Physiological strategies of Eichhornia crassipes (Mart.) Solms to tolerate Cr⁶⁺ accumulation, compared to a sensitive species Pistia stratiotes L.

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ABSTRAK

Kromium hexavalent (VI) banyak digunakan oleh industri, termasuk industri penyamakan kulit. Kromium diketahui merupakan logam yang dapat memberi dampak negatif kepada lingkungan dan makhluk hidup. Limbah pabrik penyamakan kulit yang tidak diolah dengan baik dan dibuang ke sungai berpotensi menimbulkan ganggaun terhadap kesehatan masyarakat sekitar, terutama petani yang mengairi sawah mereka dengan air sungai yang tercemar. Karena itu, pengolahan limbah sebelum dibuang ke lingkungan merupakan proses yang penting. Tujuan dari penelitian ini adalah untuk memahami strategi ekofisiologis dari tanaman eceng gondok (Eichornia crassipes) dalam mengakumulasi Cr⁶⁺ pada jaringannya, dibandingkan dengan spesies sensitif tanaman kiapu (Pistia stratiotes). Kedua jenis tanaman ditumbuhkan dalam kontainer yang berisi medium Hoagland dan diberi perlakuan variasi konsentrasi Cr6+, yaitu 0,40,80, dan 120 ppm selama 14 hari . Beberapa parameter termasuk konsentrasi enzim katalase (CAT), Ascorbate peroxidase (APX), klorofil dan prolin diukur pada kedua jenis tanaman tersebut. Yield biomassa tumbuhan yang terpapar Cr⁶⁺ bernilai negatif (-0,732 sampai -1,84 g/minggu) yang mengindikasikan terjadinya penurunan pertumbuhan E. crassipes dan P. stratiotes. Semakin tinggi konsentrasi Cr6+, semakin rendah kadar klorofil pada daun. Kadar klorofil terendah adalah pada perlakuan Cr6+ 120 ppm yakni 0,15 mg/g pada P.stratiotes dan 0,12 mg/g pada E.crassipes. Aktivitas CAT paling tinggi pada E.crassipes adalah 109% dalam 40 ppm Cr6+, sedangkan pada *P. stratiotes* adalah 76% dalam 120 ppm. Kadar prolin pada kedua jenis tumbuhan tidak berbeda signifikan. Pada umumnya, E. crassipes memiliki kemampuan adaptasi terhadap cekaman Cr6+ lebih baik dibandingkan dengan P.stratiotes yang mengalami kerusakan ketika ditumbuhkan pada konsentrasi Cr6+ tinggi. Kedua tumbuhan dapat meremediasi logam Cr6+ cukup baik (tingkat eliminasi 62-68%) selama terdedah oleh Cr⁶⁺ selama 14 hari.

Kata kunci: Eichhornia crassipes, Fitoremediasi, Kromium hexavalent, Pistia stratiotes, Respon fisiologi,

ABSTRACT

Chromium in the form of hexavalent chromium (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 cromium, 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 [Bacordit *et al.*, 2014]. The chromium in tannery wastewater is discharged into the environment and then it caused stress conditions for aquatic organisms.

Chromium is an element in the earth with various forms, i.e. Cr, Cr⁺, Cr²⁺, Cr³⁺, Cr4+, Cr5+, and Cr⁶⁺., however only Cr3+ and Cr6+ are commonly found in environment because of their stability in water and soil. Cr³⁺ has a lower toxicity than Cr⁶⁺, but both of them are still serious threats if they accumulate in high concentration in organisms. In plant, chromium is mostly accumulated in roots [Gomes, et al., 2017], and become immobile in vacuole, but it can also being transferred to other parts through xvlem [Taufikurahman et al., 2017]. Under chromium stress, plant reduce its root diameter, root surface area, and number of root hair, affect photosynthesis metabolism, since chromium is also reported to reduced the absorption of Fe, S, and P elements, which are important cofactors in photosynthesis [Gomes, et al., 2017].

Eichhornia crassipes (water hyacinth) and Pistia stratiotes (water lettuce) have been known as phytoaccumulator for Cr which accumulates in wastewater [Gomes, et al., 2017]. Our previous study also showed that E. crassipes and P. stratiotes have the ability as phytoremediator for chromium in water, although P. stratiotes growth was suffered in high Cr concentration [Dazy, 2008]. Plants can develop various strategies to adapt and maintain their lives in stress conditions [Lambers et al., 2008]. Two basic strategies of plant response are accumulators and excluders (Baker, 2008). In plants, chromium can induces phytotoxicity by interfering growth, nutrient uptake, and photosynthesis. Plants tolerate Cr toxicity via various defense mechanism such as complexation by organic ligands, compartementation into the vacuole, and scavenging ROS via antioxidative enzymes (Shahid et al., 2017). In this study we investigated ecophysiological strategies of E. crassipess to tolerate Cr6+ accumulation in its tissue, compared to sensitive species of *P. stratiotes*.

2. Materials and Method

Cultivation of *E. crassipes* and *P. stratiotes* was carried out in 10% strength Hoagland solution using 15-L container, with 3 replicates for 14 days (March-April 2019). The K_2 CrO₇ was dissolved in water with various concentration: 0 (control), 40, 80, and 120 ppm. Acidity of medium was adjusted in the range of

5.5-6.8. All parameters were measured on the 14th day, including: fresh weight, number of leaves, chlorophyll content, CAT enzyme activity, APX enzyme activity, and proline content. 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),

Chlorophyll $\left(\frac{mg}{g}\right) = \frac{8,02A_{663}+20,2A_{645}}{1000 x wet weight} x V_{Aseton}$ (1) CAT activity was measured by changes in

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 μ mol H₂O₂ per minutes at pH 7 and 25°C.

Volume activity CAT (unit/mL) = $\frac{\Delta A x v q}{0.0436 x Vs}$ (2)	Volume activity CAT (unit/mL)	$=\frac{\Delta A \ x \ V q}{0.0436 \ x \ V s}$	(2)
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Enzyme activity CAT (unit/mg) = $\frac{volume \ activity}{fresh \ weight}$ (3)

Volume activity APX (unit/mL) =
$$\frac{\Delta A \times Vq}{2,8 \times Vs}$$
 (4)

Enzyme activity APX (unit/mg) = $\frac{volume \ activity}{fresh \ weight}$ (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.

Component	Blank	Sample	
50 mM buffer	920 μL	920 μL	
Deionized water	70 µL	-	
Sample	-	70 µL	
H ₂ O ₂	10 µL	10 µL	

Table 2. Composition of solution for APX activity test

Component	Blank	Sample
50 mM buffer	890 μL	890 μL
Ascorbic acid	2 μL	2 µL
Sample	-	30 µL
H_2O_2	20 µL	20 µL

Proline content was measured using Bates method (1973) by extracting leaves and roots in liquid nitrogen with 3% sulfosalicylic 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.

Proline
$$\left(\frac{\mu mol}{g FW}\right) = \frac{\mu g P x mL toluene}{115,5} \times \frac{5}{g sample}$$
 (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.

$$\% removal = \frac{([Cr medium]_{initial} - [Cr medium]_{end}) \left(\frac{mg}{L}\right)}{[Cr medium]_{initial} \left(\frac{mg}{L}\right)}$$
(7)

$$BCF = \frac{Cr \ concentration \ in \ plant \ (\frac{mg}{kg})}{Early \ Cr \ concentration \ in \ medium \ (\frac{mg}{L})}$$
(8)

$$TF = \frac{Cr Concentration in leaves \left(\frac{mg}{kg}\right)}{Cr concentration in root \left(\frac{mg}{L}\right)}$$
(9)

$$TI = \frac{Plant\ biomass\ in\ treatment\ (g\ FW)}{Plant\ biomass\ in\ control\ (g\ FW)}$$
(10)

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 Cr⁶⁺ [Hayat, 2012]. Chromium can enter plant cell through active transport mechanism [Shanker, 2017], and the accumulation of Cr⁶⁺ increase ROS in plants will affect biosynthesis of chlorophyll [Lu, 2011]. In *E. crassipes*, this damage was not visibly seen in all Cr⁶⁺ concentrations, while in *P. stratiotes* chlorosis already occurred in plants grown at low concentration of Cr⁶⁺ (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 Cr⁶⁺ 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 Cr⁶⁺ 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 Cr6+ concentrations was not significantly different with control (P<0.05). This indicate that E. crassipes is tolerant to Cr6+ accumulation.



Gambar 1 Morphology of *P. stratiotes* (up) and *E. crassipes* (bottom) after 14 days of cultivation in Hoagland medium treated with various concentration of Cr⁶⁺

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 Cr⁶⁺

Plants	Parameter -	Chromium concentration (ppm)				
		0	40	80	120	- P value
P. stratiotes	Relative growth rate (g/ week)	$1.71 \ 2 \pm 0.39^{a}$	-0.732 ± 0.86^{b}	-1.84 ± 2.55 ^b	-0.196 ± 0.43 ^b	0.01
	Total leaves number	13.33 ± 4.93^{a}	5 ± 1.53 ^b	4 ± 2^{b}	6 ± 1 ^b	0.011
	Chlorophyll content (mg/g)	0.42 ± 0.20^{a}	0.22 ± 0.10^{ab}	0.20 ± 0.11^{ab}	0.15 ± 0.08^{b}	0.064
E. crassipes	Relative growth rate (g/ week)	9.41 ± 0.05^{a}	0.161 ± 2.26 ^b	-6.87 ± 1.56 ^b	-4.59 ± 3.53 ^b	0.02
	Total leaves number	7 ± 1	7 ± 1	7 ± 1	6 ± 1	0.265
	Chlorophyll content (mg/g)	0.22 ± 0.2	0.18 ± 0.15	0.12 ± 0.06	0.17 ± 0.09	0.329

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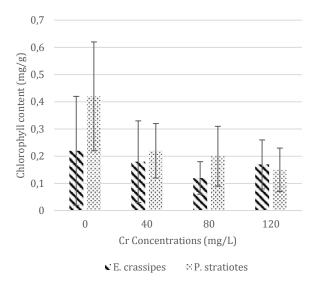


Figure 2 Chlorophyll content in *E. crassipes* and *P. stratiotes* after 14 days of cultivation in Hoagland medium treated with various concentration of Cr⁶⁺

Chromium removal efficiency of *P. stratiotes* and *E. crassipes* is not significantly different, in the range of 62-68% (Table 4). Chromium removal efficiency in *E. crassipes* reached 84% after 11 days of exposure [Mishra dan Tripathi, 2009] and chromium removal efficiency in *P. stratiotes* reached 100% after 72 days of exposure [Prajapati *et.al.*, 2012].

Table 4 The chromium removal efficiency in *P. stratiotes*

 and *E. crassipes*

Comple	Cr ⁶⁺ concentration in medium (mg/L)			
Sample	Initial End Removal Effici		Removal Efficiency (%)	
E. crassipes	40	15.233	62	
	80	25.95	68	
	120	45.233	62	
P. stratiotes	40	14.525	64	
	80	27.133	66	
	120	39.1	67	

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

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 [Sucahyo 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.

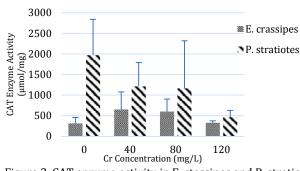


Figure 3. CAT enzyme activity in E. ctassipes and P. stratio

The same pattern also occurred in APX enzyme activity. The higher Cr^{6+} concentration in the medium, the higher of ascorbate peroxidase enzyme activity (APX) in *E. crassipes*, while the opposite occurs in *P. stratiotes* (Figure 4). The highest level of APX enzyme activity in *E. crassipes* was showed in 120 ppm Cr^{6+} (130%) followed by 80 ppm Cr^{6+} (63%). The APX enzyme has a function to reduce H_2O_2 to H_2O in ascorbate-glutaton (Asc-Glu) metabolism. The Cr^{6+} stress can induce the increasing of APX enzyme which can cause detoxification of H_2O_2 . The same mechanism was known in other metal stress, such as silica (Si) in plants [Liang *et al.*,

2003]. 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.

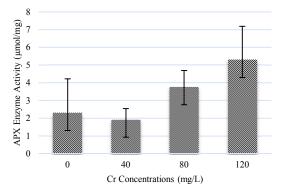


Figure 4 APX Enzyme activity in *E. Crassipes* after 14 days of cultivation in Hoagland medium treated with various concentrations of Cr⁶⁺

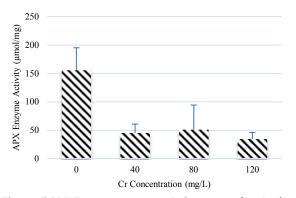


Figure 5 APX Enzyme activity in *P. Stratiotes* after 14 days of cultivation in Hoagland medium treated with various concentrations of Cr⁶⁺

The same result shown in *Brassica junce*a 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 glutahthione 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 treatment. Other study and 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].

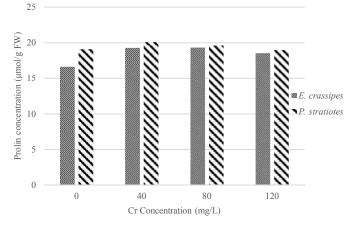


Figure 6 Proline content in E. crassipes and P. stratiotes

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 Cr6+ 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 Cr⁶⁺ concentration, so it can be concluded that E. crassipes ability has more for chromium compartementalisation 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. Table 5 BCF and TF Value of *E. crassipes* and *P. stratiotes* after 14 days exposure to various Cr⁶⁺ concentrations

Samp	le	BCF Root	BCF Leaves	BCF Total	TF
E. crassipes	0	-	-	-	2.536
	40	9.688	0.401	10.088	24.183
	80	5.459	0.204	5.663	26.714
	120	3.999	0.097	4.096	41.429
P. stratiotes	0	-	-	-	-
	40	8.141	0.777	8.918	10.477
	80	5.124	5.056	10.180	1.013
	120	4.172	6.750	10.922	0.6180

Table 6. TI Value

Sampl	e	TI	
P. stratiotes	40	0.440	
	80	0.866	
	120	0.843	
E. crassipes	40	0.911	
	80	1.298	
	120	0.771	

4. Conclusion

E. crassipes and P. stratiotes showed different physiological responses at different concentrations of Cr⁶⁺+. At all Cr⁶⁺ 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 Cr⁶⁺ concentration, while *E. crassipes* showed no visible difference between treated groups and control. Relative growth rate of both plants were affected by Cr6+ concentration in medium. Relative growth rate of *P. stratiotes* plants started to decrease even from the lowest Cr⁶⁺ concentration (40 ppm), while relative growth rate of E. crassipes started to decrease from 80 ppm. Increasing Cr⁶⁺ concentration results in a decrease in total chlorophyll content in both plants. Both species were able to remediate Cr⁶⁺ 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 Cr⁶⁺ stress. Proline concentration in E. crassipes also increased with increasing Cr6+ 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 Cr6+ concentration. High TF value was shown in E. crassipes, almost two times higher than *P. stratiotes* indicating higher adaptive ability of *E. crassipes* towads Cr⁶⁺ stress than *P.* stratiotes.

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