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# Bio-Phytoremediation of Pb-Contaminated Wastewater using Perennial Grass

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The high concentration of heavy metals in wastewater highlights the urgent need to explore alternative treatment methods. Partially treated wastewater with elevated heavy metal levels can have severe environmental consequences, ultimately affecting the food chain. This study evaluates the effectiveness of bio-phytoremediation in treating heavy metal-contaminated wastewater using perennial grasses.
The research analyzed 10 days average effluent results for Pb, comparing their removal efficiencies at an initial concentration of 10 ppm after introducing Vetiver grass (Chrysopogon zizanioides) and Elephant grass (Pennisetum purpureum). The compliance levels of different remediation approaches were assessed against South African wastewater discharge limits and World Health Organization (WHO) guidelines. The findings indicated that Vetiver grass demonstrated a higher removal efficiency for Pb as compared to elephant grass.

## 1. Introduction

Heavy metals enter the food chain as a result of their uptake by edible plants due to their non biodegradability, they accumulate at high levels in the plant after their uptake in polluted soils (Udom et al., 2004). Their accumulation in plants depends on plant species and the efficiency of the plants in absorbing metals which is evaluated by soil to plant transfer factors of the metals (Khan et al., 2008).
The heavy metals that are available for plant uptake are those present as soluble components in soil solution or easily solubilized by root exudates (Blaylock et al., 2000). Plants require certain heavy metals for their growth, excessive amounts of these metals can become toxic to plants. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structure due to oxidative stress (Assche et al., 1990).
Indirect toxic effect is the replacement of essential nutrients at cation exchange sites of plants (Taiz and Zeiger, 2002). Heavy metals have negative influence on the growth and activities of soil micro-organisms which indirectly affect the growth of plants. A reduction in the number of soil micro-organisms as a result of high concentration of heavy metals may lead to a decrease in organic matter decomposition thus a decline in soil nutrients. Heavy metals interference with soil micro-organisms may also affect the activities of some enzyme useful for plant metabolisms (Plant et al 2000); thus, affecting the growth of the plant.
When heavy metals enter food chain, they cause different health challenges such as damages to nervous system, endocrine, circulatory system, cancer, malignancy, benign prostatic hyperplasia (Yang et al 2002, Zhang et al., 2012).
Several methods have been in use to decontaminate heavy metal contaminated environment, most of these techniques are either costly or far away from their optimum performance, the chemical technologies may generate large volume sludges which may also increase cost (Rakhshaez et al., 2009). The chemical and thermal methods are both technically difficult and expensive. All these methods can also degrade the valuable components of the soil (Hinchman et al., 1995).
Conventionally, remediation of heavy metal-contaminated soil involves either onsite management or excavation and disposal to land fill site. The method has the disadvantage of shifting the contaminants to another place, transportation of contaminated soil and moving of the contaminants from land fill to adjacent environment. An

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alternative method to excavation is Soil Washing where the heavy metal contaminated soil are removed and disposed to land fill. This method is costly, the residue produced rich in heavy metal may require further treatment; more so use of the area for crop production may be affected due to the removal of the soil with all its biological activities (Gaur et al., 2004).

Rapid industrialization (e.g., dyes, paper, thermal power plants, pharmaceutical, paint, cement, sugar industries, etc.) has resulted in the generation of increasing amounts of wastewater over the last 40–50 y. Organic matter, inorganic matter, and heavy metals are abundant in discharged wastewater. Wastewater is any type of water that has had its physicochemical properties harmed by anthropogenic, or man-made, activities.

Wastewater is defined as liquid waste discharged by residential homes, commercial properties industry, and/or agriculture. Petroleum hydrocarbons, chlorinated hydrocarbons, heavy metals, acids, alkalis, dyes, detergents, and other chemicals all have a significant impact on the physicochemical properties of water (Lokhande et al., 2011)

Rapid population growth necessitates a substantial supply of products and services to sustain livelihoods worldwide (Shang et al., 2019).

The increasing demand for clean water has become a critical global issue, exacerbated by rapid industrial expansion (Al Sharabati et al., 2021). Routine human activities and industrial operations generate a range of organic and inorganic water pollutants that negatively impact aquatic ecosystems. The environmental crisis is already apparent, driven by unsustainable development, unchecked economic expansion, and a disregard for environmental sustainability (Vita et al., 2019).

Over the past ten years, significant environmental concerns have emerged regarding heavy metal contamination. Although the words "heavy metals" lacks a precise explanation, it therefore encompasses areas such as toxicological, biological, physical, and chemical aspects (Katheresan et al., 2018). It remains widely used in scientific discourse due to the absence of a suitable alternative. The presence of heavy metals in wastewater poses serious risks to ecosystems and human health, as they can accumulate in the food chain (Naseem et al., 2018).

Heavy metals are a major worry due to their ability to cause adverse impacts in humans even with extremely low concentrations (Levchuk et al., 2018). These adverse impacts include Cancer-causing potential, developmental toxicity, and genetic mutations, even with low-level or sub-chronic exposure. Proper disposal of heavy metal-containing waste is essential to prevent ecosystem contamination (Masinire et al., 2020). While some heavy metals (such as Cu, Fe, Mn, Zn, and Mo) play vital roles in biological growth at trace levels, excessive amounts can negatively impact development and progression (Raji et al., 2023).

Naturally occurring as part of the Earth's composition and found in various ores, heavy metals are indestructible. Their xenobiotic concentrations pose significant environmental risks due to their toxicity, nonbiodegradable (El-Naggar et al., 2018).

In this context, selecting plants capable of absorbing heavy metals with minimal risk of biomagnification is highly beneficial. Therefore, Vetiver grass (Chrysopogon zizanioides) was chosen for this study. This research examines the effectiveness of perennial grass in the removal of heavy metals, specifically evaluating its response to Pb. Due to its high toxicity, accumulation potential, and persistence, Pb is considered one of the most hazardous environmental pollutants (Nazik et al., 2023; Jia et al., 2020). Major sources of Pb contamination in water include traffic emissions, mining activities, batteries, and a range of industrial operations (Ahmad et al., 2022; Jin et al., 2019).

Various methods have been employed to eliminate toxic materials, including microprocesses and ion exchange (Tchounwou et al., 2012), membrane filtration processes (Alirzayeva et al., 2017), and absorption (Sorayyaei et al., 2021; Ahmad et al., 2023). Among these, adsorption is widely recognized for its efficiency in wastewater treatment, effectively removing hazardous contaminants (Suhail et al., 2020; Naseem et al., 2023).

The appeal of this method lies in its numerous advantages, including ease of operation, simple design, and the ability to produce clean, safe water (Iqbal et al., 2019).

Given the widespread use and release of heavy metals in both domestic and industrial activities, along with their severe environmental impacts, this research primarily focuses on these contaminants (Ashrafi et al., 2017; Khan et al., 2019). Additionally, the study explores methods to enhance the tolerance of perennial grasses, discussing their benefits and limitations. Furthermore, it highlights the role of perennial grasses as model vegetation in phytoremediation research. This research supports Sustainable Development Goals (SDG) 6 which targets to ensure the supply and sustainable control of water and sanitation for all.

## 2. Materials and methods

Vetiver grass slips were supplied by Free Choice Progressive Learning (Pty) Ltd (White River, South Africa) while, Elephant grass was obtained from SMR Africa (Pty) Ltd in Bronkhorstspruit, South Africa. Lead nitrate, $Pb(NO_3)_2$) was used to prepare the synthetic Pb solutions. This was because Pb is soluble in water.

To determine the desired concentration of 10 ppm, the following formulas were used to calculate the mass for the Pb compound.

Mass = **M** x **V** x **Molar Mass**. **(1)**

Where M is the molarity, V is the volume in litres, and the molar mass of $Pb(NO_3)_2$ is 331.2g/mol.

Water sample was collected using a 50mL syringe and put stored in a 100mL glass beaker. To prevent Pb from adhering to the wall of the glass beaker, 0.5mL of nitric acid HNO3 was added to the sample to lower the pH to <2. The sample was filtered using a 0.45μm filter just to separate dissolved Pb from suspended solids. The water sample was mixed with 0.1mL of hydrochloric acid (HCL) to break down the organic matter and release Pb. The mixture was gently heated in a microwave until the solution became clear, and then cooled and diluted with deionized water. Analysis was conducted using Atomic Absorption Spectroscopy (AAS).

### 2.1 Elephant and Vetiver grass

Elephant and Vetiver grass, which were originally planted in soil, were moved and introduced to water. To ensure that the roots remained intact with little if no damage to the roots, the roots were handled with care. Both grasses were washed and left in water for 3 days for acclimatization. To ensure the growth of roots and shoots to the desired length, nutrients such as P, K, N, and Ca were added to the water. The shoot length was adjusted to a height of 20 cm, which is when acclimatization was observed. To start with the experiment, vetiver and elephant grass which were in the water, was then transferred to 2 transparent buckets with 4Liters of 10ppm concentration of Pb solutions. The decrease in water level in each bucket over time was attributed to evaporation and uptake by the vetiver and elephant grass. It was presumed that the evaporating water did not contain Pb, and water was topped to each bucket before sampling. This was to ensure that the change in concentration was not due to evaporation. Water samples were collected once every day for analysis for 10 days.

*Figure 1: Elephant grass in Pb Solution*

*Figure 2: Vetiver grass in Pb Solution*

*Table 1: Results after elephant and vetiver grass were introduced into Pb solution for period of 10 days*

| Time/d | Pb Effluent (mg/L) | Elephant in Pb Solution (mg/L) | Vetiver in Pb Solution (mg/L) | WHO Guidelines (mg/L) |
|---|---|---|---|---|
| Day 1 | 0.12 | 0.03 | 0.05 | 0.01 |
| Day 2 | 0.11 | 0.04 | 0.05 | 0.01 |
| Day 3 | 0.11 | 0.04 | 0.06 | 0.01 |
| Day 4 | 0.12 | 0.04 | 0.05 | 0.01 |
| Day 5 | 0.10 | 0.06 | 0.08 | 0.01 |
| Day 6 | 0.12 | 0.07 | 0.08 | 0.01 |
| Day 7 | 0.10 | 0.08 | 0.09 | 0.01 |
| Day 8 | 0.10 | 0.07 | 0.09 | 0.01 |
| Day 9 | 0.10 | 0.08 | 0.09 | 0.01 |
| Day 10 | 0.10 | 0.08 | 0.09 | 0.01 |

Pd were measured to assess the efficiency of Vetiver grass wastewater treatment. Calculating removal efficiency of these parameters were made using removal efficiency equation (Eq. 1):

$$x = \frac{C_0 - C_t}{C_0} \times 100, \quad \textbf{(2)}$$

where $C_0$ is the initial concentration of parameter, $C_t$ is the final concentration of parameter at time t (de la Luz-Pedro et al., 2019). The results obtained are presented in the table 1 and table 2 above.

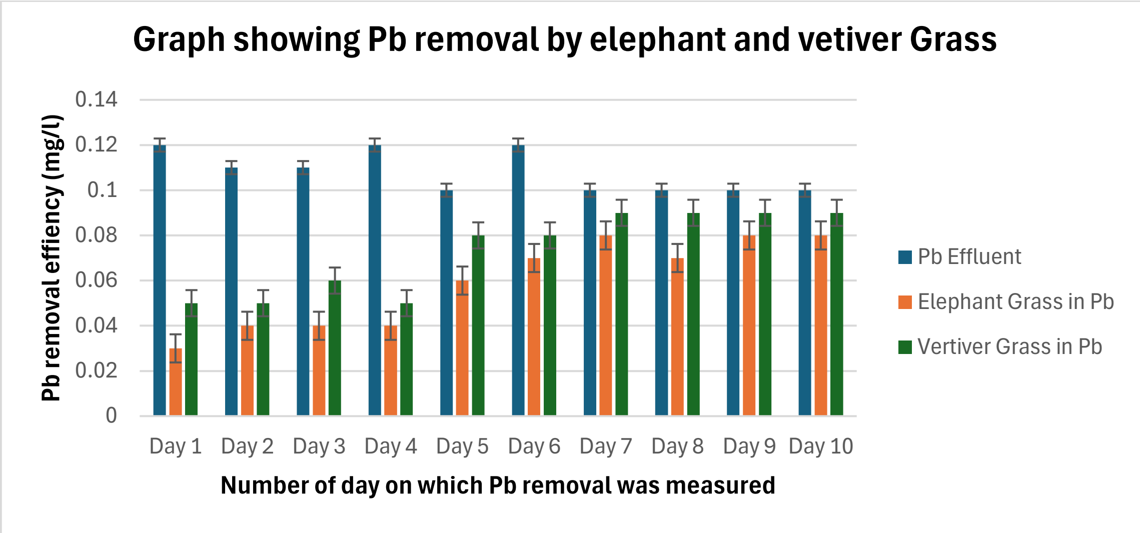


*Figure 3. Shows Pb removal by elephant and vetiver grass removal over the period of 10 days.*

## 3. Conclusion

Among the different methods available for remediation of heavy metal contaminated environment, phytoremediation appears to be more effective due to its cost effectiveness, environmentally friendly. Vetiver grass (*Vetiveriazizanioides*) stands out as one of the best plant species for remediation of heavy metal contaminated wastewater as compared to elephant grass. This is because Vetiver grass has high biomass with fast growth rate with the ability to accumulate heavy metals in their tissues. Combining the plants with micro-organisms or organic manures enhances the ability of the plants to accumulate heavy metals in their tissues.
The results indicated that Vetiver grass proved to have the most removal efficiency for Pb as compared to elephant grass. Though both grasses showed a good Pb removal rate, they did not meet both the South African wastewater discharge limits into the resource or the World Health Organization (WHO) guidelines. Elephant grass removal efficiency for Pb was between 25% and 80%, while that for vetiver grass was between 41% and 90%. Overall, Vetiver grass had the highest removal efficiency on Pb as compared to elephant. This is because vetiver has an extensive root network that enhances wastewater stabilization and increases contact with contaminants. This facilitates greater Pb uptake because of its high tolerance to Pb stress, allowing it to thrive in Pb-contaminated wastewater. Further research on a pilot study assessing the removal efficiency of Pb by the two grasses over a longer period is recommended.


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