# Bioremoval of Heavy Metals (Mn, Pb, Zn) from Wastewater Chemistry Laboratory, Satya Wacana Christian University Using Water Fern (Azollapinnata R.Br.)

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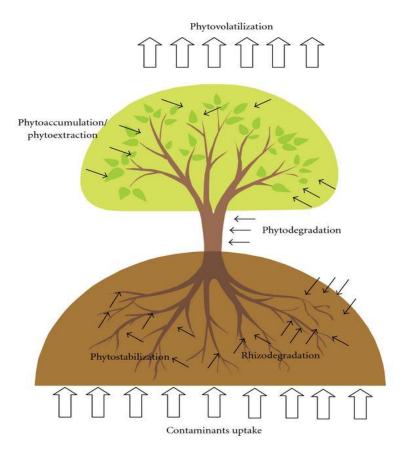
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**Abstract**. The objective of this study were: Firstly,to determine the optimal absorption of manganese (Mn), zinc (Zn) and lead (Pb) based on determined the optimum of Water Fern (A. pinnata) population densities, and secondly to determine the effectiveness of Mn, Zn and Pb absorption by different population densities of A. pinnata. The waste water obtained from Chemistry Laboratory, Satya Wacana Christian University, Salatiga. Heavy metals analysed in plants and in waste water were measured using AAS (Perkin Elmer, 3110). Data were analysed by Randomized Completely Block Design (RCBD), 6 treatments and 4 replications. As the treatments were various percentage surface area coverage of the plastic cups by A. pinnata, which were: 0%, 12.5%, 25%, 37.5%, 50%, and 62.5%, respectively. To test the differences between treatment means, the Honestly Significant of Differences (HSD) were used with 5% level of significant. The result of this study showed that : Firstly, optimal absorption of manganese (Mn), zinc (Zn), and lead (Pb), respectively, could be performed by 62.5% population densities of A. pinnata. Secondly, within 4 days effectively of A. pinnata absorb heavy metals were as follow: Mn 95.11%; Pb 90.90%, and Zn 87.04%.

#### 1. Introduction

Laboratory wastewater can be categorized as Hazardous Toxic Materials because most of the elements that are harmful contained in the laboratory wastewater is heavy metal such as iron (Fe), manganese (Mn), chromium (Cr), and Mercury (Hg) and others. In addition, there is also total dissolved solids (TDS), ammonia (NH<sub>3</sub>), nitrite (NO<sub>2</sub>), and the effect of the degree of acidity or pH (Said, 2009). The results of the study show the concentration of iron (Fe) 46.4 mg/l and manganese (Mn) 3.91 mg/l in wastewater Laboratory Environment Agency Banjar district; the concentration of zinc (Zn) 33.63 mg / l and lead (Pb) 6.70 mg/l in the wastewater Laboratory of Physical Chemistry, Inorganic Chemistry and affiliates Department of Chemistry, University of Indonesia [1, 15]. Furthermore, the range of heavy metal concentrations of wastewater laboratory are as follows: mercury (Hg) 77.6 - 391.6 mg / l; silver (Ag) 2.6 - 9.1 mg/l, and chromium (Cr) 11.3 - 21.9 mg/l, of course, it will be harmful if discharged directly into water bodies without being processed first.

Phytoremediation is one of methods that rely on the role of plants to absorb, degrade, transform, and immobilize heavy metal pollutants [11]. Further on, the advantages of phytoremediation compared to other waste treatment technology are: it's a natural process, synergistic relationship between plants, microorganisms, and living environment or habitat, as well as unnecessary high technology [13]. Phytoremediation mechanism comprising: phytoextraction, rhizofiltration, phytostabilization, rhyzodegradation, phytodegradation, and phytovolatization (Figure 1).



**Figure 1.** Mechanism of phytoremediation [22]

Some species of aquatic plants can be used as phytoremediation agents which are: floating aquatic fern (*Salviniamolesta*), water hyacinth (*Eichhorniacrassipes*), water spinach (*Ipomeaaquatica*), lesser duckweed (*Lemna minor*), and water fern (*Azollapinnata* R.Br).

The toxic dose depends on the type of ion, ion concentration, plant species, and stage of plant growth. Tolerance to metals is based on multiple mechanisms such as cell wall binding, active transport of ions into the vacuole, and formation of complexes with organic acids or peptides. Metal ions are adsorbed first to the cell surface by interactions with metal-functional groups as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide group, etc., and then penetrate the cell membrane and enter into the cell [24]. Furthermore, plants are susceptible to heavy metal toxicity and respond to avoid detrimental effects in a variety of different ways [14]. One of the most important mechanisms for metal detoxification in plants appears to be chelation of metals by low-molecular-weight proteins such as metallothioneins and peptide ligands, the phytochelatins. Azolla has the ability to absorb heavy metals such as chromium (Cr), zinc (Zn), nickel (Ni), Cadmium (Cd), copper (Cu), even Uranium (U) [16]. The research of Thayaparaet al. (2013) showed that A. pinnata is capable of absorbing lead (Pb) in amount of 1,383 mg/kg dry weight after four days of treatment and the concentration of lead (Pb) in the growth media has been reduced 83%. The  $Pb^{+2}$ ,  $Cd^{+2}$  and  $Cu^{+2}$  accumulated in azolla fronds after 30 days of growth period were highly significant compared with the control treatment. The highest accumulations of Pb<sup>+2</sup>, Cd<sup>+2</sup> and Cu<sup>+2</sup> were 270.11, 255.20 and 209.50 mg/m2, respectively, at the concentration of 2.0 ppm at 30-day growth period [1].

Based on the above mentioned matters, the purpose of this study are:

- 1. To determine the optimum of coverage area ratio of *A. pinnata* in the absorption of Mn, Zn, and Pb from Chemistry Laboratory wastewater.
- 2. To determine the effectiveness of *A. pinnata* in the absorption of metal Mn<sup>2+</sup>, Zn<sup>2+</sup>, and Pb<sup>2+</sup> from Chemistry Laboratory wastewater.

# 2. Method

### 2.1. *Time and Place of the Study*

The research has been conducted in the Laboratory of Environmental Chemistry, Department of Chemistry, Faculty of Science and Mathematics (FSM), Satya Wacana Christian University (SWCU), Salatiga. The study started from August 2014 to May 2015.

### 2.2. Materials and Equipments

*Materials.A. pinnata* obtained from the rice fields located in SidorejoLordistrict ,Salatiga. Wastewater was collected from liquid waste of Chemistry Laboratory, FSM-SWCU, Salatiga. The chemicals used were ferrous ammonium sulfate (FAS) 0.1 N, HgSO<sub>4</sub>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 0.25 N, AgSO<sub>4</sub>, MnSO<sub>4</sub>, Alkali-Iod solution, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.025 N, AlluVer 3 Alluminium Reagent, Chromaver 3 Reagent, Ferrover Iron Reagent.

*Equipments*. Spectrophotometer HACH DR / EL 2700, HACH DR / EL 2000, pH meter, Balance analytical (Mettler H 80) at Laboratory of Environmental Chemistry, FSM-SWCU, and the AAS (Atomic Absorption Spectroscopy) (Perkin Elmer, 3110) at Wahana Tata Laboratory, Semarang-Indonesia.

First of all, liquid wastewater of Chemistry Laboratory, FSM-SWCU was characterized on the physico-chemical characteristics, which were: temperature, color, turbidity, conductivity, TDS (Total Dissolved Solids), pH, alkalinity, COD (Chemical Oxygen Demand),  $Mn^{2+}$ ,  $Pb^{2+}$ , and  $Zn^{2+}$ , respectively.

### 2.3. Laboratory waste treatment using A. pinnata.

A. *pinnata* plants were grown in a plastic cup of 8.1 cm in diameter containing wastewater of Chemistry Laboratory. Each plastic cup filled with wastewater of Chemistry Laboratory with a concentration of 1.25%. The water ferns (*A. pinnata*) were grown with different coverage area ratios (%), it's were as follow: 0; 12.5; 25; 37.5; 50, and 62.5%, respectively, in the surface area of the plastic cup. The physico-chemical characteristics of wastewater were analyzed on the  $2^{nd}$ ,  $3^{rd}$ , and  $4^{th}$  days, while the growth rate of *A. pinnata* were calculated on the  $4^{th}$  day and based by the wet and dry weight [10].

*Analysis of heavy metals* in wastewater were measured using a spectrophotometer HACH DR / EL 2700 Chemistry Laboratory, Department of Chemistry, FSM-SWCU, Salatiga and Atomic Absorption Spectroscopy (Perkin Elmer, 3110) at the Wahana Tata Laboratory- Semarang.

*Data Analysis* [19]. Data sorption of heavy metals Mn, Zn and Pb were analyzed by Randomized Completely Block Design (RCBD): 6 treatments and 4 replications. As the treatment was the different percentage of coverage area ratios of *A. pinnata*, those were: 0; 12.5; 25; 37.5; 50, and 62.5%, repectively, and as the block was the time. analysis to test the different of treatment means, Honestly Significant Differences (HSD) were used with 5 % of significance level.

## 3. Results and Discussion

## Initial Characterization of Wastewater Chemistry Laboratory and A. pinnata.

The initial characterization of wastewater Chemistry Laboratory and *A. pinnata* are presented in Table 1 and Table 2. Table 1 showed that the heavy metals concentration in Chemistry Laboratory were Mn: 8.148 ppm; Pb : 6.135 ppm; and Zn: 18.240 ppm, respectively. Chemical Laboratory heavy metal content is very high even for Class IV Water Quality Criteria even above the threshold. Class IV water quality is allowed for a maximum of 4,000 ppm TDS and wastewater Laboratory doubled, that is 8,300 ppm [8]. Especially for Pb 1 ppm, while the laboratory wastewater reaches 6,135 ppm; the maximum threshold only 5 ppm Mn, whereas the results of laboratory analysis of wastewater 8.148 ppm, Zn has maximum value of 10 ppm, while the concentration of Zn in the wastewater to 18,240 ppm.

Parar	Content				
Physical					
Temperature (°	26				
TDS (ppm)		8,300			
Electrical	Conductivity	16,600			
(µS/cm)					
Color (PtCo)	3,000				
Turbidity (FTU	1,000				
<u>Chemical</u>					
pН		2.1			
COD (ppm)		60,000			
Pb (ppm)		6.135			
As (ppm)		2.319			
Hg (ppm)	0.265				
Cd (ppm)	4.340				
Cu (ppm)	3.890				
Cr (ppm)		3.620			
Mn (ppm)		8.148			
Ni (ppm)		0.937			
Zn (ppm)	18.240				
Al (ppm)	2.150				
Fe (ppm)	11.235				

**Table 1**. Initial Characterization of Chemistry

 Laboratory Wastewater as Growing Media

From Table 2 it showed that *A. pinnata* contained different levels of heavy metals, ranging from 0.006 mg/kg to 0.162 mg/kg. Analysis of heavy metals in *Azollapinnata* before used as agents of bioremediation is considered important to point out that original plants also have done the bioaccumulation of heavy metals, both essential for biochemical plant cell (Mn and Zn), and which has no role in the process (Pb). These three heavy metals (Pb, Mn and Zn) absorbed by plants in the form of Pb<sup>+2</sup>, Mn<sup>+2</sup> and Zn<sup>2+</sup>. Last two heavy metals that play a role in supporting the growth of plants and act as a micro-nutrient essential. While the initial measurement of the heavy metals content in *A. pinnata* were presented in Table 2.

Table 2. Initial Characterization of
Heavy Metal Content in A. pinnata

Parameter	Content (mg/Kg)
Pb	0.006
As	0.006
Cd	0.008
Cu	0.023
Cr	0.020
Mn	0.145
Ni	0.003
Zn	0.162
Al	0.003
Fe	0.130

Note: The water content of A. pinnata was 94.19%

In Table 3 it appears that the average contents of Mn, Pb and Zn on the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> days observation varied between heavy metal's species. The mean concentrations of Mn (mg / L  $\pm$  SE) between the different levels of coverage area ratio of *A. pinnata* in period of 2-4 days ranged between 0.5393  $\pm$  0.0417 mg / L - 12.9308  $\pm$  0.1040 mg / L. The highest concentration of Mn absorbed by *A*.

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*pinnata* from Chemistry Laboratory wastewater within 2-4 days occurred in *A. pinnata* coverage area ratio of 62.5%. It means that the more surface area of *Azollapinnata* the more potential uptake area for removing heavy metals from the concentrate solution of growing medium into the bioaccumulate in plants.

Heavy Metals	Time		Area Cover Area (%)					
(mg/L±SE) (Days)		62.5	50	37.5	25	12.5	0	
		Average	0.7970±	$18.740 \pm$	27.993±	55.118±	103.688±	129.308±
	2	±SE	0.0228	0.0228	0.0620	0.2936	0.2606	0.1040
		W = 0.6255	(a)	(b)	(c)	(d)	(e)	(f)
		Average	$0.5885 \pm$	16.298±	$27.085\pm$	$34.620\pm$	91.713±	$112.098 \pm$
Mn	3	±SE	0.0357	0.0390	0.1398	0.1897	0.1925	0.1695
		W = 0.5977	(a)	(b)	(c)	(d)	(e)	(f)
		Average	$0.5393 \pm$	$12.070 \pm$	$22.148\pm$	$32.395\pm$	73.955±	$110.200 \pm$
	4	±SE	0.0417	0.0497	0.0521	0.1205	0.1928	0.1240
		W = 0.5044	(a)	(b)	(c)	(d)	(e)	(f)
		Average	$0.3505\pm$	$0.7245\pm$	$10.590\pm$	$12.530\pm$	19.128±	$27.558\pm$
	2	±SE	0.0114	0.0343	0.0277	0.0710	0.1007	0.3084
		W = 0.6279	(a)	(ab)	(bc)	(c)	(d)	(e
	3	Average	$0.2923\pm$	$06385\pm$	$10.258\pm$	$13.010\pm$	$18.393 \pm$	$24.088 \pm$
Pb		±SE	0.0477	0.0132	0.0458	0.0458	0.0395	0.0253
		W = 0.5769	(a)	(ab)	(bc)	(cd)	(d)	(e)
	4	Average	$0.1773\pm$	$05188\pm$	$0.9328\pm$	$11.420 \pm$	17.668±	19.490±
		±SE	0.0166	0.0400	0.0256	0.0869	0.0697	0.0318
		W = 0.2460	(a)	(b)	(c)	(d)	(e)	(e)
		Average	$13.493 \pm$	$26.903\pm$	$38.445\pm$	$62.678 \pm$	$85.743\pm$	$122.635 \pm$
Zn	2	±SE	0.0214	0.1203	0.0762	0.2212	0.1659	0.0796
		W = 0.5445	(a)	(b)	(c)	(d)	(e)	(f)
	3	Average	$10.700 \pm$	21.360±	31.705±	49.988±	78.170±	98.125±
		±SE	0.1957	0.0510	0.0482	0.1609	0.1711	0.3800
		W = 0.9442	(a)	(b)	(c)	(d)	(e)	(f)
		Average	$10.600 \pm$	$20.475\pm$	30.530±	$46.875\pm$	$78.093 \pm$	81.798±
	4	±SE	0.0295	0.0240	0.0389	0.2801	0.2499	0.1831
		W = 0.8285	(a)	(b)	(c)	(d)	(e)	(e)

**Table 3.** Average Concentrations of Mn, Pb and Zn in the Wastewater Chemistry Laboratory between Different A. *pinnata* Coverage Area Ratios in period of 2-4 Days

<u>Note</u> : W = HSD 5%

Number followed with the same alphabet on same arrow among the treatment showed were not significantly while the number are followed by different alphabet indicate significantly different.

The mean concentrations of Pb (mg / L  $\pm$  SE) between the different levels of *A. pinnata* coverage area ratio in period of 2-4 days ranged between 0.1773  $\pm$  0.0166 mg / L to 2.7558  $\pm$  0.3084 mg / L. It demonstrated that highest Pb concentration absorbed by *A. pinnata* occurred in coverage area ratio of 62.5% within 2 -4 days. The Pb absorption in the coverage area ratio of 62.5% began to occur in the 2<sup>nd</sup> day with the highest Pb uptake by *A. pinnata* in the amount of 0.3505  $\pm$  0.0114 mg/L. Other research showed that *A. pinnata* can absorb Pb greater than 50% from the oil waste but less than 50% of the diluted (0.5 ml / ml) concentration of wastewater [12]. Further, it is explained that no significant difference in the % absorption in the different concentrations of the wastewater shows no effect of dilution on the absorption capacity of ions by the test plant. Strong correlation between the metal ions in the solution and the plant tissue absorbed shows its bioindicating capacity of metal ions.

The mean concentrations of Zn (mg/L  $\pm$  SE) between different coverage areas ratio of *A. pinnata* in period of 2-4 days ranged between 1.0600  $\pm$  0.0295 mg / L to 12.2635  $\pm$  0.0796 mg / L. The highest Zn concentrations absorbed by *A. pinnata* from the wastewater Chemistry Laboratory within 2-4 days

occurred in 62.5% A. *pinnata*coverage ratio with the absorption effectiveness of 8.74 mg / L (89.10%) on the  $3^{rd}$  days.

Table 4 showed that *A. pinnata* area cover ratio of 62.5% is the best area cover ratio for the absorption of Mn, especially on day 4 with the effectiveness of Mn absorption of 95.11%. Effectiveness of Mn uptake by *A. pinnata* ranged from 10.48 mg/L (day 4) up to 12.13 mg/L (day 2). On the  $2^{nd}$  day effectiveness Mn uptake by *A. pinnata* high that is 12.13 mg/L (93.84%), it is associated with Mn which is one of the nutrients required by *A. pinnata* for growth.

Metals	Time	Control (mg/L)	Cover Area Ratio	Absorption (mg/L)
	(days)	Control (mg/L)	62.5% (mg/L)	Absorption (mg/L)
	2	12.9308±0.1040	$0.7970 \pm 0.0228$	12.13 (93.84%)
Mn	3	11.2098±0.1695	$0.5885 \pm 0.0357$	10.62 (94.75%)
	4	11.0200±0.1240	$0.5393 \pm 0.0417$	10.48 (95.11%)
Pb	2	$2.7558 \pm 0.3084$	$0.3505 \pm 0.0114$	2.41 (87.28%)
	3	2.4088±0.0253	$0.2923 \pm 0.0477$	2.12 (87.87%)
	4	$1.9490 \pm 0.0318$	0.1773±0.0166	1.77 (90.90%)
Zn	2	12.2635±0.0796	$1.3493 \pm 0.0214$	10.91 (89.00%)
	3	9.8125±0.3800	$1.0700 \pm 0.1957$	8,74 (89.10%)
	4	8.1798±0.1831	$1.0600 \pm 0.0295$	7.12 (87.04%)

**Table 4**. Effectiveness Absorption of Metal Zn, Pb and Zn by *A. pinnata* on Waste Water Chemistry Laboratory in The Cover Area Ratio of 62.5% During 2-4 Days

Mn is one of the esensiel metals for growth in phytoextraction process [22]. Furthermore, Mn is a micronutrient that is an essential for plant growth. *A. pinnata* contains a variety of nutrients, one of which Mn content of about 66-2,944 ppm. Mn best absorption by *A. pinnata* occurred on the 4<sup>th</sup> days (95.11%), due to the burden of toxic metals lead absorption occurs in dense populations of *A. pinnata* higher with a lower absorption rate [9]. *A. pinnata* can clean up by 65.1% Mn from liquid waste [mixture to irrigation for 10 days]. The results of this study showed a higher uptake and absorption effectiveness. In this research, Azolla media growth has micronutrients not only Mn but also Zn that can work together to support growth. The better the growth the better of heavy metal absorption capability.

Effectiveness of the optimum absorption of Pb by *A. pinnata* occured in the coverage area ratio of 62.5% in amount of 1.77 mg/L (90.90%) on the 4<sup>th</sup> day. Toxicity effect of heavy metals on plant growth follows the order as follows: Cu> Se>Pb> Cd> Ni> Cr [5]. In the point of viewed from the toxicity effect, *A. pinnata* is able to absorb lead (Pb) in conjunction with the absorption of Zn and Mn. In addition, the pH effect on the absorption of heavy metals is more acidic in the higher solubility of heavy metals, making it easily absorbed. Metal solubility decreased with increasing pH [21]. Furthermore, the absorption of heavy metals by plants is influenced by a competitor, metal, natural ligands, and artificial, characteristics and type of environment, plants, and the pH of the solution [26]. The results showed that the absorption of Pb by *A. filiculoides* L. affected by pH and the pH value of 3.5 and 4.5 is an optimal pH in the absorption of Pb [4]. Moreover, it is showed that *A. pinnata* is able to absorb Pb in the amount to 4.68% from Pb concentration of 140 ppm [7].

Azolla is able to decrease the concentration of lead (Pb) to 100% in a rice field irrigation system [12]. Furthermore, another study by Thayapara et al. (2013) showed that *A. pinnata* is capable of absorbing lead (Pb) of 1,383 mg / kg dry weight after four days and a reduction in the concentration of lead (Pb) by 83%. Effectiveness of Pb uptake in this study is much better when compared with the mentioned above results [23].

In Table 4 appears the effectiveness of Zn uptake by *A. pinnata* ranged from 7.12 mg/L (in day  $4^{th}$ ) up to 10.91 mg/L (in day  $2^{td}$ ). On the  $2^{nd}$  day, the effectiveness of Zn uptake by *A. pinnata* is high in the amount of 10.91 mg / L (89%). It is associated with Zn is one of micronutrient needed by *A. pinnata*. *A. pinnata* contains a variety of nutrients, one of which Mn content of about 66-2944 ppm. If the absorption of Zn compared with Mn on the  $2^{nd}$  day, it seems that on the  $2^{nd}$  day effectiveness Mn uptake greater than Zn. This corresponds to a radius smaller than the atom Mn, thus Zn is more difficult to remove, due to the greater the attractive force of the nucleus of valence electrons. Atomic

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radius (Å) of 1.37 Å Mn being Zn 1.34 Å [20]. Although the ionization energy is greater than the Zn, but Mn is relatively stable compared to Zn for having a full configuration. In addition, affinity electrons on metal Zn>Mn>Pb, Mn and Pb metal thus more easily absorbed than the zinc metal. But each plant metal accumulation evolved mechanisms different [25]. So that the absorption of metal Mn, Pb and Zn was dependent on the absorption mechanism of A. pinnata.

*A. pinnata* has accumulated a 2.1 ppm Zn metal to the total disappearance of 2:04 ppm, with an efficiency of 34% after a 10-day period at Dal River Ecosystem, India [17]. *A. pinnata* can reduce the concentration of Zn in two seasons (dry and wet) with an efficiency of 70.03% and 64.51% for 28 days in the domestic waste in Nigeria on artificial marsh [2]. The results of this study demonstrate the effectiveness of a higher uptake.

The pattern of uptake Mn, Pb and Zn in conjunction with *RGR*The number of colonies and Wet Weight of solid percentages among various population on day 4 are presented in Figure 2.

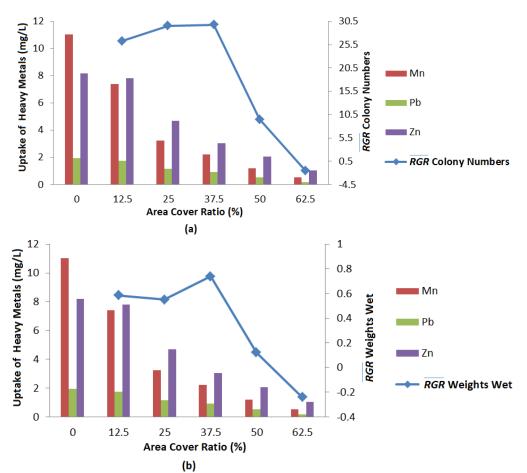


Figure 2. Uptake of Mn, Pb, Zn and growth of *A. pinnata* Based on Relative Growth Rates (*RGR*) Number of colonies (a) and Wet Weight (b) Between Various Cover Area Ratio on day 4

From Figure 2a and 2b it was shown that the average growth rate of *A. pinnata RGR* occurred in the coverage area ratio of 37.5%. This is in line with the start of the absorption of Mn, Pb and Zn. The Mean Growth Curve of *A. pinnata* showed a similar growth pattern, initially they increased and reached a maximum in the area cover ratio of 37.5%, and then declined rapidly in the coverage area ratio of 50% and 62.5%. In the area cover ratio 37.5% *A. pinnata* grows optimally under the influence of surface area to allow for the increase of *A. pinnata*'s new colonies. Another thing that affects the growth of *A. pinnata* is the content of Mn and Zn which is a micronutrient found in liquid wastewater of Chemistry Laboratory (Table 1).

Further study in relation to the effectiveness of absorption showed that on the 4<sup>th</sup> day, the coverage area ratio 62.5% of *A. Pinnata* absorbed Mn, Zn and Pb optimally. This is in line with the curve of

average growth rate of *A. pinnata* which achieved a minimum in the coverage area ratio of 62.5% on the 4<sup>th</sup> day. Accumulation of Zn and Pb by *A. pinnata*occured in the root (rhizofiltration), while in the case of Mn by the phytoextraction process. With the variety of amino acids composition, *A. pinnata* is capable of forming a metal chelating compounds to form complex compounds, and can also produce phytochelat substances which help metal absorption. Phytochelatine formed when plants are exposed to the metal. Phytochelatine (PCs) are small-derived glutathione, enzymatic synthesis of peptides, which bind metals and a major part of the metal detoxification system in plants, have a common structure ( $\gamma$ -glutamyl-cysteinyl) n-glycine, (n = 2-11) [3]. Moreover, it described that peptides/proteins involved in the most important metal accumulation and tolerance is phytochelatine (PCs) and metalotionin (MTs). Phytochelatine (PCs) and metalotionin (MTs) of plants rich in cysteine sulfhydryl groups, which bind and absorb heavy metal ions. This Metalotionin (MTs) are low molecular weight (4-10 kDa), rich in cysteine, and a protein that binds to metals through the thiol groups of cysteine residues.

The absorption of heavy metals in plants can also be affected by competition, competing with nutrient cations from metal to uptake the place [23]. So that the metal absorption decreases with increasing concentration of nutrients. The process of absorption of heavy metals by the roots is assisted by a chelate agent, namely phytosiderofor. Phytosiderofor molecules bind Zn and Mn, while Pb bound by phytochelatine, then forms a molecule in the membrane reductase roots to improve absorption. Reductase which further serves to reduce the metal is transported through special channels in the membrane of the root [18]. The tolerant nature determined by the content of glutathione (GSH), cysteine (Cys) and O-acetyl-L-serine (OAS) while the ability to accumulate heavy metals in tissues affected by the content of serine Acetyltransferase (SAT) and activity glutathione reductase [25]. Heavy metal is converted into a form that is less toxic through a chemical reaction or complex formation of secondary metabolites produced by plants, to be able to enter into tissue without poisoning the plants. Plants are generally issued thiol group as a chelating (ligand), but many also metabolites issued as a ligand depending on the type of metal to be chelat [24]. Isolation of a cDNA type 2 metalotionin called AzMT2, and concluded that Metalotionin, AzMT2, responded more to the ion Cd and Ni in Azollafiliculoide, so that it can participate in detoxification mechanisms, and AzMT2 respond more lenient to ion Zn and Cu is an essential micronutrient [6].

#### 4. Conclusions

*A. pinnata* coverage area ratio of 62.5% was the best ratio in the absorption of Mn, Pb, Zn from wastewater of Chemistry Laboratory. Effectiveness absorption of *A. pinnata* within 4 days could absorb 10.48 Mn mg/l (95.11%); Pb 1.77 mg/l (90.90%); and Zn 7.12 mg/l (87.04%) from wastewater of Chemistry Laboratory.

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