ATLANTIS PRESS
Atlantis Highlights in Engineering (AHE), volume 1
International Conference on Science and Technology (ICST 2018)

Azolla microphylla and *Pistia stratiotes* as Phytoremediator of Pb (lead)

Fida Rachmadiarti[1], Herlina Fitrihidajati, Tarzan Purnomo, Yuliani, Dwi Asih Wahyuningsih
Department of Biology
Universitas Negeri Surabaya
Surabaya, Indonesia
[1]fidarachmadiarti@unesa.ac.id

Abstract— ***Azolla microphylla* and *Pistia stratiotes* were type of plants which used as alternative to remove pollutant from contaminated water. This study aimed to determine efficiency of *Azolla microphylla* and *Pistia stratiotes* as phytoremediator of Pb. These plants were grown in 14 days under hydroponic system with type of plants were *Azolla microphylla, Pistia stratiotes,* Azolla *microphylla* and *Pistia stratiotes,* Pb concentrate (0, 5, 10, and 15 ppm). Analyze method of Pb used AAS (Atomic Absorption Spectrophotometer) and the wet biomass calculation. The data were analyzed with Statistic Program 20th Edition to calculate Anova and continued Tukey test with 5% of real level or 95% of confidence level. Efficieny of plant as a phytoremediator consecutively from low to high was *A. microphylla>P. stratiotes and A. microphylla+P. stratiotes* combination, which value of BCF>1 and TF<1. Capability of plant to remediate was also supported by growth from wet biomass, which biomass growth of plant that has concentrate high Pb metal was higher than plant that has concentrate low Pb metal. This result showed significantly difference in plant organ with various concentrate that has been used, which composition of Pb metal in roots was higher than leaves.***

Keywords— Azolla microphylla; Pistia stratiotes; BCF; TF; Pb metal

I. INTRODUCTION

Heavy metal contamination waters are causing negative impact to the aquatic biota. It is reported there are 80 types of metal, which one of them is lead (Pb) include of the big three heavy metal categories based on toxicity level [1]. Heavy metal chelated with sulfurhidril group and accumulated on water. Accumulation of heavy metal are causing mortality for aquatic biota if the level over water threshold. Excessive Pb in water destroys transpiration process, photosynthesis, respiration, inhibit enzyme activation and replace membrane permeability [2], therefore there is a way to solve water contamination by Pb.

Treatment and control of water contamination are an effort to inhibit, treat and restore water quality so that water quality accord to water quality standard [3]. One of water contamination recovery is by using phytoremediation technique. Phytoremediation is the uses of plant to transfer, remove, stabilize and decrease soil contaminant [4], water and sediment [5]. Utilization of phytoremediation by using plant which capable to accumulate organic matter or inorganic matter as lead such as utilization of aquatic fern (*Azolla microphylla)* and water lettuce (*Pistia stratiotes*). These plants were indigenous in Indonesia and have multi benefits for Indonesian people. *A. microphylla* was found at rice field while *P. stratiotes* was found at rice field or river [6].

Azolla microphylla was reported capable to accumulated toxic matter such as lead, mercury, cadmium, chromium, copper, nickel, zinc and also remove contaminants from wastewater [7]. The previous study [8] about *A. microphylla* as bioconcentrate of lead (Pb) contamination was resulted *A. microphylla* on 7th day with lead concentrate 5 ppm capable to absorb Pb up to 1.6475 ppm so that from this result Pb composition in the water was decrease, while the composition of *A. microphylla* and soil was increase on 14th than 7th day.

Tewari *et al.*, studied that *P. stratiotes* remediate a metal by using detoxification and bioaccumulation treatment in example against Arsenic [29]. Dwijayanti in her study about phytoextraction of Cu, Cr and Pb with water lettuce plant (*Pistia stratiotes* L.) gained result in 48 hours was capable to decrease Pb and Cu concentrate in textile wastewater till the level was under the wastewater quality standard. These plants were considered have potency as phytoremediation agent related its capability to concentrate Pb metal in roots and translocate to organ [10].

Based on previous study by Oktaviani was using *Hydrila* sp. and *P. Stratiotes* as phytoremediation agent in order to decrease Pb composition on water showed the optimal growth found in combination treatment [11]. Previous research about *A. microphylla* and *P. stratiotes* were studied respectively, therefore the combination treatment from these plants and determination the efficiency *A. microphylla* and *P. stratiotes* as metal phytoremediator Pb in water should be studied furthermore, so, this study aimed to determine efficiency of *Azolla microphylla, Pistia stratiotes, and combination both of plants* as phytoremediator of Pb.

II. MATERIALS AND METHODS

The study was conducted at Green House C10 Faculty of Mathematics and Life Sciences, Universitas Negeri Surabaya. The sample *A. microphylla* was collected locally from water cultivation in Sepanjang, Sidoarjo District, while *P. stratiotes* was collected locally at the rice field around Purwosari, Malang District. This study did into 3 stages, which were preparation, treatment and data collection.

Preparation started with acclimatization as long as 7 days with aquadest grown media on aquarium, pH was measured each day at range 6,8, the sample was continuing used on next stage. Grown media was made by using aquadest fulfilled into 36 aquariums up to 10 liters and labeled accord to treatment, added 20% Hoagland solution from media, also added heavy metal $Pb(NO_3)_2$ with each concentrate 0 ppm (control), 5 ppm, 10 ppm and 15 ppm into aquarium accord to treatment code.

Treatment followed by steps such as: 1) Analyze heavy metal level which composed in *A. microphylla, P. stratiotes* plant and water as its habitat, 2) *A. microphylla* and *P. stratiotes* plant weighed 100 gr and 50 gr respectively and fed into aquarium which has been given grown media accord to treatment code on aquarium, 3) Tests of heavy metal level was took after 14 days on grown media and roots of *A. microphylla* and *P. stratiotes* plant, 4) Absorption level by plant was total of heavy metal which showed difference from lead heavy metal level in biota distinguished by before and after treatment. Calculation was counted by value that showed post treatment of lead heavy metal level (ppm) on plant then reduced by value of pretreatment of lead heavy metal level (ppm) on plant, 5) Absorption level on grown media was total of heavy metal which showed difference from lead heavy metal level distinguished before and after treatment on media. Calculation was counted by value that showed post treatment of lead heavy metal level (ppm) on media then reduced by value of pretreatment of lead heavy metal level (ppm) on media.

Data collection, there are steps in this stage such as: 1) Measurement of temperature on grown media by using water thermometer at ^{0}C, pH of grown media was measured using pH probe also the light intensity was measured by lux meter, as supporting data in this study, 2) Heavy metal level which contained in roots *A. microphylla* and *P. stratiotes* was analyzed after 14 days of treatment by using AAS method, took place at Nutrition Laboratorium Faculty of Public Health Universitas Airlangga, 3) Biomass growth on pretreatment and post treatment was measured using electric scale, 4) Morphology observation both of plant, 5) Accumulation of Pb was measured with Bioconcentrate Factor (BF) which used to calculate leaves capability in order to accumulate Pb, according to calculation,

$$BCF = \frac{\text{Lead heavy metal on Roots or Leaves}}{\text{Pb heavy metal on sediment or water}}$$

Translocation Factor (TF) heavy metal that has been used to calculate heavy metal translocation process from roots to leaves, formulated as:

$$BCF = \frac{\text{Lead heavy metal on Leaves}}{\text{Pb heavy metal on Roots}}$$

The data of Pb level and growth of plant were statistically analyzed by using ANAVA two ways, two treatment factors represented by concentrate differentiation Pb and plant type, continued by DMRT Test to determine the optimal treatment in this study.

III. RESULTS

Result of DMRT Test with significant standard 0.05, gained there was real difference between BCF value and TF value on Pb concentrate of treatment 0 (control), 5 ppm, 10 ppm and 15 ppm which marked with difference of capital letter notation distribution on each average value, while TF an BCF value for treatment of plant type *A. microphylla* and *P. stratiotes* optimal combination higher than which marked with small letter notation distribution on each average value (Table I).

TABLE I. BCF AND TF OF *AZOLLA MICROPHYLLA* AND *PISTIA STRATIOTES*

Plants	Pb Concentration (ppm)	Bioconcentration Factor (BCF)	Translocation Factor (TF)
Azolla microphylla	0	$0.00{\pm}0.00^{bA}$	$0.00{\pm}0.00^{bA}$
	5	$1.35{\pm}0.008^{bB}$	$0.169{\pm}0.001^{bB}$
	10	$1.56{\pm}0.063^{bC}$	$0.185{\pm}0.043^{bC}$
	15	$1.64{\pm}0.0177^{bD}$	$0.218{\pm}0.004^{bD}$
Pistia stratiotes	0	$0.00{\pm}0.00^{aA}$	$0.00{\pm}.0.00^{aA}$
	5	$0.72{\pm}0.00^{aB}$	$0.138{\pm}0.001^{aB}$
	10	$0.78{\pm}0.00^{aC}$	$0.184{\pm}0.005^{aC}$
	15	$0.80{\pm}0.00^{bD}$	$0.196{\pm}0.000^{aD}$
Azolla microphylla and *Pistia stratiotes*	0	$0.00{\pm}0.00^{bA}$	$0.00{\pm}0.000^{bA}$
	5	$0.41{\pm}0.00^{bB}$	$0.135{\pm}0.001^{bB}$
	10	$0.47{\pm}0.00^{bC}$	$0.162{\pm}0.001^{bC}$
	15	$0.50{\pm}0.00^{bD}$	$0.170{\pm}0.000^{bD}$

Note: Number which followed by difference of alphabet notation on row and column showed real different result according to DMRT Test on standard test 0,05. Small letter notation showed treatment of plant type, while capital letter showed Pb concentration difference.

Study result showed there was an effect of Pb concentration against *A. microphylla* and *P. stratiotes* growth, optimal growth was found on Pb treatment at 10 ppm, while for effect of plant type against *A. microphylla* and *P. stratiotes* growth and combination, optimal growth was found on *P. stratiotes* and combination treatment (Table II).

TABLE II. GROWTH OF *AZOLLA MICROPHYLLA* AND *PISTIA STRATIOTES* ON DIFFERENCE PB CONCENTRATION.

Plants	Pb Concentration (ppm)	Initial biomass (gram)	Fresh biomass (gram)
Azolla microphylla	0	100	116.67±2.89^{aA}
	5	100	130.00±5.00^{aBC}
	10	100	132.67±4.62^{aC}
	15	100	115.00±4.36^{aB}
Pistia stratiotes	0	100	120.67±1.16^{bA}
	5	100	140.00±5.00^{bBC}
	10	100	137.67±8.74^{bC}
	15	100	139.00±7.94^{bB}
Azolla microphylla and Pistia stratiotes	0	100	125.00±5.00^{bA}
	5	100	139.67±4.73^{bBC}
	10	100	144.33±4.04^{bC}
	15	100	143.00±4.36^{bB}

Note : Number which followed by difference of alphabet notation on row and column showed real different result according to DMRT Test on standard test 0,05. Small letter notation showed treatment of plant type, while capital letter showed Pb concentration difference.

Pb level in roots and leaves were respectively different on *A. microphylla*, *P. stratiotes* treatment and combination. On roots, Pb level was higher than Pb level on leaves.

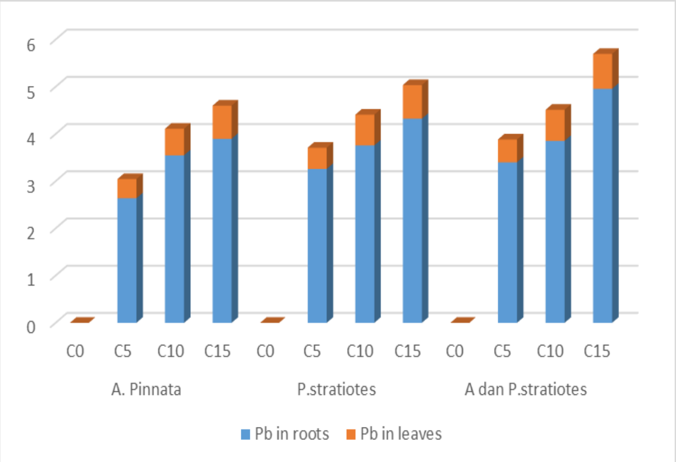

Fig. 1. Graphic of Pb Level on Roots and Leaves of *A. microphylla*, *P. stratiotes* and Combination.

IV. DISCUSSION

On 14 days of treatment, in *A. microphylla* found the higher of concentration treatment, the more higher BCF value. Value of BCF more than 1, which showed roots capability to accumulate Pb was higher than leaves and shoots. Based on BCF value, *A. microphylla* was efficient as Pb bioaccumulator. Capability of *A. microphylla* to accumulate Pb on roots confirmed furthermore by TF calculation, which showed Pb metal that translocate to bud was lower with average value of TF less than 1 (Table 1).

Evaluation from BCF and TF on this study explained that plant was capable to translocate Pb from contaminated media to roots with higher concentrate than shoots and leaves, it's because in roots zone found rhizosphere which produce exudates and enzymes, chelated with metal so that metal can absorb by roots [12], so this plant used as phytoremediation. In other words, *A. microphylla* and *P. stratiotes* were capable to concentrate Pb in roots system, so that more efficient applied to phytoremediation on contaminated water (rhizofiltration) or contaminated area in order to limit the metal absorb into water layer (phytostabilization). The condition was supported with growth of plant.

Study result which related to growth of plant showed that treatment of plant type. On *A. microphylla*, *P. stratiotes* and combination showed increase of the growth marked with the increase of plant biomass. This result was according to Petshombat *et al.*, investigated fern plant of Salvinea which contaminated Pb in concentrate 10, 20, 30, 40 mg L^{-1} as 2,4,6,8 days showed decrease of growth. As kale and yellow bur-head plants, on this study concentration treatment wasn't influence growth of clover. On water kale plant, yellow bur-head and clover which not contaminated Pb or another plant that contaminated Pb showed biomass growth and RGR. The longer day of contaminated Pb the more higher biomass growth and RGR that decreased, which showed Pb absorption in plant organ can tolerated by its plant [13]. Adaption of plant on high metal level media and tolerate against that metal has slow growth [14].

Plant that contaminated Pb influence germination and growth phase on its plant [15]. Pb effect rarely related to inhibitor and decrease of growth [16]. This mean there was possibility both of that plant were natural hyperaccumulator which more tolerant against Pb toxicity than another sensitive plants [17].

Result showed that Pb composition on roots was higher than shoots and leaves. On concentrate treatment showed difference, the higher concentrate treatment was related to the higher Pb which absorb by roots of plant [18]. The higher Cu which absorb by roots in 8 ppm concentrate of Cu on media [19]. Zou found media that contained Pb showed correlation between Pb concentration on media and time of contamination Pb in herb *dycotyledoneae carpesium abrotanoides*, *Conyza Canadensis*, *Anemone vitifolia*, monocots *Juncus effussus* and pterydophytes *Ahyrium wardii*, *Pseudocyclosorus subochthodes* [20].

Heavy metal absorption process of plant through roots, leaves and stomatal, called by translocation mechanism. Transport from cell to cell in vascular tissue so that distributed into whole of plant body. Catalysis diffusion chelated with cytoplasm which called by plasmodesmata [21]. Heavy metals which absorb by plant through ions dissolve into water such as nutrition as followed by water mass. Root plant cells contain high ion concentrate than medium, continues by water diffusion which contain Pb into roots.

Another process which helps Pb accumulation on roots was leaf water potential and ions on media. Root cells with high osmosis potential were also causing high water potential. Metal accumulation into plant roots through ligand transport on root membrane, continues by build ligand transport which penetrate into xylem till leave cells. On leaves, this transport through plasmalemma, cytoplasm and tonoplast to enter vacuole, in vacuole this complex ligand transport react with ligand terminal acceptor to build ligand complex acceptor. Ligand transport released and metal complex acceptor accumulated on vacuole which not related with plant physiology process [22].

Some researcher investigated capability of *A. michrophylla* absorbs Pb which caused by type of float plant and accumulate metal by using its submerged root [19]. *P. stratiotes* was type of water plant which capable to grow fast and has a high absorption level in organic or inorganic matter [24].

Roots capability in accumulate Pb metal was higher than leaves and shoots, related with *A. michrophylla* and *P. stratiotes* roots function as phytostabilization, which that roots immobilize toxic ion of Pb on growth media through accumulation, absorption on roots surface, and precipitate pollutant precipitate on roots zone. Roots take a role as rhizofiltration which absorb toxic ion of Pb. Compared with another organ from those three plants, roots has a potency to absorb Pb level in the form of ions and inorganic salts. Increase of Pb level in roots was causing by Pb accumulation on roots [25]. According to U.S. Environmental Protection Agency (EPA) [26] generally explained some role of roots that may take a role against Pb toxic ion.

V. CONCLUSION

Plant efficiency as phytoremediator consecutively from low to high level was *P. stratiotes*, *A. microphylla+P. stratiotes* combination, *A. microphylla*, with value of BCF >1 and value of TF<1. Capability of plant to remediate was also supported by wet biomass, which plant that has concentrated Pb in high level, not so high biomass growth. Movement of metal in media depends on roots length, plant density and metal concentrate. The more long of roots and high of density showed the great movement of metal, which caused by increase of surface area for metal absorption by roots. Results were also showed significant difference on plant organ with different concentration treatments, which is composition Pb metal on roots was higher than leaves. *A. microphylla* and *P. stratiotes* were potency as Pb phytoremediator on water which continues have implication for contaminated water treatment.

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