Physiological strategies of Eichhornia crassipes (Mart.) Solms to tolerate Cr$^{6+}$ accumulation, compared to a sensitive species Pistia stratiotes L.

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**ABSTRACT**

Chromium hexavalent (VI) has been used in some industry including leather tanning industry. The chemical has been known to be harmful to living organisms. Therefore, it is important to treat wastewater from leather tanning industry before being discharged to the environment. The aim of this study is to examine ecophysiological strategies of waterhyacinth (Eichhornia crassipes) to tolerate Cr$^{6+}$ accumulation in its tissue, compared to sensitive species water lettuce (Pistia stratiotes). The plants were cultivated in containers containing Hoagland medium and treated with some variation of Cr$^{6+}$ concentrations of Cr$^{6+}$ i.e. 0, 40, 80 and 120 ppm for 14 days. Some parameters including CAT (catalase), Ascorbate peroxidase (APX), chlorophyll concentration and proline in the plants were measured. The biomass yield of plant in Cr$^{6+}$ stress was negative (-0.732 to -1.84 g/week) which indicated both E. crassipes and P. stratiotes reduced their growth. The higher the concentration of Cr$^{6+}$, the lower the chlorophyll contents in the leaves. The lowest of chlorophyll content was in 120 ppm (0.15 mg/g in P. stratiotes and 0.12 mg/g in E. crassipes). The highest of CAT activity in E. crassipes was 109% in 40 ppm Cr$^{6+}$, while in P. stratiotes was 76% in 120 ppm. Proline content in both E. crassipes and P. stratiotes were not different significantly. In general, E. crassipes plants have the ability to adapt to Cr$^{6+}$ stress better compared to P. stratiotes which was severely damaged when grown in high Cr$^{6+}$ concentration. Both plants can remediate waste fairly well (level of elimination 62-68%) during the exposure period of 14 days to Cr$^{6+}$ solution.

**Keywords:** Eichhornia crassipes, Hexavalent chromium, Pistia stratiotes, Ecophysiology, Phytoremediation

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1. Introduction

The development of the leather tanning industry in Indonesia has both positive and negative impact to the society and environment. The positive impact is increasing economic growth for the society because leather tanning industry provides high economic value, however the discharge of untreated wastewater could severely polluting nearby river. Chromium is still the main tanner and is used by 85% of leather tanning industry worldwide [Bacorditi et al., 2014]. The chromium in tannery wastewater is discharged when the leather tanning industry wastewater reached 10 mL. Chromium content in medium, roots and leaves were measured using Atomic Absorption Spectrophotometry (AAS). For chlorophyll content measurement, 0.1 g of the plant’s fresh weight was extracted using 80% acetone. The extract was added with acetone until the volume reached 10 mL. Chlorophyll content was measured using spectrophotometry at wavelength of 663 nm and 645 nm. Total chlorophyll content was measured using the equation (1).

\[
\text{Chlorophyll (mg/g)} = \frac{0.02A_{645} + 20.2A_{663}}{1000 \times \text{wet weight} \times V_{\text{acetone}}} \quad (1)
\]

CAT activity was measured by changes in absorbance of sample at wavelength of 240 nm, while the APX activity was measured at 290 nm. Absorbance was measured every 30 seconds for three minutes. Enzyme activity in a unit was determined as the number of enzyme decomposed in 1 μmol H₂O₂ per minute at pH 7 and 25°C. Volume activity CAT (unit/mL) = \( \frac{\Delta A \times V_s}{0.0436 \times V_s \times \text{volume activity} \times \text{fresh weight}} \)  \( \quad (2) \)

Enzyme activity CAT (unit/mg) = \( \frac{\Delta A \times V_s}{2.8 \times V_s} \)  \( \quad (3) \)

Enzyme activity APX (unit/mL) = \( \frac{\Delta A \times V_s}{2.8 \times \text{volume activity} \times \text{fresh weight}} \)  \( \quad (4) \)

Enzyme activity APX (unit/mg) = \( \frac{\Delta A \times V_s}{2.8 \times \text{volume activity} \times \text{fresh weight}} \)  \( \quad (5) \)

Vq is volume reaction in cuvette (mL) and Vs is sample volume that used (mL). The blank and sample composition for measuring enzyme activity are shown at Table 1 and Table 2.

Table 1. Composition of solution for CAT activity test

<table>
<thead>
<tr>
<th>Component</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM buffer</td>
<td>920 μL</td>
<td>920 μL</td>
</tr>
<tr>
<td>Deionized water</td>
<td>70 μL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>10 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>-</td>
<td>10 μL</td>
</tr>
</tbody>
</table>

Table 2. Composition of solution for APX activity test

<table>
<thead>
<tr>
<th>Component</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM buffer</td>
<td>890 μL</td>
<td>890 μL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>2 μL</td>
<td>2 μL</td>
</tr>
<tr>
<td>Sample</td>
<td>20 μL</td>
<td>20 μL</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>-</td>
<td>20 μL</td>
</tr>
</tbody>
</table>

Proline content was measured using Bates method (1973) by extracting leaves and roots in liquid nitrogen with 3% sulfoalicylic acid. The filtrate was filtered, and two mL filtrate was reacted with 2 mL of ninhydrin acid and 2 mL glacial acetic acid for an hour at 100°C in a water bath. The solution was added with 4 mL of toluene by stirring using vortex for 20 seconds. The absorbance of solution with toluene was measured using spectrophotometry at wavelength of 520 nm. The proline content is calculated using equation 6.
The ability of *E. crassipes* and *P. stratiotes* in remediating chromium (VI) was determined by percent of removal, BCF (Bio Concentration Factor) in root and leaves, TF (Translocation Factor), and TI (Tolerance Index). BCF is defined as the concentration ratio of heavy metal in plants compared to concentration heavy metal in medium. Percent of removal, BCF, TF dan TI is determined using equation 7, 8, 9, 10.

\[
\% \text{removal} = \left( \frac{[\text{Cr}_{\text{medium}}]_{\text{initial}} - [\text{Cr}_{\text{medium}}]_{\text{end}}}{[\text{Cr}_{\text{medium}}]_{\text{initial}}} \right) \times 100
\]  

BCF = \frac{[\text{Cr} \text{ Concentration in plant}]_{\text{mg kg}^{-1}}}{[\text{Earl Cr} \text{ concentration in medium}]_{\text{mg L}^{-1}}}

TF = \frac{[\text{Cr Concentration in leaves}]_{\text{mg kg}^{-1}}}{[\text{Cr Concentration in root}]_{\text{mg kg}^{-1}}}

TI = \frac{\text{Plant biomass in treatment (g FW)}}{\text{Plant biomass in control (g FW)}}

3. Result and Discussion

Control plants of *P. stratiotes* after cultivated for 14 days showed darker green in color compared to treated *P. stratiotes* (Figure 1). As chromium concentrations increased, plants color was more yellow and showed chlorosis due to accumulation of Cr\(^{6+}\) [Hayat, 2012]. Chromium can enter plant cell through active transport mechanism [Shanker, 2017], and the accumulation of Cr\(^{6+}\) increase ROS in plants will affect biosynthesis of chlorophyll [Lu, 2011]. In *E. crassipes*, this damage was not visibly seen in all Cr\(^{6+}\) concentrations, while in *P. stratiotes* chlorosis already occurred in plants grown at low concentration of Cr\(^{6+}\) (40 ppm). Cell damage in *P. stratiotes* was worse in 80 and 120 ppm, indicated by degradation and decay of plant biomass in both concentrations and caused plant death. Observation on both plants' morphology indicates that *P. stratiotes* was much more sensitive to Cr\(^{6+}\) exposure than *E. crassipes*.

Yield in biomass of *P. stratiotes* and *E. crassipes* were significantly different (P<0.05) between control and treatment. The negative of biomass yield in stress chromium condition indicated cell damage in plant cell. Chromium concentration 100 ppm can reduce 50% of fresh weight of Vallisneria spiralis after 72 hours exposure [Vajpayee, *et al.* 2001]. Cell damage is caused by increasing chromium accumulation in cell. The accumulation of chromium causes accumulation of ROS and apoptosis of cell. ROS can cause enzyme damage and disturb cellular activity.

Chlorophyll content of *P. stratiotes* grown in Cr\(^{6+}\) concentrations was significantly different with control (Table 3). The higher chromium concentration in medium, the lower chlorophyll content. Accumulation of chromium seem to disorganize chloroplast structure. In addition, chromium may affect enzyme activity for chlorophyll biosynthesis. In other report, chlorophyll destruction also occured at 100 ppm chromium concentration [Sufia, 2014]. The accumulation of chromium can cause a lack of ferrum and zinc absorption in plant, which will affect chlorophyll biosynthesis. Chlorophyll content in *E. crassipes* grown in Cr\(^{6+}\) concentrations was not significantly different with control (P<0.05). This indicate that *E. crassipes* is tolerant to Cr\(^{6+}\) accumulation.

**Table 3.** Relative growth rate, total leaves number and chlorophyll content in *P. stratiotes* and *E. Crassipes* after 14 days of cultivation in Hoagland medium treated with various concentration of Cr\(^{6+}\)

**Gambar 1** Morphology of *P. stratiotes* (up) and *E. crassipes* (bottom) after 14 days of cultivation in Hoagland medium treated with various concentration of Cr\(^{6+}\)
days of exposure to high Cr⁶⁺ concentration, leaves and root of *P. stratiotes* were severely damaged.

Cr⁶⁺ accumulation was seem to increase catalase enzyme activity in *E. crassipes* (Figure 3). The Cr⁶⁺ concentration that can increase the highest of CAT activity was in 40 ppm (109%). An increase in Cr⁶⁺ concentration in medium causes accumulation of Reactive Oxygen Species (ROS) in plants which in turn will create cell damage, hence there is an increase of catalase enzyme activity in order to eliminate ROS. Catalase enzyme has a function in decreasing ROS [Sucha, dan Kasmiyanti, 2018]. In contrast, in *P. stratiotes* the higher Cr⁶⁺ concentration, the lower CAT enzyme activity. The lowest CAT activity was in 120 Cr⁶⁺ concentration (76%). Beside that, the CAT enzyme activity in 40 ppm and 80 ppm were 39% and 41%. A decrease in CAT enzyme activity may be caused by reaction of Cr⁶⁺ with other component [Palace, et al., 1992], for example Fe²⁺ which can be found in metabolic [Palace, et al., 1992; Vernay, et al, 2007]. In this study *P. stratiotes* plants degraded after 14 days of exposure, which indicate its inability to tolerate Cr⁶⁺ accumulation in its tissue.

An increase in plant biomass may cause an increase in removal efficiency in medium [Smolyakev, 2012]. Biomass reduction in *P. stratiotes* was higher than *E. crassipes*, especially above 40 ppm chromium concentration. *E. crassipes* did not increase its biomass so the rate of chromium removal in the medium was also lower at high initial chromium concentrations (more than 40 ppm). *E. crassipes* is more tolerant than *P. stratiotes* in chromium concentration above 40 ppm. After 14
Meanwhile, the APX enzyme activity decreased significantly in *P. Stratiotes* (Figure 5). Percentage of decrease in APX enzyme activity were 71% (40 ppm), 67% (80 ppm), and 78% (120 ppm) compared to control.

The same result shown in *Brassica juncea* L. that APX enzyme activity decreased because of an increase in Cr concentration [Diwan et al., 2010]. This indicates that *P. stratiotes* is more sensitive to Cr$^{6+}$ than *E. crassipes*. Beside CAT enzyme and APX enzyme, other enzymes such as superoxide dismutase (SOD) and glutathione reductase (GR) also plays a role in defense mechanism against ROS [Madan et al., 2017].

In *E. crassipes*, proline concentration in leaves of Cr treatment is higher than control (Figure 6). While in *P. stratiotes*, there is no difference between control and treatment. Other study showed that accumulation proline in plants increase with the increasing of metal concentration in medium [Odjegba and Fasidi, 2006]. The main function of proline is to keep osmotic cell from oxidative stress and macromolecule stability in cell [Diwan, et al., 2010]. Proline can reduce the free radical molecule which induced by metal stress and prevent ROS forming in cell [Abraham, et al., 2010 dan Gomes, et al., 2017].

Both plants’ root BCF have higher value than leaves for all treatments (Table 5). This result is the same result with previous study which *E. crassipes* use to remediate Cr$^{6+}$ from tanning wastewater [Woldemichael, et al., 2011]. Root are part of plant which is directly exposed to chromium in medium and plants have mechanism that minimizes the accumulation of ions in the leaves or stem. The accumulation of ions in the leaves can cause damage to photosynthesis process [Woldemichael, et al., 2011]. Increasing of waste concentration caused increasing of heavy metal accumulation so that the total BCF value decreased [Lu, et al., 2004].

The TF value describe the ability of plant to translocate metals from roots to leaves or stem. The high TF value is more advantageous for remediation because it can decrease waste concentration in root so that the absorption can continue without toxicity in root [Lu, et al., 2004]. In *E. crassipes*, the higher of Cr$^{6+}$ in medium, the higher TF value, while the opposite occurs for *P. stratiotes*. This result is due to the degradation of *P. stratiotes* plant in higher Cr$^{6+}$ concentration, so it can be concluded that *E. crassipes* has more ability for chromium compartmentalisation than *P. stratiotes*. *E. crassipes* is a better phytoremediator than *P. stratiotes*.

TI value describe the effect of Cr$^{6+}$ to relative growth of plant. In general, TI value of *E. crassipes* is higher than *P. stratiotes* in 40 ppm and 80 ppm (Table 6). High TI value means that the plant is tolerant to the treatment [25]. *E. crassipes* is more tolerant to Cr$^{6+}$ exposure than *P. stratiotes* at 40 and 80 ppm concentrations. TI value at 120 ppm concentration of *E. crassipes* is supposed to be higher than *P. stratiotes* because *E. crassipes* has higher TF value than *P. stratiotes*, indicating a more efficient mechanism in Cr$^{6+}$ compartmentalisation. This anomaly may be caused by the non-uniform initial plant size and mass of *E. crassipes* and the degradation of *P. stratiotes* plants in 120 ppm Cr$^{6+}$ concentration.
4. Conclusion

E. crassipes and P. stratiotes showed different physiological responses at different concentrations of Cr\(^{6+}\). At all Cr\(^{6+}\) concentration, P. stratiotes plants showed inability to tolerate metal accumulation, this is indicated by no new leaf emergence in treated groups during 14 days of exposure, even the plant was dead at 120 ppm Cr\(^{6+}\) concentration, while E. crassipes showed no visible difference between treated groups and control. Relative growth rate of both plants were affected by Cr\(^{6+}\) concentration in medium. Relative growth rate of P. stratiotes plants started to decrease even from the lowest Cr\(^{6+}\) concentration (40 ppm), while relative growth rate of E. crassipes started to decrease from 80 ppm. Increasing Cr\(^{6+}\) concentration results in a decrease in total chlorophyll content in both plants. Both species were able to remEDIATE Cr\(^{6+}\) well with %removal ranging between 62 and 68% for 14 days of exposure. Accumulation of Cr\(^{6+}\) in P. stratiotes resulted in decrease of both CAT and APX enzyme activities, while the opposite occurs in E. crassipes. The increase of CAT and APX enzyme activities indicated physiological strategies of E. crassipes to survive under Cr\(^{6+}\) stress. Proline concentration in E. crassipes also increased with increasing Cr\(^{6+}\) concentrations, but no significant difference was shown in P. stratiotes. BCF value in E. crassipes was decreased and P. stratiotes BCF value increased with increasing Cr\(^{6+}\) concentration. High TF value was shown in E. crassipes, almost two times higher than P. stratiotes indicating higher adaptive ability of E. crassipes towards Cr\(^{6+}\) stress than P. stratiotes.

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Table 5 BCF and TF Value of E. crassipes and P. stratiotes after 14 days exposure to various Cr\(^{6+}\) concentrations

<table>
<thead>
<tr>
<th>Sample</th>
<th>BCF Root</th>
<th>BCF Leaves</th>
<th>BCF Total</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. crassipes</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>2.536</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>9.688</td>
<td>0.401</td>
<td>10.088</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>5.459</td>
<td>0.204</td>
<td>5.663</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3.999</td>
<td>0.097</td>
<td>4.096</td>
</tr>
<tr>
<td>P. stratiotes</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>8.141</td>
<td>0.777</td>
<td>8.918</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>5.124</td>
<td>0.506</td>
<td>10.180</td>
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<td></td>
<td>120</td>
<td>4.172</td>
<td>0.675</td>
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Table 6. TL Value

<table>
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<tbody>
<tr>
<td>P. stratiotes</td>
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<td></td>
<td>80 0.866</td>
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<tr>
<td></td>
<td>120 0.943</td>
</tr>
<tr>
<td>E. crassipes</td>
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<td></td>
<td>80 1.298</td>
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<td>120 0.771</td>
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