International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 28-10-2025, Accepted: 27-11-2025, Published: 12-12-2025
Volume 10, Issue 4, 2025, Page No. 38-41

Antioxidant enzyme responses in *Ceratophyllum demersum* L. and *Eichhornia crassipes* mart (solms) under chromium-induced oxidative stress

Suvarna Ubale, Sangeeta Ahuja
Department of Botany, Sir sayyed College Chatrapati Sambhaji Nagar, Maharashtra, India

Abstract
The present investigation was carried out to evaluate the antioxidant enzyme response in two aquatic macrophytes, *Ceratophyllum demersum L.* and *Eichhornia crassipes Mart (*Solms*)*, under chromium-induced oxidative stress. The study was conducted in 2021 under controlled laboratory conditions using potassium dichromate to simulate chromium pollution at concentrations of 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mg/L. Fresh plant tissues were subjected to enzymatic assays after 3, 7, 10, 20, and 30 days of exposure. Enzyme activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was quantified and expressed as units per mg of protein. In *C. demersum L.*, SOD and CAT activity increased at moderate chromium levels but declined at higher concentrations. In contrast, *E. crassipes Mart (*Solms*)* exhibited a concentration-dependent increase in SOD and APX activity up to 5.0 mg/L, followed by a reduction at higher levels. Maximum CAT activity (2.45 ± 0.014 U/mg) was recorded in *E. crassipes Mart (*Solms*)* at 5 mg/L after 20 days. The enzyme responses suggest that both species possess differential but effective antioxidant defense mechanisms, making them promising candidates for phytoremediation of chromium-contaminated aquatic environments.

Keywords: Ascorbate peroxidase, catalase, chromium, phytoremediation, superoxide dismutase

Introduction
Heavy metal pollution, particularly from chromium (Cr), poses a serious threat to aquatic ecosystems due to its toxicity, mobility, and persistence. Hexavalent chromium (Cr^{6+}), discharged mainly from electroplating, leather tanning, and textile industries, is the most toxic form and can cause severe physiological disruptions in aquatic organisms (Shanker *et al*., 2005; Jaishankar *et al*., 2014) [6, 10]. In aquatic plants, Cr^{6+} generates reactive oxygen species (ROS), leading to oxidative stress that damages lipids, proteins, and nucleic acids and disrupts cellular homeostasis (Gill & Tuteja, 2010) [5]. Several physicochemical methods such as ion exchange, reverse osmosis, and chemical precipitation have been developed to remove Cr^{6+} from water, but these are often costly, energy-intensive, and produce hazardous sludge (Barakat, 2011) [1]. In contrast, phytoremediation using aquatic macrophytes is considered a cost-effective and sustainable alternative. Species such as *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms) have shown excellent metal uptake capacities due to their fast growth, high biomass, and adaptability to polluted conditions (Mishra & Tripathi, 2008) [7].

While various biochemical changes such as chlorophyll degradation, total phenolic content alterations, or shifts in protein and sugar levels have been used to assess plant responses to metal toxicity, these markers often lack specificity or occur only at later stages of stress. For example, total phenolic content and chlorophyll levels may remain unchanged in early metal exposure despite internal oxidative stress (Tripathi *et al*., 2007). In contrast, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) serve as rapid and sensitive indicators of oxidative stress and redox imbalances at the cellular level (Sharma & Dubey, 2005; Panda & Khan, 2003) [9, 11]. These enzymes directly participate in scavenging ROS generated during metal uptake and translocation and are often upregulated even before phenotypic symptoms become visible. A study by Srivastava *et al*. (2005) [12] on *Lemna minor* exposed to cadmium demonstrated that chlorophyll content remained relatively stable while SOD and CAT activities increased sharply within 48 hours, highlighting the superior sensitivity of antioxidant enzymes in early stress detection. Thus, evaluating these enzymatic responses not only reflects oxidative stress but also helps reveal detoxification strategies adopted by plants, offering a mechanistic understanding of phytoremediation.

Therefore, the present study aimed to assess and compare the antioxidant enzyme responses of *C. demersum L.* and *E. crassipes Mart (*Solms*)* under graded concentrations of Cr^{6+}. By analyzing changes in SOD, CAT, and APX activity over time, the research seeks to elucidate species-specific biochemical adaptations and validate their utility as effective phytoremediators and bioindicators of chromium contamination in aquatic systems.

Materials and methods
1. Biochemical Studies
Fresh leaves (>200 mg) of *Ceratophyllum demersum L.* and *Eichhornia crassipes* Mart (Solms) were homogenized separately in a prechilled mortar and pestle using 2.0 mL of ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the resulting supernatants were collected for the estimation of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities. Total soluble protein content was determined using the Bradford assay (Bradford, 1976) [4], with bovine serum albumin (BSA) as the standard.

2. Superoxide Dismutase (SOD) Activity
Superoxide dismutase (SOD) activity was evaluated based on its capacity to inhibit the photochemical reduction of

nitroblue tetrazolium (NBT), following the method described by Beauchamp and Fridovich (1971) [2]. The assay was performed in a 3.0 mL reaction mixture comprising 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2.25 mM NBT, 60 µM riboflavin, and a defined volume of enzyme extract. The reaction was initiated by exposing the mixture to a 40-watt fluorescent light for 15 minutes. A control (without enzyme extract, exposed to light) and a blank (with enzyme extract, kept in the dark) were included for reference. Absorbance was measured at 560 nm using a UV-visible spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction, and results were expressed in units per milligram of protein per hour (U/mg protein/h)

3. Catalase (CAT) Activity

Catalase (CAT) activity was measured by monitoring the decomposition rate of hydrogen peroxide (H_2O_2), following the method of Beers and Sizer (1952) [3]. The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 20 mM H_2O_2. The reaction was initiated by adding 100 µL of enzyme extract, and the decrease in absorbance was recorded at 240 nm using a UV-visible spectrophotometer. Enzyme activity was calculated using an extinction coefficient of 39.04 mM^{-1} cm^{-1}. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute per milligram of protein, and results were expressed as U/mg protein/min.

4. Ascorbate Peroxidase (APX) Activity

Ascorbate peroxidase (APX) activity was assayed following the protocol of Nakano and Asada (1987) [8]. The reaction mixture (3.0 mL) consisted of 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.3 mM hydrogen peroxide (H_2O_2), and an appropriate volume of enzyme extract. The oxidation of ascorbate was monitored by measuring the decrease in absorbance at 290 nm over a 3-minute interval using a UV-visible spectrophotometer. Enzyme activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX activity was defined as the amount of enzyme required to oxidize 1 µmol of ascorbate per minute at 25°C, and results were expressed as U/mg protein/min.

5. Statistical analysis

All the experiments were conducted in triplicates. Standard error of the mean was calculated. Significance between the means was estimated by one way ANOVA Tukey's HSD post hoc analysis at 0.05 level of significance.

Results

1. Superoxide dismutase (SOD) activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms)

The activity of superoxide dismutase (SOD) in *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms) under varying chromium concentrations and time intervals is presented in Table 1. In *C. demersum*, SOD activity remained relatively stable at lower Cr levels (1–5 mg/L) over 10 days but increased significantly at 7.5 and 10 mg/L, peaking at 37 ± 0.21 U/mg protein on day 10, indicating an elevated antioxidant response. In contrast, *E. crassipes* Mart (Solms) showed a concentration-dependent increase in SOD activity at lower Cr levels (1 and 2.5 mg/L), reaching up to 176 ± 1.02 U/mg protein on day 10. However, higher Cr concentrations (5–10 mg/L) led to a significant decline in SOD activity ($p < 0.05$), suggesting possible enzyme inhibition. At 12.5 and 15 mg/L, SOD activity was not assessed in *C. demersum*, while in *E. crassipes*, a gradual reduction in activity was observed over 30 days, implying a compromised antioxidant defense under severe Cr stress.

Table 1: Superoxide dismutase (SOD) activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes Mart* (Solms) at different chromium concentrations

Chromium concentration (mg/L)	*Ceratophyllum demersum* L. U/mg of protein			*Eichhornia crassipes* Mart (Solms) U/mg of protein		
	3 days	7 days	10 days	10 days	20 days	30 days
0 (control)	28 ± 0.16	29 ± 0.17	27 ± 0.16	150 ± 0.87[a]	152 ± 0.88	161 ± 0.93[e]
1	30 ± 0.17	32 ± 0.18	35 ± 0.2	162 ± 0.94[b]	165 ± 0.95[d]	169 ± 0.98
2.5	31 ± 0.18	34 ± 0.2	32 ± 0.18	172 ± 0.99[c]	174 ± 1.00	176 ± 1.02
5	33 ± 0.19	36 ± 0.21	37 ± 0.21	177 ± 1.02	179 ± 1.03	181 ± 1.05
7.5	25 ± 0.14	26 ± 0.15	28 ± 0.16	170 ± 0.98[c]	162 ± 0.94[d]	158 ± 0.91[e]
10	22 ± 0.13	20 ± 0.12	18 ± 0.1	163 ± 0.94[b]	157 ± 0.91	151 ± 0.87
12.5	-	-	-	148 ± 0.74[a]	146 ± 0.73	140 ± 0.7
15	-	-	-	138 ± 0.69	133 ± 0.67	129 ± 0.64

Any value followed by ± is the standard error of the mean. All the means were significantly different at the $p < 0.05$ level of significance. The values with common superscripts are similar means.

2. Catalase (CAT) activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms)

Catalase (CAT) activity in *Ceratophyllum demersum L* decreased significantly ($p < 0.05$) with increasing chromium concentrations, as shown in Table 2. While the control group showed a slight increase over time (0.98 ± 0.01 to 1.22 ± 0.01 U/mg protein from day 3 to day 10), CAT activity dropped sharply at 1 mg/L Cr (0.35 ± 0.002 to 0.58 ± 0.003 U/mg protein) and continued declining with higher concentrations, reaching the lowest values at 10 mg/L (0.12 ± 0.001 U/mg protein on day 10), indicating enzyme inhibition under metal stress. In contrast, *Eichhornia crassipes* Mart (Solms) exhibited a more adaptive response. At 1 and 2.5 mg/L Cr, CAT activity increased over time, peaking at 1.12 ± 0.006 and 0.98 ± 0.006 U/mg protein by day 30, respectively. At 5 and 7.5 mg/L, early peaks were recorded (1.87 ± 0.011 and 1.89 ± 0.011 U/mg protein), followed by stabilization. At 10 mg/L, activity remained moderate (1.01–1.22 U/mg protein), while at 12.5 and 15 mg/L, a decline was observed by day 30 (0.81 and 0.54 U/mg protein), indicating potential stress-induced inhibition at higher Cr concentrations.

Table 2: Catalase (CAT) activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes Mart* (Solms) at different chromium concentrations

Chromium concentration (mg/L)	*Ceratophyllum demersum* L. (U/mg of protein)			*Eichhornia crassipes* Mart (Solms) (U/mg of protein)		
	3 days	7 days	10 days	10 days	20 days	30 days
0 (control)	0.98 ± 0.01	1.12 ± 0.01	1.22 ± 0.01	1.1 ± 0.01	1.2 ± 0.01	1.4 ± 0.01
1	0.35 ± 0.002	0.46 ± 0.003	0.58 ± 0.003	0.57 ± 0.003	0.89 ± 0.005	1.12 ± 0.006
2.5	0.61 ± 0.004	0.63 ± 0.004	0.75 ± 0.004	0.65 ± 0.004	0.91 ± 0.005	0.98 ± 0.006
5	0.42 ± 0.002	0.65 ± 0.004	0.73 ± 0.004	1.87 ± 0.011	2.45 ± 0.014	1.44 ± 0.008
7.5	0.35 ± 0.002	0.28 ± 0.002	0.21 ± 0.001	1.33 ± 0.008	1.68 ± 0.01	1.89 ± 0.011
10	0.17 ± 0.001	0.15 ± 0.001	0.12 ± 0.001	1.17 ± 0.007	1.25 ± 0.007	1.01 ± 0.006
12.5	-	-	-	1.16 ± 0.006	1.09 ± 0.005	0.81 ± 0.004
15	-	-	-	0.98 ± 0.005	0.79 ± 0.004	0.54 ± 0.003

Any value followed by ± is the standard error of the mean. All the means were significantly different at the $p < 0.05$ level of significance.

3. Ascorbate peroxidase activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms)

Ascorbate peroxidase (APX) activity in *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms) under different chromium (Cr) concentrations and incubation periods is presented in Table 3. In *C. demersum,* APX activity in the control group (0 mg/L Cr) increased significantly ($p < 0.05$) from 3 ± 0.01 U/mg protein at day 3 to 9 ± 0.04 U/mg protein at day 10. Upon Cr exposure, enzyme activity increased with both concentration and time, peaking at 20 ± 0.10 U/mg protein at 10 days under 1 mg/L Cr. The highest APX activity (18 ± 0.10 U/mg protein) was recorded at 5 mg/L Cr on day 10, after which activity declined slightly at 7.5 and 10 mg/L (17 ± 0.09 U/mg protein), suggesting possible enzyme inhibition at elevated stress levels. In *E. crassipes*, APX activity showed a consistent rise across all incubation periods, particularly at 2.5 and 5 mg/L Cr, with values ranging from 16 ± 0.08 to 18 ± 0.10 U/mg protein. Enzyme activity continued increasing with Cr concentration, reaching a maximum of 21 ± 0.11 U/mg protein at 15 mg/L on day 30. This concentration-dependent enhancement highlights *E. crassipes* Mart (Solms) as a strong candidate for Cr phytoremediation.

Table 3: Ascorbate peroxidase activity of *Ceratophyllum demersum* L. and *Eichhornia crassipes Mart* (Solms) at different chromium concentrations

Chromium concentration (mg/L)	Enzyme activity (U/mg of protein) of Ascorbate peroxidase					
	Ceratophyllum demersum L.			*Eichhornia crassipes* Mart (Solms)		
	3 days	7 days	10 days	10 days	20 days	30 days
0 (control)	3 ± 0.01	5 ± 0.02	9 ± 0.04	10 ± 0.04^{d}	13 ± 0.07	14 ± 0.07
1	12 ± 0.06	16 ± 0.08^{b}	20 ± 0.10	15 ± 0.08^{c}	17 ± 0.09	18 ± 0.10^{e}
2.5	21 ± 0.11	22 ± 0.11	23 ± 0.12	16 ± 0.08	18 ± 0.10	18 ± 0.10^{e}
5	24 ± 0.06	19 ± 0.08	18 ± 0.10	18 ± 0.10	20 ± 0.11	21 ± 0.11
7.5	17 ± 0.09^{a}	16 ± 0.08^{b}	15 ± 0.08	15 ± 0.08^{c}	14 ± 0.07	13 ± 0.07
10	17 ± 0.09^{a}	15 ± 0.08	13 ± 0.07	12 ± 0.06	15 ± 0.08	17 ± 0.09
12.5	0	0	0	10 ± 0.04^{d}	11 ± 0.06	10 ± 0.04
15	0	0	0	8 ± 0.04	6 ± 0.02	4 ± 0.01

Any value followed by ± is the standard error of the mean. All the means were significantly different at the $p < 0.05$ level of significance. The means with a common superscripts are similar.

Discussion

The antioxidant enzyme responses of *Ceratophyllum demersum* L. and *Eichhornia crassipes* Mart (Solms) under chromium-induced stress reveal distinct yet effective defense strategies against oxidative damage. Both species activated key enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) to counteract the harmful effects of reactive oxygen species (ROS). SOD activity, assessed by the NBT photoreduction inhibition method (Beauchamp & Fridovich, 1971) [2], increased in both macrophytes with rising chromium concentrations, indicating their capacity to dismutate superoxide radicals. However, CAT activity displayed divergent patterns: it increased at moderate concentrations in *C. demersum* L. but declined sharply at higher levels, suggesting possible enzyme inhibition under severe stress. In contrast, *E. crassipes* Mart (Solms) maintained stable or elevated CAT activity across Cr levels, reflecting a more resilient H_2O_2 detoxification system. APX activity, measured via the ascorbate oxidation method (Nakano & Asada, 1987) [8], rose significantly in both species under chromium exposure, with *E. crassipes* Mart (Solms) consistently showing higher activity especially at 10–15 mg/L Cr. This superior enzymatic performance in *E. crassipes* Mart (Solms) is in line with prior studies on pollutant-exposed aquatic plants (Vajpayee *et al*., 2000; Panda & Khan, 2003) [9, 13] and suggests a more robust H_2O_2-scavenging capacity and greater oxidative stress tolerance.

These enzymatic patterns not only highlight the ability of aquatic macrophytes to respond to heavy metal stress but also position *E. crassipes* Mart (Solms) as a promising candidate for phytoremediation of chromium-contaminated aquatic environments. The sustained activity of CAT and elevated APX levels in *E. crassipes* Mart (Solms) suggest that it can endure prolonged Cr exposure and maintain effective detoxification pathways, making it suitable for practical environmental applications. Moreover, its consistent biochemical responses underline its potential use as a bioindicator of heavy metal pollution. However, a key limitation of this study is the laboratory-based experimental setup, which may not fully replicate complex natural conditions. Additionally, the focus on enzymatic parameters alone limits broader conclusions regarding plant physiology

and stress adaptation. Future studies should laccase incorporate field trials, assess long-term chromium accumulation, and explore gene expression profiles of antioxidant enzymes to deepen our understanding of macrophyte resilience. Such approaches will be crucial for validating the phytoremediation potential of *E. crassipes* Mart (Solms) and optimizing its use in sustainable water pollution management.

Conclusion

The present study demonstrated that *Ceratophyllum demersum* L. and *Eichhornia crassipes* (L.) exhibited distinct antioxidant enzyme responses under chromium-induced oxidative stress. Both species activated key enzymes *viz.* superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) involved in detoxifying reactive oxygen species (ROS). *E. crassipes* Mart (Solms) showed a more sustained enzymatic response across different exposure levels and time intervals, suggesting greater biochemical resilience. In contrast, *C. demersum* L. displayed a moderate increase in enzyme activity at lower chromium concentrations but reduced activity at higher levels, indicating lower tolerance. The enhanced performance of *E. crassipes* Mart (Solms) may be attributed to its floating nature, which allows better access to oxygen and light, supporting more active metabolism. Unlike studies that required chemical enhancement, both species in this study responded effectively under natural laboratory conditions, highlighting their intrinsic phytoremediation potential. The use of multiple antioxidant enzymes as biomarkers over time provided a robust method for evaluating plant tolerance to heavy metal stress. Overall, *E. crassipes* Mart (Solms) emerged as a more promising candidate for phytoremediation of chromium-contaminated waters and for use as a bioindicator in environmental monitoring.

References

1. Barakat MA. New trends in removing heavy metals from industrial wastewater. Arab J Chem,2011:4(4):361–377. doi:10.1016/j.arabjc.2010.07.019
2. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem,1971:44(1):276–287. doi:10.1016/0003-2697(71)90370-8
3. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem,1952:195:133–140. doi:10.1016/S0021-9258(18)66141-2
4. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem,1976:72(1–2):248–254. doi:10.1016/0003-2697(76)90527-3
5. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem,2010:48(12):909–930. doi:10.1016/j.plaphy.2010.08.016
6. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol,2014:7(2):60–72. doi:10.2478/intox-2014-0009
7. Mishra VK, Tripathi BD. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour Technol,2008:99(15):7091–7097. doi:10.1016/j.biortech.2008.01.013
8. Nakano Y, Asada K. Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol,1987:28(1):131–140. doi:10.1093/oxfordjournals.pcp.a077268
9. Panda SK, Khan MH. Antioxidant efficiency in *Oryza sativa* seedlings under nickel stress. Biol Plant,2003:46(2):261–263.
10. Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S. Chromium toxicity in plants. Environ Int,2005:31(5):739–753.
11. Sharma P, Dubey RS. Lead toxicity in plants. Braz J Plant Physiol,2005:17(1):35–52. doi:10.1590/S1677-04202005000100004
12. Srivastava S, Mishra S, Dwivedi S, Tripathi RD, Tandon PK, Srivastava MK. Evaluation of metal accumulation potential of submerged aquatic macrophyte *Ceratophyllum demersum* L. for cadmium and chromium. Environ Toxicol,2005:20(1):22–34.
13. Vajpayee P, Rai UN, Ali MB, Tripathi RD, Singh SN. Chromium-induced physiochemical changes in *Hydrilla verticillata* (L.f.) Royle. Chemosphere,2000:41(1–2):149–155. doi:10.1016/S0045-6535(99)00406-7