* and *amaranthus hypochondriacus* can be used as phytoextractors of Pb and Zn in soils. However, only *amaranthus hypochondriacus* can also be used for phytoextraction of Ni polluted soils. A complete list of abbreviations is shown in Appendix I.

5.1 Limitations

This research focused on evaluating of the *sphagneticola trilobata* and *amaranthus hypochondriacus* for the phytoremediation of Pb, Ni and Zn during a short period of only four months. Therefore, it is recommended to extend the investigation until the plants die.

6. Conclusion and future work

In conclusion, the two plant species, the *sphagneticola trilobata* and *amaranthus hypochondriacus*, were planted, sampled monthly, and analyzed to assess their phytoremediation capacities, the accumulation of heavy metals (Pb, Ni and Zn) in all their plant parts, and the specific phytoremediation mechanism observed. The accumulation capacity of Pb, Ni and Zn varied according to the plant species. The study demonstrated that the *sphagneticola trilobata* and *amaranthus hypochondriacus* were able to absorb and accumulate heavy metals (Pb, Ni and Zn) in all their parts (leaves, stem and roots), with strong positive correlation coefficients (r) ranging between 0.810 and 0.989, except for Nickel, which was not accumulated in the *sphagneticola trilobata*. Lastly, the findings provide insights and vital information for further investigation on the type of mechanism involved in the accumulation of heavy metals in plants studied in greenhouse pots. In the future, the work could be extended to eight months with the addition of nutrients to the soil to increase the phytoremediation capacity of *sphagneticola trilobata* and *amaranthus hypochondriacus*. involved in the accumulation of heavy metals in plants studied in greenhouse pots. In the future, the work could be extended to eight months with the addition of nutrients to the soil to increase phytoremediation capacity.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix I

S. No	Abbreviation	Description
1	AAS	Atomic Absorption Spectroscopy
2	BCF	Bioconcentration Factor
3	JKUAT	Jomo Kenyatta University of Agriculture and Technology
4	Kg	Kilogram
5	mg	Milligram
6	PSB	Phosphate-Solubilizing Bacterium
7	TF	Translocation Factor
8	WHO	World Health Organization