



Phytoremediation of Copper and Nickel Contaminated Soil Using *Eleusine indica* on Soil Properties

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Authors' contributions

This work was carried out in collaboration between both authors. Author EJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GIA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The effects of *Eleusine indica* phytoremediation on the soil properties of Copper and Nickel contaminated soil samples were investigated using standard techniques. Soil sample and seeds of *E. indica* were collected from a farmland in Obe, Nkanu West L.G.A of Enugu State. 0%, 1%, 2% and 3% potted treatments of Copper and Nickel contaminated soil were made for three samples (initial sample, sample without plant and sample with plant). The plant showed BAF and TF greater than 1. The Soil samples after the plant was harvested were subjected to soil analysis tests (Metal determination, pH, Soil moisture content, total organic carbon, and cation exchange capacity). The results from the study revealed that soil samples with plant showed a lower pH level within the range of 6.50 to 7.80 and higher percentage moisture content for both Cu and Ni contaminated soil samples, of which 1% treatment showed the highest percentage moisture of 0.679% for Cu contamination and 3.16% for Ni contamination. Heavy metal contamination of soil reduces its total organic carbon and increases its cation exchange capacity. *Eleusine indica* is a hyperaccumulator, its root stores more heavy metals and thus suitable for phytoremediation.

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1. INTRODUCTION

The sources of heavy metals contamination are geological and anthropogenic activities [1]. Anthropogenic source of heavy metal contamination includes industrial effluents, fuel production, mining, smelting processes, military operations, utilization of agricultural chemicals, small-scale industries (including battery production, metal products, metal smelting and cable coating industries), brick kilns and coal combustion [2]. One of the prominent sources contributing to increased load of soil contamination is disposal of municipal wastage. These wastes are either dumped on roadsides or used as landfills, while sewage is used for irrigation. Such wastes, although useful as a source of nutrients, are also sources of carcinogens and toxic metals. Other sources include unsafe or excess application of (sometimes banned) pesticides, fungicides and fertilizers [2].

Heavy metals pollution is of primary concern than other environmental pollutions because heavy metals can't be destroyed by degradation. Heavy metals (such as nickel and copper) have an adverse effect on the environment, specifically at high concentrations in areas with severe anthropogenic activities. According to World Health Organization [3] the permissible limit for nickel and copper contamination is 1.5 mg/kg and 10 mg/kg respectively. Levels above these concentrations are considered as pollution. Nickel and copper can be poisonous for macro- and microorganisms through direct influence on the biochemical and physiological procedures, reducing growth, deteriorating cell organelles, and preventing biological functions [4]. Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis, et al. 2001). Plants grown in high nickel containing soil showed impairment of nutrient balance and resulted in disorder of cell membrane functions [5].

Phytoremediation is a potential novel technology that utilizes plants to clean up contaminated areas. It is an inexpensive, long term, eco-friendly and aesthetical method of immobilizing/stabilizing, degrading, transferring, removing, or detoxifying contaminants, including metals, pesticides, hydrocarbons, and chlorinated solvents [6,7]. The specific plant and

wild species that are used in this technique are effective at accumulating increasing amounts of toxic heavy metals [8,9]. These plants are known as hyper-accumulators. They accumulate heavy metals at higher concentrations (≥ 100 times) above ground than do non-hyperaccumulators growing in the same conditions, without showing any observable symptoms in their tissues [10].

The availability of inorganic elements to plants in soils is majorly govern by the solubility and the thermodynamic activity of the uncomplexed ion [11] because in order for root absorption to occur; a soluble species must exist adjacent to the root membrane for some definite period. The form of this soluble species will have a strong influence on its longevity in soil solution, mobility in soils, and on the rate and extent of uptake, and perhaps, mobility and toxicity in the plant [12]. The solubility of these inorganic elements is dependent on the soil properties such as soil moisture, soil pH and soil structure. After polluted soil are decontaminated, there is need to investigate the properties of the soil so as to ascertain if the fertility of the soil has been restored, since the soil properties are the determining factors of the soil fertility.

2. MATERIALS AND METHODS

Ten kilogram of fine loamy soil was collected from a farmland in Obe, Nkanu West L.G.A of Enugu State using a hand trowel after removing 10cm the of surface soil alongside all types of plant growth. The equipment used for collecting the samples was sterilized according to Environmental Protection Agency (E.P.A) Standard Cleaning Procedure [13].

0.5 kilograms of the loam soil was measured into each 24 plastic buckets (mixed thoroughly to achieve homogeneity). Two groups of 12 buckets each for Copper and Nickel were divided. From each groups 0%, 1%, 2% and 3% treatments of copper and nickel from their salts Copper (II) sulphate pentahydrate ($\text{Cu}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$) and Nickel (II) Chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) were measured in triplicates, mixed thoroughly and labeled properly. Nickel and copper were not added to 0% treatment buckets which were used as control. *E. indica* seedlings were carefully transplanted into these buckets and kept to grow in a greenhouse for two months, at Proda Laboratory, Enugu. At a period of three months, the plants were harvested. The soil samples from

these potted media were prepared for soil analysis.

2.1 Soil pH

About 10 g of each air-dry sediment sample, passed through 2 mm sieve, was added to 20 ml 0.01 M CaCl₂ solution to form some suspension. The suspensions were allowed to stand for 30 min with occasional stirring using a glass rod. The pH of each sample was then measured using Glass-electrode pH meter.

2.2 Determination of Percentage Moisture Content

Moisture was determined by oven-drying method. 1.5 g of well-mixed soil sample was accurately weighed in clean, dried crucible (W₁). The crucible was allowed in an oven at 100-105°C for 6-12 hrs until a constant weight of the crucible was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling, it was weighed again (W₂). The percent moisture was calculated using the following formula:

$$MC = \frac{W_2 - W_3}{W_1}$$

Where

W = Initial weight of crucible + soil sample 1, W₂ = Final weight of crucible + soil sample

Note: Moisture free soil sample were used for future analysis

$$\% \text{ Moisture} = \frac{\text{Loss in weight}}{\text{Weight of Sample}} \times 100$$

2.3 Total Organic Carbon (TOC)

The TOC was determined using the Walkley-Black Method. 0.15 g of each dried soil sample was weighed into separate 250 ml conical flask and 10 ml of 1N K₂Cr₂O₇ solution accurately added into each flask and swirled gently to disperse the soil sample. 20 ml concentrated H₂SO₄ was rapidly added and swirled. The mixture was then allowed to stand for 30 min and 10 ml of O-phosphoric acid added, followed by 3 drops of N-Phenylanthranilic acid. The mixture was then titrated with a standardized 0.5 N Ferrous Ammonium Sulphate till the colour changed from green to red, indicating the end point. Blank titrations were also done in the same manner without the soil sample.

The TOC for each sample was computed using the formula below:

$$\% \text{TOC} = \frac{\text{ml Fe+for blank} - \text{ml Fe+for sample} \times \text{Normality of Fe}^{2+} \times 0.390}{\text{Weight of Sediment in g}}$$

2.4 Cation Exchange Capacity (C.E.C)

The C.E.C of each soil sample was determined from the calculated readings of the different concentrations of Na⁺, Ca²⁺, Mg²⁺ and K⁺ in each sample using AAS.

2.5 Metals Determination

2.5.1 Copper stock solution and standards

An AAS atomic absorption spectrometer (bulk scientific) equipped with a deuterium lamp for background correction and hollow-cathode lamps as radiation sources was used for copper absorbance measurements at 324.8 nm. The instrumental parameters were adjusted according to the manufacturer's recommendations. An UV-Vis spectrophotometer equipped with reduced volume plastic cells (10 mm optical path) was employed for spectrophotometric measurements.

2.5.2 Nickel stock solution and standards

One phthalate-phosphate reagent powder pillow was added to the soil samples. The mixture was shaken to dissolve. 0.5 ml of 0.3% PAN indicator solution was added. It was then inverted severally to mix. One EDTA reagent powder pillow was added to the sample and shaken to mix. The concentration of nickel was determined using AAS.

3. RESULTS

3.1 Heavy Metal Determination in Soil

The Table 1 shows the concentrations of Cu and Ni in the soil samples for the four treatments (0%, 1%, 2% and 3%).

The four treatments (0%, 1%, 2% and 3%) showed the presence of copper, which was highest in 3% sample treatment. There was an increase in the concentration of Cu from 0% treatment to 3% treatment (14.86±1.93 mg/kg). The concentration of Cu observed in all the soil samples were above WHO maximum allowed concentration. Nickel concentration present in

the soil samples for all the four treatments increased as the treatments increased (i.e. from 1% to 3%). Samples from 3% treatments had the highest concentration of Ni (10.86±1.87 mg/kg). The concentrations of Ni observed in all the soil samples were above WHO maximum allowed concentration (1.5 mg/kg).

Table 1. Copper and nickel concentrations in the soil samples

Treatment	Cu (mg/kg)	Ni (mg/kg)
0%	10.49±1.36	6.09±1.05
1%	12.65±1.65	6.09±1.05
2%	14.84±1.92	8.25±1.42
3%	14.86±1.93	10.86±1.87
WHO [3]	10.0	1.5

Results are in Mean±SE. Mean that do not share a letter within a row are significantly different

3.2 Effect of *Eleusine indica* on the pH of Heavy Metal Contaminated Soil

Table 2 shows the effect of *E. indica* on the pH of copper and nickel contaminated soil. From the Table 2, it can be seen that for 0% treatment there was a slight reduction in the pH level of sample with plant (6.59) compared to the initial sample (7.38) and samples without plant (7.85) for Cu contaminated soil. The level of pH was highest in sample without plant for all the treatments, while samples with plant had pH levels slightly below those observed in initial sample and samples without plant for both Cu and Ni contaminated soil. There was an increase in pH as the treatment increases from 0% to 3%.

For soil samples contaminated with Cu there were no significant difference ($p < 0.05$) in the mean pH levels of the control samples (0% treatment). Similarly, the differences in the pH levels for all the samples for 1% treatment were not significant ($p < 0.05$). All the samples for 2% and 3% treatment showed significant differences ($p > 0.05$) in the pH levels of samples with plant from both initial samples and samples without plant. This implies that the pH value of soil sample with plant varied significantly ($p > 0.05$) from the pH values of soil sample without plant and the initial soil sample for both 2% and 3% treatment. For soil samples contaminated with Ni there were no significant differences ($p < 0.05$) in pH for all the samples from 0% treatment, and 1% treatment. The difference in the pH of all the samples for 2% and 3% treatments were not significant ($p < 0.05$) likewise.

3.3 Effect of *E. indica* on the Percentage Moisture Content of Heavy Metal Contaminated Soil

Table 3 illustrates the percentage moisture content in heavy metal (Cu and Ni) contaminated soil remediated using *E. indica*.

The percentage moisture content observed in soil samples contaminated with copper and nickel showed highest percentage moisture for samples with plant for all the treatments (0%, 1%, 2% and 3%). Generally, the highest percentage moisture content was observed in sample with plant from 1% treatment (0.679% for Cu contaminated soil and 3.16% for Ni contaminated soil), while the least percentage moisture content was observed in initial sample for both 0% and 3% treatment (0.197% for Cu contaminated soil and 0.79% for Ni contaminated soil). The control treatment showed no significant difference ($p < 0.05$) in the moisture content of initial sample and sample without plant but varied significantly ($p > 0.05$) from the moisture content of sample with plant (for Cu contaminated soil). For Ni contaminated soil the percentage moisture content of sample with plant and without plant (for 0% treatment) showed significant difference ($p > 0.05$) with the percentages observed in initial sample. Similar level of significant difference ($p < 0.05$) as observed in control treatment (0%) of Cu contaminated soil was observed in samples from 1% treatment (for both Cu and Ni contaminated soil).

The different percentage moisture content observed in samples from 2% treatment (for Cu contaminated soil) varied significantly ($p > 0.05$) from one another. 2% treatment samples for Ni contaminated soil showed significant difference ($p > 0.05$) in the percentage moisture content between initial sample and both sample with and without plant. Also, there were significant difference ($p > 0.05$) in the percentages of moisture observed in samples from treatment 3% (Table 3).

3.4 Effect of *E. indica* on the Total Organic Carbon of Heavy Metal Contaminated Soil

Table 4 shows the effect of *E. indica* on the total organic carbon of soil contaminated with Cu and Ni. The total organic carbon in the soil sample decreased with increasing concentration of heavy metal contamination.

Table 2. Effect of *E. indica* on the pH of copper and nickel contaminated soil

Heavy metal	Treatment	Initial sample (pH.H ₂ O)	Sample without plant (pH.H ₂ O)	Sample with plant (pH.H ₂ O)
Copper	0%	7.38±0.05 ^{abc}	7.85±0.05 ^{abc}	6.59±0.08 ^{abc}
	1%	7.58±0.05 ^{abc}	8.15±0.09 ^{abc}	7.27±0.10 ^{abc}
	2%	8.20±0.05 ^{ab}	9.37±0.10 ^{ab}	7.35±0.10 ^{ac}
	3%	8.41±0.05 ^{ab}	9.79±0.10 ^{ab}	7.78±0.09 ^{ac}
Nickel	0%	7.27±0.05 ^{abc}	7.38±0.07 ^{abc}	6.41±0.08 ^{abc}
	1%	7.46±0.05 ^{abc}	7.85±0.10 ^{abc}	7.01±0.06 ^{abc}
	2%	8.07±0.05 ^{abc}	8.71±0.11 ^{abc}	7.81±0.06 ^{abc}
	3%	8.18±0.05 ^{abc}	8.84±0.12 ^{abc}	7.82±0.09 ^{abc}

Results are in Mean±SE. Mean that do not share same letter in a row are significantly different at 95% confidence level as determined by Duncan New Multiple Range Test (DNMRT)

Table 3. Effect of *E. indica* on the percentage moisture content of copper and nickel contaminated soil

Heavy metal	Treatment	Initial sample (% wt)	Sample without plant (% wt)	Sample with plant (% wt)
Copper	0%	0.197±0.21 ^{ab}	0.200±0.21 ^{ab}	0.347±0.37 ^{abc}
	1%	0.350±0.37 ^{ab}	0.353±0.37 ^{ab}	0.679±0.74 ^c
	2%	0.347±0.37 ^{abc}	0.350±0.37 ^{ab}	0.453±0.16 ^c
	3%	0.197±0.21 ^{ab}	0.243±0.36 ^{abc}	0.360±0.17 ^c
Nickel	0%	0.79±0.51 ^c	1.43±0.92 ^{bc}	1.44±0.92 ^{bc}
	1%	2.22±1.43 ^{bc}	2.23±1.43 ^{bc}	3.16±2.03 ^c
	2%	1.93±0.60 ^a	2.22±1.43 ^{bc}	2.37±1.52 ^{bc}
	3%	0.79±0.51 ^c	0.94±0.60 ^a	1.43±0.92 ^{bc}

Results are in Mean±SE. Means that do not share same letter within a row are significantly different at 0.05 level of significance

For copper contaminated soil samples, the levels of total organic carbon observed in sample with plant were higher than sample without plant. The initial sample had the highest total organic carbon in all the treatment. The levels of total organic carbon observed in all the samples were highest in samples for 0% treatment and least in samples for 3% treatment. The total organic carbon observed for sample with plant for 0%, 1%, 2% and 3% treatment were 10.56%, 9.90%, 7.66% and 7.09% respectively. While the total organic carbon observed for sample without plant for 0%, 1%, 2% and 3% treatments were 2.08%, 1.66%, 1.43% and 0.65% respectively (Table 3). For nickel contaminated soil sample, percentage of total organic carbon was highest (9.92%) in 0% and 1% treatment of the initial sample. The percentages of the total organic carbon in samples with plant were higher than the percentage observed in samples without plant but were below that of the initial samples. The percentages of organic carbon in samples with plant for 0%, 1%, 2% and 3% treatments were 9.07%, 9.08%, 8.79% and 8.78% respectively,

while the percentages of organic carbon in samples without plant for 0%, 1%, 2% and 3% were 2.79%, 2.29%, 1.13% and 0.85% respectively.

ANOVA at 95% revealed no significant difference in the levels of total organic carbon observed in sample with plant and initial sample but varied significantly ($p < 0.05$) from the levels of total organic carbon observed in sample without plant for the control (0%) treatment) in both Cu and Ni contaminated soil samples. Similarly, no significant difference ($p < 0.05$) was observed in the total organic carbon of initial sample and sample with plant for 1% treatment but sample without plant varied significantly ($p > 0.05$) from the other samples (for both Cu and Ni contaminated soil samples). The percentage of total organic carbon in all the three samples for 2% and 3% treatments varied significantly ($p > 0.05$) from each other, for Cu contaminated soil samples. While Ni contaminated soil samples show similar level of significant difference as observed in 0% and 1% treatment for both 2% and 3% treatment.

Table 4. Effect of *E. indica* on the total organic carbon of copper and nickel contaminated soil

Heavy metals	Treatment	Initial sample (% wt)	Sample without plant (% wt)	Sample with plant (% wt)
Copper	0%	11.98±1.55 ^{a,c}	2.08±0.27 ^b	10.56±1.37 ^{a,c}
	1%	10.55±1.37 ^{a,c}	1.66±0.09 ^b	9.90±1.28 ^{a,c}
	2%	9.98±1.55 ^a	1.43±0.19 ^b	7.66±0.09 ^c
	3%	9.89±1.28 ^a	0.65±0.08 ^b	7.09±0.27 ^c
Nickel	0%	9.92±1.47 ^{a,c}	2.79±1.30 ^b	9.07±1.34 ^{a,c}
	1%	9.92±1.47 ^{a,c}	2.29±0.04 ^b	9.08±1.34 ^{a,c}
	2%	9.69±1.47 ^{a,c}	1.13±0.17 ^b	8.79±1.30 ^{a,c}
	3%	9.07±1.34 ^{a,c}	0.85±0.13 ^b	8.78±1.30 ^{a,c}

Results are in Mean±SE. Mean that do not share same letter in a row are significantly different at 95% confidence level as determined by Duncan New Multiple Range Test (DNMRT)

3.5 Cation Exchange Capacity of *E. indica* on Heavy Metal Contaminated Soil

The Table 5 shows the cation exchange capacity of *E. indica* on Cu and Ni contaminated soil respectively. The cation exchange capacity in the soil samples increased with increasing concentration of heavy metal contamination.

The Table 5 showed higher cation exchange capacity of *E. indica* for treatments with higher Cu and Ni contamination. The highest cation exchange capacity of *E. indica* was observed in sample with plant for 3% treatment (3.98 meq/kg for Cu contamination and 4.16 meq/kg for Ni contamination), while the least cation exchange capacity of *E. indica* was observed in sample with plant for 0% treatment (2.52 meq/kg and 1.74 meq/kg for Cu and Ni contaminations respectively). The cation exchange capacity was higher in sample without plant than sample with plant and initial sample for both heavy metal contaminated soil samples. The cation exchange capacity in sample without plant for 0%, 1%, 2% and 3% were 3.32, 4.01, 4.82 and 5.02 meq/kg for Cu contaminated soil and 3.14, 3.90, 4.90 and 5.74 meq/kg for Ni contaminated soil; while the cation exchange capacity of *E. indica* in

sample with plant for 0%, 1%, 2% and 3% were 2.52, 2.92, 3.53 and 3.98 meq/kg respectively for Cu contaminated soil samples and 1.74, 3.14, 3.90 and 4.16 meq/kg respectively for Ni contaminated soil samples.

In Cu contaminated soil samples, ANOVA at 95% revealed significant differences ($p>0.05$) in the cation exchange capacity of *E. indica* in all the samples for 0% treatment. The CEC of initial sample and sample with plant showed no significant difference ($p<0.05$) but varied significantly ($p>0.05$) from sample without plant for 1% treatment. The cation exchange capacity of *E. indica* initial samples and sample with plant varied significantly ($p>0.05$) from the capacity observed in samples without plant for 2% and 3% treatments. For Ni contaminated soil samples, ANOVA at 95% revealed no significant difference ($p<0.05$) in the cation exchange capacity in the initial sample and samples with plant but varied significantly ($p>0.05$) from sample without plant for 0% treatment. For 1% treatment the CEC of all the three samples varied significantly ($p>0.05$) from each other. Similarly, the cation exchange capacity observed in all the samples for 2% treatment varied significantly ($p>0.05$) from one another. This is

Table 5. Cation exchange capacity of *E. indica* on copper and nickel contaminated soil

Heavy metals	Treatment	Initial sample (meq/kg)	Sample without plant (meq/kg)	Sample with plant (meq/kg)
Copper	0%	1.80±0.82 ^a	3.32±1.98 ^b	2.52±1.16 ^c
	1%	2.81±1.29 ^{a,c}	4.01±0.46 ^b	2.92±0.47 ^{a,c}
	2%	3.32±1.98 ^{a,c}	4.82±1.15 ^b	3.53±1.16 ^{a,c}
	3%	3.80±0.82 ^{a,c}	5.02±0.47 ^b	3.98±0.69 ^{a,c}
Nickel	0%	1.74±0.72 ^{a,c}	3.14±1.30 ^b	1.74±0.72 ^{a,c}
	1%	2.14±1.30 ^a	3.90±2.03 ^b	3.14±1.30 ^c
	2%	2.40±0.58 ^a	4.90±2.03 ^b	3.90±2.02 ^c
	3%	3.75±0.72 ^a	5.74±0.72 ^b	4.16±1.31 ^c

Results are in Mean±SE. Mean that do not share same letter in a row are significantly different at 95% confidence level as determined by Duncan New Multiple Range Test (DNMRT)

also similar in the cation exchange capacity observed in all the samples for 3% treatment.

4. DISCUSSION

The effect of phytoremediation of copper and nickel contaminated soil using *E. indica* on soil properties has been studied. The results obtained on factors affecting metal availability for plant uptake is in line with those Jenne and Luoma [11] who stated that the major factor governing metal availability for plant uptake is the solubility and thermodynamic activity of the uncomplexed metal ion. These properties that affect the availability of metals for plant uptake include; soil pH, soil moisture, total organic carbon, and cation exchange capacity.

According to Glick (2003) the optimum pH range for most plants is between 5.5 and 7.5. The effect of pH on heavy metal availability to plant has been reportedly researched and it is accepted that as pH decreases, the solubility of cationic forms of metals in the soil increases and therefore, they become more available to plants (Glick, 2003). Glick (2003) explained that pH has a major effect on metal dynamics because it controls the absorption and precipitation, which are the main mechanisms of metal retention to soil. In support to Glick's findings, the present study revealed a neutral pH range for uncontaminated soil (control) and an increase in pH as Cu and Ni increases in the soil samples. Similarly, Cook and Morrow [14] opined that pH is one of the factors which influence the bioavailability and transport of heavy metals in the soil, heavy metal mobility decreases with increasing soil pH.

Organic carbon plays an important role in metal binding According to Van Nevel, et al. [15] total organic carbon is the storehouse of plant nutrients and mineral recycling. In line with this, total organic carbon observed in this research decreases in the order Initial soil sample > Soil with *E. indica* > Soil without *E. indica*.

The cation exchange capacities were observed to increase with increasing heavy metal concentration. Cation exchange capacity (CEC) is a measure of how many cations that can be retained on soil particle surfaces [16]. CEC is one of the ways that solid materials in soil alter the chemistry of the soil. CEC affects many aspects of soil chemistry, as it indicates the capacity of the soil to retain several nutrients in plant-available form. It also indicates the capacity

to retain pollutant cations [16]. The findings from the investigations revealed that soil properties (pH, soil moisture content, total organic carbon and cation exchange capacity) have significant effect on phytoremediation. This lends support to Zhang, et al. [17] that the amount of heavy metals mobilized in soil environment is a function of soil pH, properties of metals, redox condition, soil chemistry, organic matter content and cation exchange capacity.

5. CONCLUSION

The root zone of *E. indica* stored more heavy metals because it has more biomass. The soil pH after phytoremediation was slightly alkaline and it falls within the optimum pH for plant growth. Generally, the result also depicted that the higher the concentration or treatment of heavy metals, the higher the pH and vice-versa. The soil parameters affected the mobility of the heavy metals for plant uptake. Phytoremediation will clearly play some role in the stabilization and remediation of many contaminated sites. This can mitigate the rate of toxic pollutants in our environment and help in sustainability of the soil ecosystem, which consists of a multitude of physical, chemical and biological entities that plays a role in the existence of many forms of life that evolved on our planet.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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