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# Green Remediation using the Monocot Grass *Vetiveria zizanoides* (L.) Nash

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## Abstract

Now-a-days our environment is laden with contaminants. Heavy metals are important among them. These are toxic and may cause threat to living organisms and the environment. The conventional methods used for heavy metal removal have their limitations because they are ineffective, economically expensive and produce large quantities of sludge. So, there is a need to develop a cost efficient and eco-friendly method to alleviate this type of pollution. The current study was carried out to evaluate the phytoremediation capacity of *Vetiveria zizanoides* for heavy metals from polluted water. The objectives of the present research were to grow the test plant in nutrient solution with different concentrations (50, 100, 150 and 200 μM) of Lead acetate for 20 days. Fresh and dry biomass of vegetative parts (above and below ground) was determined and the Bio-concentration and Translocation factor was calculated. Results revealed that most of the lead from the solution was absorbed by *Vetiveria zizanoides* till the 20$^{th}$ day. The highest lead content was recorded in the root of the plant. Control plants did not record lead content in its tissues.

**Keywords:** Lead and Environment, Phytoremediation, *Vetiveria zizanoides*

## 1. Introduction

According to Alloway and Ayres (1997) "heavy metals constitute a heterogenous group of elements with a relatively high density (approximately 6 gcm$^{-3}$) and with atomic weight more than that of iron". Heavy metals usually enter the environment through natural (weathering of minerals, erosion and volcanic eruptions) and anthropogenic (mining, smelting, electroplating, use of pesticides and fertilizers as well as bio solids in agriculture, sludge dumping, industrial discharge, atmospheric deposition) sources (Ali *et al*., 2013; Pandey *et al*., 2011; Wuana and Okieimen, 2011). Heavy metal contamination of soil and water is one of the major problems of ecosystem degradation, which has a major impact on human health and the environment (Ansari *et al*., 2015; Singh *et al*., 2020).

"Lead (Pb) is one of the toxic metal pollutants which occurs as a contaminant in all environmental compartments including soil, water and living organisms. It is known to induce a wide range of harmful effects to morphological, physiological and biochemical activities of living organisms" (Amin *et al*., 2018). "Lead retards plant growth such as root elongation, seed germination, seedling development, transpiration, chlorophyll production, lamellar organization in the chloroplast, cell division. However, the intensity of these effects depends on the heavy metal concentration, duration of exposure, intensity of plant stress, and particular organ studied". Therefore, clean-up of heavy metal contaminated soil is of extreme importance to minimize their impact on ecosystem (Giri *et al*., 2019).

Phytoremediation is an environment-friendly and ecologically responsible recent technology which needs further research. "Phytoextraction is the uptake of contaminants from soil or water by plant roots and their translocation to and accumulation in aboveground biomass" (Sekara *et al*., 2005; Yoon *et al*., 2006; Kushwaha *et al*., 2018). According to Woraharn *et al*. (2021) today there are many technologies for wastewater treatment and rhizofiltration is one of phytoremediation techniques that is very promising for cleaning large quantities of

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water contaminated with heavy metals. Removed plants, rich in accumulated pollutants, can safely be processed by drying, burning and composting. Few of the metals can be re-extracted from the ash, which further reduces the generation of toxic waste into the environment.

*Vetiveria zizanoides* (L.) Nesh (Poaceae) is of interest as an alternative technology for heavy metal removal from polluted water because it has several important features suitable for phytoremediation. The goal of this study is to observe the consequence of different concentrations of lead on growth of *Vetiveria zizanoides*.

## 2. Materials and Methods

The test plant *Vetiveria zizanoides* (L.) Nash was collected from Palliyuthu, Erode district, Tamil Nadu, India. According to Pourakbar *et al.* (2007) "the plants were surface sterilized with 0.1% $HgCl_2$ with sterilized water, for 10 minutes and washed 7 times. The seedlings were selected and pruned to have similar height of stem and length of roots. It was planted in peat, in order to get well-cultivated seedlings for one month, which can be later transferred to aqueous solutions".

The one-month old seedlings were transferred to one litre volume buckets which were half filled with distilled water and with this 0.5 L of 100% modified Hoagland Solution was added (Hoagland and Arnon, 1950). After 15 days, nutrient solution was changed and augmented with four concentrations of Lead acetate (50 μM, 100 μM, 150 μM, 200 μM). Experiment lasted for 20 days.

## 3. Digestion and Analysis of Plant Material

According to Hoenig *et al.* (1998) "after 20 days the plants were taken out from the nutrient solution and separated into underground and above ground parts. After recording the fresh weight of harvested plants after washing, they were dried at 80°C for three days, subsequently the dry weights were determined. Plant biomass was digested by dry ashing. Dried, powdered plant sample in a crucible was placed in a cold muffle furnace, the temperature was progressively elevated to 450°C over two hours and held for four hours. After cooling a drop of distilled water was added. Then 5.0 mL conc. $HNO_3$ was added to the ash. The sample was slowly heated on a sand bath for 30 minutes, at 120-130°C. To this 5.0 mL of hydrogen peroxide was added with care in small amount to avoid strong foaming. The heating was continued at that temperature, until a clear solution was got. The solution was cooled and its volume was made up to 50.00 mL by adding distilled water. The samples were analysed by AAS, to determine their lead content".

## 4. Evaluation of Phytoremediation Efficiency

The Pb concentration in the underground and aerial parts of *Cynodon dactylon* Pers. was calculated (Monni *et al.*, 2000).

$$\text{Pb Conc. ppm} = \frac{\text{AAS interpretation (reading)} \times \text{dilution factor}}{\text{Weight of plant tissues}}$$

### 4.1 Calculation of Bio-concentration and Translocation Factors

As stated by Lu *et al.* (2004) and *Mun et al.* (2008), "Bio-concentration factor was a useful parameter, to assess the potential of plants for accumulating metals. It was calculated using the formula:

$$\text{BCF} = \frac{\text{Concentration of metal in dried plant tissue } (\mu g\ g^{-1})}{\text{Initial concentration of metal in external solution } (\text{mg L}^{-1})}$$

Padmavathiamma and Li (2007) and Adesodun *et al.* (2010) stated that the Translocation factor (TF) indicates the efficiency of the plant, in translocating the accumulated heavy metals from root to shoot. It is a ratio of the concentration of the heavy metal in shoot to that in its root. It was calculated using the formula:

$$\text{Translocation Factor (TF)} = C_{Shoot} / C_{Roots}$$

Where, $C_{shoot}$ is concentration of the metal in plant shoot and $C_{root}$ is concentration of the metal in plant root"

### 4.2 Statistical Analysis

For all experiments, the values are expressed as mean of the triplicate analysis of the samples, (n = 3) ± Standard Deviation (SD).

## 5. Results and Discussion

Data presented in Table 1, indicates the lead content in the aerial and underground parts of the test plant on treatment with lead acetate. Lead content in the aerial part increased as the lead concentration in the solution was increased. Highest lead content was recorded in the root tissues and it amounted to 31.1 ± 0.12 ppm on treatment with 200μM Pb $(CH_3COO)_2$. When compared to the underground part the concentration of lead was lowest in the aerial part (28.5 ± 0.21 ppm) at 200 μM Pb $(CH_3COO)_2$ concentration. This indicates the high mobility of lead through the plant. The control plants showed the absence of lead in its tissues.

Similarly, Cule *et al.* (2012) observed highest lead content in the root and lowest in the leaves of *Canna indica.* Amin *et al.* (2018) stated that "the Pb concentration was more in root compared to stem and leaves in *Cyamopsis tetragonoloba*". On the contrary, Aransiola *et al.* (2013) proved that *Glycine max* absorbed significant concentrations of Pb in the aboveground biomass compared to concentrations in the root.

In this study, high amount of lead was present in the roots than aerial parts. "According to Sharma and Dubey, (2005) the main reason for Pb retention in the roots was the binding of Pb to ion-exchangeable sites on the cell wall and extracellular precipitation mainly in the form of Pb carbonate deposited in the cell wall". Ramana *et al.* (2021) proved that "the strong binding of Pb to the carboxyl groups of the carbohydrates in the cell wall restricts its transport via apoplast. Pb moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The casparian strips of the endodermis are reported to be the major limiting factor restricting Pb transport across the endodermis into the central cylinder tissue". Begonia *et al.* (2005) stated that "the lead accumulation capacity based on their availabilities in the soil varies greatly among different plants species and cultivars".

Table 2 depicts the growth performance of *Vetiveria zizanoides* in terms of fresh weight. The fresh weight ratio of the aerial and underground parts of the plant was on an average 40:60. This indicates that when grown in water, *Vetiveria zizanoides* tends to grow good root system and rhizome. At higher concentration (200 μM

**Table 1.** Lead content in the plant parts of *Vetiveria zizanoides* (L.) Nash. in the treatments with Pb $(CH_3COO)_2.3H_2O$ measured on the 20th day of the experiment

| Lead Concentration (ppm) | Treatment with Pb $(CH_3COO)_2$. $3H_2O$ | | | | Control |
|---|---|---|---|---|---|
| | 50 μM | 100 μM | 150 μM | 200 μM | |
| Aerial parts | 18.5 ± 0.12 | 23.2 ± 0.16 | 25.7 ± 0.19 | 28.5 ± 0.21 | 0.00 ± 0.00 |
| Underground part | 26.5 ± 0.27 | 28.1 ± 0.12 | 28.7 ± 0.01 | 31.1 ± 0.12 | 0.00 ± 0.00 |
| Whole plant parts | 45.0 ± 0.39 | 51.3 ± 0.28 | 54.4 ± 0.20 | 59.6 ± 0.33 | 0.00±0.00 |

Values are mean ± SD (n = 3)

**Table 2.** Fresh weight of the plant parts of *Vetiveria zizanoides* (L.) Nash

| Fresh Weight (g) | Treatment with Pb $(CH_3COO)_2.3H_2O$ | | | | Control |
|---|---|---|---|---|---|
| | 50 μM | 100 μM | 150 μM | 200 μM | |
| Aerial parts | 10.62 ± 0.17 | 11.06 ± 0.21 | 14.05 ± 0.12 | 17.39 ± 0.26 | 10.97 ± 0.29 |
| Underground part | 14.46 ± 0.45 | 16.08 ± 0.20 | 17.50 ± 0.07 | 18.06 ± 0.32 | 12.79 ± 0.37 |
| Whole plant parts | 25.08 ± 0.62 | 27.14 ± 0.41 | 31.55 ± 0.19 | 35.45 ± 0.57 | 23.76 ± 0.66 |

Values are mean ± SD (n = 3)

**Table 3.** Dry Weight of the plant parts of *Vetiveria zizanoides* (L.) Nash

| Dry Weight (g) | Treatment with Pb $(CH_3COO)_2.3H_2O$ | | | | Control |
|---|---|---|---|---|---|
| | 50 μM | 100 μM | 150 μM | 200 μM | |
| Aerial parts | 2.39 ± 0.14 | 2.73 ± 0.17 | 3.80 ± 0.21 | 4.10 ± 0.25 | 3.16 ± 0.29 |
| Underground part | 2.63 ± 0.32 | 3.03 ± 0.26 | 3.43 ± 0.20 | 4.04 ± 0.37 | 2.36 ± 0.42 |
| Whole plant parts | 5.02 ± 0.46 | 5.76 ± 0.43 | 7.23 ± 0.41 | 8.14 ± 0.62 | 5.52 ± 0.61 |

Values are mean ± SD (n = 3)

**Table 4.** Bioconcentration factor and translocation factor of lead in *Vetiveria zizanoides*

| BCF and TF | Treatment with Pb $(CH_3COO)_2.3H_2O$ | | | | Control |
|---|---|---|---|---|---|
| | 50 µM | 100 µM | 150 µM | 200 µM | |
| BCF Values | 7.8 ± 0.15 | 4.5 ± 0.21 | 3.1 ± 0.28 | 2.6 ± 0.35 | 0.00 ± 0.00 |
| TF Values | 0.7 ± 0.27 | 0.8 ± 0.27 | 0.9 ± 0.27 | 0.9 ± 0.27 | 0.00 ± 0.00 |

Values are mean ± SD (n = 3)

Pb concentration), the fresh weight of both the aerial and underground parts increased considerably. Maximum fresh weight was recorded in the root (18.06 ± 0.32 g) at a concentration of 200 µM lead acetate. Generally, the fresh weight of *Vetiveria zizanoides* increased considerably compared to the control which shows its tolerance to lead.

The dry weight ratio of the aerial and underground parts of the plant amounts to an average of 50:50. Maximum dry weight was recorded in the aerial part (4.10 ± 0.25 g) at a concentration of 200 µM lead acetate. Compared to control treatments, there was a substantial increase in the dry weight of *Vetiveria zizanoides* (Table 3).

Khizar *et al*. (2013) proved that wheat (*Triticum aestivum* L.) plants were tolerant when exposed to lead. Ali *et al*. (2015) showed that spinach (*Spinacia oleracea*) was tolerant to Pb, Cd and Zn. Likewise, Cule *et al*. (2012) showed that *Canna indica* plants were highly tolerant to the presence of lead and showed very good plant growth even with the highest concentration of lead in the medium. In contrast, Ignatius *et al*. (2014) reported that lower concentrations of lead increased the plant biomass of *Plectranthus amboinicus* slightly.

Fitz and Wenzel (2002) stated that "the Bioconcentration Factor (BCF) and Translocation Factor (TF) values help to identify the suitability of plants for phytoremediation (i.e., phytoextraction or phytostabilzation) by explaining the accumulation characteristics and translocation behaviours of metals in plants. Plants with BCF and TF values >1 are considered promising phytoextractor, suitable for phytoextraction, while those with BCF and TF <1 are not suitable for phytoextraction/phytostabilzation". According to Mendez and Maier (2008) "plants with BCF>1 and TF<1 are considered potential phytostabilizers suitable for phytostabilziation".

As seen in Table 4 the plants growing in 50 µM lead acetate concentration registered the highest BCF (7.8 ± 0.15) while the plants growing in 200 µM lead acetate concentration recorded the lowest BCF (2.6 ± 0.35). Our results showed that *Vetiveria zizanoides* at 50,100, 150 and 200 µM concentrations had BCF values>1, which indicated that it could be used as a lead accumulator. Similarly, Thayaparan *et al*. (2013) revealed that" BCF of *Azolla pinnata* increased with increased lead concentration". Amin *et al.* (2018) in C*yamopsis tetragonoloba* and *Sesamum indicum* showed BCF values>1 at all Pb treatments.

Ma *et al.* (2001) and Yoon *et al.* (2006) stated "the Translocation Factor (TF) is defined as the ratio of the metal concentration in the shoots to that in the roots. Plants with TF values>1 are classified as high-efficiency plants for metal translocation from the roots to shoots". In this study, all the concentrations of lead acetate showed TF value<1 which shows that *V. zizanoides* is suitable for phytostabilzation of lead. Similarly, Amin *et al.* (2018) proved that C*yamopsis tetragonoloba* and *Sesamum indicum* plants showed TF values <1 at all Pb treatments. Similar trend was noticed by Ignatius *et al.* (2014) in *Plectranthus amboinicus* where "the TF value of all treatments was below 1, signifying poor translocation of Pb from the root to the shoot".

Taken together, these findings indicate that *Vetiveria zizanoides,* a member of the family Poaceae was capable of removing heavy metals like lead from polluted water. Therefore, *Vetiveria zizanoides* could be recommended for phytoremediation of lead.

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