Original Research Article

Lemna sp. AS A CHROMIUM HEAVY METAL PHYTOREMEDIATOR ON TANNERY WASTEWATER AND ITS POTENTIAL USE AS FISH FEED

ABSTRACT

Aims: This research aims to determine the ability of *Lemna* sp. as a phytoremediation agent in absorbing chromium (Cr) in tannery wastewater.
Study design: A total of 20 fiber tubs with size 80 x 80 x 40 cm^3 and volume of 256 L were prepared and filled with 30 L each of the tannery wastewater and 1.75% bio-slurry added with a volume of wastewater as a source of nutrition for *Lemna* sp.. The number of *Lemna* sp. which is used for culture was 180 g for each fiber tube.
Place and Duration of Study: Culturing *Lemna* sp. in tannery wastewater were carried out in Ciparanje Land Fisheries Area of the Faculty of Fisheries and Marine Sciences Universitas Padjadjaran and for chromium analysis were carried out in Center for Natural Resources and Environment Research of Universitas Padjadjaran, between February and March 2019.
Methodology: This research was carried out by culturing *Lemna* sp. in tannery wastewater for five days with 20 replications and comparing Cr concentrations in tannery wastewater and *Lemna* sp. at the end and beginning of the research. Chromium analysis results on the tannery wastewater and *Lemna* sp. the beginning and end of the research on each sample was tested by paired t-test.
Results: by culturing *Lemna* sp. in the tannery wastewater as phytoremediation agent for five days the results obtained Cr concentration at the beginning of research each for tannery wastewater and *Lemna* sp. ranged from 0.180 to 0.194 mg/L with an average of 0.187±0.0034 mg/L and 0 mg/kg. While at the end of the research the concentration of Cr in tannery wastewater and *Lemna* sp. respectively ranged from 0.057-0.075 mg/L with an average of 0.068±0.0044 mg/L and 2.292-2.333 mg/kg with an average of 2.314±0.0101 mg/kg. There was a decrease in Cr concentration by an average of 64.01±1.96% in tannery wastewater and an increase in *Lemna* sp.. Paired t-test results showed that *Lemna* sp. which was cultured in tannery wastewater had a significant influence on Cr concentrations in both tannery wastewater and *Lemna* sp so that there were significant differences in Cr concentrations at the beginning (day 0) and at the end of the research (day 5).
Conclusion: Average ability of *Lemna* sp. in absorbing and accumulating chromium in the tannery wastewater in the tissue was 2.314±0.0101 mg/kg and the average reduction in Cr concentration in liquid waste was 64.01±1.96%. Utilization of *Lemna* sp. containing chromium is possible to be used as a food supplement for fish to increase growth due to reduced Cr toxicity by *Lemna* sp..

Keywords: Chromium, Lemna sp.*, Phytoremediation, Tannery wastewater*

1. INTRODUCTION

Pollution of heavy metals in the waters is one of the many problems that occur at this time. The biggest contributor to the entry of heavy metals into the waters comes from industrial activities [1]. One of the industrial activities that produce heavy metal waste is leather tanning. Tannery uses a variety of ingredients including heavy metal chromium (Cr) so that it can endanger the environment [2,3,4,5,6]. Excessive concentration of Cr in a water can cause disruption to aquatic biota and also humans that live around these waters [5,7]. The chromium used in the tanning process is not entirely absorbed into the skin so that there is residual Cr which is wasted and can pollute the environment [3]. Chromium heavy metals in tannery wastewater in the form of hexavalent chromium (Cr^{6+}) which are toxic and trivalent chromium (Cr^{3+}) which are less toxic and it is an essential micronutrient for living organism [8].

In some cases, tannery wastewater is known to have Cr^{3+}, sulfide, BOD (biological oxygen demand) and COD (chemical oxygen demand). However, it does not rule out that Cr^{3+} can be oxidized to Cr^{6+} which has toxic properties [9]. Therefore it is necessary to reduce the concentration of Cr in the tannery waste to reduce the amount of Cr concentration in the waters. There are several ways to reduce the concentration of heavy metals in waters including physical, chemical and biological [10]. One method that can be done to reduce Cr concentrations in waters is phytoremediation by utilizing aquatic plants that can become weeds and are underutilized [11].

Phytoremediation can be defined as the use of plants to remove or take up dangerous contaminants from media such as soil, water and air. Plant species were selected for phytoremediation based on the potential of plants to accumulate metals, growth and distribution and depth of the root zone [12]. Aquatic plants are known to absorb and accumulate heavy metals [11,13]. One of the aquatic plants that can be used as a phytoremediation agent for heavy metals in waters is *Lemna* sp. [14,15,16,17,18,19].

Lemna sp. is one plant that can be used as a phytoremediator for chromium heavy metals. Cr^{3+} and Cr^{6+} can be absorbed by the roots of Lemna sp. and Cr6 + will be reduced in cells to Cr3 + so that Cr toxicity is reduced [20,21,22]. Aside from being a phytoremediation agent, chromium which accumulates in the tissue of *Lemna* sp. has a great potential to be used as a supplement in increasing fish growth. As is known that *Lemna* sp. is a forage feed for fish that is cheap and easy to obtain [23,24,25,26]. Cr^{3+} accumulation in the biomass of *Lemna* sp. and administration of Cr^{3+} through *Lemna* sp. as fish feed is a form of reuse of Cr^{3+} derived from tannery wastewater, which is expected to increase the growth rate of fish.

2. MATERIALS AND METHODS

This research was conducted from February to March 2019 in the Ciparanje Land Fisheries Area, Faculty of Fisheries and Maritime, Universitas Padjadjaran and the Center for Natural Resources and Environmental Research of Universitas Padjadjaran.

The materials used in this research include: *Lemna* sp., leather tannery wastewater from leather tanning industry Sukaregang, Garut, Indonesia and bio-slurry. This research was conducted with two treatments where the first treatment is the initial Cr concentration data on day 0 and the second treatment is the final Cr concentration data on the 5th day and then compares Cr concentration at the beginning and end of the research.

Research Implementation

Leather tannery wastewater is taken as much as 50 ml for testing the chromuim content in wastewater. A total of 20 fiber tubs with size 80 x 80 x 40 cm^3 and volume of 256 L were prepared and filled with 30 L each of the tannery wastewater and 1.75% bio-slurry added with a volume of wastewater [27] as a source of nutrition for *Lemna* sp.. The number of *Lemna* sp. which is used for culture that is as much as 180 g for each fiber tube [28]. *Lemna* sp. then harvested after five days and washed with running water to clean *Lemna* sp. from waste water leftovers. Then weighed to find out the final weight of the *Lemna* sp. after the experiment. *Lemna* sp. sample is taken as much as 1 g to test the chromium content.

Chromium Testing

Chromium testing Chromium testing is done with 50 ml samples were taken and weighed for wastewater samples and 1 gram for *Lemna sp.* samples, then each sample was put into an erlenmeyer flask and 5 mL of concentrated HNO_3 and 2.5 mL H_2O_2 were added to each sample. Each erlenmeyer flask is closed with a funnel and is ordered on a hot plate for destruction. 5 mL of HNO_3 and 2.5 mL H_2O_2 were added again for each sample when the sample appeared to dissolve. Then wait until the destruction process is complete and the solution is reduced to 15-20 mL. After the destruction is complete, the sample solution is transferred and filtered using filter paper into a volumetric flask. Then aquabides are added to a volume of 50 mL solution. Tested the sample using atomic absorption spectrophotometer (AAS) with a wavelength for Cr 357.9 nm and its absorption is read.

Observation Parameters

Chromium analysis was carried out on tannery wastewater as well as on Lemna sp. using the Atomic Absorption Spectrophotometry (SSA) method according to the procedure of Indonesian standard SNI 6989.17: 2009 [29].

$$Cr\left({}^{mg}/_{kg}\right) = \frac{C \times V \times FP}{W}$$

With :
Cr = Cr concentration in solid samples (mg / kg)
C = Cr reading result concentration in liquid samples in AAS (mg / L)
V = volumetric flask (mL)
FP = Multiplier factor (1)
W = Weight of sample (gram)

The results of the chromium analysis at the beginning and end of the research in each treatment were tested by paired t-test.

3. RESULTS AND DISCUSSION

The analysis results of Cr concentrations in tannery wastewater and *Lemna* sp. at the beginning (day 0) and end of research (day 5) are presented in Table 1.

Tab. 1. Cr concentrations at the beginning and end of the study

Cr Concentration			
Tannery Wastewater (mg/L)		*Lemna* sp. (mg/kg)	
Day 0	Day 5	Day 0	Day 5
0,189	0,069	0	2,319
0,183	0,057	0	2,324
0,192	0,073	0	2,307
0,187	0,071	0	2,313
0,180	0,065	0	2,331
0,187	0,066	0	2,325
0,190	0,073	0	2,297
0,185	0,067	0	2,292
0,183	0,064	0	2,315
0,189	0,069	0	2,320
0,189	0,063	0	2,317
0,185	0,065	0	2,328
0,194	0,073	0	2,299
0,189	0,065	0	2,314
0,191	0,075	0	2,310
0,186	0,071	0	2,321
0,187	0,066	0	2,311
0,190	0,069	0	2,316
0,185	0,065	0	2,318
0,188	0,064	0	2,309

Paired t-test results showed that *Lemna* sp. which was cultured in tannery wastewater had a significant influence on Cr concentrations in both tannery wastewater and *Lemna* sp. tissue so that there were significant differences in Cr concentrations at the beginning (day 0) and at the end of the research (day 5).

Cr Concentration in Tannery Wastewater and *Lemna* sp.

Cr concentrations at the beginning of research each for tannery wastewater and Lemna sp. ranged from 0.180 to 0.194 mg/L with an average of 0.187±0.0034 mg/L and 0 mg/kg. While at the end of the research the concentration of Cr in tannery wastewater and *Lemna* sp. ranged from 0.057 to 0.075 mg/L with an average of 0.068±0.0044 mg/L and 2.292 to 2.333 mg/kg with an average of 2.314±0.0101 mg/kg, respectively. The difference in the value of Cr concentration at the beginning and end of the study was due to the transfer of Cr in the tannery wastewater into the *Lemna* sp. resulting in a decrease in the average Cr concentration of 64.01±1.96% in tannery wastewater and in *Lemna* sp. resulting in an increase in Cr concentration in the tissue (Figure 1).

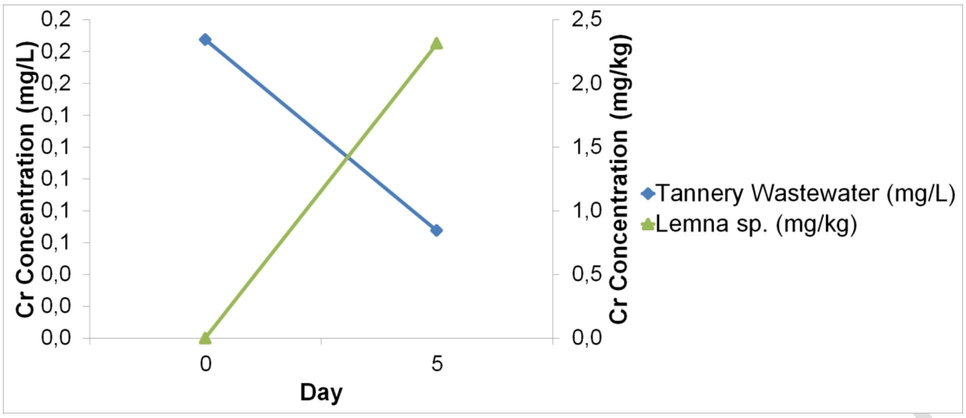

Fig. 1. Graphical representation showing changes in Cr concentration in tannery wastewater and *Lemna* sp.

This shows that Lemna sp. has the ability to absorb and accumulate Cr in the tannery wastewater into its tissue. It can be seen from the decreasing Cr concentration in tannery wastewater along with the length of time the culture is inversely proportional to the Cr concentration in Lemna sp. which continues to increase with the length of time of culture.

Conventional tannery using chrome has an impact on the environment because it carries the remaining chrome into its liquid waste [30]. Although the chrome for leather tanning is Cr^{3+}, Cr^{6+} is always present in the wastewater [31]. Thus the tannery industrial wastewater will pollute water bodies or rivers if the waste without special handling is immediately discharged into the environment [32]. The ability of *Lemna* sp. in absorbing and accumulating Cr can be used as an alternative phytoremediation agent for Cr in tannery wastewater. *Lemna* sp. very suitable for use in water quality studies to monitor heavy metals in waters [33] and is considered a better alternative to other aquatic plants and have been recommended for wastewater treatment because *Lemna* sp. is more tolerant of cold temperatures than water hyacinth, easier to harvest than algae, and is able to grow quickly [19]. Besides that, *Lemna* sp. can survive on a medium with 4 mM Cr^{6+} and is able to reduce Cr^{6+} to Cr^{3+} which has nontoxic properties [21].

In the process of absorption, Cr is absorbed by *Lemna* sp. will bind to organic complexes in the *Lemna* sp. tissue like proteins, DNA and fats that cause Cr^{6+} to be reduced to the organic form of Cr^{3+}. In the condition of culture media (in this case tannery wastewater) with a concentration of Cr which is not too high or relatively low, *Lemna* sp. can absorb and reduce Cr6+ quickly, thereby reducing Cr toxicity and can grow well. Conversely, in conditions of high Cr concentration, the growth and development of *Lemna* sp. will be inhibited and can cause death caused by oxidative reactions in tissues as a result of high concentrations of Cr which can damage the binding of proteins, DNA and fat which can further disrupt photosynthesis [21,34,35,36]. In this research the concentration of Cr in tannery wastewater is still relatively low when compared with the WHO standard 1998 regarding the maximum limit of Cr concentration in the waste that is equal to 1 mg/L.

Potential of *Lemna* sp. Containing Chromium as Fish Feed

Culturing *Lemna* sp. in tannery wastewater causing *Lemna* sp. accumulate Cr in the tissue. Use of *Lemna* sp. in phytoremediation of Cr can reduce toxicity and convert Cr in tannery wastewater into organic Cr. Organic Cr^{3+} which accumulates in *Lemna* sp. has a great potential to be used as a supplement in increasing fish growth. As is known that *Lemna* sp. is a forage that is cheap and easy to obtain. Cr^{3+} accumulation in the biomass of *Lemna* sp. and administration of Cr^{3+} through *Lemna* sp. as fish feed is a form of Cr^{3+} reuse originating from tannery wastewater, which is expected to increase the rate of fish growth.

Organic chromium is a micronutrient for fish that plays a role in the formation of chromodullin which is an oligopeptide that is very important for normal metabolism of carbohydrates and fats and plays a role in increasing the potential performance of insulin in mobilizing glucose into cells, stimulating glycogenesis, lipogenesis, and transporting and taking amino acids by cells through increased insulin receptor sensitivity [37,38].

Some research results show that organic Cr can increase fish growth rate include administration of organic Cr as much as 0.5 ppm producing the best growth rate in carp [39], giving Cr-pic as much as 0.8 ppm producing best growth rate on grass carp seeds [40] and rohu fish seeds [41], giving 0.5 ppm Cr-pic to tilapia was producing best growth rate [42], giving as much as 1.5 yeast Cr-pic ppm in giant gouramy can increase growth rate of giant gouramy [43] and Cr-pic administration of 1-2 ppm in red tilapia resulting in increase of growth rate of red tilapia [37]. Thus the administration of *Lemna* sp. containing chromium to fish with a certain concentration is possible to increase the rate of fish growth without causing toxic effects considering the toxicity of Cr has been reduced by *Lemna* sp., especially in herbivorous fish that use plants (carbohydrates) as an energy source.

4. CONCLUSIONS

Average ability of *Lemna* sp. in absorbing and accumulating chromium in the tannery wastewater in the tissue is 2.314±0.0101 mg/kg and the average reduction in Cr concentration in tannery wastewater is 64.01±1.96%. Utilization of *Lemna* sp. containing chromium is possible to be used as a food supplement for fish to increase growth due to reduced Cr toxicity by *Lemna* sp.

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