International Journal of Development and Sustainability
ISSN: 2186-8662 – www.isdsnet.com/ijds
Volume 13 Number 6 (2024): Pages 515-528
ISDS Article ID: IJDS24021801

Phytoremediation potential of four wetland plants for the sustainable treatment of highly acid mine drainage water in Tanzania

Alexander Marwa *

Department of Civil and Environmental Engineering, School of Engineering and Environmental Studies, Ardhi University, P. O. Box 35176, Dar-es-Salaam, Tanzania

Abstract

Phytoremediation is a passive technology that uses plants to clean up industrial wastewater. Highly toxic effluents from mining operations, such as acid mine drainage (AMD), are concerning due to their potential to contain high concentrations of AMD. Four wetland plants were used to address this issue. The study showed that all plants, including *Typha latifolia, Phragmites mauritinus, Pennisetum purpureum,* and *Cyperus imbricatus*, survived in low concentrations of AMD and removed Zn, Cu, and Fe at a rate of >97%. However, two of the plants, T. latifolia and P. mauritinus, died in high concentrations of AMD. On the other hand, *P. purpureum* and *C. imbricatus* exhibited a high tolerance for AMD and showed a remarkable ability to remove heavy metals in the following sequence: Fe > Cu > Zn. Interestingly, both plants tended to accumulate more zinc in their leaves than in their roots. For instance, *P. purpureum* accumulated 689 mg/kg of zinc in its roots and 1545 mg/kg in its leaves, while C. imbricatus accumulated 50 mg/kg of zinc in its roots and 201 mg/kg in its leaves. Additionally, *P. purpureum* demonstrated a higher capacity for accumulating heavy metals compared to C. imbricatus. Overall, these results suggest that *P. purpureum* and *C. imbricatus* have the potential to be effective candidates for phytoremediation of high concentrations of AMD.

Keywords: : Acid Mine Drainage; Phytoremediation; Heavy Metals; Wetland Plants; Tanzania

Cite this article as: Marwa, A. (2024), “Phytoremediation potential of four wetland plants for the sustainable treatment of highly acid mine drainage water in Tanzania”, *International Journal of Development and Sustainability*, Vol. 13 No. 6, pp. 515-528.

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* Corresponding author. *E-mail address:* alex.marwa@gmail.com

1. Introduction

Mining activity can disturb and damage the surrounding environment due to the release of potential hazards such as AMD (Azizi et al., 2023). Environmental impacts associated with metal mining are an issue in both developed and developing countries (Singh et al., 2010). AMD is considered to be one of the most significant challenges associated with mining activities due to its magnitude of environmental, ecotoxicological, and socioeconomic impacts (Masindi et al., 2022).

AMD is one of the major causes of water pollution worldwide and can be produced from both active and inactive mining sites (Xie et al., 2022). Other technologies have been used for the treatment of AMD and mine-contaminated sites, including chemical immobilization using chemical reagents and excavation of polluted areas. However, the use of phytoremediation can provide a cost-effective phytotechnology that is environmentally friendly, long-lasting, and easily rehabilitates contaminated sites (Penalver-Alcala et al., 2021; Krasavtseva et al., 2023).

The treatment of wastewater using aquatic plants in phytoremediation systems has been shown to be extremely successful on a global scale (Faizan et al., 2018). Phytoremediation is a promising green technology for treating contaminated water by using plants to remove and immobilize pollutants (Oh et al., 2014). Various studies have reported the use of aquatic plants such as *T. latifolia, P. mauritianus, P. purpureum,* and *Cyperus spp*. worldwide for the treatment of various contaminated wastewater (Ujang et al., 2021; Alquwaizany et al., 2022; Hamad, 2023; Taufikurahman et al., 2023). In addition, the selection of plants for use in phytoremediation is critical as the selected plants must be able to tolerate toxicity. Therefore, the use of various wetland plants for the treatment of acid mine drainage has been reported by various researchers. For example, the use of T. latifolia and Cyperus spp. has been documented (Hamad, 2020; Putri and Moersidik, 2021; Singh and Chakraborty, 2021). Similarly, the use of P. mauritinus and P. purpureum has also been reported by Etika and Hasan (2016), Kowitwiwat and Sampanpanish (2020), and Nabuyanda et al. (2022) for the treatment of acid mine drainage. Most aquatic plants naturally absorb pollutants such as heavy metals through their root and shoot systems (Kim, 2015).

Hundreds of plant species have been considered as prospective candidates for phytoremediation technology so far (da Conceicao Gomes et al., 2016). The plant root tissue is the first to be exposed to toxins; therefore, it serves as an escape route to other plant tissues (Maestri et al., 2010). In the process of phytoremediation, the plant shoot is responsible for accumulating the maximum metal concentration (Visioli and Marmiroli, 2011).

The accumulation of heavy metals in plant tissue has a wide variety of negative effects on plant growth (Sharma and Dubey, 2005). The root zone is the area that will be most toxically exposed when dealing with heavy metal stress (Branquinho et al., 1997). Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants (Cho-Ruk et al., 2006). However, the higher concentration of metal ions could lead to the destruction of plant physiological systems, impose inhibitory consequences on plant metabolism, and reduce plant growth (Giri, 2012). Different plant capacities, for example, hyperaccumulators. High biomass and elongated roots can influence the performance of phytoremediation (Pang et al., 2023).

Once the phytoremediation is completed, the uptaken metals can be harvested and well disposed of (Brissn and Chazarenc, 2009). Therefore, the issue of AMD in mining operators can be resolved to the fullest extent possible through the employment of phytoremediation of plants for treating AMD (Wu et al., 2022). In addition, there is an immediate need to establish an efficient and sustainable mine drainage water remediation program to reduce the risks associated with the mobility, transportation, and ecotoxicity of heavy metals from mining areas to the environment (Azizi et al., 2023). The objective of this study was to employ four aquatic plants (*Typha latifolia, Phragmites mauritianus, Pennisetum purpureum,* and *Cyperus imbricatus*) for phytoremediation potential of highly acid mine drainage by focusing on heavy metal removal, uptake by each plant and their growth characteristics at different concentrations of AMD.

2. Materials and methods

2.1. Preparation and planting of plants

The *Cyperus imbricatus, Typha latifolia, Phragmites mauritianus,* and *Pennisetum purpureum* plants (Figure 1) used in this study were collected from the Ruvu River in Dar Es Salaam, Coast Region. The young plants of these species were collected using suitable equipment and then transported to Ardhi University for transplantation and experimental setup. After arriving from the field, the young plants were immediately transplanted into plastic bottle containers filled with washed sand. To ensure uniformity of plant size, each setup contained only one plant, and the plant was measured for fresh weight both above and below ground mass before planting. This was done to maintain equal environmental conditions for all plants, as adopted from Lu et al. (2017). The containers were left for one week to allow the plants to acclimatize to the local conditions. All the young plants were grown under the same conditions and for the same duration before being used. The plants were allowed to grow to ensure adequate development of their root system.This study utilized synthetic AMD due to its ease of obtaining different concentrations, providing low, medium, and high levels of AMD for assessing the phytoremediation potential of selected plant species.The distilled water used for the preparation of AMD was collected from the Environmental Engineering Laboratory at Ardhi University. The tested metals were added as a solution to zinc chloride, copper (II) sulphate and ferric chloride salts to obtain Zn, Cu, and Fe as artificial AMD. This procedure of solution preparation was adopted from Muhammad et al. (2015). In addition, the sand media used to support plants during the experiment was collected at Ardhi University. It was placed in appropriate plastic bags and thoroughly washed to remove any unwanted materials. Powdered solutions of Zn, Cu, and Fe were dissolved in 1.0 L of distilled water, and then poured into 7 kg of washed sand for the experiment. The synthetic AMD was prepared with constituents of zinc, copper, and iron at various concentrations as indicated in Table 1.

Table 1. Preparation and AMD characteristics used in the experiment

Treatment	Initial concentration of AMD		
	Zn (mg/L)	Cu (mg/L)	Fe (mg/L)
Low AMD	5	1	1
Medium AMD	300	60	80
High AMD	1000	150	200

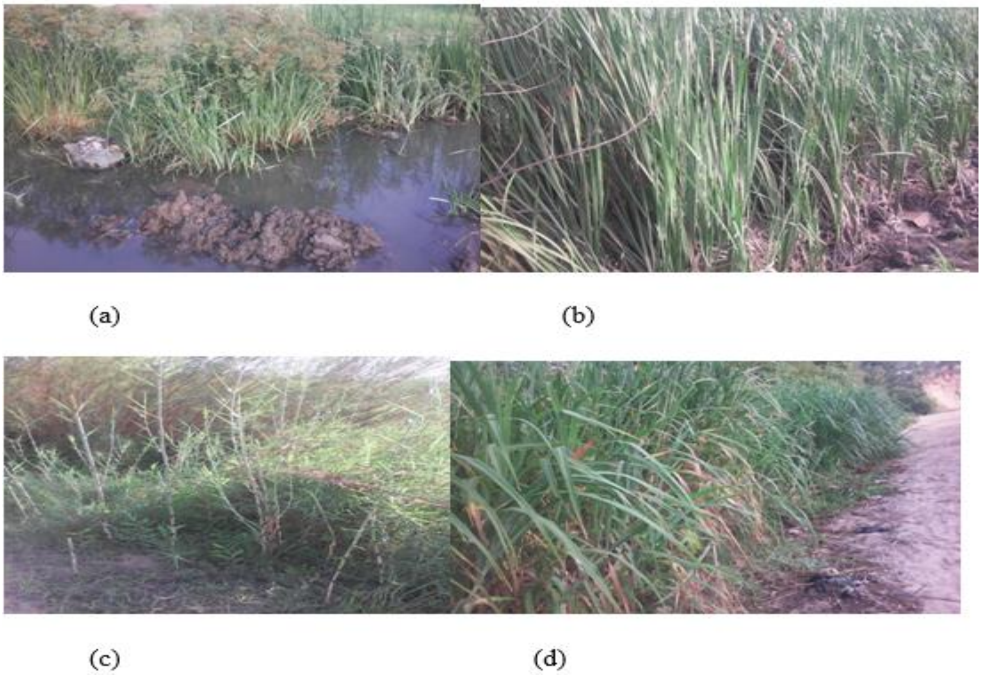

Figure 1. Typical plants used for experimental setup (a) *Cyperus imbricatus*, (b) *Typha latifolia*, (c) *Phragmites mauritianus*, (d) *Pennisetum purpureum* were collected from the Ruvu River in Dar Es Salaam, Tanzania

2.2. Experimental design

The experiment was carried out using a 15-liter plastic container. The batch experimental run was conducted with Typha Latifolia, Cyperus imbricatus, Pennisetum purpureum, and Phragmites mauritianus. All plants were planted in identical environmental conditions within plastic bottle reactors filled with sand and synthetic AMD. All experimental sets contained varying concentrations of zinc, copper, and iron. Each plant was grown in a solution containing different initial concentrations to test their potential, as shown in Table 1. Each plant was tested with three setups with low, medium, and high levels of concentration. The monitoring was conducted over a period of 10 weeks from the day of planting. After treatment, the plants were harvested after a few days and then dried in an oven at 80°C until a constant weight was achieved, following the method described by Munawar (2011). Each treatment unit has been set up with a control group, which was planted with distilled water for all types of plants. Before the start of the experiment, the plants were chosen to ensure uniformity in size, as adopted from Lu et al. (2017), and all treatments were replicated four times. After a period of treatment, the plants were harvested and then dried in the oven at 80°C until a constant weight was achieved.

2.3. Determination of heavy metals uptake by plants

For the purpose of determining the uptake of heavy metals such as Cu, Zn, and Fe in different parts of the plants (roots and leaves), plant samples were collected, thoroughly washed with distilled water, and dried at a temperature of 1030°C–1050°C for 24 hours. After drying, the plants were left to cool. Subsequently, they were measured and digested using aqua regia, a mixture of hydrochloric acid and nitric acid in a 3:1 ratio. A total of 0.5 g of dried plant material was weighed and placed into a graduated test tube, followed by the addition of 2 mL of aqua regia. The sample solutions were digested on a hot plate at 95°C for one hour. The samples were then allowed to cool to approximately room temperature and diluted with 10 mL of deionized water. Finally, the samples were left to settle overnight, and then the supernatant was analyzed using atomic absorption (AA) for Cu, Zn, and Fe.

2.4. Heavy metal removal

Removal percentage of heavy metals from AMD was calculated as follows

$$\%Removal = \frac{Co - Ce}{Co} \times 100$$

where *Co* is the initial concentration of heavy metal t parameter in AMD and *Ce* is the final concentration of the heavy metal parameter in AMD. The unit of this calculation is percentage

2.5. Statistical analysis

One-way analysis of variance (ANOVA) with a significance level of p-value < 0.05 was used to determine significant differences among the treatment units. This was followed by the Tukey test to compare these differences. The Tukey test is applied to assess the significance of differences between treatment units, as it is a follow-up to one-way ANOVA. Therefore, if the treatments showed significant differences, their mean treatment effects were tested using Tukey's HSD (Honestly Significant Difference) test.

3. Results and discussion

3.1. Heavy metals removal percentage

Results of metal removal efficiency at different initial metal concentrations of the plants are presented in Tables 2, Figure 2, and Figure 3. At high initial metal concentrations, the highest removal efficiency was observed in iron for both plants (*P. purpureum and C. imbricatus*). The lowest removal efficiency was observed in zinc, with 59% in *P. purpureum*, and 57% in *C. imbricatus*. The performance of *C. imbricatus* at a medium initial metals' concentration of AMD for zinc removal was 72%, while that for copper was 91% and for iron was 90%. The percentage of plant removal in metal at low initial metal concentration was determined as follows: *P. mauritianus* removed 96% of zinc, 94% of copper, and 98% of iron. In addition, the performance of *T. latifolia* in zinc removal was 96%, in copper was 98%, and in iron was 99%.

Zinc removal in *C. imbricatus* at a low initial concentration was 96%, copper removal was 95%, and iron removal was 99%. It was further observed that the zinc removal of P. purpureum was 98% at a final concentration of 0.09 mg/L, while that of copper was 97% and iron was 99%. As shown in Tables 2, and Figure 2 to 3, the efficiency of metal removal decreases as the initial concentration of the metal increases. It is also shown that a high initial metal concentration reduces the performance of plants in terms of removal efficiency. The removal of iron was high, reaching 90–94% for both plants (*P. purpureum* and *C. imbricatus*) at high concentrations of AMD. In addition, it was observed that the removal of iron at low concentrations of AMD was over 98% for all plants (Figure 3). The statistical significance test was conducted to compare iron removal between low and high concentrations of AMD. The results confirmed that there is a significant statistical difference ($p < 0.0006$). This study observed that at low concentrations of AMD, the removal efficiency of plants increased, while at high concentrations of AMD the removal efficiency decreased.

Table 2. Plants metal removal efficiency of high concentration of AMD

Initial Metal Concentration(mg/L)	Plant Species	Removal Efficiency					
		Zn		Cu		Fe	
		(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)
Zn-1000 Cu-150 Fe-200	*P.purpureum*	393	59	22	85	12	94
	C. imbricatus	408	57	25	81	16	90
	*P.mauritianus**						
	T. latifolia*						

** Plants die within 7 days of batch experiment*

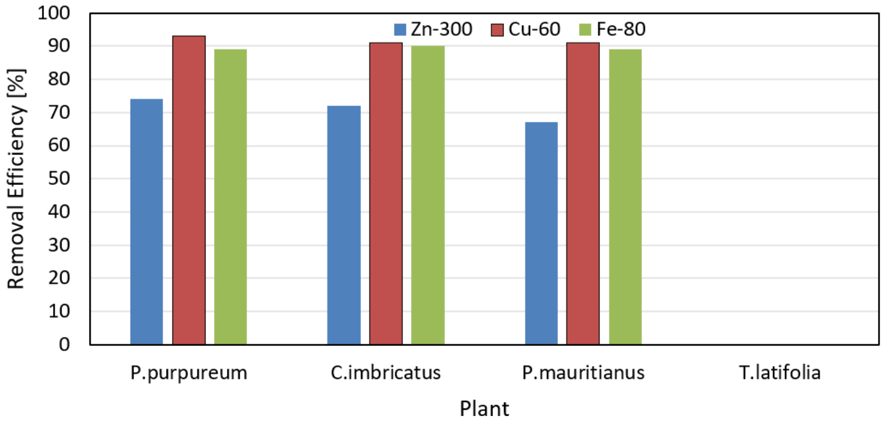

Figure 2. Plants metal removal efficiency of medium concentration of AMD

In Tables 2, Figure 2, and Figure 3, this study found that as the level of AMD concentration increased, some plants such as *T. latifolia* and *P. mauritianus* did not survive. The results of this study could be associated with excessive toxic contaminants in plant cells, which caused the plants to die in the treatment unit. This observation is supported by Arif et al. (2016), who reported that an increase in heavy metals in plants can cause direct or indirect toxic impacts. For example, it can lead to the generation of oxidative stress, which can intensify the inhibition of cytoplasmic enzymes and cause damage to cell structures. In addition, at high concentrations, it is toxic to plants, causing stunted growth (Arif et al., 2016). In the study by Riyazuddin et al., (2021) it was reported that a higher concentration of heavy metals can adversely affect cellular metabolism, particularly inhibiting photosynthesis which can result in overall damage to plant growth. Additionally, other studies (Dutta et al., 2018) have commented that heavy metal ions can cause damage to the structural and enzymatic components of plant cells, ultimately leading to negative impacts on plant growth and development. The analysis of metal removal efficiency at high concentrations of AMD was performed, and the results showed that *P. purpureum* > *C. imbricatus*. Zinc exhibited the lowest metal removal compared to copper and iron. Other studies (Noller et al., 1994; Ferniza-Garcia et al., 2017) have reported that the low removal of zinc could be attributed to the desorption process of zinc by plants. Other studies have reported that the removal of zinc is influenced by the pH level in mine drainage (Salim et al., 2003; Adamczyk-Szabela et al., 2015) because it affects zinc solubility and sorption.

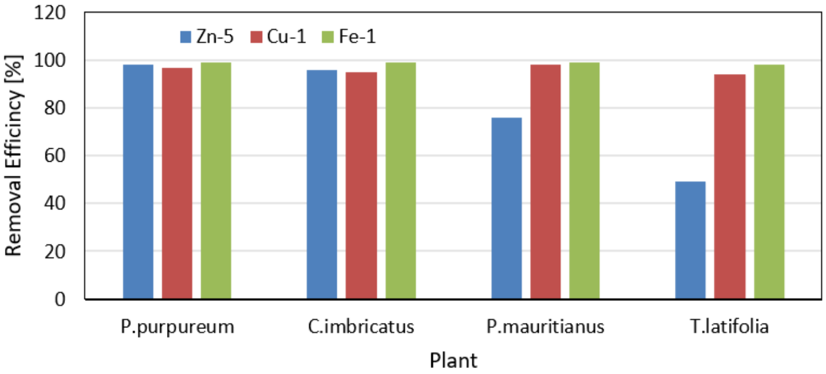

Figure 3. Plants metal removal efficiency of low concentration of AMD

Generally, a high metal removal efficiency was observed at low concentrations of AMD in the plants (*C. imbricatus* and *P. purpureum*), and they were also able to withstand high concentrations of AMD (Table 1) during the experiment. Ma et al. (2015) reported that *Cyperus* species are among the plants responsible for remediating AMD and were acid-tolerant up to pH 2.4. However, according to Usharani and Vasudevin (2017), there is not enough literature reported on a single plant species exposed to different concentrations of heavy metal mixtures.

3.2. Heavy metals uptake by plants

The accumulation of heavy metals in the plant parts at different initial metal concentrations of AMD was investigated to determine the potential of each plant. The results are presented in Table 3 to 5. At low initial concentrations of AMD, *P. purpureum* accumulated 380 mg/kg of zinc in the roots and 480 mg/kg in the leaves. *T. latifolia* accumulated 212 mg/kg of zinc in the roots and 419 mg/kg in the leaves. Additionally, zinc was accumulated in *C. imbricatus* roots and leaves at 10.2 mg/kg and 119 mg/kg, respectively. The accumulation of iron in plant parts (roots and leaves) of *C. imbricatus* was the highest. Iron accumulated 486 mg/kg and 958 mg/kg in the roots and leaves, respectively, while *P. mauritinus* accumulated 350 mg/kg and 295 mg/kg in its roots and leaves, respectively. This study observed that heavy metal accumulation in the plants varied among different plants and metals. For example, in the low concentration of AMD, the plant *P. purpureum* accumulated higher levels of iron (Fe) in the roots than in the leaves, while other metals such as zinc and copper showed higher accumulation in the leaves than in the roots (Table 4).

Table 3. Heavy metal accumulation in the plants (roots and leaves) at low concentration of AMD

Plant Species	Plant Part	Zn (mg/kg)	Cu (mg/kg)	Fe(mg/kg)
P.purpureum	Roots	380	20	280
	Leaves	480	30	155
C.imbricatus	Roots	10.2	8	486
	Leaves	119	63	958
P.mauritianus	Roots	210	290	350
	Leaves	180	35	295
T.latifolia	Roots	212	23	144
	Leaves	419	46	160

Table 4. Heavy metal accumulation in the plants (roots and leaves) at medium concentration of AMD

Plant Species	Plant Part	Zn (mg/kg)	Cu (mg/kg)	Fe(mg/kg)
P.purpureum	Roots	648	68	1190
	Leaves	845	128	1038
C.imbricatus	Roots	40	14	745
	Leaves	185	116	1352
T.latifolia	Roots	525	369	520
	Leaves	641	237	1126

Table 5. Heavy metal accumulation in the plants (roots and leaves) at high concentration of AMD

Plant Species	Plant Part	Zn (mg/kg)	Cu (mg/kg)	Fe(mg/kg)
P.purpureum	Roots	689	88	1255
	Leaves	1545	318	1125
C.imbricatus	Roots	50	15	954
	Leaves	201	132	1504

The accumulation of zinc, copper, and iron at medium initial concentrations was performed as follows: zinc accumulated in *P. purpureum* roots was 648 mg/kg and in leaves was 845 mg/kg, while iron accumulated in the leaves of *C. imbricatus* was 1352 mg/kg, the highest amount recorded. The plant (*Cyperus ssp*), identified in a study by Ashraf et al. (2011), exhibited similar behavior by accumulating the highest concentration of metals (1990.44 mg/kg). This suggests that this plant can be added to the list of species known as hyperaccumulators. Furthermore, *C. imbricatus* accumulated 50 mg/kg of zinc in its roots and 201 mg/kg in its leaves. It also accumulated 15 mg/kg of copper in roots and 132 mg/kg in leaves, while iron levels were 954 mg/kg in roots and 1504 mg/kg in leaves. In the study by Wenzel and Jockwer et al. (1999) also reported similar observations of higher metal concentrations in leaves than in roots. McLaughlin et al. (2011) pointed out that heavy metals enter plant tissues mainly through the roots, with root uptake being the primary pathway. It was further observed that *P. mauritianus* at the low level of AMD accumulated more metals (Cu, Zn, and Fe) in the roots than in the leaves (Table 4). The results of this study are similar to the study on landfill leachate treatment by constructed wetlands conducted by Peverly et al. (1995), who observed similar trends in *Phragmites spp*. Encountered a very high level of iron and other metals in the roots. However, other studies by Munir et al. (2021) have commented that the accumulation of heavy metals in plant tissues varies depending on the plant species and the specific metal elements.

3.3. Plants biomass production

The total biomass of the plants is presented in Figure 4. This study showed that four plants, planted in different initial concentrations of AMD, indicated that higher biomass production was observed in *C. imbricatus* (31.63g) in high concentrations of AMD. In medium AMD, *T. latifolia* exhibited a higher total biomass (38.11g) compared to other plants. Conversely, in low concentrations of AMD, *C. imbricatus* showed the highest biomass production with 47.4g. In addition, this study indicated that *T. latifolia* and *P. mauritianus* were not grown in high concentrations of AMD. In this study, it was also observed that as the concentration of AMD increased, some plants died.

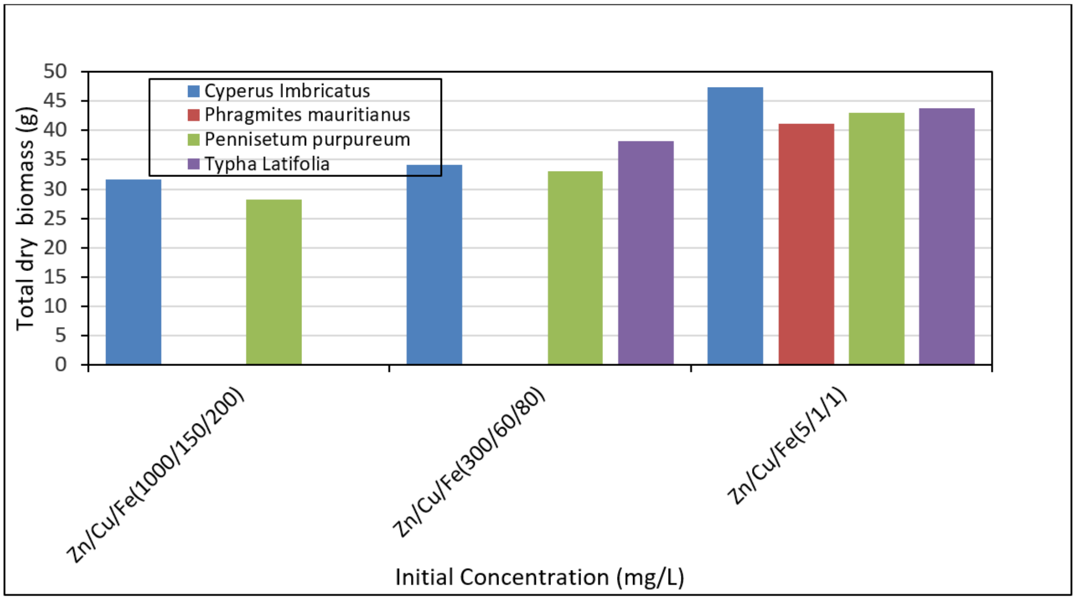

Figure 4. Effects of heavy metals on biomass production during the treatment of different concentrations of AMD

It was observed in Figure 1 that *P. mauritianus* was able to survive only at low concentrations of AMD. In the study by Sheldon and Menzies (2005), it was indicated that an excess of certain metals above the level of accumulation can disturb the health of a particular plant or even cause it to die. Another study by Ashraf et al. (2011) recommended that growth patterns change in high metal concentrations compared to low metal concentrations. In addition, an excessive concentration of heavy metals in plants can lead to oxidative stress and stomatal resistance (Fayiga et al., 2004; Shah et al., 2009). Based on the total biomass production, it was observed that only *C. imbricatus* and *P. purpureum* were sustained in high concentrations of AMD (Figure 4). These plants could be useful candidates for phytoremediation in areas with high concentrations of AMD. In the study conducted by (Pang et al., 2023), it was suggested that different plant capacities, such as hyperaccumulators and high-biomass plants, influence the phytoremediation performance of plants. Other studies considered plants with high metal accumulation were grouped as hyperaccumulator plants (Cho-Ruk et al., 2006). Therefore, the result obtained from this particular study shows that these two plants (*C. imbricatus* and *P. purpureum*) have the potential to be hyperaccumulators.

4. Conclusion

The objective of this study was to evaluate the potential of plants for highly concentration of AMD phytoremediation. Different plants respond differently when it comes to AMD remediation, as demonstrated by the phytoremediation of various AMD concentrations. In this study, the removal efficiency of heavy metals from four plants at different initial metal concentrations increases in the following order: low initial metal concentration > medium initial metal concentration > high initial metal concentration. Only two out of the four plants were able to withstand high concentrations of AMD, and their removal efficiency was ranked as follows: *P. purpureum* > *C. Imbricatus*. The accumulation of heavy metals in various plant parts was also explored in this study. It was revealed that different plants and metals have varying phytoremediation potential in leaves and roots at all levels of AMD concentration. However, this study showed that only two plants, *P. purpureum* and *C. imbricatus*, survive and accumulate heavy metals in high concentrations, while other plants (*P. mauritianus* and *T. latifolia*) die in the high concentration of AMD. Further research is needed to reduce the toxicity of heavy metals in plants in order to enhance phytoremediation of highly concentrated levels of AMD.

Acknowledgement

Without the grant from North Mara Gold Mine, which initially funded this research, this study would not have been possible. I also want to thank Dr. Stephen Mbuligwe and Prof. Gabriel Kassenga for their constant guidance and support throughout the process of preparing this publication. Additionally, I am grateful to Ardhi University's School of Engineering and Environmental Studies for granting me permission to use their lab space.

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