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Phytoremediation of Acid Mine Drainage with *Melaleuca cajuputi*, *Nauclea orientalis*, and *Vetiveria zizanioides* in Floating Treatment Wetland

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ABSTRACT

The formation of acid mine drainage (AMD) is a common environmental problem in the mining industry. Its passive management through wetland construction has gained more consideration in recent years. However, the application in the field is constrained by the large area and relatively shallow depth. Indonesia has no passive technology to neutralize AMD in deep water. One solution is to apply a floating treatment wetland (FTW) system. Therefore, this study aimed to determine the ability of several hyperaccumulator plants, such as *Melaleuca cajuputi, Nauclea orientalis*, and *Vetiveria zizanioides*, to neutralize AMD using a floating system by conducting FTW trials. The method used was a Completely Randomized Design (CRD) with 3 treatments and control/without plants. Each treatment had 3 replications, resulting in 12 experimental units. The results showed that the FTW with or without plants could increase pH and decrease dissolved Mn by 75.31-90.74%. Heavy metals were chelated by organic matter, absorbed by plants, and deposited in the form of metal sulfides. The results also indicated that besides having a positive effect on pH and heavy metal reduction, the organic-based floating wetland increased biological oxygen demand (BOD) from 61.08-79.71%.

1. Introduction

Acid mine drainage (AMD) has become a global problem in various parts of the world, causing damage to both the environment and living organisms (Simate and Ndlovu 2014). It is formed during mining activities due to the exposure of mineral rock containing pyrite to water and oxygen. Oxidizing bacteria, such as *Acidithiobacillus*, also act as a catalyst to increase the rate of pyrite oxidation. Subsequently, sulfates and heavy metals such as iron, copper, lead, nickel, manganese, cadmium, aluminum, and zinc are released into the water, lowering the pH of water and soil (Moodley *et al.* 2017). The use of wetlands as a passive treatment method has been widely developed and shown to improve the quality of AMD (Sheridan *et al.* 2013; Tuheteru *et al.* 2016; Yusmur *et al.* 2019).

The several factors that determine the success of AMD remediation with a wetland system, include plant selection. It is necessary to select the hyperaccumulator plant with high biomass productivity, adapting to AMD inundated conditions. In addition to producing litter in the long term, it can absorb high amounts of metal. *Kayu putih* (*Melaleuca cajuputi* L. Powell.) is a type of perennial plant that grows well and adapts to highly acidic soils, flooded to dry ground (Mansur 2010), and can absorb heavy metals in contaminated soil (Mohd *et al.* 2013). *Lonkida* is another recommended perennial plant (*Nauclea orientalis* L.) with a phytoremediation potential because under AMD inundated conditions, it can accumulate iron in the roots and have a massive, fast-growing root system and high biomass (Tuheteru *et al.* 2016). *Akar wangi* (*Vetiveria zizanioides*) is a fast-growing perennial grass with high biomass

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productivity, can grow at pH 3.0-11.5, as well as tolerates and accumulates metals (Kiiskila *et al.* 2019; Pidatala *et al.* 2018). It can grow sustainably in hydroponics, including acidic waters like AMD (Kiiskila *et al.* 2019; Roy Chowdhury *et al.* 2015).

The application of wetlands in the field is constrained by the large area and relatively shallow depth due to the adjustment of water flow to the height of the plants in the system. There is no passive technology to neutralize AMD in the deeper water column, such as water treatment ponds and voids. One solution is applying a floating treatment wetland (FTW) system, where plants will be cultivated on floated media but still play an optimal role in reducing AMD pollutants. Therefore, this study aims to determine the ability of *Melaleuca cajuputi*, *Nauclea orientalis*, and *Vetiveria zizanioides*, to support the neutralization of AMD using the FTW system.

2. Materials and Methods

2.1. Materials

A synthetic AMD was used to ensure consistent water quality throughout the study. It was prepared by mixing various chemicals, as shown in Table 1.

As shown in Table 2, AMD characteristic analysis was conducted to determine the difference in its quality before and after treatment. The analysis shows that pH parameters and Mn metal do not meet environmental quality standards, thereby neccesiating AMD management.

Table 1. Composition of synthetic acid mine drainage (AMD)

Element	Chemical used	Amount of chemicals added per liter (mg)*
Fe	$FeSO_4.7H_2O$	50.00
Mn	$MnSO_4$	42.50
Al	$Al_2SO_4.18H_2O$	40.00
Zn	$ZnSO_4$	44.16
Cu	$CuSO_4$	40.00
SO_4	H_2SO_4	100.00 (ml)

*Modified from Cocos *et al.* (2002)

Table 2. Characteristics of AMD before treatment

Parameters	Synthetic AMD*	Original AMD**	Quality standards***
pH	3.01	3.83	6-9
Fe (mg/L)	0.83	8.24	7
Mn (mg/L)	16.19	7.06	4
TSS (mg/L)	111.00	61.00	400
BOD (mg/L)	2.99	12.20	30

*Service laboratory SEAMEO BIOTROP, **PT Bukit Asam (2022), ***minister of environment and forestry No. 5 of 2022 concerning wastewater treatment for mining businesses and activities using the artificial wetland method

The plants used in this study were *lonkida*, *Akar wangi*, and kayu putih. The seedlings of *kayu putih* and *lonkida* were obtained from seeds sown, while those of *Akar wangi* were obtained from SEAMEO BIOTROP nurseries, Verina 1.

2.2. Research Procedure

The study was conducted using a 70 cm × 50 cm × 37 cm box container and styrofoam frames were used to float organic-based planting media. The type of organic matter used refers to previous research. This included a mixture of fresh oil palm empty fruit bunch (EFB) and cow manure at a ratio of 2:1. Each container is filled with 75 liters of AMD and the FTW contained 7.5 kg of organic matter, including 5 kg for EFB and 2.5 kg for cow manure. Furthermore, there were 6 plants in each FTW, and the method used was a Completely Randomized Design (CRD) with 3 treatments and control/without plants. Each treatment had 3 replications, resulting in 12 experimental units.

2.3. Data Measurement

The water quality parameters such as pH, redox potential (ORP), concentration of heavy metals Fe and Mn, total suspended solid (TSS), biological oxygen demand (BOD), and population of sulfate-reducing bacteria (SRB), were observed using pH meter, ORP meter, the Atomic Absorption Spectrophotometry (AAS), gravimetric, Winkler, and MPN methods, respectively. Furthermore, the analysis of heavy metals Fe and Mn in sediment and organic matter was also conducted at the end of the observation period.

At the end of the observation period, the plants were harvested, the roots and shoots were separated, then dried in an oven at 60°C for 72 hours to reach a constant weight. The concentrations of Fe and Mn metals in each plant part were analyzed using the Atomic Absorption Spectrophotometry (AAS) method. The results of which were then applied as the basis for calculating the value of the Translocation Factor (TF) using the following equation: TF = Caerial/Croot, where Caerial and Croot represent the metal content in the shoots (stems and leaves) and roots, respectively (Wang *et al.* 2005).

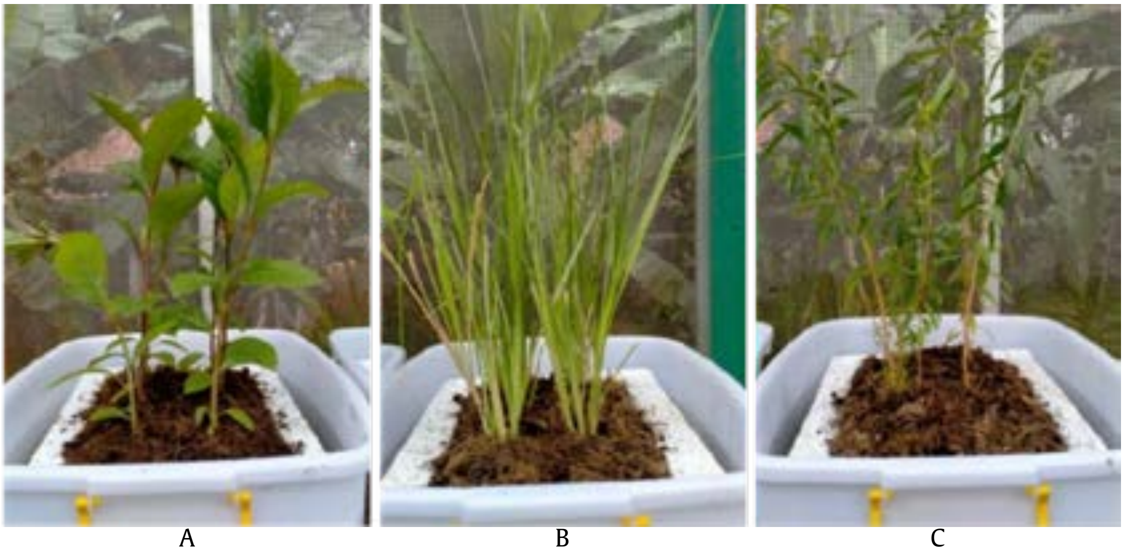


Figure 1. Floating treatment wetland (FTW) with the three species of plants: (A) *Lonkida (Nauclea orientalis)*, (B) *Akar wangi (Vetiveria zizanioides)*, (C) *Kayu putih (Melaleuca cajuputi)*

2.4. Data Analysis

Analysis of variance with a 5% confidence level was performed on the data using SPPSS v16 software. The data were subjected to the Duncan Multiple Range Test (DMRT) with a confidence level of 5% due to the significant effects of treatments.

3. Results

3.1. pH, ORP, and Enumeration of Sulfate Reducing Bacteria

Figure 2 shows the results of the daily pH measurement during the first week of study, and then weekly until the 12th week. It indicates that the treatments tested could increase the pH from 3.01 to 7-7.13 within 1 day then stabilize in the following weeks. Statistical analysis showed that the different plant treatments had no significant effect on the results (P>0.05).

The main caused of the increase in pH was the application of organic matter, which could create reduction conditions due to decomposition. The reduction reaction can be proven by a significant decrease in the value of the ORP in AMD over time, as shown in Figure 3.

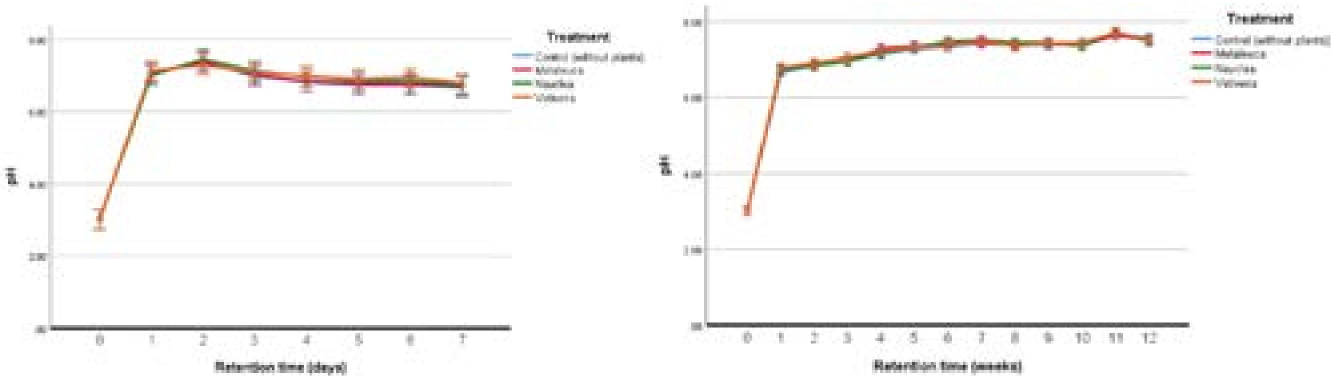
Redox potential (ORP) describes the condition of oxygen deficiency in the water, which is caused by its high consumption due to increased bacterial metabolic processes in the aquatic system. Organic carbon supply creates anaerobic conditions to reduce sulfate to sulfide. Table 3 shows the number of SRB populations in AMD at 4, 8, and 12 weeks after treatment.

Table 3 showed that all treatments were able to increase the SRB population during the first 4 week, but it decreased over time. This is because the longer the observation time, the more the carbon source from organic matter decreases, thereby reducing the growth of SRB. Despite being in a reduced condition, the decrease in the availability of carbon sources also impacts increasing the value of Eh, as shown in Figure 3.

3.2. Metal Removal

Increasing pH and decreasing heavy metal toxicity by SRB depend on organic matter's ability to offer a suitable carbon source and low redox environment for the sulfate reduction process. The dissolved metal's value in AMD is shown in Figure 4.

The data on Table 3 and Figure 4 showed that in the first 4 weeks, after the SRB population increased, the dissolved Mn decreased. This metal depletion was caused by the deposition of metal sulphides supported by reduction conditions, sulfate removal, high amounts of SRB, and increased alkalinity. Figure 4 indicates that the tested treatment reduced the dissolved Mn in AMD, specifically, the control could be reduced by 90.74%, while the treatment plant with kayu putih, *lonkida*, and akar wangi reduced by 82.7%, 75.31%, and 87.04%, respectively. Despite the control (without plants) showing a greater reduction in Mn, statistically, the presence of plants did not have a significant effect (P>0.05) on the dissolved Mn in AMD. There was an increase in dissolved Fe



A B

Figure 2. Variation of pH during the initial 7 days (A) and weekly (B)

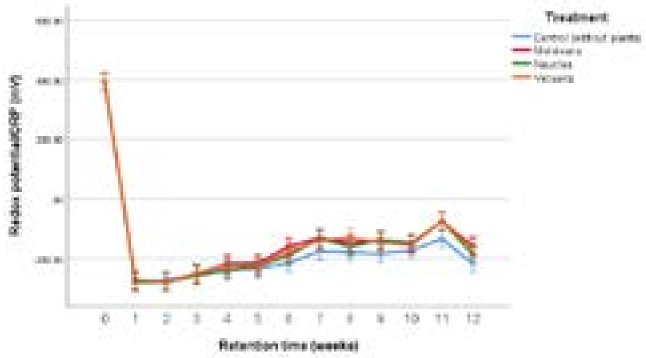


Figure 3. Effect of plants on redox potential (ORP)

Table 3. Enumeration of SRB in water at various observation times

Sulfate-reducing bacteria (MPN/ml)	A	B	C	D
Initial	<3	<3	<3	<3
4 weeks	11 × 10⁴	2.4 × 10⁴	11 × 10⁴	9.3 × 10³
8 weeks	15 × 10²	4.3 × 10²	9.3 × 10²	21 × 10²
12 weeks	43	15	43	15

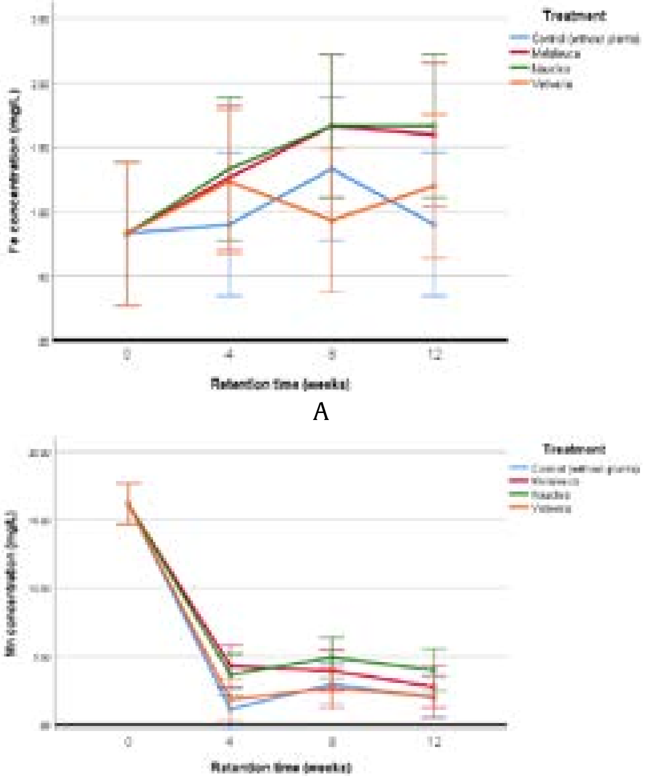
Source: ICBB Laboratory

A: control (without plants), B: planted with *kayu putih*, C: planted with *lonkida*, D: planted with *akar wangi*

between 0.9-1.7 mg/L in all treatments during the 12 week observation period. This remains below the specified quality standard of 7 mg/L.

3.3. TSS and BOD

Total Suspended Solid (TSS) consists of mud, fine sand, clay, metal oxides, sulfides, and microorganisms such as algae, bacteria, fungi, and detritus. Table 2 showed that the TSS value was initially below the quality standard of 400 mg/L. Figure 5 presents a 90% decrease in TSS in the control and the treatment plant with *kayu putih*, *lonkida*, and *akar wangi* by 70.81%, 63.69%, and 81.53%, respectively.



A

B

Figure 4. Reduction of heavy metals Fe (A) and Mn (B) in AMD

The addition of organic matter into the water caused an increased in the BOD, as shown in Figure 6. Biological oxygen demand illustrates the amount of oxygen needed by aerobic microbes to oxidize organic matter into carbon dioxide and water. Its water value before treatment was about 2.99 mg/L but increased in all treatments ranges 61.08 to 79.71% at 4th and 8th week of the study period, then decreased at 12th week of observation.

3.4. Metal Accumulation

Heavy metal content in organic matter and sediment is shown in Figure 7, while the proportion in plants tissues is shown in Figure 8.

Kayu putih, *lonkida*, and *akar wangi* had high heavy metal uptake. This was because the plant roots were able to penetrate the floating media, hence, there was a direct contact with AMD. The root types of kayu putih and akar wangi tend to be more resistant to waterlogging and stickiness. This was different with *lonkida* which tightly binded particles of organic matter and played a role in stabilizing the aggregate, such that its roots tend to be more fibrous and shorter, as shown in Figure 9.

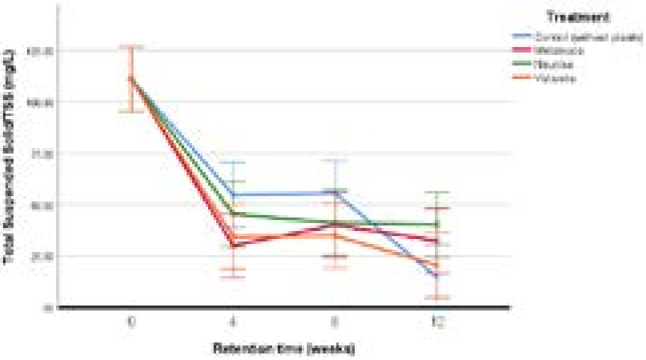


Figure 5. Effect of plants on TSS in AMD

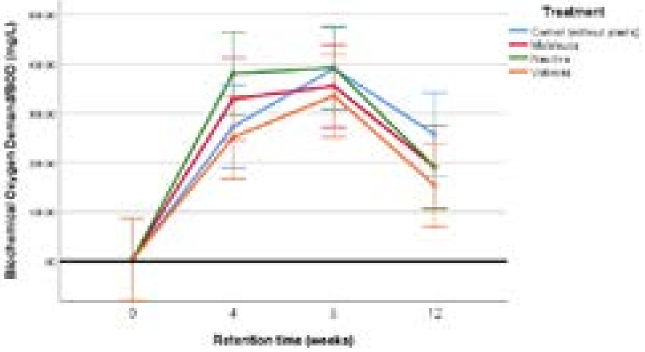


Figure 6. Effect of plants on BOD in AMD

To determine the mechanism of plants in absorbing heavy metals, the translocation factor (TF) was calculated, as indicated on Table 4.

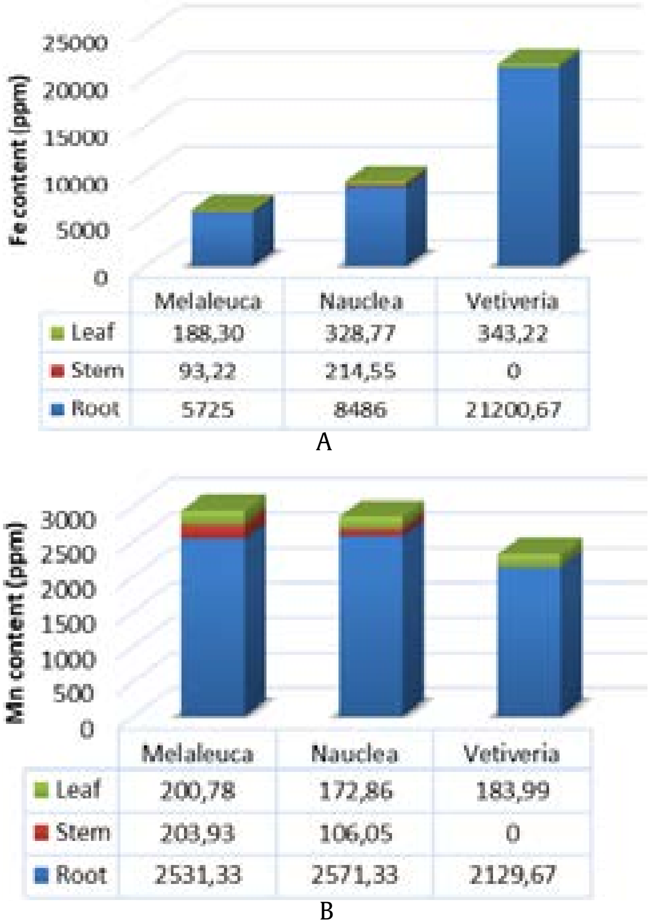


Figure 8. Heavy metal content (A) Fe and (B) Mn per plant part at 12 weeks after planting

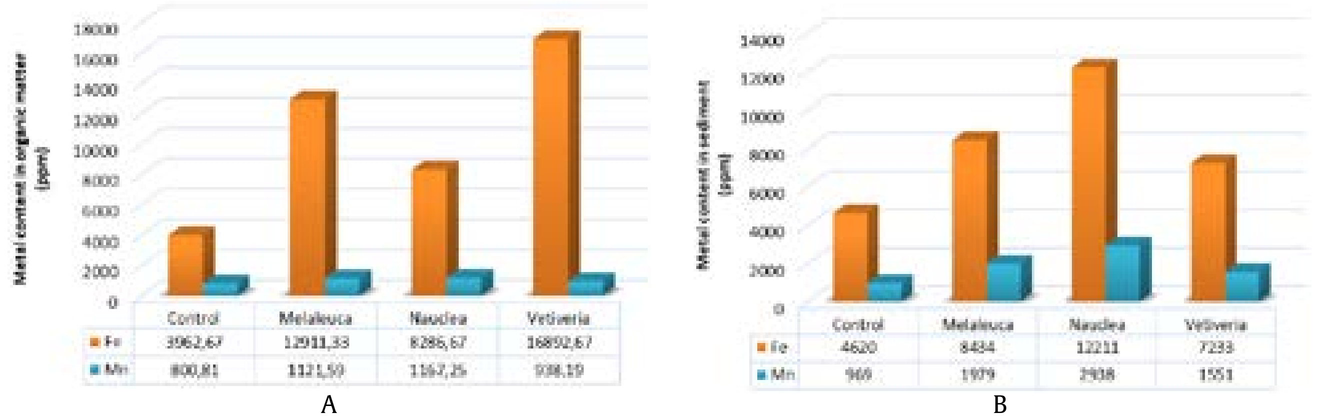


Figure 7. Total heavy metal content in (A) organic matter, (B) sediment after 12 weeks of observation

Figure 9. Root form of (A) *Kayu putih (Melaleuca cajuputi)*, (B) *Lonkida (Nauclea orientalis)*, (C) *Akar wangi (Vetiveria zizanioides)* grow on floating wetland

Table 4. Translocation factor (TF) of several metals by *kayu putih (Melaleuca cajuputi)*, *lonkida (Nauclea orientalis)*, and *akar wangi (Vetiveria zizanioides)*

Translocation factor (TF)	Fe	Mn
Melaleuca cajuputi	0.049	0.160
Nauclea orientalis	0.064	0.108
Vetiveria zizanioides	0.016	0.086

mean ± SD (n = 3), numbers followed by the same letter were not significantly different according to DMRT test at 95% confidence interval

4. Discussion

4.1. pH, ORP, and Enumeration of Sulfate-Reducing Bacteria

The presence of plants in the FTW had no significant effect on changes in AMD pH compared to the control. Madaniyah (2016) stated that the presence of aquatic plants such as *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta* did not significantly impact changes in pH. Also, Mawaddah *et al.* (2012), reported that the presence of *kayu putih* and *lonkida* had no significant effect on increasing the pH of AMD. This is because the primary caused of the elevation in pH was the application of organic matter, which could created reduction conditions due to the decomposition process (Headley and Tanner 2006). The low redox potential reduces the originally ferric (Fe^{3+}), to be reduced to ferrous ion (Fe^{2+}) and releases one molecule of OH- which plays a role in increasing the pH of the water.

$$Fe(OH)_3 + e^- \longleftrightarrow Fe(OH)_2 + OH^-$$

Redox potential (ORP) plays an important role in creating anaerobic conditions by the needs of SRB. The requirements include anaerobic and anoxic conditions with ORP in the -300 mV range (Prasad *et al.* 1999). The application of organic matter reduced the value to -270 mV (Figure 3). The decrease also affects the reduction reactions that occur in the system. At ORP values ranging from -100 to -200 mV, ferric to ferrous will be reduced. Subsequently, sulfate will be reduced to sulfite when the value decreases to -300 mV. The sulfate reduction mechanism occurs because SRB can use sulfate, sulfite, or thiosulfate ions as electron acceptors to obtain energy in the metabolic process and organic matter as electron donors. The metabolism of SRB requires low molecular weight organic compounds such as acetate as a carbon source (Hards and Higgins 2004).

4.2. Metal Removal

Based on Figure 4, it can be seen that there was an increase in dissolved Fe between 0.9-1.7 mg/L in all treatments during the 12-week observation period. The same was experienced by Herniwanti *et al.* (2013), which stated that Ipomea aquatic and Pistia stratiotes increased Fe by 13% and 65%, respectively. The increase in Fe occurs because wetland plants that release oxygen through their roots can change redox conditions in the small zone of the rhizosphere (Headley and Tanner 2006). Metal measurements was performed on the surface, hence, an increase in Fe metal can occur from a state that was originally reduced due to the role of organic matter turning into oxidation as a result of oxygen released by plant roots, such that ferrous ions (Fe^{2+}) are oxidized to ferric ions (Fe^{3+}), which is more mobile and more soluble in water.

Despite the increase in the solubility of Fe metal in water, the solubility of Mn decreased. In this study, the decrease in Mn was 1.7-12.6 times higher than the average value reported by Herniwanti *et al.* (2013). The 5 different plants used are *Eleocharis dulcis, Cyperus odoratus, Hydrilla vercilata, Ipomea aquatic*, and *Pistia statatiotes* on a mini-project scale at the PT Jorong Barutama Greston with a Mn decrease of 6-55%. *Akar wangi* in this study was able to reduce the Mn 3.2 times higher than that in Kiiskila *et al.* (2020). The reduction of this metal by *lonkida* was 11.8 times higher than those planted in wetland in research of Tuheteru *et al.* (2016). In general, the followings are several possibilities leading to a decrease in dissolved metal concentrations:

- The interaction between sulfide (S^{2-}) produced in the sulfate reduction process with metal cations forms metal sulfide which precipitates
- The process of metal absorption by plant tissues
- The process of metal adsorption by organic matter
- The process of metal biosorption by microorganisms in the wetland environment

4.3. TSS and BOD

The concentration of TSS can be removed by sedimentation processes and the activity of microorganisms and plants (Wood 1993). Metal oxides are discovered in AMD primarily in metal cations suspended or floating in acidic water. The control treatment reduced TSS due to the role of organic matter that could adsorb metal oxides floating in AMD. Furthermore, comparing the three plants, akar wangi and kayu putih can decrease TSS higher than *lonkida*. This is related to the length of the plant roots, especially those that come out of the floating media and directly touch the AMD. The longer the plant roots in the water, the lower the TSS value in the aquatic system. This decrease is due to plants having positively charged root hairs that attract colloidal particles with opposite charges, such as suspended solids. These particles stick to the roots and are slowly absorbed and assimilated by plants and microorganisms (Singh *et al.* 2012).

There was an increase in BOD at the 4th and 8th week of the study period, which then decreased at the 12 weeks of the observation, with the results presented in Figure 6. This was because the treatment was performed by adding organic matter to the floating media, so increase the oxygen needed by aerobic microbes to decompose the organic matter. But, it was observed that BOD decreased with the length of time the study. This indicated that the source of organic matter that needed to be decomposed gradually would decrease.

4.4. Metal Accumulation

The presence of plants can increase the amount of heavy metal uptake by organic matter. This is because they can form a layer of organic particulate and litter which will adsorb these metals (Marchand *et al.* 2010). The ability of organic matter to adsorb heavy metals is influenced by the value of cation exchange capacity (CEC). A high CEC indicates that the material can absorb or exchange cations, in this case being able to remove dissolved metal cations in AMD (Munawar and Riwandi 2010). Metals chelated by organic matter (humus layers of organic matter) tend not to move effectively, especially under anoxic or reducing conditions (Gambrell 1994). Metals linked to the surface through cation exchange processes tend to be weakly bound and rapidly mobilized by redox conditions and pH changes. However, when the bindings between organic matter and metal are broken, the metal can be deposited in a new form, such as metal sulfide. The released sulfide will react with cationic metals such as Fe and Mn to form metal sulfides that precipitate (Drury 1999; Zagury *et al.* 2006). The anoxic zone of the sediment supports chemical and microbial reduction processes, converting previously soluble metals and sulfates to insoluble sulfides (Fennessy and Mitsch 1989).

In addition to heavy metals being absorbed by organic matter and deposited in sediments, plants can also absorb them. The best performance for Fe was shown in *akar wangi*>*kayu putih*>*lonkida*, meanwhile, for Mn, it was shown by *kayu putih*>*lonkida*>*akar wangi*. The highest average metal content was identified in roots, then varies in stems and leaves depending on the type of metal and plant. The value of translocation factor indicates this (TF)<1 (Table 4). This implies that the metal content is lower in the shoots and stored in the roots. The mechanism used by lonkida, kayu putih, and akar wangi to remove Fe and Mn is the rhizofiltration technique. It removes contaminants in water by plant roots through adsorption or absorption, followed by metal storage in the roots (Benavides *et al.* 2018; Dhir 2013). Plants for rhizofiltration can produce a widespread root system and can accumulate heavy metals in high concentrations (Kushwaha *et al.* 2018).

In conclusion, treatment of FTW can improve the quality of AMD, which can increase pH and reduce dissolved metals and TSS (Table 5). In addition to growing well in AMD inundated condition, kayu putih, lonkida, and akar wangi can absorb high amounts of Fe and Mn by rhizofiltration mechanism. This involves the removal of contaminants in water by plant roots through adsorption or absorption followed by metal storage in the roots. In addition to being absorbed by plants, heavy metals are also chelated by organic matter and deposited as metal sulfides in sediments.

Table 5. Comparison of characteristics of AMD before and after treatment

Parameters	Before treatment*	After treatment*	Quality standards**
pH	3.01	7.52	6-9
Fe (mg/L)	0.83	1.30	7
Mn (mg/L)	16.19	2.70	4
TSS (mg/L)	111.00	27.00	400
BOD (mg/L)	2.99	197.90	30

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