



Effects of single and binary applications of bisphenol A and NaCl on *Ceratophyllum demersum*

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ARTICLE INFO

ABSTRACT

Research Article

Received : 23/05/2020

Accepted : 01/07/2020

Keywords:

Bisphenol A

NaCl

Ceratophyllum demersum,

Physiological effects

Nutrient content

Determining the physiological effects of single and binary applications of BPA and NaCl on the *Ceratophyllum demersum* L., which is a rootless submerged aquatic macrophyte, is the aim of the present study. For this purpose, the macrophyte was collected from a local pond that was not exposed to any pollution. Macrophytes were acclimatized in 10% nutrient solution for 14 days, then they were treated with 17.2 mg/L BPA; 10 and 100 mM NaCl; 17.2 mg/L BPA plus 10 mM NaCl and 17.2 mg/L BPA plus 100 mM NaCl. The macrophyte without added BPA or/and NaCl served as control (without treatment). Increasing concentrations of NaCl induced a progressive accumulation of Na in the macrophyte tissues. On the other hand, BPA application partially reduced Na uptake. Nutrient uptake was affected differently by the applications. The contents of some nutrients such as Cu, Zn, Mn, K and Mg were generally reduced, whereas the Fe and Ca contents were increased. In general, increases in contents of total carbohydrate, total phenolic and non-protein sulfhydryl groups were found, when compared to control. Protein and photosynthetic pigment contents, on the contrary to these, were decreased. According to findings, the increase in H₂O₂ and MDA levels showed that single and combined applications of BPA and NaCl in *C. demersum* tissues induced oxidative stress.

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Introduction

The vital processes of organisms living in aquatic and terrestrial ecosystems are adversely affected by waste materials given to the environment by anthropogenic activities. Bisphenol A, among these substances, is an environmental pollutant (BPA; 2,2-bis(4-hydroxyphenyl) propane or 4,4'-isopropylidenediphenol), which was used in the production of polycarbonates, epoxy resins and other products. (Huang et al., 2012). BPA is solid at room temperature and has moderate water solubility, low vapor pressure, and low volatility. (Tsai, 2006). Significant quantities of waste containing BPA, some of which are discharged into environments, generated by manufacturing facilities (Dorn et al., 1987). There are many studies on the effects of BPA on living organisms. However, studies on plants are still insufficient. On the other hand, there are some studies on the effects of especially high BPA concentrations on the germination, growth and

development, physiological and biochemical processes of the plants (Dogan et al., 2010; Qiu et al., 2013; Sun et al., 2013). Mammal sex hormones and estrogen receptors are also present in plants. Therefore, effects of BPA in plants can be as complex as in mammals (Janeczko and Skoczowski, 2005; Janeczko et al., 2008).

Salinity significantly limits the growth and development of plants, which is one of the most important environmental (Botella et al., 2005). Many physiological and biochemical processes in plants are affected by salinity stress. Water stress, nutritional disorders, ion toxicity, genotoxicity, changes in metabolic processes and membrane structure, reduction of cell division and development are some of the processes affected by salinity stress (Hasegawa et al., 2000; Munns, 2002; Zhu, 2007). Besides, oxidative stress could be caused by salinity stress in plants due to the excessive production of reactive

oxygen species (ROS). Consequently, ROS can damage lipid, carbohydrates, membranes, DNA, and proteins (Sevindik et al., 2017; Mohammed et al., 2018; Mohammed et al., 2019). There are many processes for salinity stress responses in plants including various compatible solutes, transport of ion, and compartmentalization of toxic ion (Sairam and Tyagi, 2000).

Plants may encounter one or more stress factors in the environment and the effects of multiple stress factors on plants are reported in the literature. (Mozafar and Oertli, 1990; Dogan and Gultekin, 2017). However, there is no study on the physiological effects of single and combined applications of BPA and NaCl in aquatic macrophytes. Thus, the objective of this study was to determine the physiological effects of BPA, NaCl, and their combination on *C. demersum*, a submerged rootless aquatic macrophyte.

Materials and Methods

C. demersum was collected from the Karapinar pond (Yavuzeli-Gaziantep, Turkey). The macrophytes were acclimatized in 10% nutrient solution (Ozturk et al., 2003) (2.0 mM Ca(NO₃)₂, 0.88 mM K₂SO₄, 0.25 mM KH₂PO₄, 0.1 mM KCl, 1 mM MgSO₄, 1 μM H₃BO₃, 0.5 μM MnSO₄, 0.2 μM CuSO₄, 1 μM ZnSO₄, 0.02 μM (NH₄)₆Mo₇O₂₄ and 00 μM Fe-EDTA), at a climate chamber (Snijders Scientific, Netherlands) (light/dark regimes of 16/8 h, light level 120 μE.m⁻².s⁻², temperature 24±1 °C) for 14 days. 6.8-7.1 g of healthy macrophyte was placed in 500 ml glass vessels. Each application was carried out in three replicates. *C. demersum* was treated with 17.2 mg/L BPA, 10 mM NaCl, 100 mM NaCl, 10 mM NaCl plus 17.2 mg/L BPA and 100 mM NaCl plus 17.2 mg/L BPA as supplied with 10% nutrient solution. The macrophyte without added BPA or/and NaCl served as control. The test media were changed after 2 days, and the treatments were replenished. Macrophytes were harvested after 5 days, because of observation of toxicity symptoms such as detachment of some leaves and chlorosis.

Fresh macrophytes were homogenized in 80% acetone to determine the photosynthetic pigment contents. After the supernatants were separated, the samples were read in a spectrophotometer (CINTRA 202, Australia) at 662, 645 and 450 nm. Chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and carotenoid contents were calculated according to Lichtenthaler and Wellburn (1985). To determine the total soluble carbohydrate content of the macrophyte, anthrone method was used (Plummer, 1998). The carbohydrate contents were calculated using the standard glucose curve. The content of total phenolics in macrophyte tissues was determined by Folin-Ciocalteu reagent according to the method of Ratkevicius et al. (2003). Gallic acid was used as standard. Lipid peroxidation level was determined by determining the amount of malondialdehyde (MDA) using the method proposed by Zhou (2001). The Lowry method was used to determine protein content (Lowry et al., 1951). The content was determined using the calibration curve of BSA (bovine serum albumin). H₂O₂ content of the macrophyte tissues was determined according to Sergiev et al. (1997).

To determine Na, Ca, K, Mg, Fe, Zn, Mn and Cu contents, the macrophyte samples were dried in an oven at 80 °C until constant weight. The samples were mineralized in 14 M HNO₃ and residues were dissolved in 1 M HCl. After mineralization, element content of samples was determined using an atomic absorption spectrometer (Perkin Elmer AA400).

Statistical analysis of the data was performed using SPSS 11.0. The differences between the mean values were determined using the LSD (Least Significant Difference) test.

Results and Discussion

Sodium contents of *C. demersum* tissues were increased with increasing NaCl concentrations (Figure 1.A). The content at 10 and 100 mM NaCl concentrations was increased by 3.4 and 5.5 fold, respectively, when compared to the control. Similar findings were also obtained in previous studies in aquatic plants (Rout and Shaw, 2001; Jampeetong and Brix, 2009). However, these increases in 10 mM NaCl plus 17.2 mg/L BPA and 17.2 mg/L BPA plus 100 mM NaCl combinations were 3.0 and 5.1 fold, respectively. That is, BPA application was partially found to reduce the uptake of Na.

Macro and micro nutrients are required for growth, development and biosynthetic processes in plants. Stress factors can affect the absorption and transport of these elements (Dogan and Gultekin, 2017; Xia et al., 2017). Macro and micro nutrient uptake of the macrophyte was influenced differently by the applications (Figure 1B, Figure H). Contents of Mg ve K were decreased by the treatments. The maximum reduction was found for Mg in 17.2 mg/L BPA concentration as 29.91% (P<0.05) and for K in 100 mM NaCl plus 17.2 mg/L BPA as 12.1% (P>0.05), when compared to the their controls. In contrast, macro nutrient Ca concentrations were increased by applications, except for 17.2 mg/L BPA plus 100 mM NaCl. The highest increase was found in 100 mM NaCl concentration as 52.1% (P<0.05). Besides that Ca concentrations were higher in individual NaCl concentrations than BPA plus NaCl combinations. Although contents of Mn and Fe were increased by the applications, Cu and Zn contents were decreased, except for Mn and Cu at 17.2 mg/BPA. According to these, Mn and Fe contents were increased up to 100.5% (P<0.05) and 155.7% (P<0.05), respectively, at 17.2 mg/L BPA plus 100 mM NaCl and 17.2 mg/L BPA plus 10 mM NaCl combinations. In addition, Cu and Zn contents were decreased in 48.4% and 60.9% BPA plus 100 mM NaCl. However, Cu and Zn contents were decreased up to 48.4% (P<0.05) and 60.9% (P<0.05), respectively, at 17.2 mg/L BPA plus 100 mM NaCl combination. According to our findings, BPA, NaCl and BPA plus NaCl applications affected absorption of the elements in a different way. Accordingly, Fe, Ca and Cu contents were increased by 17.2 mg/L BPA, while Mg, Zn, K and Mn contents were decreased. Ferrara et al. (2006) stated that BPA could selectively inhibit the uptake of some nutrients (e.g., K, Mg and Mn) and promote uptake of Cu and Ca, as determined in our study. 10 and 100 mM NaCl applications differentially affected the mineral contents of *C. demersum*.

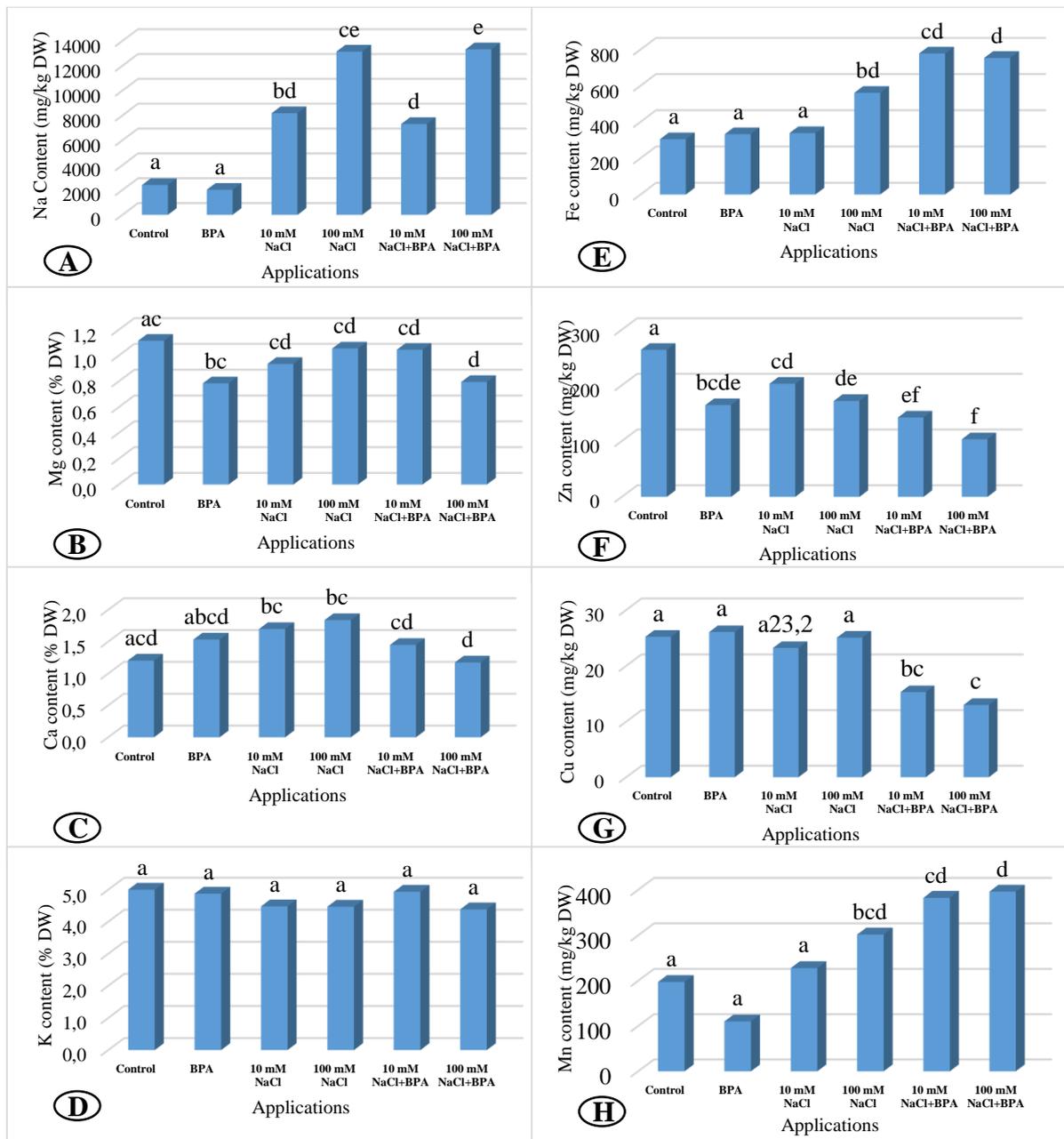


Figure 1. Na and nutrient contents of *C. demersum* after the applications.

Control had only 10% nutrient solution. Bars indicate mean of three replicates. Bars with different letters are significantly different ($P < 0.05$) based on the oneway ANOVA, separated by LSD test.

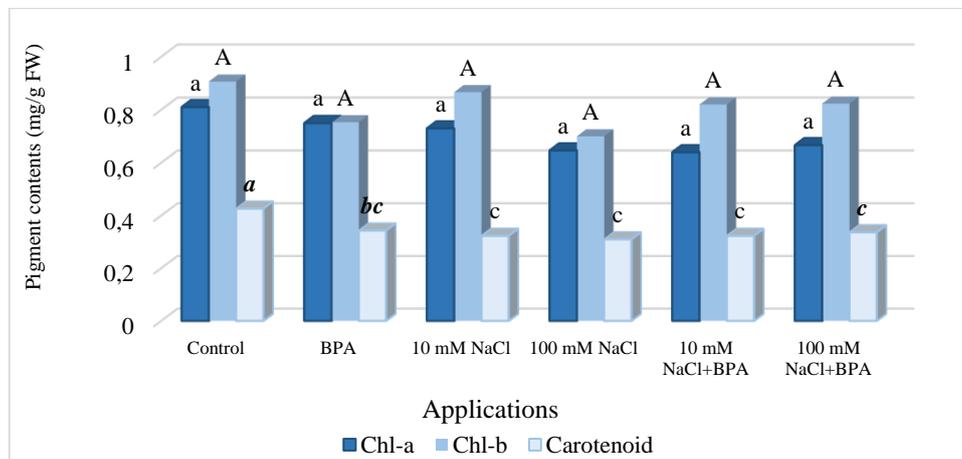


Figure 2. Photosynthetic pigment contents of *C. demersum* tissues after the applications.

Control had only 10% nutrient solution. Bars indicate mean of three replicates. Bars with different letters are significantly different ($P < 0.05$) based on the oneway ANOVA, separated by LSD test.

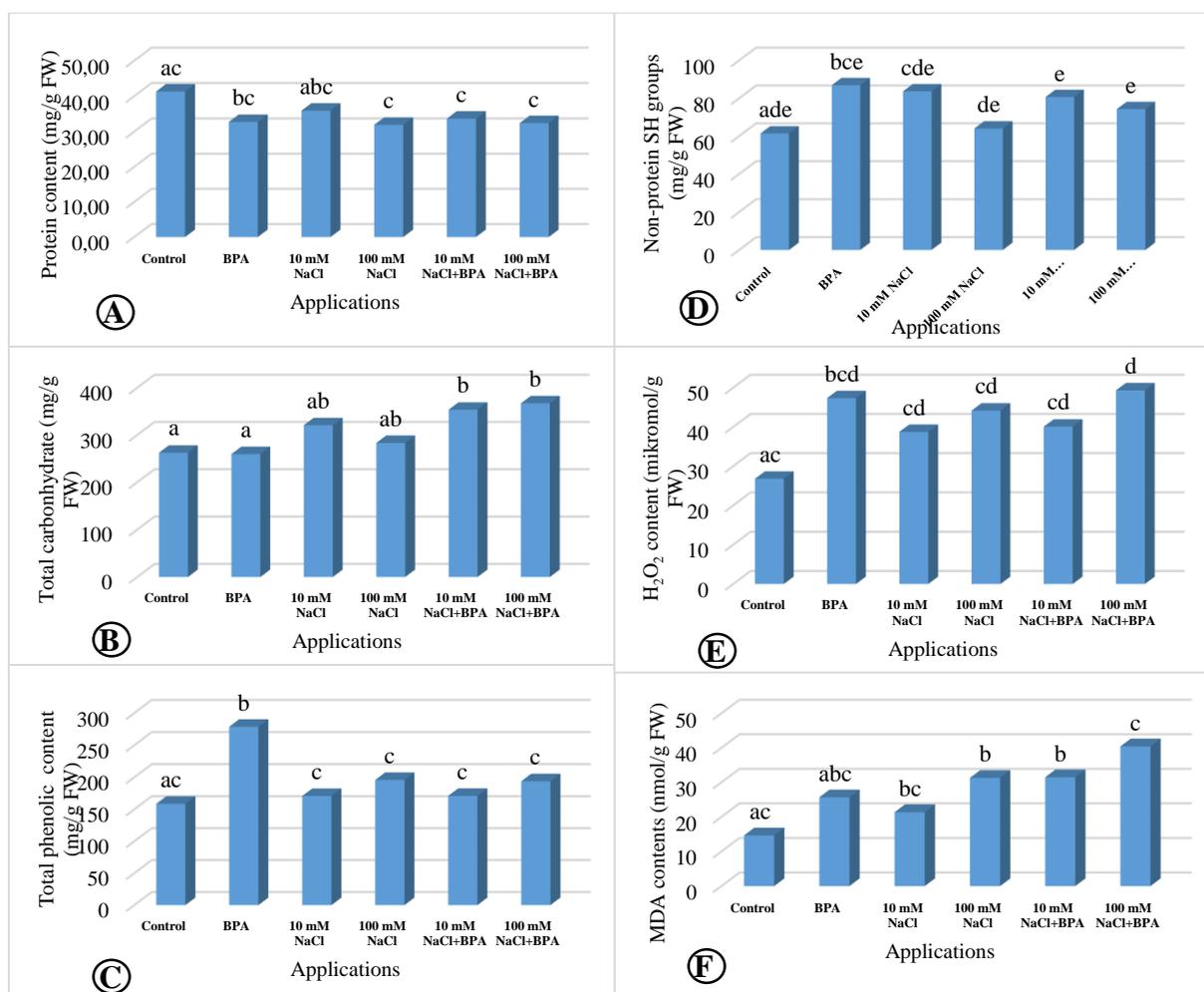


Figure 3. Some biochemical changes of *C. demersum* tissues after the applications.

Control had only 10% nutrient solution. Bars indicate mean of three replicates. Bars with different letters are significantly different ($P < 0.05$) based on the oneway ANOVA, separated by LSD test

The contents of Fe, Ca and Mn increased in individual NaCl applications, but Mg, Zn, Cu and K contents were decreased. The results obtained from NaCl applications may probably due to the competition of Na^+ and Cl^- with macro and micronutrients. In addition, BPA plus NaCl combinations increased Mn and Fe uptake compared to individual BPA and NaCl applications, but Cu and Zn further decreased. As a result, BPA, NaCl and BPA plus NaCl applications caused nutrient imbalances in the macrophyte.

Chl-a, Chl-b and carotenoid contents of the macrophyte have been reduced by treatments (Figure 2). Chl-a contents were found to be decreased by 7.41%, 9.88%, 20.16%, 20.99% and 90.48%, respectively, under the influence of 17.2 mg/L BPA, 10 mM NaCl, 100 mM NaCl, 10 mM NaCl plus 17.2 mg/L BPA and 100 mM NaCl plus 17.2 mg/L BPA. The maximum reductions in Chl-b and carotenoid contents were calculated as 23.08% and 27.99% in 100 mM NaCl concentration, respectively. The minimum decreases were 4.76 and 20.16% in 10 mM NaCl and 17.2 mg/L BPA, respectively, with respect to their controls. One of the most important processes affected by salt stress in plants is photosynthesis (Stepien and Klobus, 2006). Many studies have reported that NaCl salinity causes photosynthetic pigment loss in aquatic macrophyte (Rout et al., 1998; Cheng 2011). The decrease in the pigment contents in NaCl concentrations can possibly be

due to changes in the lipid protein ratio of pigment-protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996). On the other hand, Qiu et al. (2013) inferred that reduction of Chl in BPA application may be due to peroxidation of chloroplast membrane lipid, as obtained our study.

The protein content of the macrophyte tissues was reduced by the applications (Figure 3A). The maximum reduction was found in 100 mM NaCl concentration as 22.7% ($P < 0.05$), when compared to the control, whereas the minimum decrease was found in 10 mM NaCl concentration as 13.1% ($P > 0.05$). As previously reported, BPA and NaCl cause oxidative stress

(Chitra et al., 2003; Khan and Panda, 2008; Dogan et al., 2010). Therefore, the stress may be triggered by the destruction of proteins in *C. demersum* tissues. In addition, NaCl-induced ionic toxicity and/or nutrient disturbances may also be a cause of a reduction in protein content. In contrast, total carbohydrate contents were increased except for 17.2 mg/L BPA (Figure 3B). This reduction in 17.2 mg/L BPA was statistically insignificant as 1.13% ($P < 0.05$). The highest increase was calculated as 39.81% ($P < 0.05$) in 17.2 mg/L BPA plus 100 mM NaCl, when compared to the control. In addition, BPA plus NaCl combinations increased total carbohydrate content compared to individual NaCl applications. Under salinity stress, the accumulation of compatible solutes as well as

carbohydrates allow plants to maintain their cellular turgor pressure; such compounds also act as osmoprotectants (Ruiz-Carrasco et al., 2011). Increased carbohydrate content in *C. demersum* under NaCl stress may be due to these reasons.

Total phenolic content of the macrophyte tissues was increased by applications (Figure 3C). The highest increase was calculated in 17.2 mg/L BPA concentration as 75.6% ($P < 0.05$). Besides, total phenolic contents were higher in BPA plus NaCl combinations than individual NaCl concentrations. There may be increases in the level of phenolic compounds during stress. On the other hand, the phenolics can directly scavenge ROS caused by oxidative stress (Michalak, 2006). According to our findings, the fact that the applications caused oxidative stress also explains this situation. Similarly, the highest increase in non-protein sulfhydryl groups was calculated as 41.6% in 17.2 mg/L BPA concentration ($P < 0.05$) (Figure 3D). In the other applications, increases in the amounts of the non-protein sulfhydryl groups were also found. As phenolic compounds, increase in the thiol levels of the macrophyte tissues may be another defense mechanism against oxidative stress induced by NaCl and BPA.

As previously mentioned, BPA and NaCl may induce reactive oxygen species (ROS). H_2O_2 and MDA contents were analyzed in order to determine whether the applications were causing oxidative stress (Figures 3E and 3F). Accordingly, H_2O_2 contents were found to be 1.77, 1.45, 1.65, 1.50 and 1.84 times higher at 17.2 mg/L BPA, 10 mM NaCl, 100 mM NaCl, 17.2 mg/L BPA + 10 mM NaCl and 17.2 mg/L + BPA 100 mM NaCl, respectively, when compared to control ($P < 0.05$). In addition, MDA content was found to be up to 2.76 times increased at 100 mM NaCl. Besides that MDA contents were higher in BPA plus NaCl combinations than individual NaCl concentrations. According to the findings, the applications clearly showed that oxidative stress increased level of lipid peroxidation.

Conclusions

The effects of individual stress factors were shown by many studies. The interactions between stressors have not been account although many stressors co-exist in the environment. Some physiological and biochemical effects of BPA, NaCl, and BPA plus NaCl in *Ceratophyllum demersum* grown under controlled conditions were determined. Increasing concentrations of NaCl induced a progressive accumulation of Na in the macrophyte tissues. Conversely, NaCl and BPA concentrations distributed nutrient balance and the BPA application partially reduced Na uptake. Besides, NaCl applications caused specific ion toxicity. Some contents such as photosynthetic pigments and protein were adversely affected. There was an increase in total carbohydrate contents, except for 17.2 mg/L BPA concentration. Also, the contents of non-protein sulfhydryl groups and total phenolics were increased by the applications. It is assumed that the increases in the H_2O_2 and MDA in the macrophyte tissues are the results of provoked oxidative stress. Further investigations may be useful to reveal the details of the mechanisms of BPA, NaCl, and BPA plus NaCl in the aquatic plants.

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