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Effects of inorganic arsenic species on the antioxidant enzyme system of the Amazon Sword Plant (*Echinodorus amazonicus* Rataj)

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ABSTRACT

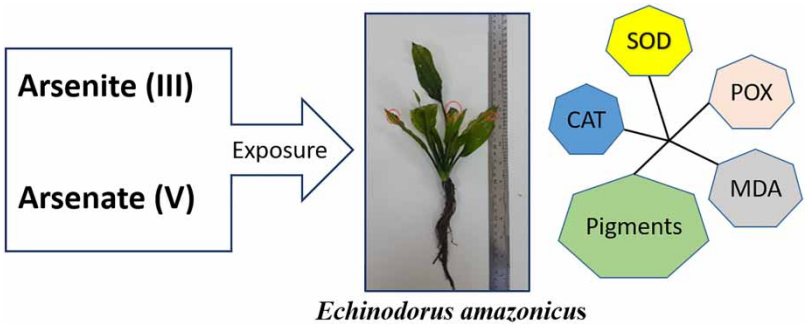
This study aims to examine the effects of Arsenite (As^{+3}) and Arsenate (As^{+5}) on the aquatic macrophyte Amazon Sword Plant (*Echinodorus amazonicus* Rataj). To this aim, different concentrations of As^{+3} and As^{+5} (0, 6, 18 and 54 μM) were analyzed. At the end of the trail, photosynthetic pigment contents, total protein amounts, the enzymatic antioxidants superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activities and the amount of malondialdehyde (MDA) in the leaf samples of *E. amazonicus* were investigated. The antioxidant enzyme activities increased at low concentrations (32.13% for SOD, 185% for CAT and 201.5% for POX in the groups of 6 μM As^{+5}), but decreased at high concentrations (64.98% for SOD, 21.64% for CAT and 21.29% for POX in the groups of 54 μM As^{+3}). MDA increased in all the treatment groups. The highest MDA contents were observed as 96% for 54 μM As^{+3} and 71.50% for 54 μM As^{+5}. Photosynthetic pigment contents and the amount of protein were decreased with higher concentrations. The most significant decreases in protein content were 65% for 54 μM As^{+3} and 34.9% for 54 μM As^{+5}. As a result, the toxicity of As^{+3} was higher and the toxic effect increased at higher concentrations.

Key words: antioxidant enzymes, aquatic macrophyte, inorganic arsenic, malondialdehyde

HIGHLIGHTS

- Oxidative stress caused by inorganic arsenic on aquatic plants was determined.
- At high concentrations of arsenic ions, both antioxidant enzyme activity and photosynthetic pigment contents were decreased.
- It was found that the As^{+3} ion is more toxic than the As^{+5} ion.
- The results of this study showed that plants will not survive if exposed to arsenic for a long period.

GRAPHICAL ABSTRACT



INTRODUCTION

Arsenic is one of the elements that can be found in water and has a high toxic effect, especially in inorganic forms. USEPA has classified arsenic and arsenic compounds as carcinogenic to humans (USEPA 1998a; WHO 2011; Jang *et al.* 2016; Sarkar & Paul 2016). Many terrestrial and aquatic ecosystems around the world are contaminated with arsenic from either

anthropogenic or natural sources (Mandal & Suzuki 2002). Arsenic enters the ecosystem from natural sources such as rocks, hot springs, volcanic activities or anthropogenic activities such as agricultural activities, coal burning, oil refining, paint industry, copper smelting and mining activities (USEPA 1998b; USEPA 2002; Rose *et al.* 2007; Yabanlı 2010; Juncos *et al.* 2016).

Arsenic is found at higher levels in aquatic ecosystems than in terrestrial areas because it has a very high solubility in water and can derive from rocks rich in arsenic by the waves (Rose *et al.* 2007). Especially As^{+3} and As^{+5} forms of arsenic, which are generally found in the inorganic form in water resources, are more dominant (Viraraghavan *et al.* 1999; Ötleş & Çağındı 2010). For all organisms, including photosynthetic microorganisms, inorganic arsenic species are generally more toxic than organic arsenic species, and As^{+3} is 5–10 times more toxic than As^{+5} (Rossman 2003; Cordos *et al.* 2006; Jang *et al.* 2016).

The toxic effect of arsenic on plants depends on its oxidation state (Duman *et al.* 2010). As^{+3} and As^{+5} forms are bioavailable forms for plants and are easily taken up by plant roots (Rahman & Hasegawa 2011; Finnegan & Chen 2012). Given that As^{+5} is chemically similar to PO_4^{-2}, it is easily incorporated into the cell by active uptake with phosphate transporters and prevents the passage of phosphate (Rosen *et al.* 2011). In this way, it can interfere with important cellular events such as ATP synthesis, oxidative phosphorylation and consumes cell energy (Duker *et al.* 2005; Tripathi *et al.* 2007). As^{+3} enters the cell via passive uptake pathways with aquaporins (Rahman & Hasegawa 2011). As^{+3}, taken into the cell, reacts with the sulfhydryl groups (–SH) of enzymes and tissue proteins, causing inhibition of cellular function and death, thereby preventing tissue growth (Meharg & Hartley-Whitaker 2002). The interaction of As^{+3}, with the cell membranes of plants by inactivating microbial enzymes results in necrosis in the leaves. On the other hand, As^{+5} does not react with sulfhydryl groups, so it has no direct effect on membranes, but it affects phosphorylation in mitochondria (Sizova *et al.* 2002). Inhibition of proteins, higher solubility, faster cellular uptake and slower excretion rate compared to arsenate increase the toxicity of arsenite (Rossman 2003; Gupta 2018; Coelho *et al.* 2020).

The accumulation of non-essential arsenic by plants negatively affects the processes of cellular metabolism, leading to the production of reactive oxygen species (ROS) and thus oxidative stress (Meharg & Hartley-Whitaker 2002; Yu *et al.* 2012; Andrade *et al.* 2016; Abbas *et al.* 2018). The excessive increase in the amount of ROS under stress conditions leads to lipid and protein oxidation in cell membranes, degradation of chlorophyll, inhibition of enzymes, damage to DNA and RNA, and as a result, cell death (Cakmak 2000; Mittler 2002; Leão *et al.* 2014; Andrade *et al.* 2016). ROS causes lipid peroxidation and the release of malondialdehyde (MDA), which is a toxic product (Hartley-Whitaker *et al.* 2001; Zhang *et al.* 2007). Evaluation of lipid peroxidation can be done by determining the amount of MDA (Hu *et al.* 2007).

Plants have a variety of enzymatic and non-enzymatic antioxidant systems to eliminate the increased ROS under oxidative stress conditions (Blokhina *et al.* 2003; Ren *et al.* 2021). Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) increase under the oxidative stress conditions and have an important role in the scavenging of ROS (Das & Roychoudhury 2014; Sarker & Oba 2018).

Echinodorus amazonicus, also known as The Amazon Sword, is a submerged aquatic plant that can reach 40 cm in length and usually grows in wetlands (Leekroh 2017; AquaticCommunity 2020). In previous studies, heavy metal tolerance and accumulation of *E. amazonicus* were investigated (Sapci & Ustun 2012; Yang & Ye 2015). This plant was used as a test material in the present study such as heavy metal tolerance and their accumulation because it easily adapts to different environmental conditions and for this it is widely used by aquarists. This study was aimed to reveal the effects of As^{+3} and As^{+5} ions at different concentrations on the antioxidant enzyme activity, lipid integrity, chlorophyll and carotenoid content of *E. amazonicus*.

MATERIALS AND METHODS

Plant material and metal treatment

The samples of *E. amazonicus* were obtained from a company selling aquarium products (Aquainturkey company, Turkey). In order to remove the organisms (e.g., bacteria) on the plants, they were rinsed with distilled water and the deformed leaves were removed by sorting. For the adaptation of the plants to the laboratory conditions, a 45-day acclimatization period was applied in 40-l plastic aquariums in the Water Quality Laboratory (Faculty of Fisheries, Mugla Sıtkı Kocman University, Turkey). During the acclimatization period, water with a temperature of 24–28 °C and pH in the range of 7–8 was used. Photoperiod application was carried out with 14:10 light:dark (Yang & Ye 2015). 20% Hoagland solution was used as nutrient solution (Hoagland & Arnon 1950; Li *et al.* 2011).

After the acclimatization period, the plants were rinsed with distilled water before starting the experiments. Then, they were placed in plastic aquariums filled with 40 l of water, with their roots buried in the sand. Plants were exposed to 0, 6, 18 and 54 μM concentrations of As^{+3} and As^{+5} ions separately for 17 days in three repetitions. As^{+3} and As^{+5} ions were applied by taking certain amounts from stock solutions prepared from $NaAsO_2$, $HAsNa_2O_4{\cdot}7H_2O$, respectively. Necrosis and chlorosis were observed in the leaves of the plants in the groups where high concentrations (54 μM) were applied, and therefore, the plants were harvested on the 17th day. In order to perform protein, chlorophyll, MDA and enzyme analysis, undamaged leaves were taken and stored in a deep freezer at −20 °C for further analysis. All analyses were performed considering fresh weight (F.W.).

Determination of MDA contents

MDA contents in the samples were measured according to the thiobarbituric acid (TBA) method determined by Heath & Packer (1968) and Du & Bramlage (1992). According to this method, 0.5 g of fresh leaf samples were weighed and homogenized in 10 ml of 0.1% TCA (trichloroacetic acid) and then centrifuged at 10,000 $\times g$ (relative centrifugal force) for 10 min. After centrifugation, 1 ml of the supernatant was taken and 4 ml of 0.5% TBA prepared in 20% TCA was added. The mixture was kept in a water bath at 95 °C for 30 min, and the reaction was terminated by quickly placing it into ice. This mixture was centrifuged again at 10,000 $\times g$ (relative centrifugal force) for 15 min. Absorbance values were recorded at 532 and 600 nm wavelengths in the spectrophotometer. The concentration of MDA was calculated from the difference in absorbance at 532 and 600 nm using an extinction coefficient of 155 $mM^{-1}\ cm^{-1}$. MDA content expressed as μmol g^{-1} F.W.

Determination of protein contents and antioxidant enzymes

0.5 g of leaf samples were homogenized in 5 ml of 50 mM potassium phosphate buffer (pH: 7) containing 1 mM of disodium EDTA and 2% (w/v) polyvinylpyrrolidone. These homogenates were centrifuged at +4 °C 10,000 $\times\ g$ (relative centrifugal force) for 20 min (Peixoto *et al.* 1999; Zhang *et al.* 2007). Obtained supernatants were used for enzyme analysis. The protein content of the samples was determined according to Bradford (1976) using bovine serum albumin as a standard. The results were expressed as mg g^{-1}. SOD activity was analyzed by measuring the ability of nitroblue tetrazolium to inhibit photochemical reduction at 560 nm, as determined by Beauchamp & Fridovich (1971). POX activity was determined by the change in absorbance of guaiacol oxidation at 470 nm according to the method determined by Chance & Maehly (1955). Buffer solutions were prepared by using the appropriate pH for each enzyme (50 mM phosphate buffer with a pH of 6.5 for POX, pH of 7 for CAT and pH of 7.8 for SOD, respectively). According to the method determined by the method of Aebi (1984), the decomposition of H_2O_2 was observed by decline at 240 nm for the CAT activity. POX and CAT activities are expressed as unit mg^{-1} protein.

Determination of chlorophyll and carotenoids

0.5 g of the plant leaves was extracted in 90% chilled acetone and chlorophyll and carotenoid contents were calculated according to the formulas determined by Arnon (1949) and Lichtenthaler (1987). Pigment contents are expressed as mg g^{-1} F.W.

Statistical analysis

STATISTICA Stat 7.0 program was used for the statistical analysis of data obtained from the experimental groups, and analysis of variance (ANOVA) test followed by Post Hoc Tukey test were used to reveal the differences between the groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of As^{+3} and As^{+5} ions on photosynthetic pigments

In this study, significant decreases in photosynthetic pigment content was observed with increasing arsenic concentrations when compared to the control group (Table 1). The most significant decreases were observed in the groups treated with the highest concentrations (54 μM) of As^{+3} and As^{+5} ions. Compared to the control, these decreases were found to be 52 and 27% for As^{+3} and As^{+5} for chlorophyll a, 57% and 36.5% for chlorophyll b, 48 and 37% for total chlorophyll, 42 and 31% for carotenoids, respectively. As a result of daily observations, necrosis and chlorisis were observed in the plant leaves in the groups treated with 54 μM As^{+3} and As^{+5} (Figure 1).

Table 1 | Effects of As^{+3} and As^{+5} on photosynthetic pigments (mean ± SE) of *Echinodorus amazonicus* at different concentrations

Treatment group	Chlorophyll a (mg g^{-1} F.W.)	Chlorophyll b (mg g^{-1} F.W.)	Total chlorophyll (mg g^{-1} F.W.)	Carotenoid (mg g^{-1} F.W.)
Control	1.34 ± 0.05^{a}	1.07 ± 0.05^{a}	2.44 ± 0.05^{a}	0.66 ± 0.08^{a}
6 μM As^{+3}	1.17 ± 0.05^{b}	0.81 ± 0.11^{ab}	2.12 ± 0.05^{ab}	0.58 ± 0.02^{ab}
18 μM As^{+3}	1.03 ± 0.31^{c}	0.75 ± 0.24^{abc}	1.86 ± 0.31^{b}	0.52 ± 0.01^{b}
54 μM As^{+3}	0.63 ± 0.13^{d}	0.46 ± 0.01^{cd}	1.27 ± 0.13^{c}	0.38 ± 0.01^{c}
6 μM As^{+5}	1.26 ± 0.08^{e}	0.96 ± 0.08^{ae}	2.30 ± 0.08^{ae}	0.64 ± 0.05^{ad}
18 μM As^{+5}	1.18 ± 0.07^{f}	0.88 ± 0.14^{aef}	2.12 ± 0.07^{aef}	0.54 ± 0.01^{de}
54 μM As^{+5}	0.97 ± 0.01^{g}	0.68 ± 0.02^{efg}	1.52 ± 0.01^{g}	0.45 ± 0.01^{ef}

The difference between the values indicated with different letters in each column is statistically significant at the $p < 0.05$ level.

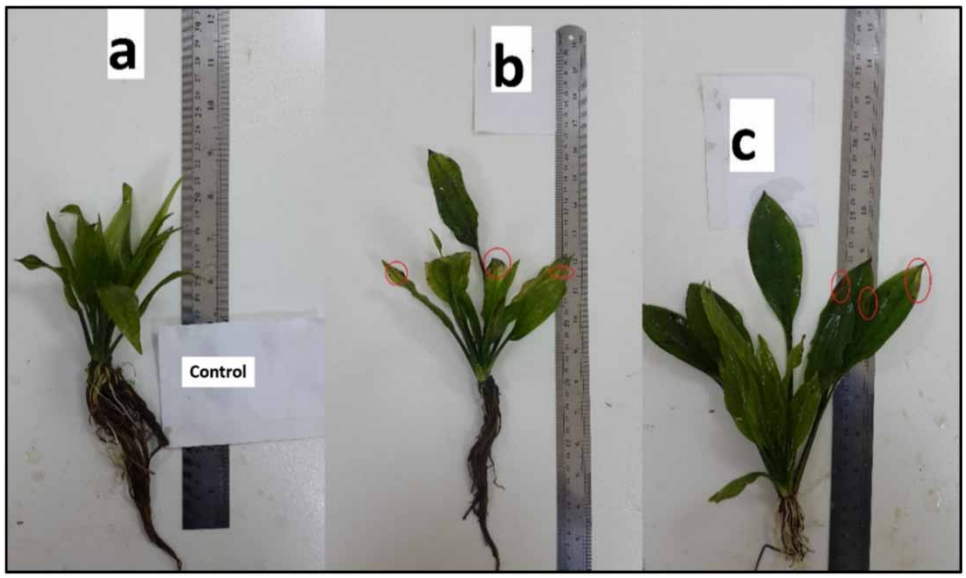


Figure 1 | Necrosis and chlorosis seen in 54 μM applied groups. (a) Control, (b) 54 μM As^{+3}, (c) 54 μM As^{+5}.

Similar to this study, Meneguelli-Souza *et al.* (2016) reported that chlorosis and necrosis were observed at the end of 20 days in *Eichhornia crassipes* plant treated with 20 mg l^{-1} of As^{+5}, and there were significant decreases in photosynthetic pigment content compared to the control (a decrease around 51.17% for chlorophyll a, 42.72% for chlorophyll b, 49.04% for total chlorophyll and 7.77% for carotenoids). However, unlike the current study, researchers observed an increase in photosynthetic pigment contents in the group treated with 0.025 mg l^{-1} of As^{+5}. This increase can be due to hormesis, known as the stimulating effect of low doses of toxic substances on photosynthetic pigment contents (Callbrese & Baldwin 2003; Duman *et al.* 2010). In the current study, decreases were observed in the photosynthetic pigments content in the groups treated with the lowest concentration (6 μM). Divergence in the results may be due to the higher concentration used in the present study (6 μM) if compared with that reported by Meneguelli-Souza *et al.* (2016) (0.025 mg l^{-1}). Similarly, Malar *et al.* (2016), reported that the photosynthetic pigment contents of *E. crassipes* plant decreased with increasing Pb concentrations and that chlorosis and drying occurred in the plant after 10 days in the group treated with the highest concentration (1,000 mg l^{-1}). ROS that increase with oxidative stress cause DNA, protein and lipid damage, as well as necrosis and chlorosis in plant leaves by affecting metabolic processes and photosynthesis (Nath *et al.* 2014; da-Silva *et al.* 2017; Roychowdhury *et al.* 2018). The elements that form the basis of the pigment structure of the plants and heavy metals are replaced, and because the light uptake is prevented due to the deterioration of the pigment structure, photosynthesis cannot be performed and this causes the death of the plant (Prasad 1998).

Effect of As^{+3} and As^{+5} ions on protein content and antioxidant enzymes

The effects of As^{+3} and As^{+5} ions on protein and antioxidant enzymes are presented in Figure 2. As a result of this study, with increasing concentrations of As^{+3} and As^{+5} ions, significant decreases were observed in the protein amounts. The most significant decreases were found to be around 65% (1.64 ± 0.02 mg g^{-1} F.W.) in the group treated with 54 μM As^{+3}, and around 34.9% (3.05 ± 0.07 mg g^{-1} F.W.) in the group treated with 54 μM As^{+5}. There were statistically significant differences ($p < 0.05$) between these observed decreases and control group. The decrease in protein amounts may be due to the oxidative stress created by As^{+3} and As^{+5} ions in the plant, resulting in increased ROS such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) causing major damage to cell membranes, DNA and proteins (Meharg & Hartley-Whitaker 2002; Tripathi *et al.* 2014). Especially in the As^{+3} group, the high amount of protein decrease may be due to the inhibition of this ion by directly binding to the sulfhydryl (–SH) groups of the proteins (Mishra *et al.* 2008; Rahman & Hasegawa 2011). Similarly, decreases in the protein contents of *Lemna minor* and *Najas indica* exposed to As^{+3} and As^{+5} ions were observed in the groups with the highest concentration, and the decreases were more severe especially in the As^{+3} group (Duman *et al.* 2010; Tripathi *et al.* 2014). The major decreases of protein content observed in the group treated with As^{+3} can be explained by faster cellular uptake, higher solubility and inhibition of proteins by binding to the –SH groups (Meharg & Hartley-Whitaker 2002; Wang & Mulligan 2006).

As a result of the antioxidant enzyme analysis, SOD enzyme activity decreased in all As^{+3} applied groups compared to the control. The most significant decrease was 64.98% (0.43 ± 0.05 units mg^{-1} protein F.W.) in the 54 μM As^{+3} groups. This decrease was found to be statistically significant when compared to the control ($p < 0.05$). The continuous decrease in SOD activity may be due to the fact that it is the first line of defense against the toxicity of ROS and the longer treatment period (Khan *et al.* 2009; Leão *et al.* 2017). An increase of 32.13% (1.61 ± 0.03 units mg^{-1} protein F.W.) of SOD activity was observed in the group treated with 6 μM As^{+5} compared to the control. Furthermore, SOD activity was almost at the

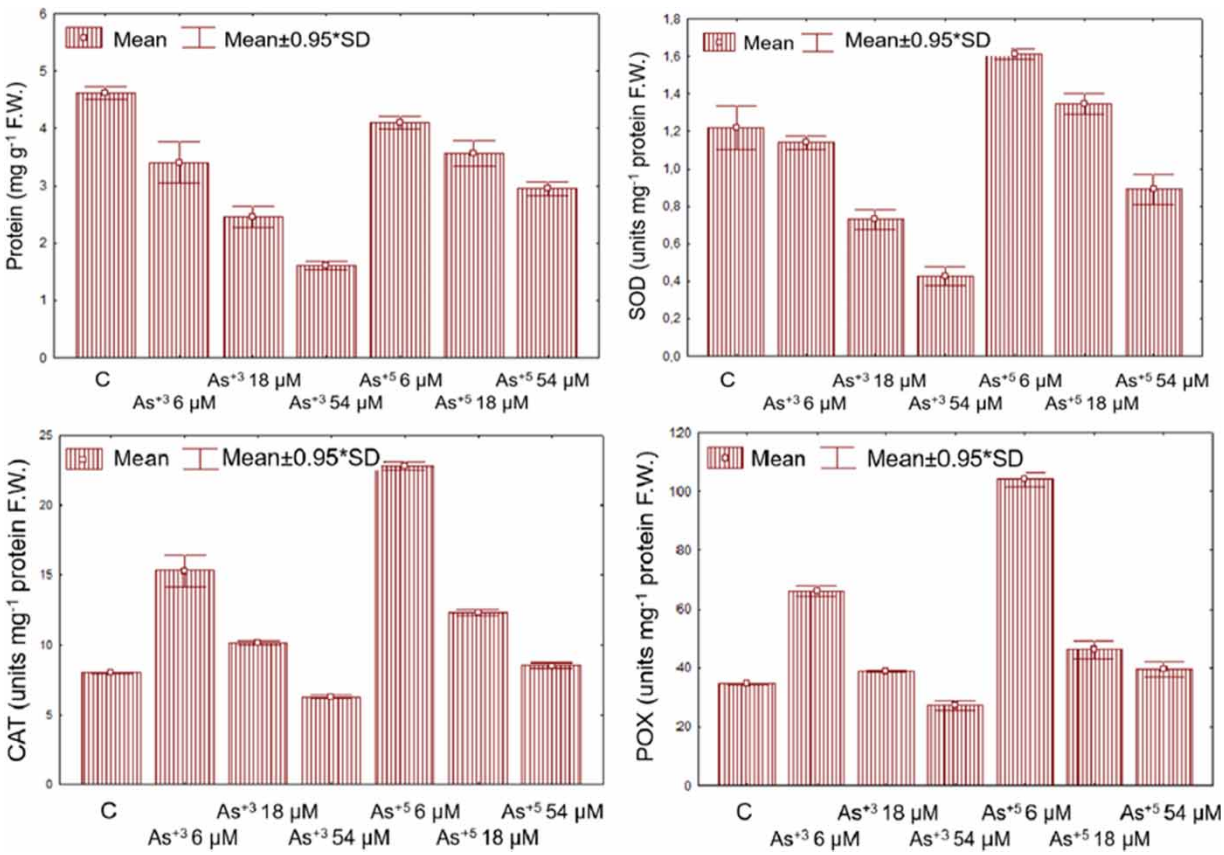


Figure 2 | Effects of As^{+3} and As^{+5} ions on protein content and antioxidant enzyme activities of *Echinodorus amazonicus* (C, control group).

same level as the control in the group treated with 18 μM. A decrease of 26.96% (0.89 ± 0.09 units mg^{-1} protein F.W.) was detected in the activity of SOD for the group treated with 54 μM As^{+5} compared to the control group ($p < 0.05$). The SOD enzyme is the first line of defense against environmental stress factors and converts the superoxide radical to hydrogen peroxide (Singh *et al.* 2018). The decreases observed in all the groups treated with As^{+3} in this study may be due to inhibition of the proteins (Srivastava *et al.* 2007). As^{+5} does not bind to protein groups. Therefore, the increase in the group treated with 6 μM As^{+5} may be due to the defense mechanism occurred under the oxidative stress conditions, and the decrease in the group treated with 54 μM As^{+5} may be due to the oxidation of proteins, damage to nucleic acids and enzyme inhibition caused by ROS, which increased due to the long treatment period (Sizova *et al.* 2002; Sharma *et al.* 2012).

For the CAT enzyme acitivity, the highest increase was around 185% (22.83 ± 0.30 units mg^{-1} protein F.W.) in the group treated with 6 μM As^{+5} and followed by the group treated with 6 μM As^{+3} with an increase of 91.3% (15.31 ± 1.22 units mg^{-1} protein F.W.). The enzyme activity decreased in other application groups, and the CAT activity in the group treated 54 μM As^{+3} was at a lower level than the control group, with a rate of 21.64% (6.27 ± 0.14 units mg^{-1} protein F.W.).

The POX enzyme activity was increased around 201.5% (104.04 ± 2.62 units mg^{-1} protein F.W.) in the group treated with 6 μM As^{+5} compared to the control group, and there was an increase of around 91.45% in the group treated with 6 μM As^{+3} compared to the control (66.28 ± 1.95 units mg^{-1} protein F.W.) ($p < 0.05$). In the groups treated with 18 and 54 μM, the enzyme activity was higher than the control, but the enzyme activity decreased compared to the 6 μM applied group. In the samples treated with 54 μM As^{+3}, a decrease of 21.29% (27.25 ± 1.78 units mg^{-1} protein F.W.) was detected in the activity POX compared to the control group.

Similarly to this study, Tripathi *et al.* (2014) reported that SOD enzyme activity increased in the first 2 days and decreased in the following days in *N. indica* aquatic plant treated with different concentrations of As^{+3} and As^{+5} for 7 days. Unlike the current study, the decrease in SOD enzyme activity in all groups treated with As^{+3} may be due to the fact that it is the first line of defense against the toxicity of ROS and the longer treatment period (Khan *et al.* 2009; Leão *et al.* 2017). Also Farnese *et al.* (2013) stated that the SOD activity of the water lettuce (*Pistia stratiotes* L.) treated with different concentrations of As^{+5} (0, 5, 10, 15 and 20 μM) increased at the group of 10 μM and decreased at higher concentrations (15 and 20 μM). The increase in CAT and POX activities observed at low concentrations can be evaluated as the plant's ability to cope with arsenic stress by keeping the increased H_2O_2 radicals under control (Praveen *et al.* 2019). While SOD catalyzes superoxide radicals to H_2O_2 and O_2, CAT and POX enzymes break down H_2O_2 radicals into harmless molecules O_2 and H_2O (Zhang *et al.* 2007; Ighodaro & Akinloye 2018). Like the current study, da-Silva *et al.* (2017) found that CAT activity increased at low concentration (0.5 mg l^{-1}) and decreased at high concentrations (1, 1.5 and 2 mg l^{-1}) in *Spirodela intermedia* aquatic macrophyte, which they treated with As^{+5} for 24 h. Duman *et al.* (2010) found that there was a 65% increase in CAT enzyme activity at the end of the 6th day in the 1 μM As^{+3} group, and 84% increase in the 16 μM As^{+5} applied group at the end of the 4th day in duckweed (*L. minor* L.). In the present study, CAT activity increased around 185% in the group treated with 6 μM As^{+5} and around 91.3% in the group treated with 6 μM As^{+3}. The increase or decrease level of antioxidant enzyme activities in plants under oxidative stress may vary according to metal and plant species, metal exposure time and concentrations (Radić *et al.* 2010). The decreases in the antioxidant enzyme activity at high concentrations are due to the fact that ROS, which increase with oxidative stress, bind to the active sites of proteins and inactivate enzyme structures (Khan *et al.* 2009; Tripathi *et al.* 2014; Leão *et al.* 2017).

Effect of As^{+3} and As^{+5} ions on lipid peroxidation

As a result of the treatment with As^{+3} and As^{+5} ions, MDA contents of *E. amazonicus* was increased significantly. The highest increase rates in MDA contents were found as 96% (27.53 ± 0.59 μmol g^{-1} F.W.) for 54 μM As^{+3} and 71.50% (24.08 ± 0.21 μmol g^{-1} F.W.) for 54 μM As^{+5}. These increases were found to be statistically significant when compared with the control and each other ($p < 0.05$). The increased MDA contents are due to the inhibition of antioxidant enzymes and thus increased free radicals causing lipid peroxidation. The increased free radicals disrupts the structure of cell membranes and released MDA, a toxic product of lipid peroxidation (Shri *et al.* 2009; Li *et al.* 2013). The amount of MDA was found to be higher in the group treated with As^{+3} compared to As^{+5} (Figure 3). The higher MDA contents in As^{+3} applied groups can be explained by the higher affinity of As^{+3}, which has a higher cellular uptake, to thiol groups and therefore directly affecting the cell membrane with inhibition of enzyme activities (Duester *et al.* 2011; Podder & Majumder 2016). Similarly, both Jung *et al.* (2019) and Yadav & Srivastava (2020), reported an increase in MDA content in rice plant (*Oryza sativa*) exposed to As^{+3}. Similar to the results of the current study, Srivastava *et al.* (2007) revealed that the MDA content of *Hydrilla verticillata* plant was

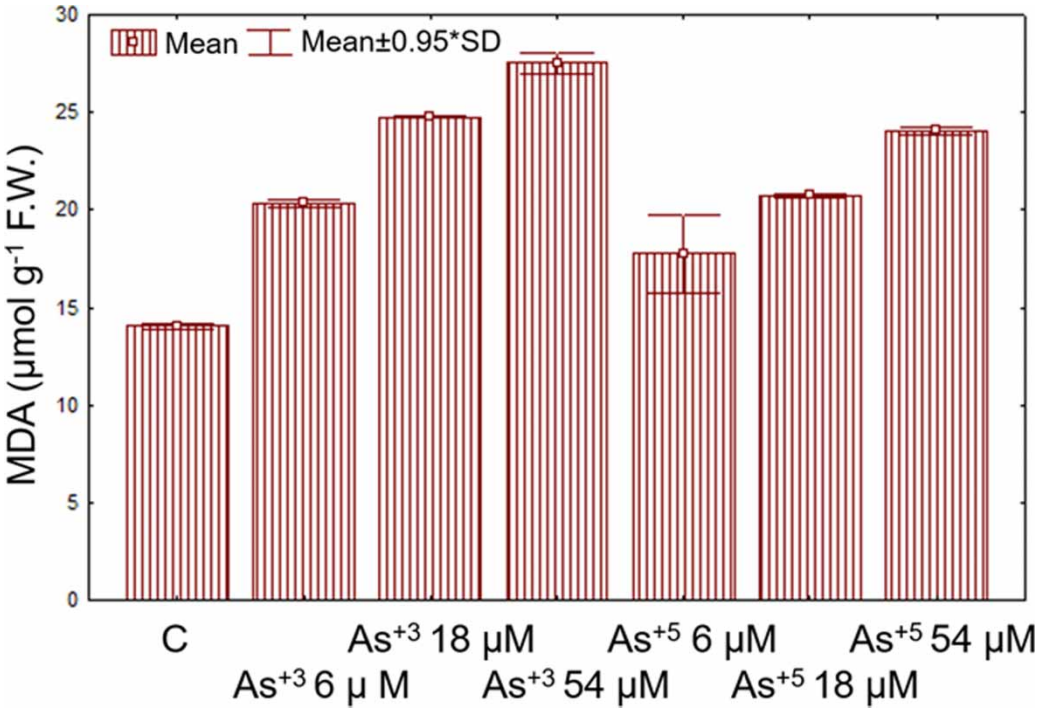


Figure 3 | MDA contents of *Echinodorus amazonicus* treated with different concentrations of As^{+3} and As^{+5} ions (C, control group).

increased by 213% in the group treated with 25 µM As^{+3} at the end of the 7th day, and 144% in the group treated with 250 µM for As^{+5} compared to the control group.

CONCLUSION

As a result of the study, it was observed that there were morphological and physiological changes at high concentrations in *E. amazonicus* aquatic macrophyte treated to different concentrations of As^{+3} and As^{+5} ions. A significant decrease in protein, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents was observed with increasing of arsenic concentrations. The antioxidant enzymes SOD, CAT and POX act as a defense mechanism of the plant against stress. The antioxidant defense system of *E. amazonicus* dealed with the oxidative stress conditions at low concentration (6 µM) of inorganic arsenic, but at high concentrations (18 and 54 µM), the defense system was not effective against increased ROS. The amount of MDA, which is an indicator of lipid peroxidation, increased in all the groups of treated with As^{+3} and As^{+5} ions. The increase in the As^{+3} group was higher than in the As^{+5} group. As^{+3} and As^{+5} ions were compared with each other and the toxicity of As^{+3} was higher. Compared to other studies, the decrease observed in SOD activities in all the groups treated with As^{+3} even at the lowest concentration (6 µM) shows that As^{+3} is more toxic, and the antioxidant defense system loses its effectiveness as the treatment time gets longer. Considering the natural environment conditions, it is suggested that plants will not survive if exposed to arsenic for a longer period of time. In conclusion, the findings of current studies contribute to the understanding of the effects of pollutants on living things in nature and the mechanisms of possible reactions to these effects, thus helping the survival of living organism in aquatic ecosystems. Since the consequences of adverse events occurring in aquatic ecosystems will cause the disruption of the natural balance, there may be danger to all the living organisms.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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