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Chromium Phytoremediation of Tannery Wastewater using *Ceratophyllum demersum*

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Abstract

Tanning industry produces liquid waste containing heavy metals, especially chromium – harmful for ecosystems and human health. Phytoremediation is a technique that utilizes the physiologicalpotential of plants to transform contaminants to be less or non-hazardous. The aim of the research is to determine the efficiency of *Ceratophyllum demersum* L. to remediate chrome in tannery wastewater. The research used 84 strands of *C. demersum* compound leaves of 30 g wet weight. The treatments consist of the use of 7.74 mg/L, 11.30 mg/L, 17.00 mg/L and 23.73 mg/L concentrations of chromium and a control. The research was conducted using a static method. The design of the experiment was the complete random design with 3 replication of treatment in 14 days. The parameters observed were the efficiency of chromium phytoremediation, water turbidity, BOD, and total chlorophyll level of the leaves. The results showed that the highest efficiency was at the concentration of 7.74 mg/L with 1.7% chromium, 17.3% water turbidity, and 46% BOD. Meanwhile, the highest efficiency of total chlorophyll level was 3.88 mg/L, reached at the concentration of 17.00 mg/L. In conclusion, *C. demersum* is good to use as a *phytoremediator* of tannery wastewater at the concentration of 7.74 mg/L, subsequently, these results can be used as a basis for the consideration of the application implementation in the process of liquid waste reduction.

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INTRODUCTION

Tanning industry is one of the industries using hazardous and toxic materials in the production; that is chromium (Cr). Among the well-known centers of tanning industry is the one located in Sukaregang Village of Garut Sub-district of Garut Regency. Based on the survey and sampling conducted by the Ministry of Environment on 4-6 July 2002, Ciwalen river is highly polluted, exceeding the water quality-based effluent limits (Kementrian Lingkungan Hidup, 2002).

Cr in waters is a potential pollutant for the ecosystem. The bioaccumulation of heavy metal (Cr) in the tissue of water plants could chronically affect their growth by slowing it down (Shanker et al., 2005), as well as cause kidney disease and lung cancer in humans (Wilbur et al., 2012), if consumed. In plants, the Cr can affected to reduction of seedling growth on *Sorghum bicolor* (L.) Moench species (Kasmiyati et al., 2016). Therefore, decreasing its level in waters is necessary.

One of the ways to decrease the level of Cr in waters is by phytoremediation, which is the utilization of plants with their biosorption ability. Besides, phytoremediation is easy to conduct, as well as offers lower budget and simpler technology than the so-called engineering-based methods (Hidayati, 2005).

Ceratophyllum demersum (coontail) is a water plant that could be used as a phytoremediator. It could absorb heavy metals with its root tissue. Among the previous studies is the one conducted by Maryam et al. (2011). The study remediates ferrous metals (Fe) for 18 days by 40% using treated municipal wastewater (TMW) method and 67.5% using raw municipal wastewater (RMW) method. It also remediates nickel (Ni) for 14 days by 50% (Chorom & Jaafarzadeh, 2012), as well as absorbs Cd, Pb, Zn, Co, Cu, and Ni metals as much as 2.35 mg/L, 208.71 mg/L, 1172.8 mg/L, 23.5 mg/L, 96.3 mg/L, and 48.09 mg/L respectively. Other serults show that *C. demersum* can used for remediation of heavy metals such as Cadmium (Cd) (Al-Ubaidy & Rasheed, 2015), Cu, Pb & Zn in river sediment (Fawzy et. al., 2012). In addition, the plant is easy to find in Indonesia and able to adapt well in a polluted environment.

The use of *C. demersum*as a phytoremediator is expected to decrease the level of Cr in the river polluted by the liquid waste of the tanning industry in Sukaregang, Garut to a non-hazardous level. For the society, the result of the research could be one of the solutions to the problems of tannery waste, both individually and in groups.

METHODS

The materials used were approximately 80 strands of mature *C. demersum* compound leaves of 30 g wet weight, taken from aquascape shop in Jakarta. The liquid waste was taken from the tanning home industry in Sukaregang, Garut Regency.

The research used static method. It means that there was no addition of materials during the observation/experiment. The research used complete random design, consisting of four treatments by using waste concentrations of 7.74 mg/L (K1), 11.30 mg/L (K2), 17.00 mg/L (K3), and 23.73 mg/L (K4), as well as the control (water) (K0), 1 liter each. The treatmentsare repeated three times.

*Ceratophyllum demersum*is acclimated for seven days by cutting the tip of the stems to broaden the absorption surface. The concentrations of tannery liquid waste of 25%, 50%, 75%, and 100% as well as the control are put into 1500 ml-sized water bottles. The experiment was conducted in 14 days in accordance with the doubling time. The measured parameters were the efficiency of chrome phytoremediation (*SNI* 06-6989.17-2004, *AAS* method), the water turbidity (*SNI* 06-6989.25-2005, using nephelometer), BOD (*SNI* 6989.2-2009, spectrophotometry method), and the total chlorophyll level of the leaves (Arnon-1949 method, 96% alcohol solvent, 649 nm and 665 nm λ spectrophotometer). The measurement of chrome, water turbidity and BOD was observed at the Health Laboratory of West Java in Bandung. Mean while, the measurement of total chlorophyll level was conducted at the Aquatic Ecology Laboratory of Biology Department of Science and Technology Faculty of Sunan Gunung Djati State Islamic University, also in Bandung. The research was conducted from June to November 2015.

The efficiency value of the phytoremediation of tannery wastewater was determined by using the equation: $E = \frac{C_0 - C_i}{C_0} \times 100\%$, with E=Efficiency (%), C_0=initial level (mg/L), C_i=final level (mg/L). Data were analyzed using analyses of variance and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The initial levels of chrome in the tannery wastewater in this research were 7.74 mg/L, 11.30 mg/L, 17.00 mg/L, and 23.73 mg/L. *Ceratophyllum demersum* shows the potential to remediate chrome in the concentration of 7.74 mg/L and

11.30 mg/L, meaning that there was a positive value of efficiency. However, potential remediation was not found in the concentration of 17.00 mg/L and 23.73 mg/L, meaning that the efficiency value was negative (Figure 1). The highest efficiency level of chrome phytoremediation was shown in the 7.74 mg/L waste concentration, which is 1.7% in 14 days. This phytoremediation ability is in line with the research conducted by Teymouri et al. (2013) which uses *C. demersum* in a NaCl-modified chrome solution. The highest result of Teymouri's research was gained in the condition where the pH is 2, the biosorbent dosage is 8 g/L, the treatment is in 60 minute contact, and there was 10.20 mg/L maximum adsorption capacity of *C. demersum*.

According to Priyanto (2006), the liquid waste taken from the joint waste storage pond belonging to a number of tanning home industries in Sukaregang Village of Garut Regency contains 1,265.05 mg/L of BOD and 8,554.05 mg/L of chrome. Two metal ions found there are Cu (cooper) and Cr (chromium). The highest amount of metal ion found is chromium. Chromium (VI) is known to be a thousand times more toxic than Chromium (III); and Chromium (VI) is found more than Chromium (III) in the tanning industry (Villegas et al., 2008). Chromium could cause problems of respiratory system, skin, blood vessels, and kidney (Tchounwou et al., 2012). Contact with the skin could cause irritation, and it could result in stomachache and vomiting if swallowed. The negative effects of heavy metals on plants are weakened organism activities and degraded soil fertility. As a result, the production quantity also decreases. The most fatal effect of heavy metal pollution on plants is the contaminated food chain (McGrath et al., 2002). Cr (VI) could affect the growth of mung beans (Turner & Rust, 1971), lettuce, wheat and tomato (Moral et al., 1995), *Albizialebbek*, *Acasialebbek*, and green beans (Sharma & Sharma, 1993) as well as rice and beans (Prasad & Freitas de Oliviera, 2003). Those results show that Cr (VI) – on some specific concentrations – affects the growth of roots, leaves, and seeds.

Chrome accumulated in *C. demersum* decreases the level of chlorophyll. It is resulted by the hampered photosynthesis process marked by chlorosis of the leaves. Moreover, there is a loss of leaf buds, marked by the amount of leaf buds floating on the wastewater. *Ceratophyllum demersum* experiences oxidative stress caused by over-limit chrome toxicity in its tissues – and Reactive Oxygen Species (ROS) such as H_2O_2 occurs. According to Panda & Patra (1997), the accumulation of chrome in plants could hamper their growth, cause chlorosis in new leaves, reduce the pigment content, change the enzymatic functions, damage root cells, and cause ultrastructural modification of chloroplast and cell membrane. Heavy metals in a plant would trigger ROS as a result of the deactivation of antioxidant enzymes such as s*uperoxidedismutase* (SOD), catalase (CAT), and glutathione peroxidase (GPOD). ROS occurring in a plant is also caused by oxidative stress. ROS would easily damage peroxide fat in the lipid-membrane, cell membrane of the phospholipid and lipoprotein by spreading through chained reaction (Hazra et al., 2010). ROS itself could affect any kinds of biomolecule such as nucleic acid, protein, and amino acid; disturbing the cell metabolism.

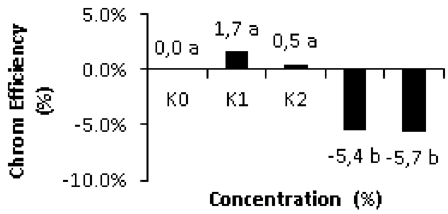

Figure 1. The efficiency of *Ceratophyllum demersum* in remediating chrome in tannery wastewater (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

A plant develops a complex mechanism where they could control the absorption and accumulation of heavy metals. This mechanism involves chelation and execution of some specific class of heavy metals. They would compose *Phytochelatin* Currency (PC) and *metallothienins* (MTs) (Cobbet, 2000).

The other indicator of potential phytoremediation of tannery wastewater by *C. demersum* is the remediated water turbidity. Its potentials in the waste concentrations of 7.74 mg/L, 11.30 mg/L, 17.00 mg/L and 23.73 mg/L were positive (Figure 2). The efficiency value of the water turbidity phytoremediation is decreased as the concentration was increased: 17.3% in the waste concentration of 7.74 mg/L, 2.7% in the concentration of 11.30 mg/L, 0.8% in the concentration of 17.00%, and 0% in the concentration of 7.74 mg/L. The highest efficiency was shown in the concentration of 23.73 mg/L of tannery wastewater.

The phytoremediation efficiency by *C. demersum* on the water turbidity was below 20%; it means the turbidity level of the wastewater was still high (Figure 3). In the concentration of 25%

liquid waste, the level of turbidity was between 180-370 NTU/L. Even with the highest value of efficiency, the water clarity was still below standard because the turbidity is more than 20 NTU/L. According to Yusuf (2008), water withover 20 NTU/L turbidity is dangerous for the biota living in it, as it could interfere with their activities and metabolisms.

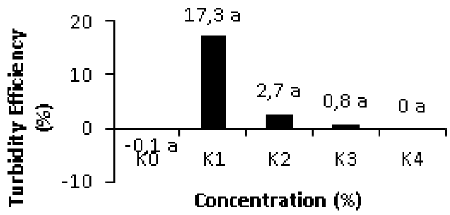

Figure 2. *C. demersum* efficiency in remediation of tannery wastewater turbidity (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

The higher the concentration of tannery wastewater is, the lower the turbidity efficiency value becomes. *C. demersum* could remediate the turbidity best in the treatments on the 7.73 mg/L concentration. Meanwhile, in the treatments onthe 11.30 mg/L, 17.00 mg/L and 23.73 mg/L concentrations the efficiency values were low. It happened because particles in the liquid waste clog the pores of the cut leaf stems that they could not optimally absorb the contaminant.

Remédios et al., (2012) points out that the deposition and colloidal in the water would prevent the sunlight to go through. It would not reach the bottom. That is why the photosynthesis of *C. demersum* is hampered. As a result, the lives of the microorganisms are bothered. Water turbidity restrains the incoming light. It occurs as there are floating materials and decomposition of certain substances, such as organic materials, microorganisms, muds and other minute floating things (Bilotta & Braizer, 2008).

The initial condition of the researched tannery waste water BOD in the treatments 7.70 mg/L, 11.30 mg/L, 17.00 mg/L, and 23.73 mg/L were 228.07 mg/L, 412.80 mg/L, 530.43 mg/L, and 647.40 mg/L respectively. The *C. demersum* showed that its phytoremediation efficiency on BOD in 14 days was fluctuating (Figure 4). The value decreased in the 7.70 mg/L and the 11.30 mg/L concentrations, and increased in the 17.00 mg/L and the 23.73 mg/L concentrations. The efficiency values of phytoremediation using *C. demersum* on the BOD from the highest to the lowest waste concentrations respectively were 46%, 14%, 25%, and 29%.

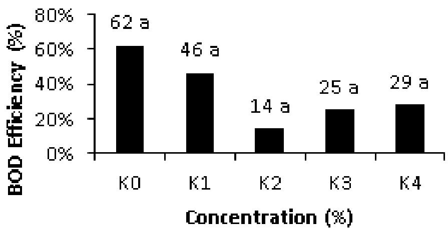

Figure 4. *C. demersum* efficiency in the remediation of tannery wastewater BOD (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L)

During the phytoremediation process, the efficiency value of BOD decreases in the different concentrations. It is supposed to be caused by several reasons, among which is water turbidity. The higher the level of turbidity is, the more difficult the *C. demersum* does photosynthesis as the light coming through the water is limited (Remédios et al., 2012). The result of photosynthesis is oxygen. It is the one used by microorganisms to move aerobically.

The thicker concentration of the liquid waste is, the higher the BOD becomes. The high contents of BOD in the treatments would increase the needs of polluting microorganisms in decomposing organic substances aerobically (Safitri et al., 2015). On the contrary, the amount of oxygen resulted from the photosynthesis process would decrease.

The increasing needs of microorganisms influence the amount of oxygen needed by aquatic organisms like *C. demersum* to do their metabolic processes (photosynthesis and cell respira-

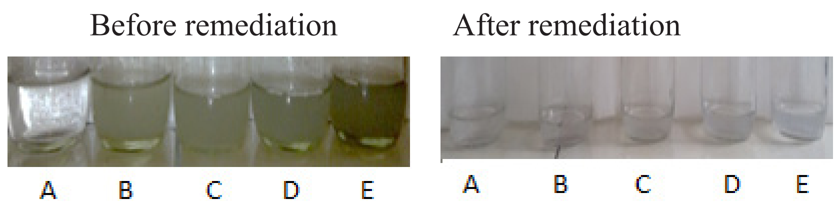

Figure 3. Before and after phytoremediation of tannery wastewater using *C. demersum* (A=control, B=7.74 mg/L, C=11.30 mg/L, D=17.00 mg/L, E=23.73 mg/L)

tion). If the metabolic processes are hampered, the plant's ability in remediating BOD weakens.

Water temperature also influences the amount of oxygen needed by microorganisms for the aerobic decomposing process. The rise of temperature would result in the rise of biological activities, and in turn, the needs of more oxygen. The rise of water temperature would decrease the level of oxygen solubility, so that it lowers the aquatic organisms' ability to use available oxygen to sustain the biological processes in the water (Safitri et al., 2015).

Meme et al., (2014) states that the level of BOD have direct relation with the other parameters component of water quality such as water temperature and pH in the water. Temperature influences most biochemical reactions. Biological activities increase as the temperature goes up to 60°C. Organisms reorganizing organic materials would be able to adapt in the range of 6.8-8.3 acidity. The process of organic waste decomposition occurs through the oxidation process by microorganisms with aerobic bacteria. Organic waste is broken and decomposed into carbon dioxide (CO_2), water and ammonia (NH_3). The resulted ammonia is the one causing bad smell of the water polluted by organic waste.

The levels of chlorophyll-a, chlorophyll-b and total chlorophyll found in *C. demersum* used as phytoremediator of tannery wastewater showed various conditions (Figure 5). Generally, the level of chlorophyll-a was higher than chlorophyll-b and the total chlorophyll in all treated waste concentrations. The highest level of chlorophyll-a as much as 9.88 mg/L was found in the 11.30 mg/L concentration; of chlorophyll-b as much as 8.21 mg/L is found in the 17.00 mg/L concentration; and of total chlorophyll as much as 4.88 mg/L was found in the 17.00 mg/L concentration.

For 14 days, the chlorophyll in *C. demersum* increased although being exposed to an extreme environment. It is because *C. demersum* have the ability to survive extreme condition. Based on a research by Chorom & Jaafarzadeh (2012), the level of chlorophyll and wet weight of *C. demersum* still increased in an extreme condition of 23.73 mg/L nickel-contaminated treatment. However, the result showed that the levels of total chlorophyll decreased in each treatment. Its level in the 100% concentration was totally different from the one in the control. It proves that the higher the concentration of liquid waste is, the lower the level of clorophyll in *C. demersum* becomes. According to Panda and Patra (2002), a plant accumulating heavy metals would experience an oxidative stress, including decreased level of chlorophyll. The decreases of total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoid happens because chrome has the ability to lower the ∂-aminolevullinic acid dehydratase. It is a very essential enzyme for the biosynthesis process of chlorophyll that it affects the utilization of ∂-aminolevullinic acid (ALA). Besides, chrome-VI is able to change most Mg^+ ions and drain the chlorophyll content.

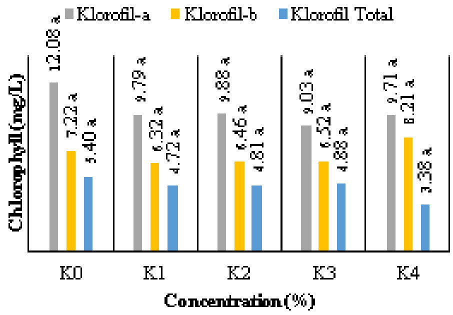

Figure 5. The level of total chlorophyll of *Ceratophyllum demersum* leaves as phytoremediator of tannery wastewater (K0=Control, K1=7.74 mg/L, K2=11.30 mg/L, K3= 17.00 mg/L, K4=23.73 mg/L).

Panda & Patra (2002) also stated that the decreased level of chlorophyll in a heavy metal-accumulated plant is caused by chrome exposure in a micromolar range, which results in ultrastructural change of chloroplast and thylakoid structures that hampers photosynthesis. Chrome also blocks the Hill reaction, affecting the light and dark reactions. Those reactions are essential stages of photosynthesis. According to Panda & Choudhury (2005), *Lemna minor* and *Pistia sp.* with chromium stress experience chlorosis in their new leaves.

Chromium stress is an important factor influencing CO_2 fixation, electron transport, photophosphorylation, and photosynthesis enzyme activities. According to Vazquez et al. (1987), the effects of chrome to the hampered electron transport is caused by the change of enzyme in the Calvin cycle. Chromate is used as the hill reagent by the isolated chloroplast. On the other hand, Bishnoi et al. (1993) pointed out that chrome affects photosystem I (PS I) in the chloroplast activity, so that it gets isolated in photosystem II (PS II). It is proven in their research on peas with high chromium content where the level of chlorophyll decreases drastically. Krupa and Baszynski (1995) describe a number of hypotheses on the possible mechanisms of chromium toxicity in photosynthesis; they are the reduction of carbon essential for the process, the disorganization of chloroplast ultrastructure, and the obstruction of electron transport as the chrome divertsthem from PS I side to PS II side. The obstruction results in degrading photosynthesis ability as chrome induction occurs. There is a possibility that not all electrons resulted in the photochemical process are used for carbon fixation as shown by the degrading photosynthesis performance.

According to Shanker (2003), chrome toxicity on photosynthesis happened because it is able to shrink the size of peripheral parts of the antenna. The decrease of chlorophyll is also caused by protein destabilization and degradation in the peripheral part. Enzyme inactivation involved in the chlorophyll biosynthesis fascia also affects the decrease of chlorophyll level in plants with chromium oxidative stress.

The phytoremediation potential of *C. demersum* could be used in a bigger scale. The design management could consider the aspects of material volume, the amount of phytoremediator, and the time constraint. The volume of tannery wastewater could range from 25 to 1000 L, by using 30 g × (25-1000) of *C. demersum*, for 14 days. Containers with 25-1000 L capacity or more could be used for the treatment.

CONCLUSIONS

Ceratophylum demersum is good for remediating chrome in tannery wastewater of 7.74 mg/L concentration by using static method. The remediation potential is accompanied by the ability to reduce the levels of water turbidity and BOD. The use of *C. demersum* as phytoremediator of tannery wastewater results in the decrease of its chlorophyll level so that their growth is hampered.

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