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RESEARCH ARTICLE

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The Effectiveness of Lotus (*Nelumbo nucifera* G.) with Various Growing Media for Phytoremediation of Acid Mine Drainage

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ABSTRACT

Acid mine drainage (AMD), characterized by high acidity and concentrations of heavy metals that can damage aquatic ecosystems, poses a serious environmental problem. This research aimed to analyze the effectiveness of *Nelumbo nucifera* Geartn. grown using a Floating Wetland System (FTW), treated with topsoil or bokashi, in altering pH and reducing heavy metals in coal mine AMD. The experiment was conducted for 14 days in a sedimentation pond of post-mining land at PT Bukit Asam, Palembang, Indonesia. Two FWS units were installed on the pond's surface: one was enriched with topsoil, while the other was with bokashi. Fifteen *N. nucifera* plants were grown in each floating reactor, with plants grown directly in the AMD without the FWS used as the control group. Plant growth, media pH, and heavy metal contents were monitored during and after treatment. The results indicate that the system was capable of increasing the initial highly acidic AMD pH (pH 2.8) to a range close to neutral (6.5–6.9). The concentrations of Fe and Mn metals were significantly reduced through the absorption mechanism of roots, stems, and leaves, with an efficiency of more than 90%. XRD analysis also revealed the formation of secondary mineral phases that support vegetative growth in both reactors. These findings confirm that the FWS installed with the bokashi ameliorant and *N. nucifera* has great potential as a sustainable solution for acid mine drainage remediation.

Introduction

Coal mining activities, while contributing significantly to economic growth and energy supply, also have serious environmental impacts, one of which is the formation of acid mine drainage (AMD) [1]. Mine acid drainage is a waste product resulting from the oxidation of sulphide minerals exposed to air and water, which is highly acidic and contains dissolved heavy metals in high concentrations, such as leachate, seepage, or drainage. If not managed effectively, AMD can contaminate surface water bodies, damage aquatic habitats, and disrupt the balance of aquatic ecosystems [2].

In Indonesia, open-pit mining remains the primary method used in coal exploitation activities. This situation exposes sulphide-bearing rocks directly to atmospheric oxygen and water, accelerating oxidation reactions that produce acid mine drainage [3]. AMD typically has a very low pH (<4) and contains dissolved heavy metals such as iron (Fe) and manganese (Mn) at high concentration exceeding environmental quality thresholds. Managing AMD is a major challenge in the post-mining phase, given its potential to contaminate aquatic ecosystems. Various strategies have been implemented, both through active chemical-based approaches and passive approaches involving biological processes, such as vegetation systems. According to the Decision of the Minister of Environment of the Republic of Indonesia No. 113 of 2003, the maximum permissible limits for metal content in liquid waste from coal mining activities are set at 7 mg/L for iron (Fe) and 4 mg/L for manganese (Mn) [4].

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Phytoremediation is one of the waste management approaches based on the use of plants, with the advantages of being environmentally friendly [5], cost-effective, and sustainable [6,7]. This method is based on the biological ability of plants to absorb, accumulate, stabilise, or transform pollutants from contaminated environments, including in the context of managing mining waste such as acid mine drainage (AMD). One practical application of this technology is the floating treatment wetland (FTW) system, which allows plant roots to hang directly in the water column, thereby enhancing the efficiency of contact between the plant tissue and dissolved toxic compounds [8]. Another advantage of implementing FTW is the ease of replacing new plants on the raft when they reach maturity, ensuring that heavy metals have been absorbed into the plant body [9]. In AMD management, there are two main approaches: active treatment and passive treatment. Active treatment typically involves adding alkaline compounds to neutralise acidity and precipitate metals, but this method has limitations in terms of high operational costs and intensive maintenance requirement [10]. In contrast, passive treatment, such as the use of constructed wetland systems with vegetation, offers advantages in terms of simplicity, long-term efficiency, and relatively low costs. If designed and managed optimally, plant-based passive treatment systems can provide effective and sustainable solutions for tap-watering AMD at the mine landscape scale [11].

Various studies have reported the ability of several aquatic plant species to absorb and stabilise heavy metals from contaminated environments, making them potential phytoremediation agents. Some of these include *Nelumbo nucifera* [12,13], *Typha latifolia* [14], *Eichornia crassipes* [15], *Hydrilla verticillate* [16], *Lemna minor* [17], *Typha angustifolia* [18], *Marsilea crenata* [19], *Eleocharis dulcis* [20], *and Pistia stratiotes* [21], each of which demonstrates a specific capacity to accumulate heavy metals through its root systems and vegetative tissues. *Nelumbo nucifera* G. is an ecologically adaptive species due to its ability to optimally adapt to various aquatic environmental condition, rapid growth rate, and strong accumulative capacity for various types of heavy metals such as Fe, Mn, Pb, and Cr. Its dense and long root system allows for an increase in the contract area between the plant and the contaminated medium [12]. Previous studies have also shown that heavy metals can be distributed throughout the plant's tissues, including roots, stems, rhizomes, and seeds, without causing noticeable toxicity symptoms, thereby supporting the long-term potential of *N. nucifera* in the passive and sustainable tap-water of acid mine drainage [22].

The success of phytoremediation is influenced by the growing medium used, as it determines the availability of nutrients, microbial activity, and the chemical buffering capacity of the environment. Several phytoremediation media have been commonly used, such as sand, compost, or artificial substrates. Bokashi is a fermented compost that can be a nutrient source for biological soil improvement. However, the use of bokashi combined with topsoil has been limited, especially when combined with lotus as a phytoremediation agent. Bokashi contains organic matter and active microorganisms that can improve nutrient cycling [23]. Furthermore, enhancing the quality of phytoremediation can be achieved by combining topsoil to provide a natural matrix with good buffering capacity, efficiency, and low cost. The ease of cultivation of lotus also strengthens the use of this combination in the phytoremediation process. In this research, lotus was planted on a raft so that the roots dangling into the water functioned as a biological filter [24]. In several previous studies, floating raft systems have been implemented as an environmentally friendly method for treating various types of polluted water [25]. However, the use of lotus in floating raft systems supplemented with bokashi and topsoil are still limited. Therefore, this study aims to investigate the effectiveness of combining organic (bokashi) and mineral (topsoil) media in supporting the phytoremediation capacity of lotus toward heavy metal reduction in acidic mine drainage, using a floating raft system directly applied at an active mine site. This research aims to analyze the effectiveness of *Nelumbo nucifera* G. in altering pH, reducing heavy metals (Fe and Mn), plant growth response, and metal accumulation in tissues for rehabilitating acidic mine drainage. This research is the basis for developing vegetation-based tap-water technology using local vegetation and integrated media in mining locations.

Materials and Methods

Research Location

The research site is located in the operational area of PT Bukit Asam Tbk in Tanjung Enim, South Sumatera Province, Indonesia, with coordinates of 03°43'37.704"N and 103°48'55.350"E (Figure 1). The phytoremediation was conducted in situ in a sedimentation pond (7 m x 5 m x 2.5 m), which serves as the main storage medium for acid mine drainage. The study used floating raft system made of PVC pipes and equipped with polyethene nets as a planting medium retainer as depicted in Figure 2. The floating raft was designed using 4-inch diameter polyvinyl chloride (PVC) pipes and elbows and equipped with polyethene (PE) nets to support the planting media. Each raft unit had dimensions of 6 m x 4 m, with a planting media thickness of 12 cm, and was installed in a compartment pond with a depth of 2.5 m. To regulate the flow rate and maintain stable air circulation during the experiment, a stop valve was installed at the outlet of the pond.

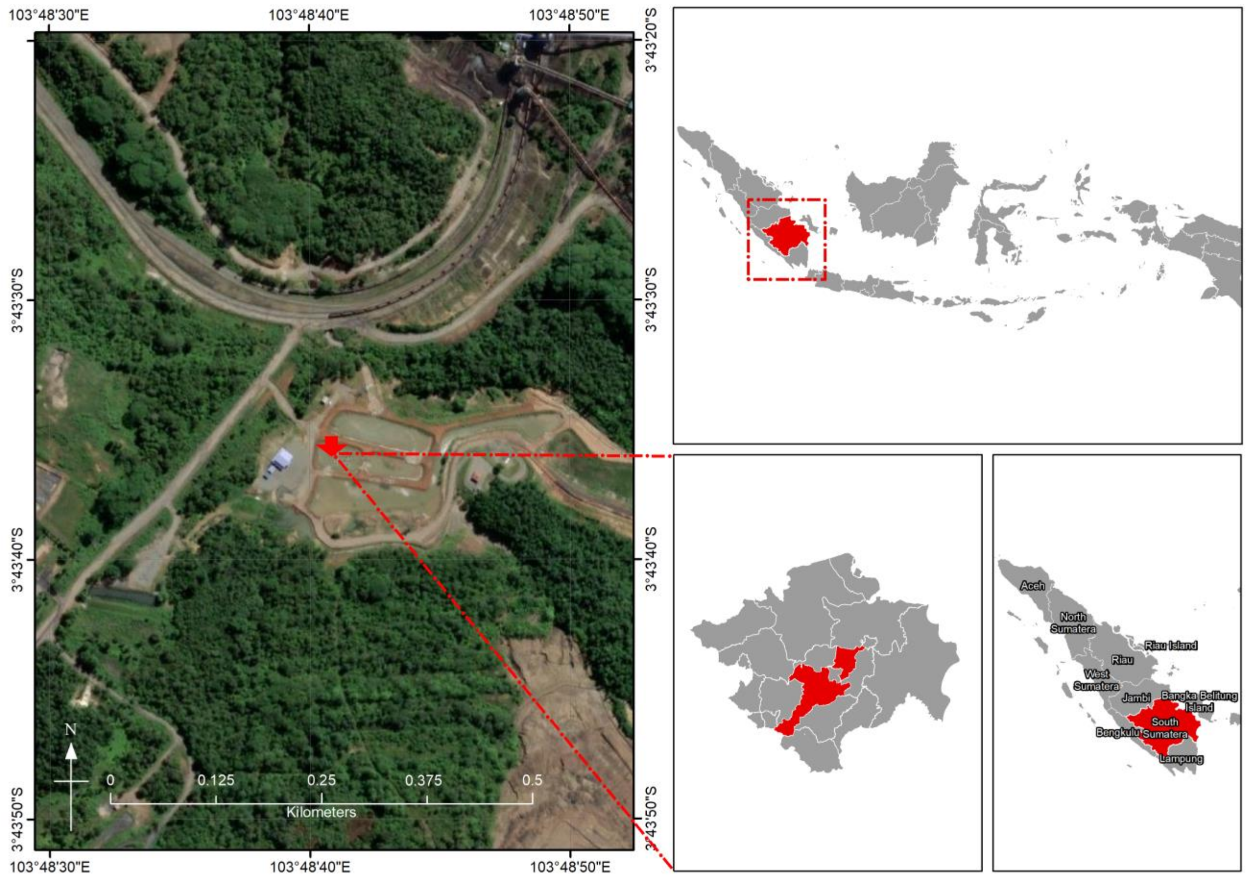


Figure 1. Research study area at Pit 03 Banko Barat 04 PT Bukit Asam Tbk., Muara Enim, South Sumatera Province, Indonesia.

Procedure

N. nucifera seeds were obtained from a population of mother plants that grew naturally in Muara Belida Regency, South Sumatra Province, Indonesia. To stimulate uniform germination, the seeds were scarified by slightly scraping the outer skin (testa) using a scalpel to accelerate water penetration into the embryo. After this process, the seeds were soaked in settled water (chlorine-free) and placed under natural sunlight at an optimal temperature of 25–30 °C for 4 to 7 days. The soaking water was changed twice a day to prevent the growth of pathogenic microorganisms and to maintain aerobic conditions that support early physiology, until the emergence of radicles and plumules. After 30th day, the lotus seedlings entered the floating leaf formation phase (Figure 2 and Figure 3). This duration was determined based on physiological considerations referring to previous studies [26]. Then the seedlings were grown for 30-days acclimatization before the plants were applied to the planting medium. After the acclimatization period was completed, the plants were transferred into a floating bed system installed above the sludge settling pond, which receive the flow of acid mine drainage (Figure 2).

The media used were topsoil, bokashi, and tap-water using tap-water. Each raft unit was planted with 15 *N. nucifera* individuals that had undergone germination and acclimatisation for 30 days. In addition, the planting media were composed of a mixture of topsoil (72 kg) and bokashi fertilizer (72 kg), which were each tested on different rafts (Figure 2). As an initial step, 420 kg of dolomite lime was added to the pond to increase the initial pH of the acid mine drainage. As a tap-water, a floating raft without vegetation and planting media was used to compare the effectiveness of the system in reducing heavy metal concentrations and pH. Periodic observations were conducted to monitor changes in water quality, biomass growth, and metal accumulation in plant tissues as indicators of remediation effectiveness over 14 days.

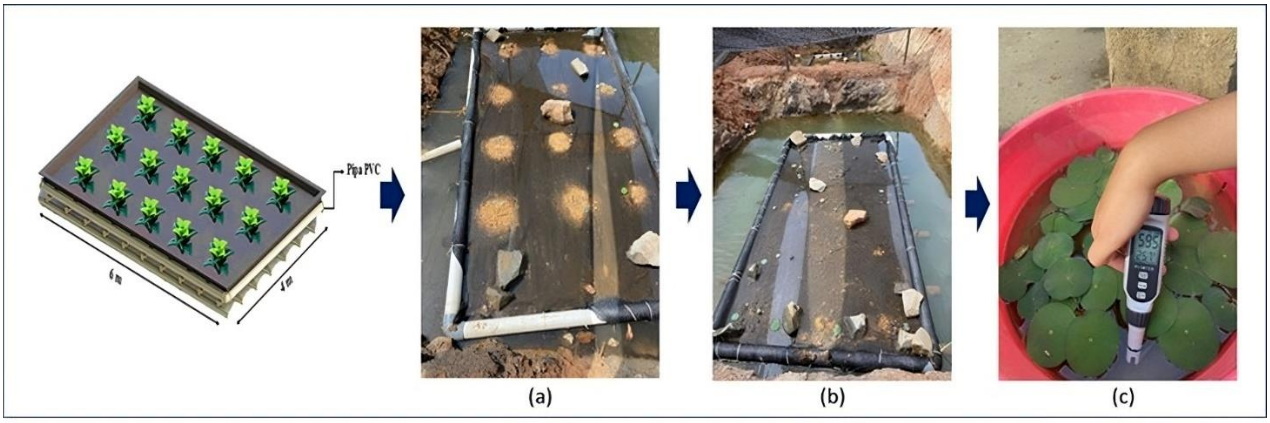


Figure 2. Design of the floating treatment wetland (FTW) raft constructed for two treatments, where the *Nelumbo nucifera* were grown: (a) an FTW treated using topsoil and (b) another FTW treated using bokashi, while control plants were grown in plastic buckets using tap water (c).

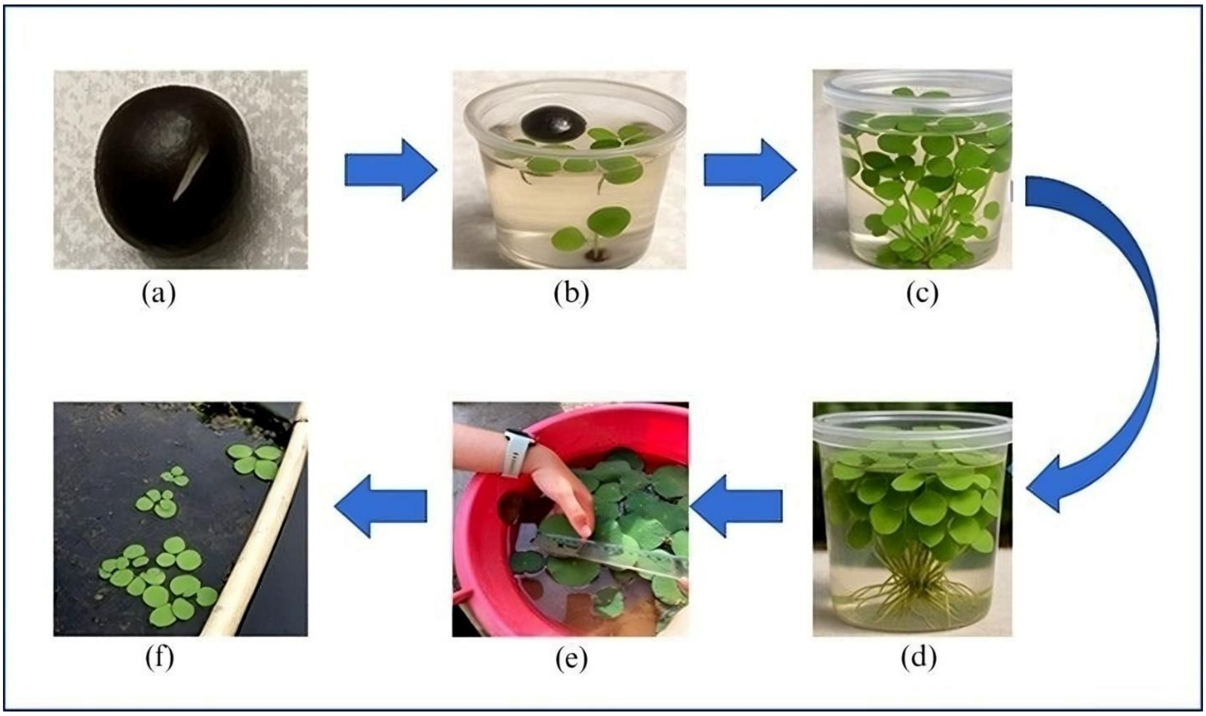


Figure 3. *Nelumbo nucifera* preparation before planting in the FTW starting from seed until the plants ready to transplant. (a) *Nelumbo nucifera* seed was treated by scarification to improve seed germination, (b) seed soaking process until germination, (c) initial development of leaves and roots, (d) plant after 14 days showing significant root and leaf growth, (e) measurement of plant height and leaf width, (f) plant condition which was ready to be transferred to the phytoremediation pond.

The main parameters considered in the management of acid mine drainage are presence of Fe and Mn metals, given that both are dominant elements that are consistently found in high concentrations in coal mine waste. The selection of these two parameters is based on national quality standards regulations, specifically Minister of Environment Decision No. 113 of 2003, which sets maximum limits of 7 mg/L for Fe and 4 mg/L for Mn.

To evaluate efficacy of acid mine drainage (AMD) phytoremediation, ten specimens of lotus (*Nelumbo nucifera*) were randomly selected from each experimental treatment group. The phytoremediation monitoring process was carried out periodically for 14 days, with observations focused on the neutralisation of acidity (pH), as well as the concentration of Fe and Mn metal ions, of acid mine drainage. After the 14-day phytoremediation period, all plant biomass was carefully harvested for further analysis of metal accumulation and translocation in plant tissues, which aimed to calculate the overall phytoremediation efficiency.

Data Collection

Water quality measurements were carried out using a portable pH meter that had been calibrated using standard buffer solutions (pH 4, 7, and 10) before each field measurement. Analysis of heavy metal concentrations (Fe and Mn) in acid mine drainage was performed periodically from each treatment unit using Atomic Absorption Spectroscopy (AAS), while metal accumulation in plant tissues was analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Plant sample preparation involved washing with distilled water, drying, grinding, and homogenization before analysis. To minimise potential bias, blank analyses and certified reference standards were used as quality tap-waters. All analytical procedures followed the Standard Methods for the Examination of Water and Wastewater [27] and the USEPA heavy metal analysis guidelines [28]. Removal efficiency is calculated as the percentage of Fe and Mn metals in plants and can be determined using the equation below.

$$\eta = \frac{C_0 - C_e}{C_0} \ x \ 100\% \quad (1)$$

Where :

ŋ = Percentage reduction of metal concentration (%)

C_0 = Initial concentration of iron (Fe) and manganese (Mn) (mg/L)

C_e = Final concentration of iron (Fe) and manganese (Mn) (mg/L)

Statistical Analysis

Statistical analysis began with a normality test using the Shapiro-Wilk method to evaluate the distribution of data in each treatment group. The test results showed that most of the data were not normally distributed ($p < 0.05$), so the non-parametric Kruskal-Wallis test was used to compare the differences between treatments. The Kruskal-Wallis test was applied to test the differences between treatment groups based on the mean ranks. Interpretation was based on the statistical value H and the significance level ($p < 0.05$). All analyses were performed using IBM SPSS Statistics software version 22.0.0.1. The presentation of results was intended to enable other researchers to replicate the analysis procedure under similar conditions and research designs.

Results and Discussion

Results

Chemical Interpretation of the Dynamics of pH of Heavy Metal Concentrations in the Growth Medium

Evaluating the capacity of growing media to influence the chemical parameters of acid mine drainage, particularly pH, is a crucial initial step in assessing the overall effectiveness of phytoremediation. Water pH not only reflects the acidity of the environment but also serves as an indicator of chemical reactivity that influences the solubility of heavy metals and the activity of microorganisms in floating wetland systems.

Changes in pH values during the treatment period indicate active chemical interactions between the growing media and acid mine drainage. The addition of topsoil and bokashi media plays a role neutralizing acidic conditions through the release of base cations and the decomposition of organic matter by microorganisms. The dynamics of pH changes during the 14-day observation period are presented in Figure 4. The effectiveness of metal accumulation was calculated to determine the percentage of reduction in Fe and Mn metal concentrations in acid mine drainage (AMD) after the phytoremediation process using *N. nucifera* in the experimental pond system. Based on the measurement results, *N. nucifera* plants planted in topsoil media were able to accumulate Fe metal up to 87.58% and Mn by 73.71% (Table 1), indicating high phytoremediation effectiveness in the media.

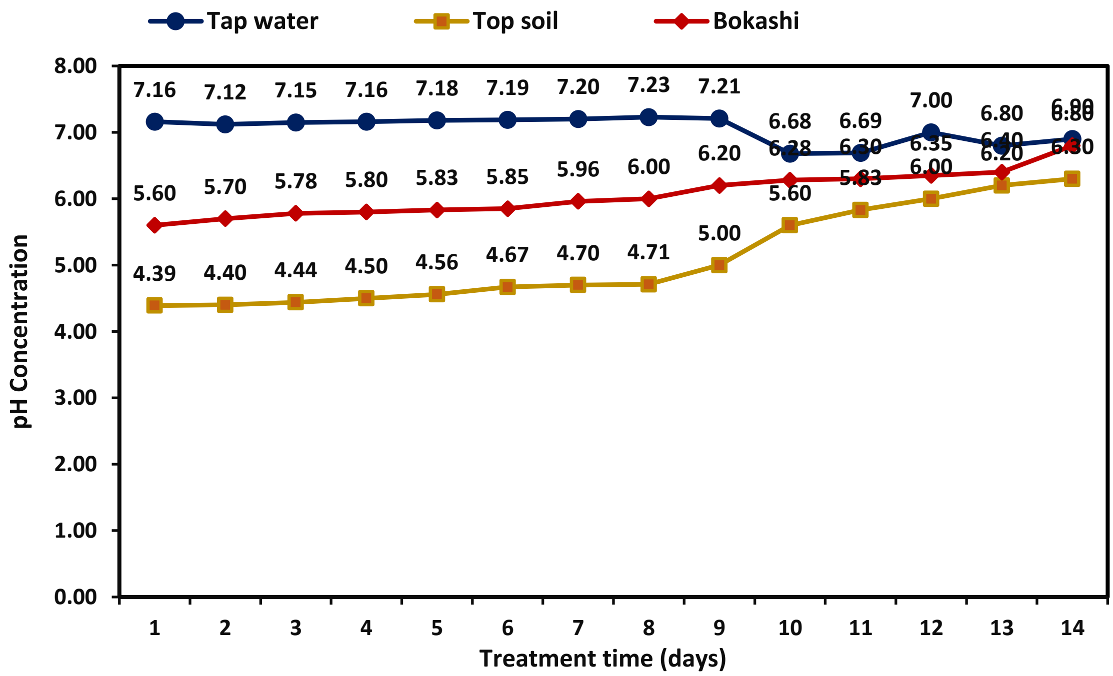


Figure 4. The changes pH values of acid mine drainage during 14 days with different treatments, i.e.: bokashi (red color), topsoil (yellow-brown color), and tap-water treatments (blue color). Both treatment (bokashi and topsoil) were able to increase the pH of the media after 14 days.

One of the main parameters in evaluating the effectiveness of phytoremediation systems is the ability of the growing medium to reduce the concentration of dissolved heavy metals in acid mine waste. Heavy metal concentrations, such as iron (Fe) and manganese (Mn), are often used as indicators of pollution levels and the effectiveness of remediation interventions due to their high solubility and potential toxicity to aquatic biota (Figure 5).

A significant decrease in Fe and Mn concentrations was observed in all treatments after the phytoremediation process lasted for 14 days. In the topsoil medium, the Fe concentration decreased sharply from an initial value of 13.69 mg/L to 2.41 mg/L, indicating similar effectiveness despite the initial value being slightly lower than in topsoil. For Mn metal concentration, the decrease was relatively uniform in both media, with final values of 14.78 mg/L in topsoil and 15.27 mg/L in bokashi, indicating that both media have nearly equivalent capabilities in reducing Mn levels.

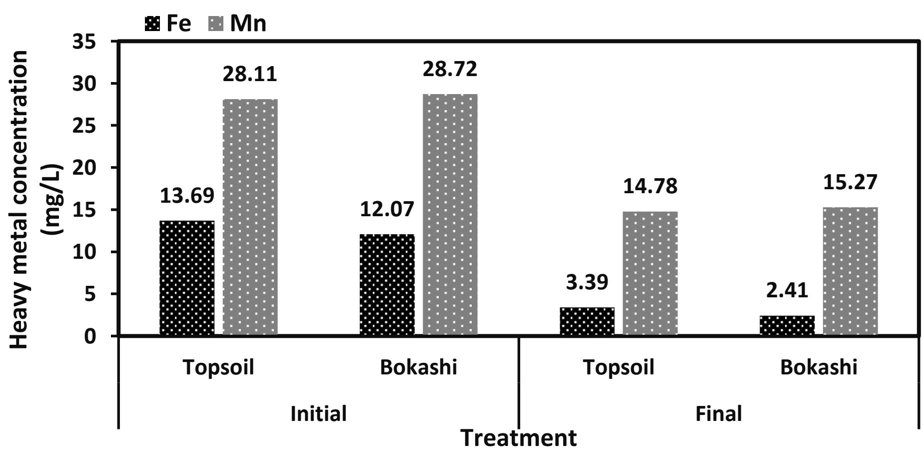
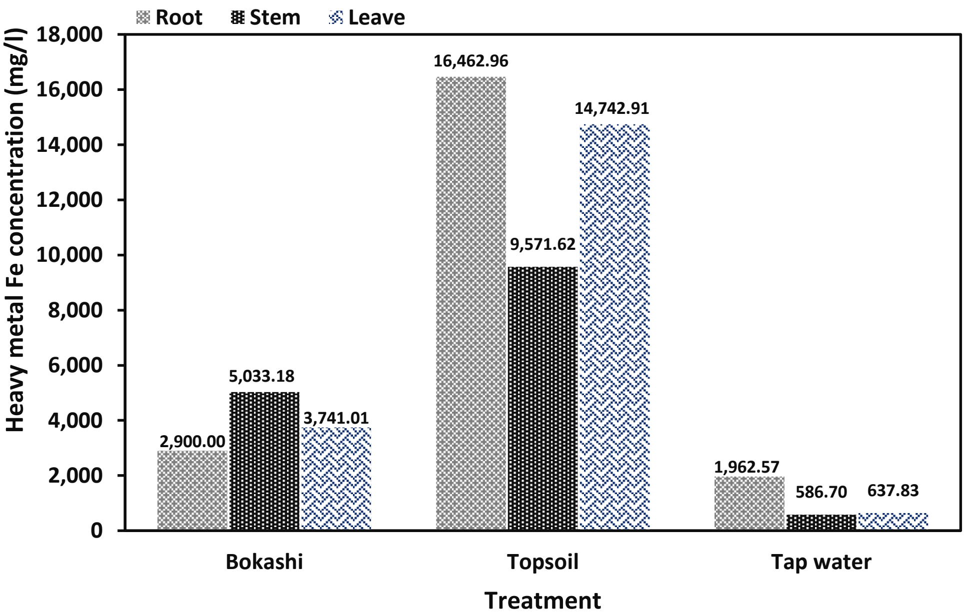


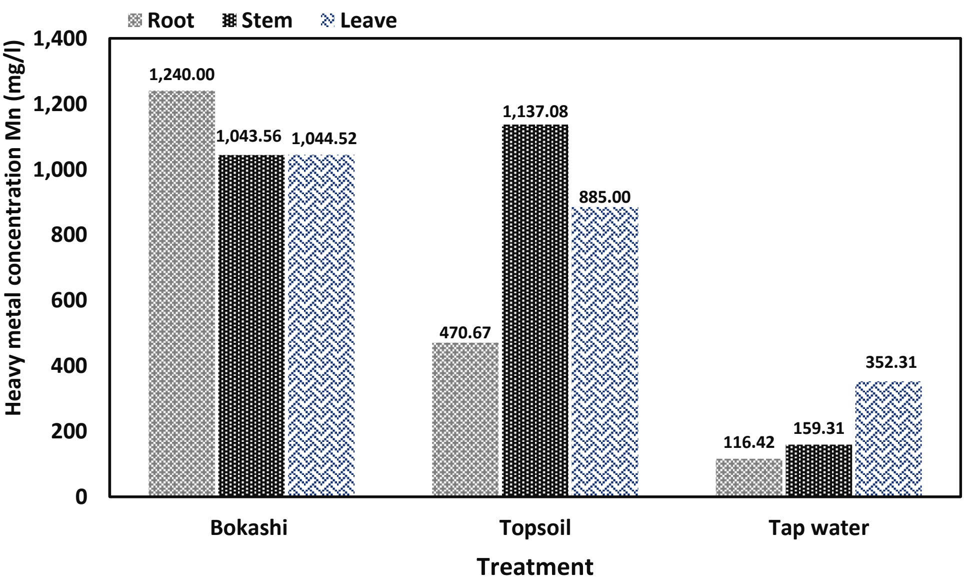
Figure 5. Heavy metal concentration (Fe and Mn) in acid mine drainage (AMD) measured at initial treatment (left figure) and after 14 days of the treatment (right figure), suggesting that the treatment using bokashi and topsoil can reduce metal content after 14 days.

Distribution of Heavy Metal Accumulation in Nelumbo nucifera Plant Parts During Phytoremediation

Distribution of Fe and Mn metal concentrations in the roots, stems, and leaves of plants treated with different planting media against acid mine drainage (AMD). The pattern of metal accumulation in plant tissue showed significant variations between types of planting media and other plant parts (Figure 6).



(a) Fe concentration in roots, stems, and leaves after phytoremediation



(b) Mn concentration in roots, stems, and leaves after phytoremediation

Figure 6. Concentration of heavy metals Fe and Mn in roots, stems, and leaves after phytoremediation in all treatments (bokashi and topsoil) as compared to control plants grown in the tap water.

In the topsoil treatment, the highest Fe concentration was recorded in the roots (16,462.96 mg/kg), followed by leaves (14,742.91 mg/kg) and stems (9,571.62 mg/kg). This indicates that the topsoil media tends to increase Fe bioavailability and supports high accumulation, especially in the root system. In contrast, in the bokashi treatment, Fe accumulation was relatively lower and more evenly distributed between the stems (5,033.18 mg/kg) and leaves (3,741.01 mg/kg), with lower accumulation in the roots (2,900 mg/kg). In the tap-water treatment, the lowest Fe concentration was observed in all plant organs, with a maximum value of 1,962.57 mg/kg in the roots (Figure 6a).

The distribution of Mn showed a different pattern (Figure 6b). In the bokashi treatment, the concentration of Mn was almost evenly distributed throughout the plant organs, with a value of around 1,240–1,044 mg/kg. This distribution reflects the relatively more mobile nature of Mn compared to Fe, as well as the possible influence of microbial activity from bokashi on Mn mobilisation. For the topsoil treatment, Mn showed the highest concentration in the stem (1,137.08 mg/kg), followed by leaves (885 mg/kg) and roots (470.67 mg/kg). This pattern supports the nature of Mn as a metal that is easily translocated through the plant vascular system, especially under oxidative conditions. In the tap-water treatment, the concentration of Mn was lower but showed a significant increase in the leaves (352.31 mg/kg).

Absorption Efficiency

N. nucifera has a good capacity to accumulate Fe and Mn, with significant variations between plant organs and influenced by the type of growing medium used (Table 1). The efficiency of Fe accumulation in topsoil treatments reached nearly 100% in all organs, i.e.: roots (99.09%), stems (99.06%), and leaves (99.53%). This condition reflects the role of roots as the primary organ in contact with metal-rich sediments, as well as the presence of a mechanism for translocating metal ions to stem and leaf tissues through the vascular system.

The use of bokashi media resulted in relatively high metal accumulation efficiency, ranging from 94.83% to 99.01%. This high efficiency can be explained by two main mechanism: the presence of organic matter in bokashi increases cation exchange capacity and forms organo-metal complexes that are more easily absorbed by roots, and the presence of microbial activity. In addition, in the tap-water treatment, metal accumulation efficiency was slightly lower, especially in the stems (84.66%) and leaves (89.03%). However, efficiency in the roots remained relatively high (92.36%). This phenomenon indicates that even though the medium was not enriched with organic matter, *N. nucifera* has a natural capacity to absorb metals from acid mine drainage (AMD) solutions.

Table 1. Efficiency of Fe and Mn metal absorption in *Nelumbo nucifera* G. during phytoremediation of acid mine drainage with different growing media i.e.: bokashi, topsoil, and tap water.

Plant parts	Metal absorption efficiency (%)					
	Bokashi		Topsoil		Tap water	
	Fe	Mn	Fe	Mn	Fe	Mn
Root	94.83	91.94	99.09	78.75	92.36	14.10
Stem	99.01	92.33	99.06	92.96	84.66	49.78
Leave	98.13	91.86	99.53	90.40	89.03	75.87

The efficiency of Mn accumulation by *Nelumbo nucifera* indicates a more complex dynamic than Fe, characterised by fluctuating distribution patterns between plant organs and between growing media treatments. The highest values in topsoil treatments, particularly in stem tissue (92.96%) and leaves (90.40%) can be interpreted as a consequence of Mn's relatively higher chemical mobility in soil solutions compared to Fe. Mn tends to exist in the form of divalent ions that are more easily translocated through the xylem to photosynthetic tissues, resulting in greater accumulation in above-ground organs.

Statistical Analysis of Metal Accumulation in Nelumbo nucifera

Statistical analysis was performed to evaluate differences in heavy metal accumulation between plant parts and between types of growing media. The Shapiro-Wilk normality test showed that some data groups did not meet the assumption of normal distribution ($p < 0.05$), so an alternative nonparametric method was used, namely the Kruskal-Wallis test, which does not require normal distribution or homogenity of variance. Based on the results of the Kruskal-Walllis test, no statistically significant differences were found between treatments for the analyzed variables ($H = 1.483$; $p = 0.476$) (Table 2). However, the pattern of variation in average response rankings between groups showed a biological response trend that was not strong enough to produce statistical significance, so further investigation with a larger data set or a more sensitive experimental design is needed.

Table 2. Results of Kruskal-Wallis test assessing the effect of heavy metal content on the efficiency of FTW-based phytoremediation in achievement drainage. The table compares variation across different growing media and metal types, indicating whether heavy metal concentrations were significantly influenced by the remediation processes.

Variable	Average rating	Test statistics	Metal concentration
Types of plant organs			
Root	19.50	Kruskal-Wallis H	0.284
Stem	18.75	Asymp. Sig. (p)	0.868
Leave	17.25		
Type of treatment			
Tap water	15.67	Kruskal-Wallis H	1.483
Bokashi fertilizer	19.00	Asymp. Sig. (p)	0.476
Topsoil	20.83		

Growth Response of Nelumbo nucifera (Height and Leaf Width)

The survival rate of *N. nucifera* indicated a significant response to differences in growing media only 33.3% of individuals were able to survive in the tap-water group, reflecting the plant's limited ability to adapt to extreme environmental pressures (Table 3). The tap-water medium, which was not modified, tended to have less supportive chemical characteristics, marked by low pH due to acid influence, as well as limited availability of macro and micronutrients. These factors caused excessive osmotic and ionic stress on plant tissues, thereby disrupting basic physiological functions such as water absorption, nutrient ion availability, and cell membrane stability.

Table 3. Survival rate of *Nelumbo nucifera* after 14 days phytoremediation of acid mine drainage using FTW under different growing media i.e.: bokashi, topsoil, and tap water.

No	Treatment	Initial plants (n)	Surviving plants (n)	Survival rate (%)
1	Tap water	15	5	33.3
2	Topsoil	15	6	53.3
3	Bokashi	15	10	66.7

Observations of the morphological parameters of *N. nucifera* in this study provide a comprehensive picture of the physiological response of plants to differences in growing media over a period of 14 days. Leaf height and width were used as the main indicators because they reflect physiological activities closely related to photosynthesis, biomass distribution, and plant adaptability to environmental conditions. The results indicate that bokashi media produced the most significant increase in plant height, from 14.5 cm in the initial phase to 23.6 cm after the treatment period. Meanwhile, the topsoil medium also showed a substantial increase, from 11.6 cm to 20.4 cm, although it was still below the bokashi achievement. In addition, the treatment using tap-water showed very limited growth, only increasing from 5.1 cm to 8.6 cm. These differences in growth patterns indicate that the growing medium plays a major role in the successful adaptation and morphological development of *N. nucifera*.

The growth of *Nelumbo nucifera* leaves showed different responses to the types of planting media used. The bokashi treatment resulted in the most significant increase in leaf width, from an average of 5.32 cm to 15.21 cm. The topsoil treatment showed a moderate increase from 4.55 cm to 7.92 cm, while the tap-water group experienced the lowest growth, from 2.69 cm to 4.72 cm (Figure 7). This condition differs from topsoil, which, although it provides inorganic minerals, is still limited in terms of the availability of active organic matter and pH buffering capacity. This explains why the increase in leaf width in surface soil is moderate compared to bokashi.

Leaf diameter measurements were carried out on 15 *N. nucifera* leaves, taking into account their unique growth characteristics, where each leaf develops on a single stalk (petiolus) in an alternating pattern. Before placement in the sedimentation pond, the average leaf width was recorded at 2.69 cm in the tap-water treatment, 4.55 cm in the topsoil medium, and 5.32 cm in the bokashi treatment. After 14 days of maintenance in the sedimentation pond, there was an increase in leaf width in all treatments, namely to 7.00 cm (tap-water), 11.45 cm (topsoil), and 15.21 cm (bokashi) (Figure 8).

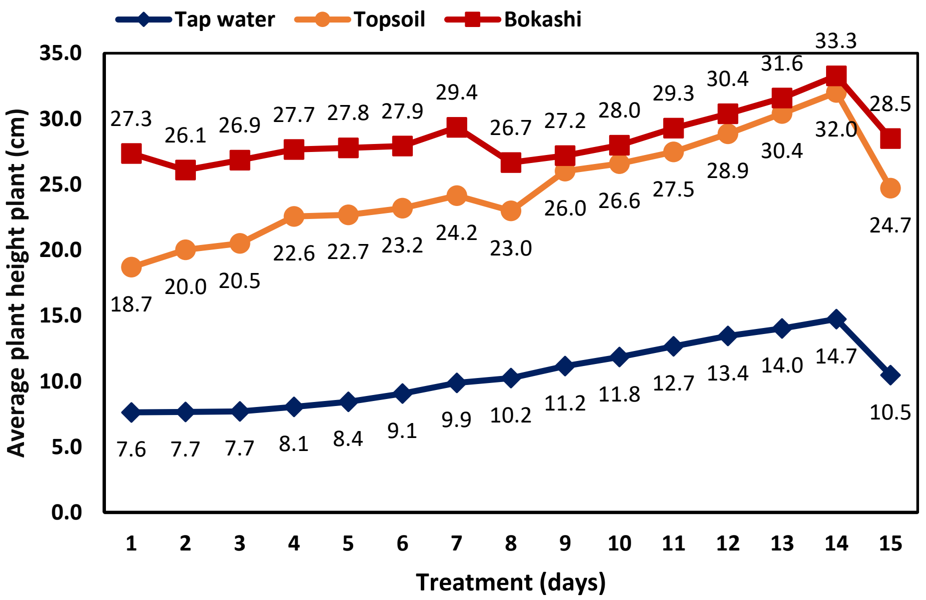


Figure 7. The average of height of *Nelumbo nucifera* G. during phytoremediation process in acid mine drainage (AMD) using floating treatment wetland treated with different growing media i.e.: bokashi (red color), topsoil (yellow-brown color) as compared to the control plant using tap water (blue color).

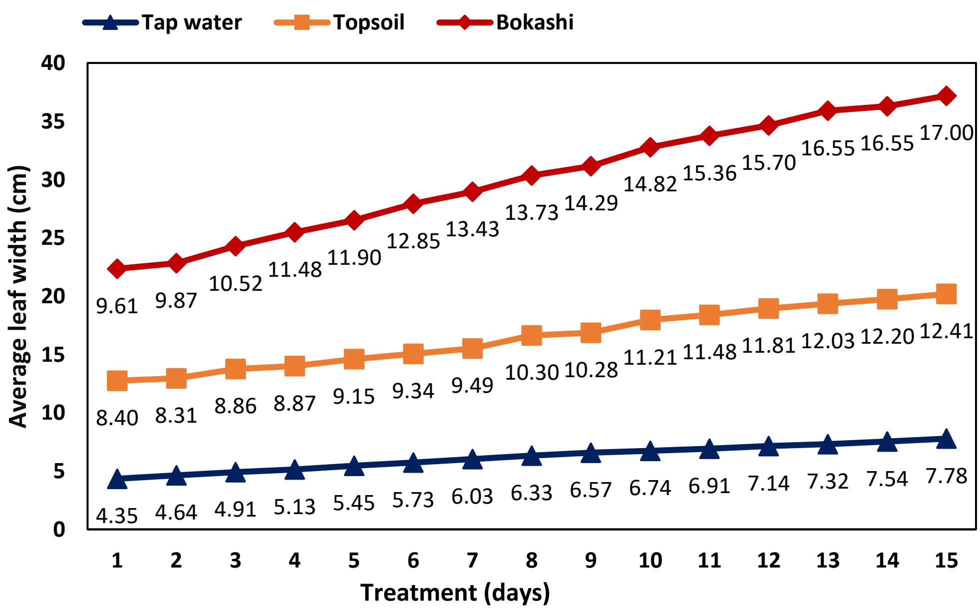


Figure 8. The average leaf width of *Nelumbo nucifera* G. during phytoremediation process in acid mine drainage (AMD) using floating treatment wetland treated with different growing media i.e.: bokashi (red color), topsoil (yellow-brown) as compared to the control plant using tap water (blue color).

Mineral Structure of Media and Nelumbo nucifera Tissues Based on XRD Analysis

The XRD pattern of *Nelumbo nucifera* tissues (roots, stems, and leaves) and planting media (topsoil and bokashi) are depicted in Figure 9. The spectrum of plant roots in the topsoil media shows the highest intensity with the main peak seen at 20° to 22°, indicating the characteristics of organic compounds such as $C_9H_{10}O_3$ (phenolic) and SiO_2 (quartz). The stem spectrum shows that the topsoil indicates complex crystalline compounds, which allows the accumulation of secondary metabolites or compounds resulting from metal complexation (Figure 9b). This pattern shows a more organised structure compared to bokashi and tap-water. The plant stems in the bokashi treatment detected organic compounds $C_{20}H_{14}N_4O_4$, which are associated with microbial activity and decomposition of organic matter. While in the tap-water, the pattern was flatter with the dominant compound being $C_{15}H_{16}O_3$, indicating a low level of crystallinity.

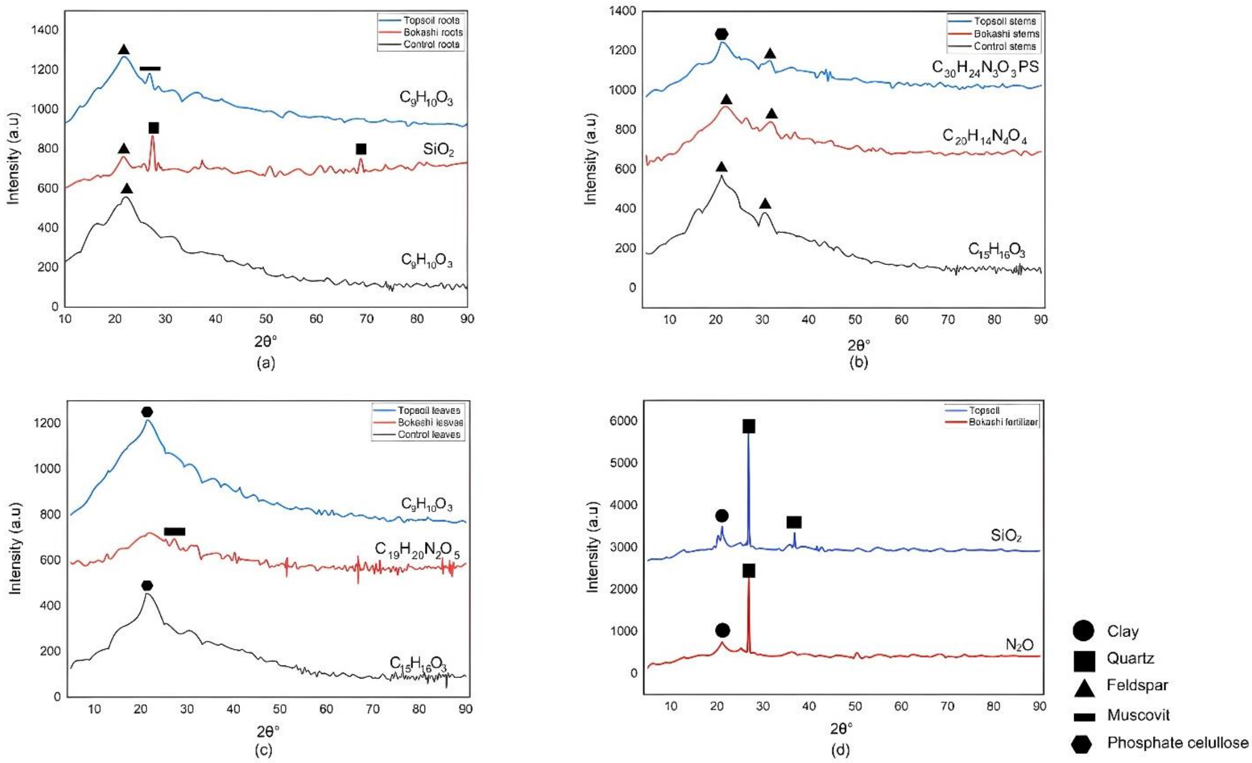


Figure 9. X-ray diffraction patterns of *Nelumbo nucifera* G. 14 days after phytoremediation process in acid mine drainage (AMD) using floating treatment wetland treated with different growing media i.e.: bokashi (red color), topsoil (blue color), and tap water (black color). The graph shown for roots (a), stems (b), leaves (c), and growth media (d). Growth media was only compared bokashi and topsoil.

The diffraction patterns of *Nelumbo nucifera* leaves showed a similar trend (Figure 9c). The samples from the topsoil media produced the highest peaks with the presence of muscovite, quartz, and phenolic compounds $C_9H_{10}O_3$, which are associated with the physiological response of plants to the environment. Bokashi showed the presence of $C_{19}H_{20}N_2O_5$, which can be associated with complex organic nitrogen compounds. Leaves from the tap-water treatment showed the most amorphous structure, indicating suboptimal growth conditions and limited accumulation of crystalline compounds. XRD analysis of the growing media showed that the topsoil was dominated by silicates (SiO_2), feldspar, muscovite, and quartz, which supported a stable mineral structure and supported root growth (Figure 9d). In contrast, bokashi showed lower intensity, but contained complex organic compounds such as N_2O, as well as small amounts of quartz and clay components, reflecting the dominance of decomposed organic matter and high microbiological activity.

Discussion

The Dynamics of pH and Heavy Metal Concentrations in Treatment Ponds

The lower pH of acid mime drainage (AMD) is among the critical problem faced by mining industry which should be resolved by various treatments. This research attempts to address the acidity level of the media through the application of *N. nucifera* and the addition of soil ameliorants to the floating raft. The increase of pH in the topsoil and bokashi treatments indicates that both media contribute to neutralizing the acidic conditions in acid mine drainage (AMD). Topsoil plays a role through its natural buffering capacity and mineral content that can neutralize acid ions, so that even though it initially has the lowest pH, this medium is able to increase the pH significantly after the 9th day. This is possible due to the release of base cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) from both media that can improve the pH of medium [29]. Meanwhile, the bokashi treatment showed a more consistent increase in pH from the start, which is likely due to the activity of microorganisms and the mineralization process that occurs in organic matter. Microbes in bokashi have the potential to produce metabolites that support the bioremediation process, as well as increase the availability of bases that neutralize H^+ ions. Marra et al. [30], indicated that the fermentative microbial activity in bokashi has a role in this dynamic, as it produces ammonia, humic acid, and bicarbonate ions that support gradual neutralisation. These differences in mechanisms have important implications for mine waste management strategies. The integration of both media has the potential to deliver a synergistic remediation strategy, combining the rapid neutralisation reaction of mineral soil with the long-term stability.

Additionally, the increase in pH can be attributed by the activity of fermentative microorganisms that dominate the bokashi system, where anaerobic biodegradation produces organic acids in the early stages, followed by the formation of alkaline compounds during the stabilisation phase. Dissolved H^+ ions in the surface soil medium cause root and microbial activity that alters the chemical conditions of the medium. Consistent with the findings of te Pas et al. [31], Guo [32], Shrestha et al. [33], indicating that changes in pH indicate the weathering of silicate minerals and the release of base cations from fine soil fractions previously bound in the soil, there is microbial activity in the medium that produces organic acids from the biodegradation process, and dissolved H^+ ions cause microbial activity in the roots.

Distribution of Heavy Metal Accumulation in Nelumbo nucifera Parts During Phytoremediation

The distribution of heavy metals within plant organs is a key indicator of phytoremediation efficiency, as it reflects both uptake capacity and internal translocation mechanisms. Heavy metal accumulation in *N. nucifera* tissue showed different dynamics between topsoil, bokashi, and tap-water treatments (Figure 6). The increase in pH due to topsoil buffering minerals and the decomposition of bokashi organic matter contributed to a decrease in dissolved Fe and Mn concentrations through precipitation, as Fe^{2+} and Mn^{2+} are more stable in acidic conditions and precipitate as hydroxides when the pH increases. Microbial activity in bokashi also supports redox transformations and the formation of organic complexes that can bind metals, thus reducing the dissolved fraction [34].

The highest Fe accumulation occurred in topsoil, particularly in the roots (16,462.96 mg/kg), a more than 100-fold increase compared to the initial conditions, with translocation to the leaves (14,742.91 mg/kg) and stems (9,571.62 mg/kg). In contrast, bokashi resulted in a more even distribution of Fe in stems (5,033.18 mg/kg), leaves (3,741.01 mg/kg), and roots (2,900 mg/kg), possibly due to the role of organic matter in forming organometallic complexes that retain some Fe outside the plant tissue [34]. The tap-water treatment showed the lowest accumulation (maximum 1,962.57 mg/kg in the roots), confirming the limitations of uptake without media improvement.

For Mn, bokashi promoted the highest accumulation in the roots (1,240 mg/kg) with a relatively balanced distribution across stems (1,043.56 mg/kg) and leaves (1,044.52 mg/kg). This pattern suggests the role of organic acids from bokashi decomposition in increasing Mn availability. In contrast, topsoil resulted in lower accumulation in roots (470.67 mg/kg) but higher in stems (1,137.08 mg/kg) and leaves (885 mg/kg), indicating faster translocation to photosynthetic tissues. In the tap-water, all tissues showed the lowest accumulation, at 116.42 mg/kg (roots), 159.31 mg/kg (stems), and 352.31 mg/kg (leaves), respectively. Comparatively, bokashi was more effective in increasing Mn accumulation evenly across all tissues, while topsoil predominantly promoted translocation to leaves and stems. This difference in mechanisms is important for determining remediation strategies, whether to retain metals in the roots to reduce movement in the food chain or to enhance translocation to facilitate biomass harvest and metal extraction [34,35].

Absorption Efficiency

Evaluating absorption efficiency reveals how effectively *N. nucifera* captures and redistributes Fe and Mn across its tissues under different ameliorant treatments. Soil amendments significantly affected the uptake and distribution of Fe and Mn in *N. nucifera*. In topsoil pH adjustment increases Fe^{2+} availability, resulting in strong accumulation in the roots (99.09% efficiency). In addition, bokashi supports a more widespread distribution of Fe to the stems and leaves (94.83%), which is likely influenced by microbial activity and organic exudates that play a role in nutrient mobilization. This phenomenon is related to the role of plant growth-promoting microbes in facilitating Fe dissolution through the release of siderophores and the secretion of organic acids, thereby increasing the efficiency of Fe uptake and translocation throughout plant organs in the microbe-soil-plant continuum [36]. In tap water, roots showed relatively high Fe absorption (92.35%), and translocation to upper organs indicated low biologically active Fe availability under acidic conditions. However, the limited translocation to upper tissues (stems and leaves) under tap-water conditions indicates barriers in xylem transport mechanisms due to low metabolic energy availability or limitations in natural chelators.

A different pattern was observed for Mn. Bokashi supported uniform absorption across tissues (≈92%), suggesting that microbial processes and humid substances enhance Mn^{2+} mobility, while topsoil promoted higher efficiency in stems and leaves (>90%) but lower in roots (78.75%), indicating dominant translocation. The tap-water showed very low Mn uptake in roots (14.10%) and uneven distribution, reflecting poor availability in unamended media. These findings highlight that soil conditioners determine not only micronutrient absorption efficiency but also internal allocation, with direct implications for enzymatic metabolism and photosynthesis [37].

Growth Response of Nelumbo nucifera (Height and Leaf Width)

The survival rate of *N. nucifera* varied markedly among treatments, reflecting the impact of soil amelioration on stress tolerance. Only 33.3% of plants survived in the tap-water, indicating that the absence of soil fraction and organic matter caused plants to experience essential nutrient deficiencies, thereby disrupting physiological processes and new tissue formation, while survival increased to 66.7% with topsoil and 86.7% with bokashi, indicating strong sensitivity to acidic and nutrient-poor conditions. The effectiveness of bokashi in improving plant survival is indicated through its combined roles as a nutrient provider, active microbiological agent, and source of buffer compounds such as bicarbonate, which stabilises the pH of the rhizosphere. When faced with acidic conditions, plants adopt adaptive strategies in the form of pH regulation, secretion of organic acids (citric, malic, oxalic), and a significant increase in antioxidant enzyme activity that suppresses the effects of oxidative stress caused by metals. This process supports the physiological function of the roots but also strengthens the plant's capacity to adapt to extreme environmental pressures [38].

Topsoil also improved survival compared to the tap-water through the provision of alkaline minerals and enhanced media porosity, in line with Vélez-Bermúdez and Schmidt [39], but its effect was lower than bokashi due to the absence of biological amendments. This suggests that topsoil primarily offers abiotic buffering, while bokashi integrates biotic and abiotic factors for stronger amelioration.

Vegetative growth further confirmed these patterns, with bokashi producing the greatest plant height and leaf width, followed by topsoil and tap-water. Similar observations were reported by tap-water. This study confirms similar findings but highlights that the increased growth and stability of the rhizosphere is formed from the synergy of biological and chemical mechanisms in bokashi. The presence of fermented organic matter is identified as a key management strategy in supporting the adaptability of aquatic plants in highly acidic environments [34].

Mineral Structure of Media and Nelumbo nucifera Tissues Based on XRD Analysis

XRD analysis revealed distinct crystalline and organic phases in media and tissues linking mineral composition to plant resilience. The XRD pattern revealed similar general diffraction patterns across treatments, but with notable differences reflecting biochemical responses of *N. nucifera* (Figure 9). In roots, topsoil showed diffraction peaks at 2θ ≈ 20–22°, associated with muscovite, quartz, and phenolic compounds ($C_9H_{10}O_3$), which play roles in secondary metabolism, chelation, and antioxidant defence [40]. Bokashi and topsoil treatments also displayed dominant peaks around 2θ ≈ 22°, indicating semi-crystalline cellulose that supports tissue stability, whereas tap-water plants exhibited a flatter pattern with low crystallinity (2θ ≈ 32°) and simpler compounds ($C_{15}H_{16}O_3$), consistent with weaker structural development [41]. In contrast, bokashi tissues contained more complex compounds (e.g., $C_{20}H_{14}N_4O_4$) linked to microbial decomposition, while topsoil supported larger multifunctional molecules ($C_{30}H_{24}N_3O_3PS$), suggesting stronger adaptive lignocellulosic formation [42].

Leaf spectra also reflected treatment effects. Topsoil produced crystalline peaks (2θ = 22°) with muscovite, quartz, and phenolics, while bokashi showed peaks at 2θ = 26° containing cryptotanshinone ($C_{19}H_{20}O_3$) and nitrogen-rich compounds ($C_{19}H_{20}N_2O_5$), indicating high metabolic activity. Tap-water leaves, dominated by osthol ($C_{15}H_{16}O_3$), showed amorphous patterns aligned with minimal growth. These findings suggest that bokashi enhances microbial-driven metabolite synthesis through a priming effect, whereas topsoil contributes to mineral-supported structural stability, and tap-water conditions limit functional tissue development.

XRD analysis of the media confirmed that the media was dominated by silicate minerals (quartz, feldspar, muscovite), supporting aeration and nutrient transport, while bokashi spectra indicated lower crystallinity but enrichment with decomposed organics and clay, reflecting microbial activity and strong nutrient retention. Collectively, the differences in diffraction patterns highlight the close link between microbial activity, nutrient availability, and secondary metabolite synthesis, which together strengthen plant resilience and physiological function [43].

Conclusions

This study demonstrates that ameliorated media significantly enhance the phytoremediation performance of *Nelumbo nucifera* installed in floating wetland for acid mine drainage (AMD). Both topsoil and bokashi effectively neutralised acidity, raising pH from highly acidic conditions to near-neutral levels within 14 days. Dissolved Fe and Mn concentrations decreased sharply (>90%), with distinct uptake dynamics. Topsoil promoted strong Fe accumulation in roots and translocation to aerial tissues, while bokashi supported more uniform Mn distribution across plant organs through microbial-driven mobilisation. XRD analysis confirmed that mineral-supported crystallinity in topsoil and complex organic phases in bokashi contributed to tissue stability and strengthening plant resilience. Bokashi provided the highest survival (86.7%) and growth rates, reflecting the synergistic role of nutrients and active microorganisms, while topsoil mainly offered rapid mineral buffering. Bokashi emerged as the most effective amendment, as its organic–microbial synergy not only reduced acidity and metal concentrations but also enhanced plant growth and resilience. Future research should extend observation periods to validate long-term performance, while policy efforts should prioritise bokashi-based floating wetlands as a low-cost and sustainable strategy for AMD management in mining regions.

Author Contributions

MNR: Conceptualization, Methodology, Investigation, Data Curation, Writing, Original Draft Preparation; **IRM**: Validation, Writing - Review & Editing, Supervision; **HAM**: Investigation, Supervision, Data Curation, Writing - Review & Editing.

AI Writing Statement

The authors did not use any artificial intelligence assisted technologies in the writing process.

Conflicts of Interest

There are no conflicts to declare.

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