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A Comparative Study of Phytoremediation Performance of Four Native Plant Species for an Indian Copper Mine Acid Mine Drainage

Seeramreddy Shyamala[1*], Kaushik Dey[1] and Jayanta Bhattacharya[1,2]

[1]Department of Mining Engineering, Indian Institute of Technology, Kharagpur - 721302, West Bengal, India; samsreddy.iit@gmail.com

[2]School of Environmental Science and Engineering, Indian Institute of Technology, Kharagpur - 721303, West Bengal, India

Abstract

Acid Mine Drainage (AMD) is a critical environmental concern arising from mining operations, leading to soil degradation through acidification, salinization, and soil and water contamination with metals and metalloids. Phytoremediation, using plants to clean up contaminated environments, offers a promising solution for restoring post-mining lands in an economically feasible manner. This study involved a three-month growth chamber experiment to evaluate the performance of four plant types: Phyllanthus urinaria (Chamber bitter), Cleome rutidosperma (Fringed Spider Flower), Impatiens balsamina (Rose balsam), and Oldenlandia corymbosa (Flat top mille grains). The study examined the changes in mineral composition of the substrate before and after the experiments, with particular attention to shifts in metal concentrations and pH levels in the drainage. No amendments were added to stabilizing metal (loid)s (Al, Co, Fe, Mn, Zn) in AMD-impacted soil from a copper mine in Eastern India. The findings indicated that all the species can sustain in acidic environments with a pH value ranging from 2.6 to 3.2. BCF was highest for Olden. Also, an excellent increase in the biomass. Fe, Mn, Mg, and Zn translocation from roots to shoots, which has been observed in all the species. The research highlighted that the acidity in the rhizosphere was neutralized through a series of geochemical processes, including: (1) carbonate dissolution, (2) the dissolution of aluminum and iron (oxy) hydroxides. Novelty of this work is these species has not been used for AMD treatment. The research shows that these species are efficient in remediating soils impacted by AMD through phytoremediation. This is probably attributable to significant alterations in rhizosphere chemistry and species-specific physiological and molecular adaptations.

Keywords: *Acid Mine Drainage, Biomass, Environment, Phytoremediation, Metals, Remediation*

1. Introduction

Acid Mine Drainage (AMD) is effluent generated from mining activities. It arises when sulfide-bearing minerals, mainly pyrite (FeS_2), undergo oxidation upon exposure to air and water[1-4]. The oxidation process produces an acidic solution abundant in sulfuric acid (H_2SO_4) and ferric iron (Fe^{3+}), facilitating the leaching of hazardous metals and metalloids from adjacent rocks[5-7]. Countries like Australia, Canada, China, India, South Africa, and the United States are major mining hubs[8,9]. The primary environmental concerns related to AMD are its high acidity and metal(loid) contamination, posing significant risks to human health and ecosystems. Humans can be exposed to harmful and carcinogenic compounds by consuming crops grown in AMD affected soil and water[6,10]. Furthermore, AMD causes acidification, salinization, and sedimentation, which are processes that degrade soil and water[11,12]. These environmental repercussions pose significant obstacles to sustained socioeconomic development, as do their implications on public health and vital food and water security systems[13,14].

AMD is a critical global issue for mining industries. Recently, the United Nations identified AMD as the second most pressing environmental challenge after global warming, underscoring its urgency, which has become of utmost importance in addressing this environmental challenge[15]. The global production of AMD from approximately 20,000 to 50,000 mines is resulting in severe impacts on nearly 6,400 kilometers of rivers and 8,000 to 16,000 kilometers of streams[13]. Furthermore, AMD has severely affected approximately 19,300 kilometers of freshwater, 72,000 hectares of lakes and reservoirs, and about 15,000 to 23,000 kilometers of streams[16-18]. Remedial measures are crucial since the extensive effects of AMD frequently surpass the financial resources of mining firms, the legal frameworks of governments, and the boundaries of mining regions[19]. Consequently, it is essential to restore AMD-affected situations.

Currently, AMD mitigation techniques as mentioned in (Table 1), concentrate on lowering acidity and eliminating high sulfate and metal(loid) concentrations, occasionally to recover valuable components. However, these approaches are often costly, requiring significant materials, labor, and technical expertise, and fail to comprehensively address the environmental needs of AMD-affected areas[18]. These areas include diverse ecosystems in soils, water, and sediments, all of which support various land uses[20]. Therefore, it is imperative to develop more effective and sustainable remediation strategies for such environments, ensuring that these solutions are both cost-effective and beneficial for land use[14].

Treatment method	Type (Passive/ Active)	Mechanism	Drawbacks	Phytoremediation comparison	References
Bioreactors	Active	Uses microbial processes to reduce sulfates and precipitate metals as sulfides.	High operational costs; requires constant monitoring; performance decreases at low temperatures.	Phytoremediation is more cost-effective, low-maintenance, and environmentally friendly in the long term.	(21,22)
Chemical precipitation (Lime/Caustic soda)	Active	Addition of chemicals (lime, caustic soda) increases pH to precipitate metals.	High operational costs; large amounts of sludge generated; hazardous sludge disposal required.	Phytoremediation avoids chemical sludge production and is more sustainable for long-term application.	(23,24)
Reverse Osmosis	Active	Membrane filtration separates dissolved metals and sulfates from water.	Very high costs; membrane fouling; energy-intensive; produces concentrated brine as waste.	Phytoremediation is far less energy-intensive and avoids the generation of hazardous brine.	(25,26,27)
Ion Exchange	Active	Resins exchange metal ions for other ions like Na^+ or H^+ in the water.	High operational and maintenance costs; requires regeneration of resins; produces secondary waste.	Phytoremediation is more cost-effective and does not produce secondary waste that requires disposal.	(28,29)
Electrochemical Treatment	Active	Electrodes precipitate metals via electrical current.	Energy-intensive; expensive equipment; limited scalability; produces hazardous by-products.	Phytoremediation is a more scalable, energy-efficient, and natural method.	(30,31,32)

Limestone Drains	Passive	Limestone neutralizes AMD by raising pH, leading to precipitation of metal hydroxides.	Clogging due to the formation of metal hydroxides; ineffective for highly acidic water; frequent maintenance required.	Phytoremediation does not involve clogging issues and can treat a wider range of contaminants over time.	(33,34)
Constructed Wetlands	Passive	Wetland plants and anaerobic bacteria promote metal precipitation and sulfate reduction.	Requires large areas; slow treatment process; less effective for high metal concentrations; potential for wetland contamination.	Phytoremediation can be applied in smaller areas, and certain hyperaccumulator plants can handle higher metal loads.	(35,36)
Anoxic Limestone Drains (ALDs)	Passive	Limestone neutralizes AMD in an anoxic environment to prevent iron hydroxide precipitation.	Limited to waters with low oxygen and iron concentrations; clogging due to metal deposition.	Phytoremediation can handle a broader spectrum of metals and pollutants without clogging.	(37,38)

Phytoremediation, which utilizes plants to treat contaminated soil, sediment, and water, has emerged as a cost-efficient and resource-effective alternative to traditional remediation methods[20]. According to studies, certain plant species that accumulate metal(loid) and can withstand acidity are very good in stabilizing pollutants when AMD criteria are met. (Table 2) discusses about some of the previous studies conducted for AMD treatment using Phytoremediation Technique. For instance, some aquatic plants facilitate the precipitation and adsorption of metals onto sediments, while others filter and retain metals within their tissues through processes such as cation exchange[39], adsorption, and interactions with root-associated bacteria. This symbiosis with inter-root bacteria can also lead to the formation of iron oxide layers, which trap additional metals. Direct metal uptake by plant roots is another important mechanism of phytoremediation.

Selecting plants with high resistance to stress and effective treatment capabilities is essential for creating successful wetland systems for wastewater treatment. Plant roots play a key role by enhancing the plant–microbial–filler complex, aiding in adsorption, filtration, and environmental modification. A review of 35 experimental studies by Brisson and Chazarenc revealed considerable variability in the pollutant removal efficiency of different plant species in constructed wetland systems, highlighting the importance of choosing the right species for specific contaminants. Additionally, some of the soil amendments like compost municipal[40], fertilization urea[40] that stabilize metal(loid)s or temporarily adjust pH levels can support plant growth and enhance remediation efforts. In some cases, bioenergy crops can be cultivated in these conditions, providing an additional benefit by repurposing contaminated land for productive use[20,41]. By combining these strategies, it is possible to address the complex and multifaceted challenges posed by AMD, moving toward more sustainable and efficient remediation practices.

In phytoremediation systems, using locally available plant species, especially those that thrive in mining areas, has shown promise in increasing plant biomass, enhancing metal(loid) tolerance, and improving soil bacterial diversity, organic matter, and nutrient availability[45-47]. The Bioconcentration Factor (BCF) and Translocation Factor (TF) are crucial for evaluating plant efficiency in phytoremediation of Acid Mine Drainage (AMD). BCF measures the plant's ability to accumulate metals from soil or water, while TF indicates the metal's movement from roots to shoots. Compared to earlier studies on AMD, current research focuses more on selecting hyperaccumulator species with high BCF and

Table 2. Summary of the previous study for AMD treatment using phytoremediation technique

S. No	Mine name, Location	Plant name	Heavy metal treated	Soil/Water	Lab scale/ Field Scale	Duration	Removal Efficiency	Remarks/ Observation	References
1.	Beaver Township, North Lima, OH, USA	*Phragmites Australis* (common reed)	Al, Fe & Mn	Aqueous media	Lab scale	3 months	Fe (57%), Mn (21%) & Al (0.60 %) were accumulated in roots	Biodegradable chelator citric acid (CA), increasing CA dosage and treatment time increased metal accumulation in plants.	[41]
2	Tab-Simco mine site in southern Illinois	*Chrysopogon zizanioides* (Vetiver grass)	Al, Cr, Cu, Fe, Mn, Ni, Pb, SO_4^{2-}Zn,	Aqueous media	Mesocosm, Lab scale hydroponic	12 months	Fe (81%) and Pb (81%), Ni (38%), Zn (35%), SO_4^{2-} (28%), Mn (27%), Cr (21%), Al (11%) and Cu (8.0%)	Difference in the metal accumulation rate differed due to the root growth in small scale & large-scale setup.	[42]
3	Dabaoshan mine located in Guangdong province, China	*Ludwigia prostrata*	Cu, Pb, Zn, Cd	Soil	On site study	-	The removal efficiency was 1.58, 130.76 and 454.72 mg kg^{-1} of Cu, Pb and Zn	The weeds which sustained in the AMD impacted field were selected and they have good heavy metal accumulation.	[43]
4	Gold mine of Gauteng province, South Africa	*Chrysopogon zizanioides* (Vetiver grass) and *Medi-cago truncatula* (Barrel clover)	Cr, Zn, As and Rare Earth Element (REE)	Soil	Pot experiment	3 months	The removal efficiency for Cr, Zn and As are 80%, 32%, 54%	Co-cropping helped in the overall heavy metal uptake capacity in the plants.	[44]

TF for targeted metal removal, enhancing remediation efficiency[48,49].

Based on an extensive literature review and field surveys conducted in a mining region, four plant species naturally adapted to such environments were selected for this study. These plant species were never used for AMD treatment, hence to find out the removal efficiency and withstanding capacity was used in the experiments also these species were found growing in mine area. Pot experiments were designed to assess their growth, resilience, and ability to uptake and transport metals such as aluminum (Al), cobalt (Co), iron (Fe), manganese (Mn), magnesium (Mg), and zinc (Zn) when irrigated with AMD. Utilizing weeds and ornamental plants in AMD remediation holds great potential, addressing both water scarcity and drainage pollution in mining areas. This approach can significantly contribute to AMD treatment while allowing for its comprehensive use. Whether it is used to safely cultivate other beneficial crops, extract valuable metals through phytoextraction, or produce high-biomass plants for biofuel, phytoremediation is an emerging technique that supports lucrative land use. Nevertheless, more research is still needed on the possible applications of weed species and ornamental plants in phytoremediation techniques for soil areas contaminated by AMD.

2. Materials and Methods

2.1. Soil Sampling

The soil samples were collected from the garden area of the Mining Engineering department, IIT Kharagpur, air-dried, ground using a mortar and pestle, and then sieved through a nylon mesh with openings smaller than 2 mm. A composite sample was prepared by combining equal weights of the individual soil samples to ensure homogeneity.

2.2. Lab Scale Greenhouse Experiment

An experimental study was conducted in the Department of Mining Engineering, IIT Kharagpur, for three months, utilizing a greenhouse environment. Four plant species, namely Chamber bitter (*Phyllanthus urinaria)*, Fringed Spider Flower (*Cleome rutidosperma),* Rose balsam (*Impatiens balsamina*), and Flat top mille grains (*Oldenlandia corymbosa*), were selected for investigation. Five treatment groups were established, denoted as S0 (control), S1 (Phyllanthus urinaria), S2 (Cleome rutidosperma), S3 (Impatiens balsamina), and S4 (Oldenlandia corymbosa). Uniform-height seedlings were chosen for the study and transplanted into designated pots. The pots, diameter is 19.5 cm and height is 15 cm were filled with 1.5 kg of soil. Four replicates of each treatment were arranged in a completely randomized manner. Throughout the experiment, the greenhouse

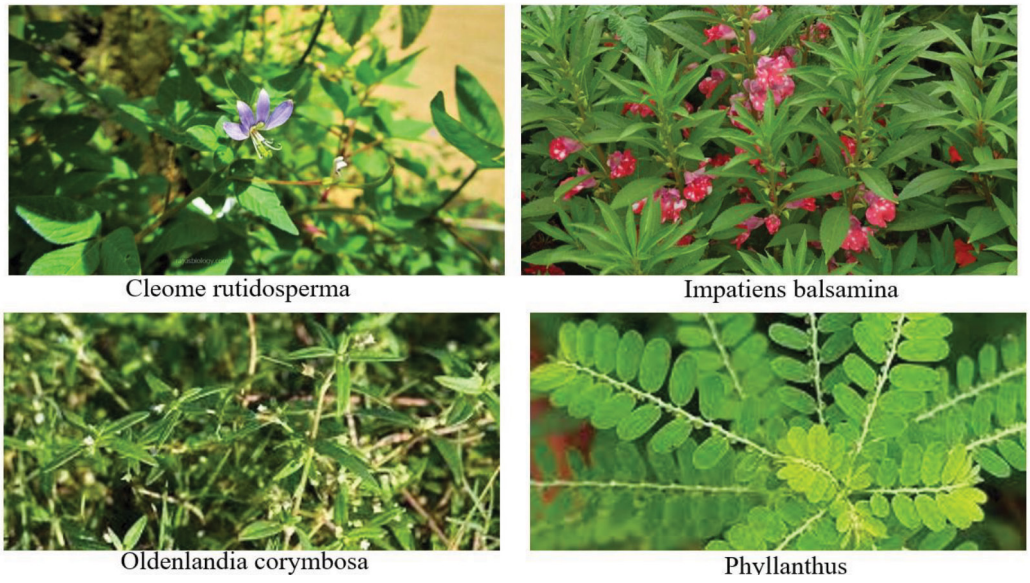


Figure 1. Plant species used for the experiment.

maintained a daytime temperature of 36±3 °C and a nighttime temperature of 22±2 °C. After 90 days of plant growth, samples of both soil and plants were collected for analysis. Plant species used are shown in (Figure 1).

2.3. Mine Effluent Characterization

The phytoremediation is aimed mainly at the target contaminants: As, Hg, Pb, Cd, Cr, Cu, Zn, Ni, Se, F, P, S, Mn, Fe, and V. In the laboratory, simulated AMD was prepared to replicate the average concentration of pollutants observed in the mine area of copper mine in Jharkhand. This simulation aimed to model the typical composition of AMD, including acidity, sulfate, and various metals. Specifically, metal concentrations (introduced as metal sulfate salts) such as Fe, Mn, Al, Co, Zn, and Mg were maintained at mean values of 100±1.8, 6.02±0.1, 25±1.02, 1.00±0.01, 12.05±0.01, and 2.10±0.02 mg/L, respectively. The specification of characteristics of mine effluent is given in (Table 3).

2.4. Plant Harvesting and Sample Collection

After the 3-month growth period, plant tissues were harvested, and soil samples were collected for analysis. The above-ground biomass (shoots) and root tissues were separated manually for each replicate and species by cutting the plants at the stem base with a scalpel. Roots were carefully extracted using tweezers and by hand, with special attention to avoid mixing species, particularly in pots where roots were tightly interwoven. Roots at the bottom of the pots were excluded due to the difficulty in distinguishing the fine root hairs. Both shoots and roots were rinsed once with tap water and then three times with deionized water to remove any remaining soil particles, following the method outlined by[50]. The chemicals used were purchased from Merck. The plant tissues were then oven-dried at 50°C for 72 hours, and their weights were recorded before and after drying. The dried plant material was stored in polyethylene containers and manually

Table 3. Mine effluent characteristics

Source	Component	Concentration (mg/L)
$FeSO_4.7H_2O$	Fe	100
$MnSO_4.H_2O$	Mn	6
$Al_2(SO_4)_3.16H_2O$	Al	25
$CoSO_4.7H_2O$	Co	1
$ZnSO_4.7H_2O$	Zn	12
$MgSO_4.7H_20$	Mg	2

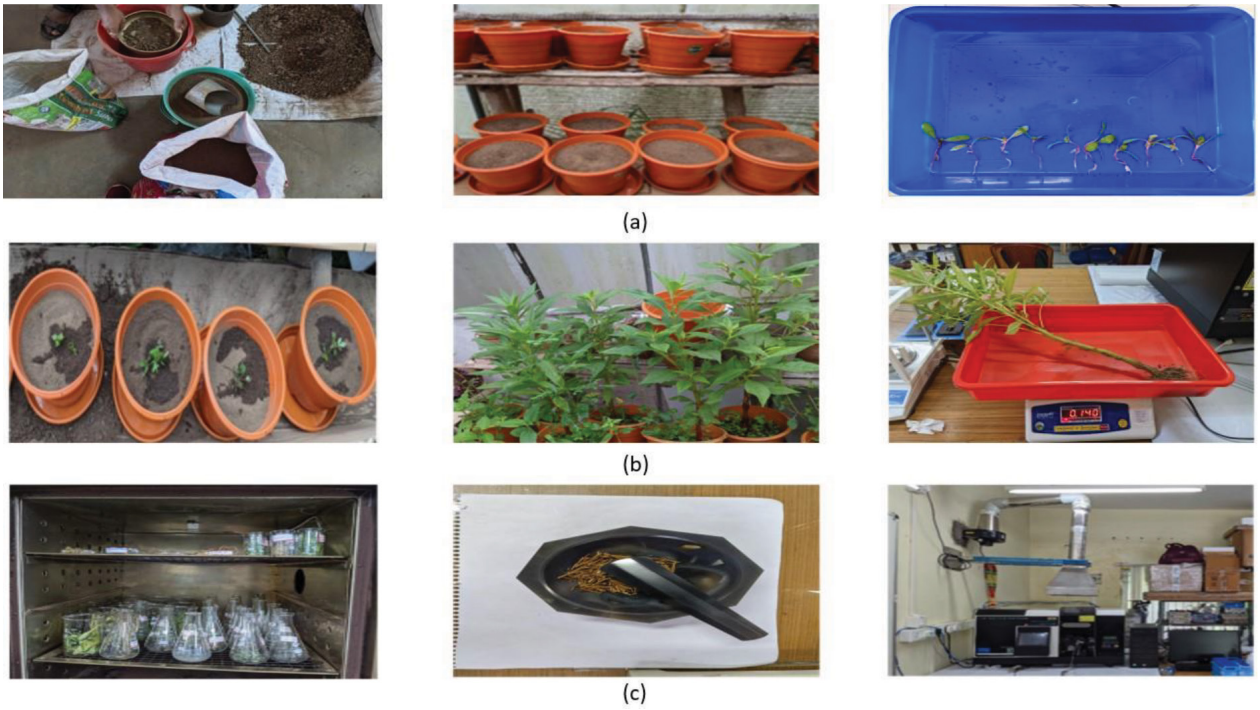


Figure 2. Experimental images (a) initial, (b) during, (c) after harvest.

ground. After plant extraction, the soil was air-dried, crushed, sieved to less than 2 mm, and homogenized for each replicate pot.

2.5. Soil and Plant Analysis

Soil pH was determined using a pH meter with an electrode, following a 1:2.5 soil-to-distilled water ratio, and allowing 30 minutes for equilibration. Both soil and plant samples were digested using a Milestone microwave digestion system. For total metal analysis, 100 mg of soil was combined with 3 ml of 70% nitric acid and 1 ml of 98% hydrochloric acid, followed by digestion. The digested samples were then filtered and analyzed using Atomic Absorption Spectroscopy (Motras, AAS). The entire process, from soil sieving to plant harvesting and sample analysis, is illustrated in (Figure 2).

2.6. Analysis of the Experimental Data

Statistical analysis was carried out. The results are presented as the average of triplicate samples. For plant samples, the data are expressed in milligrams per kilogram of dry weight (mg kg^{-1} DW), whereas soil sample values are reported in milligrams per kilogram of soil (mg kg^{-1}). Additionally, the Bioconcentration Factor (BCF) and Translocation Factor (TF) were calculated to evaluate the phytoremediation potential of the plants, as described by the following equations.

$$TF = \frac{\text{metal concentration in shoot}\left(\frac{mg}{kg}dw\right)}{\text{metal concentration in the root}\left(\frac{mg}{kg}dw\right)} \quad (1)$$

$$BCF\ shoot = \frac{\text{metal concentration in shoot}\left(\frac{mg}{kg}dw\right)}{\text{metal concentration in soil}\left(\frac{mg}{kg}dw\right)} \quad (2)$$

$$BCF\ root = \frac{\text{metal concentration in root}\left(\frac{mg}{kg}dw\right)}{\text{metal concentration in soil}\left(\frac{mg}{kg}dw\right)} \quad (3)$$

3. Results and Discussion

3.1. Plant Growth

The initial and final lengths of the root and shoot parts of the plants were measured using a scale. Amongst all the plant species, the root and shoot biomass of *Oldenlandia corymbosa* was greatest than the other three. The growth of plant parts is presented in (Table 4) and is graphically represented in (Figure 3) and (Figure 4).

3.2. Metal Concentration in Root and Shoot Tissues of Plants

The metal concentrations in the root and shoot tissues of the four plant species. Overall, the plants exhibited higher metal accumulation in their roots compared to the shoots. The variations in metal concentrations between the species may be due to differences in their physiological and ecological traits. Among the plants, Olden showed the highest concentrations of Zn and Mg in its shoots. Similarly, Phyllanthus displayed notable Zn uptake, consistent with previous studies on soil contaminated by spent engine oil. Fe and Mn accumulation was greatest in Impatiens, while trace amounts of Al were detected in the roots of Cleome and Impatiens. None of the species accumulated Co. There was noticeable change in pH it

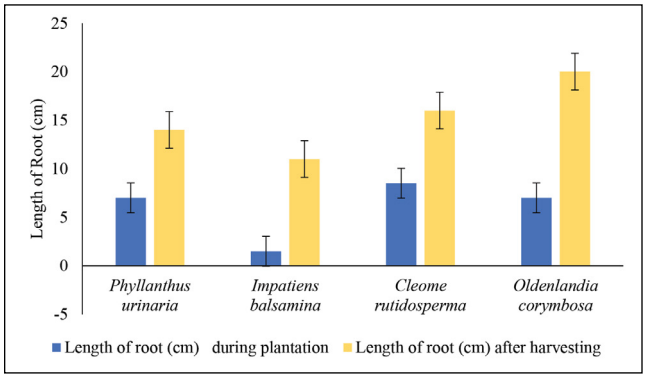


Figure 3. Root growth in plants.

Table 4. Root length before and after the experiment

Plant	Length of root (cm) during plantation	Length of root (cm) after harvesting
Phyllanthus urinaria	7	14±2
Impatiens balsamina	1.5	11±2
Cleome rutidosperma	8.5	16±2
Oldenlandia corymbosa	7	20±2

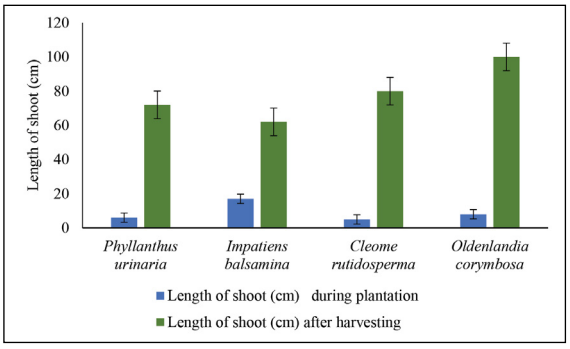


Figure 4. Shoot growth in plants.

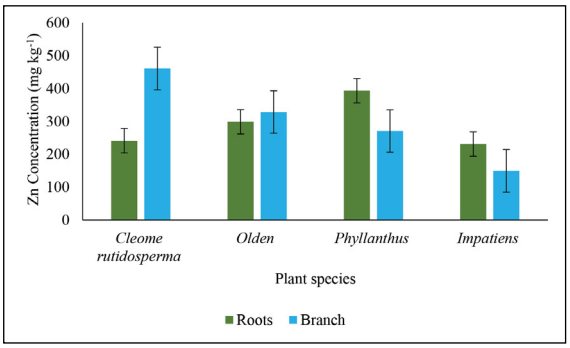


Figure 5. Zn accumulation in root and shoot tissues of the plants.

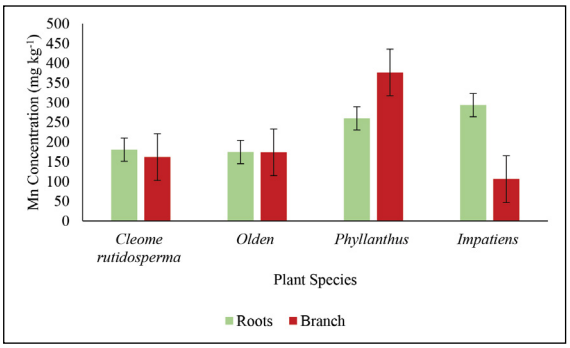


Figure 6. Mn accumulation in root and shoot tissues of the plants.

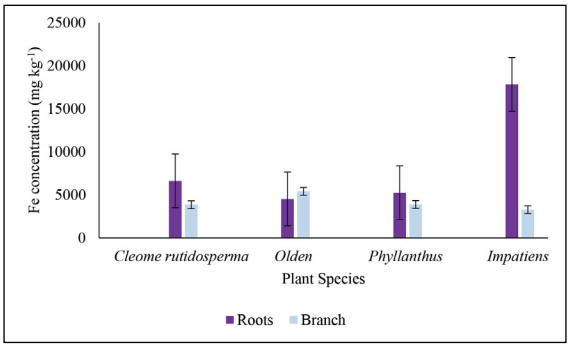


Figure 7. Fe accumulation in root and shoot tissues of the plants.

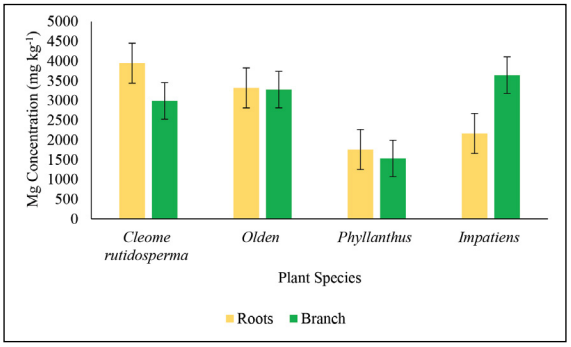


Figure 8. Mg accumulation in root and shoot tissues of the plants.

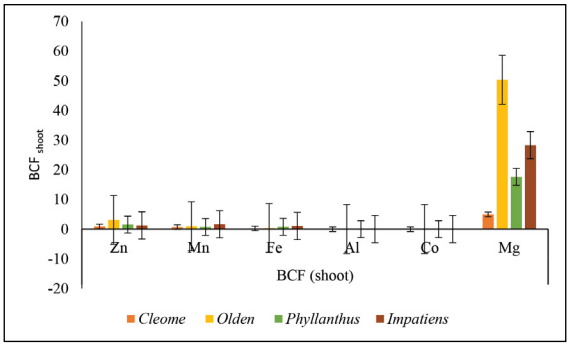


Figure 9. Bio-concentration factor of plant species.

has increased when compared to the pH in the starting of the experiment. The accumulation patterns of Zn, Mn, Fe, and Mg are illustrated in (Figures 5, 6, 7, 8).

3.3. Bio-Concentration Factor (BCF)

BCF is also called the phytoextraction coefficient. BCF can be well defined as the accumulation capacity of the plant from the substrate into its shoot[51]. If the BCF value is ≥ 1, that plant can be considered for phytoremediation[51]. The BCF values for the shoot of the plant species are presented in (Table 5), and its pictorial representation is shown in (Figure 9).

Olden showed a high accumulation of Zn among all four species, followed by *Phyllanthus, Impatiens, and Cleome*. The Mn was observed high in the shoot parts of *Impatiens,* followed by *Olden, Phyllanthus, and Cleome*. Fe was accumulated more in the shoot parts of *Impatiens* in the order *Phyllanthus, Olden, and Cleome*. Al was below the detection limit for all four species. This might be due to plants being intolerant to Al and having formed barriers

Table 5. Bioconcentration factor of the plant species

Plant species	BCF (shoot)					
	Zn	**Mn**	**Fe**	**Al**	**Co**	**Mg**
Cleome	0.91	0.69	0.25	NA	NA	4.96
Olden	3.10	0.97	0.38	NA	NA	50.31
Phyllanthus	1.54	0.72	0.75	NA	NA	17.63
Impatiens	1.24	1.64	1.07	NA	NA	28.30

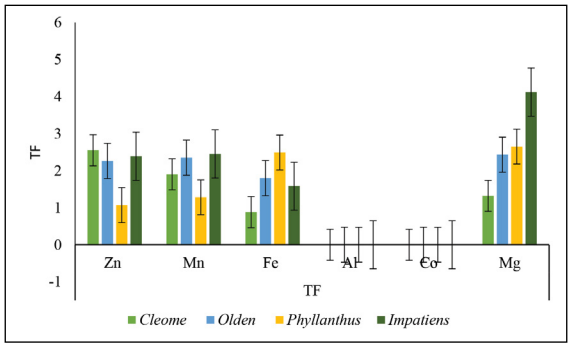


Figure 10. Translocation factor of plant species.

at roots so that plants could not accumulate them[52]. Co is a trace element; species accumulation depends on their availability and selective uptake[53]. Magnesium uptake was higher in *Olden* > *Impatiens* > *Phyllanthus* > *Cleome*. *Olden* performed well in accumulating Zn and Mg, among other species.

3.4. Translocation Factor (TF)

TF measures a plant's ability to translocate a contaminant from its roots to its above-ground tissues, such as the shoot or leaves[53]. It quantifies the degree of translocation of a contaminant within the plant. The TF values of the plant species are represented graphically in (Figure 10).

Cleome has a higher TF value for Zn, followed by *Impatiens* > *Olden* > *Phyllanthus*. The TF value for Mn is high in *Impatiens* > *Olden* > *Cleome* > *Phyllanthus*. The TF for Fe *is Phyllanthus* > *Olden* > *Impatiens* > *Cleome*. *Impatiens* has the highest TF for Mg, followed by *Phyllanthus* > *Olden and Cleome*. For Mn and Mg, Impatiens have high TF. TF and BCF are essential tools to estimate if a plant is efficient for phytoremediation. If the BCF value is ≥ 1 and the TF value is ≤ 1, such a plant is considered a phytostabiliser[54]. Also, if the BCF and TF value is ≥ 1, those plants can be regarded as phytoextractors[55]. If the TF value is < 1, the plant species is a metal excluder[56].

4. Conclusions

The results showed that all plant species are sustainable in an acidic environment with a pH value (2.60-3.20). These species can grow naturally without the supply of additional nutrients or additional substrate. Ferrous ions are consumed in a maximum quantity ranging from 4 g kg^{-1} to 30 g kg^{-1} of biomass, and the minor metal ion accumulated is Mn in the range from 0.1 g kg^{-1} to 0.7 g kg^{-1} of biomass. This might be due to the formation of Mn plaque around the plant species roots. None of the four plant species accumulated Al and Co. All species are considered phytoextractors for Mg. Impatiens exhibit as phytoextractors for Zn, Mg, and Fe. *Cleome* as a metal excluder. *Olden* and *Impatiens* exhibit massive accumulations of Mg and can be suitable phyto-remediating species for TDS and hardness removal. The study showed that these species could be good phytoremediators for treating AMD polluted water and soil among all Olden is the best. Further study is essential to know the efficiency of the species by increasing the ion concentration and varying environmental conditions.

5. Acknowledgments

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6. Declaration of Competing Interest

The authors declare that there are no known financial or personal conflicts of interest that could influence the results or interpretations of this study.

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