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The Use of Water Lettuce (*Pistia stratiotesas)* as Phytoremediator for Concentration and Deposits of Heavy Metal Lead (Pb) Tilapia (*Oreochromis niloticus*) Gills

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Abstract. This study discusses the ability of *P. stratiotes* to reduce the concentration of heavy metal in water so that it can reduce lead absorption in tilapia gills. This study used an experimental method with a complete random design. the main ingredients used were water lettuce (*Pistia stratiotes*), Pb $(NO_2)_3$ and tilapia (*Oreochromis niloticus*). Analysis of lead concentration was carried out with *Atomic Absorption Spectrophotometry*. The percentage of absorption capacity of *P. stratiotes* was up to 99.9% after 15 days of maintenance in media with 1 ppm lead contamination. The decrease in lead concentration in the media with *P. stratiotes* causes the decrease in lead absorption in the gills, and at the same time also increase the lead concentration found in the roots. The concentration of lead absorbed by the gills was reduced to 34.7%, lower than the treatment without the plants. The results of this study indicated that *Pistia stratiotes* can reduce lead concentration in water media. Therefore, this plant can be used as a phytoremediator for lead-laden wastewater in wider scoop.
Keywords. *Pistia stratiotes*, *Oreochromis niloticus*, phytoremediation, Pb, gills

1. Introduction

The emergence of industrial activities and overall human activities leads to the increase in the concentrations of heavy metals found in nature (da Silva *et al.*, 2013). Heavy metals from the industry comes from it being used as raw materials and in the mixtures of various products (Sudarmaji et al., 2006). Heavy metal lead (Pb) is widely used in the mining, textile, battery manufacturing and paint industries (Olgulín *and* Sánchez-Galván, 2012). Heavy metals that infiltrate the aquatic environment may accumulate in biota (Setiawan and Subiandono, 2015), one of which is tilapia. Heavy metals enter the body of the fish through the gills, body surface (skin) and digestive tract (intestine) (Leopold *et al.*, 2015). According to Aldogachi *et al.* (2016) the absorption of heavy metals by fish is mainly found in the gills, making it the main target organ of heavy metal contamination.

Hence, environmentally friendly and cost effective technology is critical to reduce heavy metal pollutants in order to improve water quality (Sood *et al.*, 2012). Phytoremediation is an alternative to reduce pollutants in water and soil using plants. One plant that can be used as phytoremediator is

Water lettuce (*Pistia stratiotes*) (Hanks *et al.,* 2015). The roots of *P. stratiotes* plays an important role in absorbing pollutants from heavy metals contaminated water (Lu *et al.,* 2011). *P. stratiotes* has phytochelatin content is necessary to bind heavy metals (Vesely *et al.,* 2012). Phytochelatin which bond with lead will form a PC-Pb complex stored in the vacuole (Yadav, 2010). This cause a decrease in lead concentration in water and increase lead concentration at the root of water lettuce due to the absorption process.

The purpose of this study was to determine the effect of *P. stratiotes* on the concentration of heavy metal lead in water and to find out the optimal biomass to reduce the concentration of heavy metal lead in water. This study was expected to provide information about the use of *P. stratiotes* as aquatic phytoremediators and the optimal biomass to reduce lead concentrations in water.

2. Equipments and methods

2.1. The equipments

The equipment used in this study was *P. stratiotes* obtained from Giri Village, Gresik Regency, *O. niloticus* measuring 5-7 cm, fresh water, chlorine, pellet feed, Pb $(NO_3)_2$ and aquades.

2.2. The tools

The tools used in this study were 20 aquariums of 30x20x20 cmsize^3, thermometers, pH meters, DO meters, ammonia *test kits*, digital scales, surgical instruments, hoses and aeration, volume pipettes, beaker glass 1000 ml, spatulas, filters , plastic tubs, vial bottles, cool boxes, *Atomic Absorbance Spectrophotometric* (AAS).

2.3. The method

The research was conducted in August-September 2017 at the Laboratory of the Faculty of Fisheries and Marine Universitas Airlangga Surabaya. The lead metal concentration test was carried out at the Surabaya Industrial Research and Standardization Center Laboratory.

The method used in this study was experimental method using a complete random design (CRD) with five treatments and four replications. The biomass of *P. stratiotes* used in this study were 25, 50 and 75 grams (Hernayanti and Proklamasiningsih, 2004). The treatments given were:

P1 (negative control) : media without lead + without *P. stratiotes* + *O. niloticus*
P2 (positive control) : media with a lead concentration of 1 ppm + without *P. stratiotes* + *O. niloticu*
P3 : media with lead concentration 1 ppm + 25 grams *P. stratiotes* + *O. niloticus*
P4 : media with a lead concentration of 1ppm + 50 grams *P. stratiotes* + *O. niloticus*
P5 : media with a lead concentration of 1 ppm + 75 grams *P. stratiotes* + *O. niloticus The*

The study began with sterilization of the aquarium, the hose and the aeration rocks. Acclimatization of *O. niloticus* and *P. stratiotes was* carried out by keeping it separately in a plastic tub filled with fresh water for seven days. Maintenance after administration of the treatment was carried out for 15 days. During maintenance, the fish were fed with in adlibitum way. Feed was given twice a day in the form of floating pellet administered at 08.00 and 16.00 WIB.

The data was the concentration of heavy metal lead on the roots of water lettuce, gills of tilapia and the water which was taken before and after treatment, as well as water quality (temperature, pH, DO, ammonia) and the behavior of the tilapia. Tests on lead concentration in the roots of water lettuce, tilapia gill, and water were carried out with *Atomic Absorption Spectrophotometry* (AAS). The data obtained was analyzed using ANOVA analysis of variance test and further tested with Duncan's Multiple Distance Test to find out the best treatment (Kusriningrum, 2010). Data analysis was performed with the SPSS 16.0 program.

3. Results and discussion

3.1. Results

Table 1. Average decrease in lead concentration in water for 15 days.

Treatment	initial Pb concentration (ppm)	Final Pb concentration (ppm)	Percentage of Pb Decrease (%)
P1	<0.0012	<0.0012	0
P2	1	0.018	98.2
P3	1	0.004	99.6
P4	1	0.003	99.7
P5	1	<0.0012	99.9

Description: P1 = negative control (without Pb, without *P. stratiotes*), P2 = positive control (1 ppm Pb, without *P. stratiotes*), P3 = 25 grams *P. stratiotes*, P4 = 50 grams *P. stratiotes*, P5 = 75 grams *P. stratiotes*.

Table 2. Average lead concentration in gills after 15 days of.

Treatment	Pb concentration Initial (mg / kg)	Final Pb concentration (mg / kg)	Percentage of Pb Absorption (%)
P1	2.11	3.9	45.9%
P2	2 , 11	18.8	88.8%
P3	2.11	11.9	82.3%
P4	2.11	8.5	75.2%
P5	2.11	4.6	54.1%

Description: P1 = negative control (without Pb, without *P. stratiotes*), P2 = positive control (1 ppm Pb, without *P. stratiotes*), P3 = 25 grams *P. stratiotes*, P4 = 50 grams *P. stratiotes*, P5 = 75 grams *P. stratiotes*.

Table 3. The average of lead concentration in the root after 15-day.

Treatment	Pb concentration Initial (mg / kg)	Final Pb concentration (mg / kg)	Percentage Pb Absorption (%)
P1	-	-	-
P2	-	-	-
P3	4,57	152.4	97%
P4	4.57	222.1	97.9%
P5	4.57	534.2	99.1%

Description: P1 = negative control (without Pb, without *P.*stratiotes),P2 = positive control (1 ppm Pb, without *P. stratiotes*), P3 = 25 grams *P. stratiotes*, P4 = 50 grams *P. stratiotes*, P5 = 75 grams *P. stratiotes*. Sign (-) = does not test the concentration of lead in plants.

Table 4. Data on water quality during maintenance.

Treatment	Temperature(◦C)	pH	DO (mg / L)	Ammonia (mg / L)
P1	27.1	7.7	5.96	0.3
P2	27.3	7.9	5.91	0.36
P3	27.2	7.9	5.85	0.12
P4	27.2	7.8	5.86	0.19
P5	27.1	7.8	5.93	0.19

Remarks: P1 = negative control (without Pb, without *P. stratiotes*), P2 = positive control (1 ppm Pb, without *P. stratiotes*), P3 = 25 grams *P. stratiotes*, P4 = 50 grams *P. stratiotes*, P5 = 75 grams *P. stratiotes.*

3.2. Discussion

Based on the results of the concentration test on water on the 15th day it was found that the lead concentration had decreased. This shows that the presence of *P. stratiotes* influences lead concentration in water media. The P5 treatment (75 grams) revealed the highest decrease compared to other treatments which was up to 0.001 mg / L. Giving 75 grams of biomass tend to produce a higher decrease in lead concentration up to 99.9%, compared to the administration of 50 grams of biomass which was revealed to be 99.7% and 25 grams which (99.6%). This shows that the administration of *P. stratiotes* is effective in reducing lead concentration compared to not using *P. stratiotes*. The more *P. stratiotes* biomass given, the higher is the decrease in lead concentration produced.

The administration of *P. stratiotes* also affects the concentration of lead in fish gills. Metals enter the fish's body through the gills, body surface (skin) and digestive tract (intestine) (Leopold *et al.*, 2015). According to Ekeanyanwu *et al.* (2015) the gills are the organs that are affected by pollutants the most. During 15 days of maintenance, there was an increase in lead concentration found in the gills. The lead is concentrated in gills due to the continuous contact of the gills with the surrounding environment (Low *et al.,* 2011). P5 treatment (75 grams) was revealed to yield the lowest concentration compared to other treatments. This is because the lead in the water was absorbed by *P. stratiotes*. Consequently, the lead does not accumulate much in the gills. P2 treatment (positive control) without *P. startiotes* exhibited the highest lead concentration among other treatments in which absorption reached 88.8%. This is consistent with Kalay and Calay (2000) which stated that the highest accumulation tends to be found in the gills. The high concentration of lead in the gills is in line with the amount of the final concentration of heavy metal lead in water which has decreased to 0.018 mg / L. The absence of *P. startiotes* in this treatment caused the high concentration of lead in the gills.

The results of the lead concentration test on the root of *P. stratiotes* after each treatment revealed the same tendency, the increase in concentration. This means that the absorption of lead by *P. stratiotes* is prevalent. Variations in the biomass used leads decrease in different concentrations. The decrease in lead concentration in water media is proportional to the increase in lead concentration in the roots of *P. stratiotes*. The same results were also exhibited in Hernayanti and Proklamasiningsih (2004) which stated that the more plant biomass used, the higher the absorption that occurred. Each plant has the ability to absorb or accumulate heavy metals in varying amounts (Irwanto et al., 2015). The number of concentrations that can be absorbed by plants depends on the concentration of heavy metals and the types of plants used (Memon and Schröder, 2009). *P. stratiotes* has been shown to be able to accumulate lead heavy metals from waters (Tewari *et al.,* 2008) and has good tolerance to lead (Espinoza-quinones *et al.,* 2009). *P. stratiotes* has phytochelatin which binds heavy metal ions in a stable complex form (Ali *et al.,* 2013). Phytochelatin is known to be able to bind various heavy metals including lead. Phytochelatin which binds with heavy metals is stored in vacuoles as a phytochelatin-heavy metal complex (Nguyen *et al.,* 2017). Phytochelatin which binds with lead metal will form a PC-Pb complex (Yadav, 2010). Storing heavy metals in vacuoles is one way to minimize toxic effects. Hence, their interactions with cellular metabolic processes can be reduced (Ali *et al.,* 2013). 75 gram of biomass tends to absorb more lead concentration with 99.1 percentage compared to giving 50 grams of biomass absorb 97.9% and 25 grams which absorb 97%.

The accumulation of heavy metals depends on the concentration and duration of exposure as well as several other factors such as salinity, temperature and pH (Kalay *and* Canli, 2000). The average results of measurement of water quality of maintained media during the study were at 27.1◦C, pH 7.8; DO 5.9 mg / L and ammonia 0.23 mg / L. *O. niloticus* can live optimally at temperatures between 26-32 ◦C (Suresh *and* Bhujel, 2012) and *P. stratiotes* can live optimally at a temperature range of 15-35 ◦C (Srivastava *et al.,* 2008). Increasing temperatures may cause increase in the solubility of heavy metals in water (Jobling, 1995). *O. niloticus* can live optimally at pH between 6.5-8.5 (Suresh *and*

Bhujel, 2012) and *P. stratiotes* can live optimally at a pH range between 6-9 (Srivastava *et al.,* 2008). According to Palar (2008), the lower the pH, the higher is the solubility of heavy metals. THis situation is caused by *O. niloticus* requires DO of> 3 mg / L and <1 ppm ammonia range (Suresh *and* Bhujel, 2012). The amount of ammonia increase with the length of maintenance. This is thought to be caused by feeding and the presence of fish stool. Based on measurements of water quality in the form of temperature, pH, DO and ammonia, it is known that in general the water quality during maintenance in each treatment did not affect the treatment because it was still in the optimum range.

The content in water media may cause organisms living in it to respond to changes in the environment with their various abilities. One of them is a behavioral response (Yulan et al., 2013). Parameters that may be used to determine changes in fish behavior are swimming movements and appetite (Sabullah *et al.,* 2015). Lead concentration on maintenance media causes differences in fish behavior. P2 treatment as the form of a positive control showed a change in swimming movement in fish. The fish in P2 treatment are not actively moving and more often stay in the area below the aquarium. This is different from treatment P1 and generally in other treatments which do not show much behavior change.

Besides the fish swimming movement, this study also observed fish appetite. It is prevalent on day 1 that the appetite in P2, P3, P4 and P5 treatments is similarly low. Scott *and* Sloman (2004) states that the presence of heavy metals in the waters can cause fish appetite to decrease. Fish appetite in treatment P4 and P5 returned to high on day 3 of maintenance while in treatment P3 it was on day 8. The fish in P2 treatment stayed to have low appetite until day 9 of maintenance.

Although in treatments P3, P4 and P5 has the same lead concentration as treatment P2, the fish that live in P3, P4, and P5 exhibited different behavior. The presence of *P. stratiotes* which function as phytoremediators for heavy metals lead gives affect the fish differently. This change in behavior is thought to be caused by changes in the environmental conditions in which the fish live.

4. Conclusions and suggestions

4.1. Conclusions

The application of Water Lettuce (P. stratiotes) affects the concentration of heavy metal lead (Pb) found in the water and the gills of tilapia. Providing *P. stratiotes* biomass as much as 75 grams produces the best effect in reducing lead (Pb) concentrations in water and tilapia gills.

4.2. Suggestion

Further research is expected to discuss the use of different concentrations of heavy metals with different lengths of exposure in order to determine the maximum ability of *P. stratiotes*.

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