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ORIGINAL ARTICLE

Impacts of Different Water Pollution Sources on Antioxidant Defense Ability in Three Aquatic Macrophytes in Assiut Province, Egypt

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The present study was undertaken to evaluate the impacts of surface water pollution with wastes coming from sewage effluents (Site 2), agricultural runoff (Site 4) and oils and detergents factory (Site 3) on the stability of leaf membrane (measured as injury %), hydrogen peroxide (H₂O₂), ascorbic acid (Asc A), lipid peroxidation, chlorophyll (Chl) content, soluble sugars (SS), soluble proteins (SP) and total free amino acids (TAA) of *Cyperus alopecuroides*, *Persicaria salicifolia* and *Echinochloa stagnina*. Concentration of H₂O₂, MDA and TAA were higher in the three plants collected from polluted sites as compared with those of plants grown in control Nile site (Site1). The opposite was true for Asc A, SS and SP where their concentrations reduced significantly in response to water pollution. Leaf membrane was more damaged (high injury %) in plants exposed to wastes from different sources than in plants growing at control site. The results of this study indicated that water pollution reduced the oxidative defense abilities in the three plants through reduction of Asc A activities, enhancement of H₂O₂ production and increasing MDA accumulation. In addition it impaired the metabolic activity through lowering the SS and SP contents and enhancement of TAA accumulation and increase membrane injury. The over production of hydrogen peroxide by the studied aquatic plants under water pollution could be used as an oxygen source needed to oxidize the more resistant organic and inorganic pollutants and used for pollution control and municipal and industrial wastewater treatment.

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Key words: Antioxidant, Industrial wastes, Hydrophytes, Membrane stability, Sewage.

Abbreviation: Asc A = ascorbic acid, Chl = chlorophyll, H_2O_2 = hydrogen peroxide, LP = lipid peroxidation, MDA = malonydialdehyde, SS = soluble sugars, PEG = polyethylene glycol, ROS = reactive oxygen species, SP = soluble proteins, TAA = total free amino acids.

Rapid industrialization and addition of toxic substances to the environment are responsible for altering the ecosystems (Oketola *and* Osibanjo, 2007). In various periods of the world history, scarcity and pollution of natural resources made societies aware of the fact that the economy depends on energy and ecological services provided by these resources (Ward, 2006 *and* Gürlük, 2009).

Water is undoubtedly the most precious natural source that exists on our planet. The lack of clean water has always been an issue of environmental concern of all over the world. The main sources of water pollution are industrial, municipal and agricultural. Pollutants, of all degrees of degradability, when discharge into aquatic environment accumulates primarily in water and sediment and in time translocates in various portions on a much wider scale.

Under the influence of water pollution, the plants exhibit changes in parameters related to photosynthesis, respiration, enzyme activities, syntheses of lipids, proteins and other metabolites (Steinbacchová-Vojtisková *et al.*, 2006; Warriar *and* Saroja, 2008). Many plants are very sensitive to water pollution, and pollutants can damage their leaves, impair plant growth, and limit primary productivity. Visible injuries, significant decrease in growth and loss in vitality can be observed in plants when exposed to toxicant environment for either a long or a short period of time depending on the concentration of toxicants (Karatas *et al.*, 2009).

It is well documented that various abiotic stresses lead to the overproduction of reactive oxygen species

(ROS) in plants which are highly reactive and toxic and ultimately results in oxidative stress. ROS or free radicals are generated extra or intra-cellularly, which can exert toxic effects to the cell. These species may affect cell membrane properties and caused oxidative damage to lipids and proteins (Gill *and* Tuteja, 2010). Among ROS species, H₂O₂ is also an important signaling molecule (Wood *et al.*, 2003), which is produced by the chloroplast and involved in the response of the plant to different types of environmental stressors (Perez-Ruiz *et al.*, 2006). The major water canals within Assiut area of Egypt suffer from uncontrolled disposal of increased amounts of wastes generated by various human and industrial activities.

Since plants provide not only a cheap but also an easy way to monitor the different sources of environmental pollution, their different parts were used as bioindicators of these contaminations. Aquatic macrophytes are considered useful tools in long-term studies to follow pollutants variation in some environment (Dantas *and* Locligiani, 2003; Calheiros *et al.*, 2007; Maddisn *et al.*, 2009). The information available on the effects of water pollution on oxidative parameters in hydrophytes were very scarce or negligible. The majority of studies focus on the capacity of hydrophytes to remove and accumulate metals and ions from polluted water. Accordingly, in our study, we wanted to provide information on oxidative defense ability of three aquatic macrophytes exposed to different liquid wastes coming from oils and detergents factory, sewage effluents and agricultural runoff. A further aspect considered in this

paper was the role of these wastes had on the stability of leaf membrane, chlorophyll content and some soluble carbon and nitrogen fraction found in plants.

MATERIALS AND METHODS

Plants and water sampling

Three emergent macrophytes included; *Persicaria salicifolia* (Brouss Ex Willd) Assenov, *Echionochloa stagnina* (Retz.0 p. Beauv.) and *Cyperus alopecuroides* Rottb were collected from four sites, one at the River Nile (site 1 unpolluted, control), two at irrigation canals (Sites 2 and 3) and one at drainage canal (Site 4). Surface water at three sites was suffered from wastes coming from sewage effluents (Site 2), oils and detergents factory (Site 3) and agricultural runoff (Site 4). Plant samples were collected in triplicates and were rinsed with tap and distilled water and subdivided into shoots and roots. The plant organs were dried in an aerated oven at 70° C to constant mass.

Water samples were brought to the laboratory, filtrated through a Whatman 3-mm filter paper, and stored at 4° C before assaying. Each water sample was assayed for pH, electric conductivity, and concentration of a number of soluble cations and anions. Sampling was done in December 2012.

Plant analysis

Membrane stability index

The stability of leaf membranes was assessed by determining electrolyte leakage from leaf segments exposed to dehydration (40% PEG) and heat (51 °C) stresses (Blum and Ebercon, 1981). The degree of membrane injury (based on electrolyte leakage) was calculated according to the following formula:

$$\% \text{ injury} = 1 - \left[\frac{1 - (T1 / T2)}{1 - (C1 - C2)} \right] \times 100$$

where T1 and T2 represent the first and second conductance measurements on the treatment sample and C1 and C2 the first and second measurements on the control.

Chlorophyll determination

Chlorophyll a and b contents were measured spectrophotometrically (Wellburn, 1994).

Determination of the malonydialdehyde (MDA)

The level of lipid peroxidation in plant tissues was measured by determination of MDA (Madhava Rao and Sresty, 2000). MDA content was determined with thiobarbituric acid (TBA) reaction.

Determination of hydrogen peroxide and ascorbic acid contents

Hydrogen peroxide and ascorbic acid concentrations in the leaves were assayed according the method of Velikova *et al.* (2000) and Jagota and Dani (1982), respectively.

Determination of Soluble carbon and nitrogen metabolites contents

In hot water plant extract the contents of soluble sugar, soluble proteins and total free amino acids in shoot and root systems were measured according to Buysse and Merckx (1993), Lowry *et al.* (1951) and Lee and Takahshi (1956), respectively.

Water analysis

The pH values of water samples were determined by pH meter (model pH-206 Lutron). The specific electrical conductance in water samples was measured by means of conductivity meter (model 4310 JEN WAY).

The contents of Ca^{2+} and Mg^{2+} were determined volumetrically by EDTA titration method (Schwarzenbach and Biederman, 1948). Na^+ and K^+ were analyzed by using flame photometer type M7P. Chlorides were determined by the silver nitrate titration methods described by Jackson (1973).

The significance of differences between the plants in response to the presence of pollution sources were determined by analysis of variance (F value). Dunnett test was used for comparison between means to evaluate the effects of different pollution sources on the parameters tested, using SPSS for Microsoft Windows (Ver. 10.0, SPSS Inc., USA).

RESULTS

Hydrogen peroxide (H_2O_2)

Data of Fig. 1A indicate that water pollution with wastes coming from sewage effluent, agricultural runoff and oils and detergents factory caused an increase in H_2O_2 concentration in shoots and roots of the studied plants. Industrial waste water (Site 3) was more effective in H_2O_2 elevation than the other pollution sources. The effect of wastes was more pronounced in roots than in shoots. For example, concentration of H_2O_2 in roots of *P salicifolia* collected from Site 3 was about 5-fold that of plants collected from control site (Site 1).

Malonydialdehyde (MDA)

Lipid peroxidation (Fig. 1C), measured as malonydialdehyde, increased significantly in shoots and roots of the studied plants growing at polluted sites in comparing with their analogous collected from the control ones (Site 1). Maximum increase (about 5-fold) was recorded in roots of *P salicifolia* grown in

site exposed to oils and detergents factory waste water.

Ascorbic acid

Concentration of ascorbic acid (Fig. 1B) in shoots and roots of the three studied plants showed opposite response trend to that of H_2O_2 and MDA. Regardless to pollution sources, surface water pollution resulted in a significant decrease in the concentration of Asc A except at site 3 (roots) and site 4 (shoot) in *C alopecuroides* where the concentration showed slight increase in response to surface water pollution with industrial and agricultural wastes.

Stability of leaf membrane

Exposure of leaf discs excised from control plants (Fig. 2A) to heat stress (51°C) caused 31.05, 55.27 and 56.47 % injury in leaf membrane of *C alopecuroides*, *P salicifolia* and *E stagnina*, respectively. Water pollution with the different wastes significantly increase membrane injury in the three studied plants except in *P salicifolia* exposed to agricultural wastes.

Dehydration stress (40% PEG) caused 33.27 (*P salicifolia*), 9.53 (*C alopecuroides*) and 32.47 % (*E stagnina*) injury. Sewage waste water reduced membrane injury in *P salicifolia* and *E stagnina* but it increased the injury percent in leaf membrane of *C alopecuroides*. Water pollution with wastes coming from agricultural drainage increased the membrane injury in the three studied plants. The same was true for oils and detergents factory waste water for *E stagnina* plants.

Chlorophyll content

The response of Chl a and Chl b contents to water

pollution (Fig. 2B) varied according to pollution source differences as well as plant species. The three waste water caused significant decrease in Chl a and b content in *P salicifolia*. On the contrary, contents of Chl a and b were higher in *C alopecuroides* and *E stagnina* plants grown in polluted sites than those in plants collected from control site (Chl b in *E stagnina* at S2 was an exception).

Soluble sugars

Changes in soluble sugars contents (Fig. 3A) showed irregular response to water pollution according to plant and pollution source differences. In general, significant differences ($P < 0.05$) in SS of shoots and roots of the studied plants were recorded between plants growing in control (Site 1) and polluted (Site 2, Site 3, Site 4) sites. The three waste water caused significant increases in SS contents of *C alopecuroides* shoots and roots. Contrarily, the same pollution sources reduced the contents of SS in *E stagnina* and *P salicifolia* shoots and roots.

Soluble proteins

In general soluble proteins (Fig. 3B) were higher in shoots and roots of *C alopecuroides* collected from polluted sites than in those of control site. On the other hand SP were often lower in *P salicifolia* and *E stagnina* plants growing at polluted sites in comparing with those of control site. Both changes (enhancing or reduction) in SP contents were statistically significant.

Total free amino acids

Total free amino acids (Fig. 3C) showed reversed trend in their response to water pollution to that noticed for soluble proteins. *P salicifolia* and *E stagnina* plants growing in polluted sites had higher

TAA in their shoots and roots than in plants collected from control site. The reverse held true in *C alopecuroides* where the three waste water caused a considerable reduction in the contents of TAA of shoots and roots (roots at Site 2 was an exception). The increase or decrease in TAA was significant ($P < 0.05$).

Water analysis

Water samples collected from the study sites were analyzed for their physico-chemical properties (Tab. 1). The pH values were fluctuated in neutral and slight alkaline range depending on type of water body (Nile, irrigation and drainage canals). The lowest pH value (7.17) was recorded at site 2 and the highest value (8.88) was at site 3 polluted with wastes of oil and detergents factory.

Conductivity yields a measure of a water capacity to convey an electric current. It is clear that EC of irrigation and drainage canals was generally higher than those recorded for water sampled from the Nile. The EC values ranged between 107 mScm^{-1} at site 1 and 1032 mScm^{-1} at site 4.

Generally Na^+ contents were higher than those of K^+ in water samples collected from different water bodies. The highest value of Na^+ (49.03 mg L^{-1}) was noticed at site 2 of irrigation canals and the lowest (27.67 mg L^{-1}) was at site 3. The maximum value of K^+ (0.34 mg L^{-1}) was recorded at site 1 and the minimum one (0.13 mg L^{-1}) was at site 3. Calcium fluctuated between 13.33 mg L^{-1} at site 3 and 30.67 mg L^{-1} at site 1. Chlorides contents of different water bodies decreased in the order of drainage canals > irrigation canals > River Nile. Their concentration

varied from 0.11 mg L⁻¹ at site 1 to 0.26 mg L⁻¹ at site

properties, as indicated by analysis of variance (F

4. Generally, changes in the above mentioned water

value), were statistically significant.

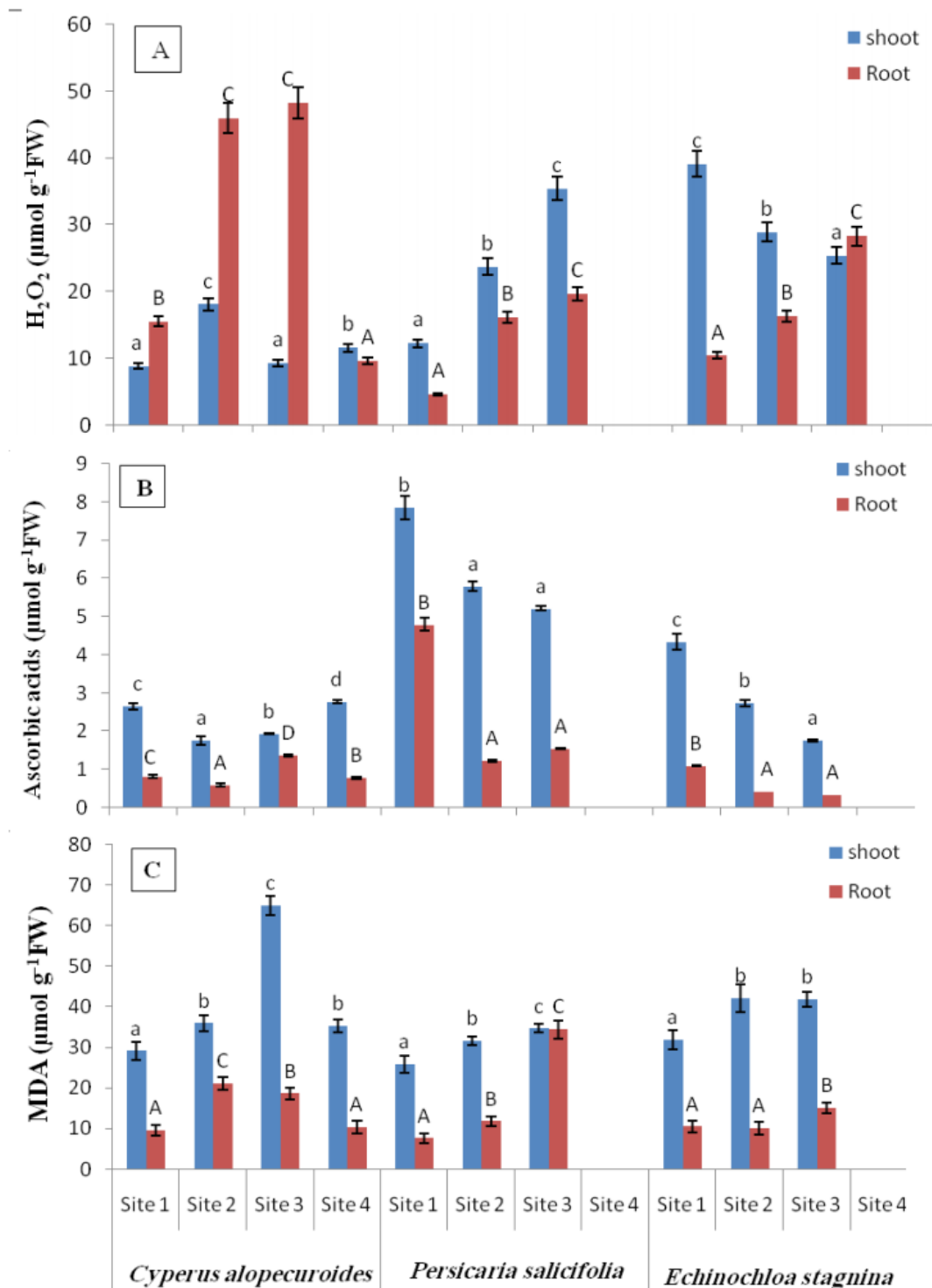


Figure 1. Changes in (A) Hydrogen peroxide, (B) ascorbic acid (Asc A), (C) malonyldialdehyde (MDA) contents (µmol g⁻¹FW) of shoots and roots of *Cyperus alopecuroides*, *Persicaria salicifolia*, *Echinochloa stagnina* plants sampled from different polluted sites. Vertical bars represent standard deviation. Different letters denote significant differences between different treatments at p ≤ 0.05 (DMRT). Site1: Control Nile site, Site 2: Sewage effluents, Site 3: Oils and detergents factory, Site 4: Agricultural runoff.

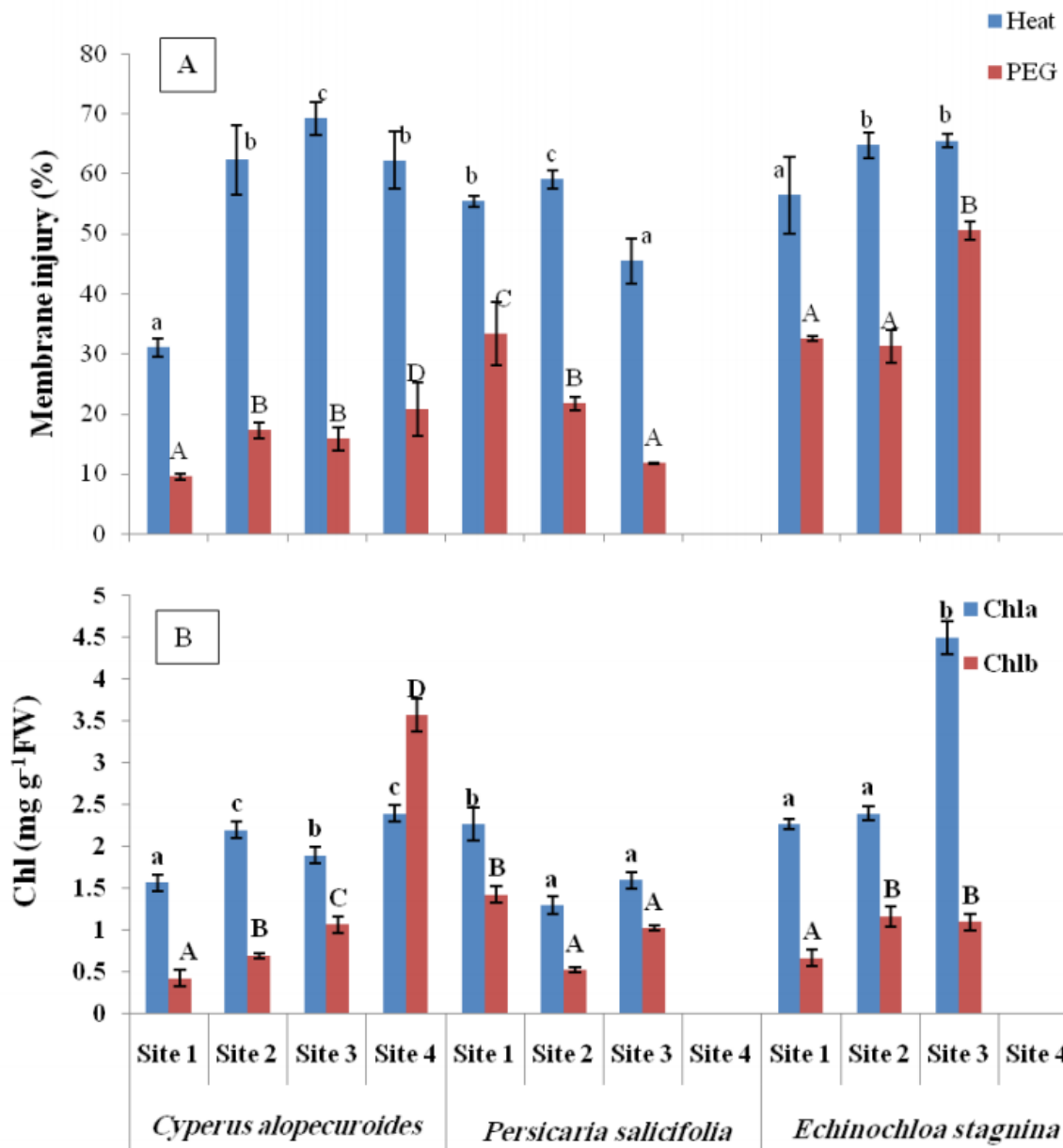


Figure 2. Changes in (A) the stability of leaf membranes to both heat stress (51°C) and dehydration (40% PEG) measured as % of membrane injury and (B) chlorophyll (Chl a, Chl b) contents (mg g⁻¹ FW of leaves) of *Cyperus alopecuroides*, *Persicaria salicifolia*, *Echinochloa stagnina* plants sampled from different polluted sites. Vertical bars represent standard deviation. Different letters denote significant differences between different treatments at $p \leq 0.05$ (DMRT). Site1: Control Nile site, Site 2: Sewage effluents, Site 3: Oils and detergents factory, Site 4: Agricultural runoff.

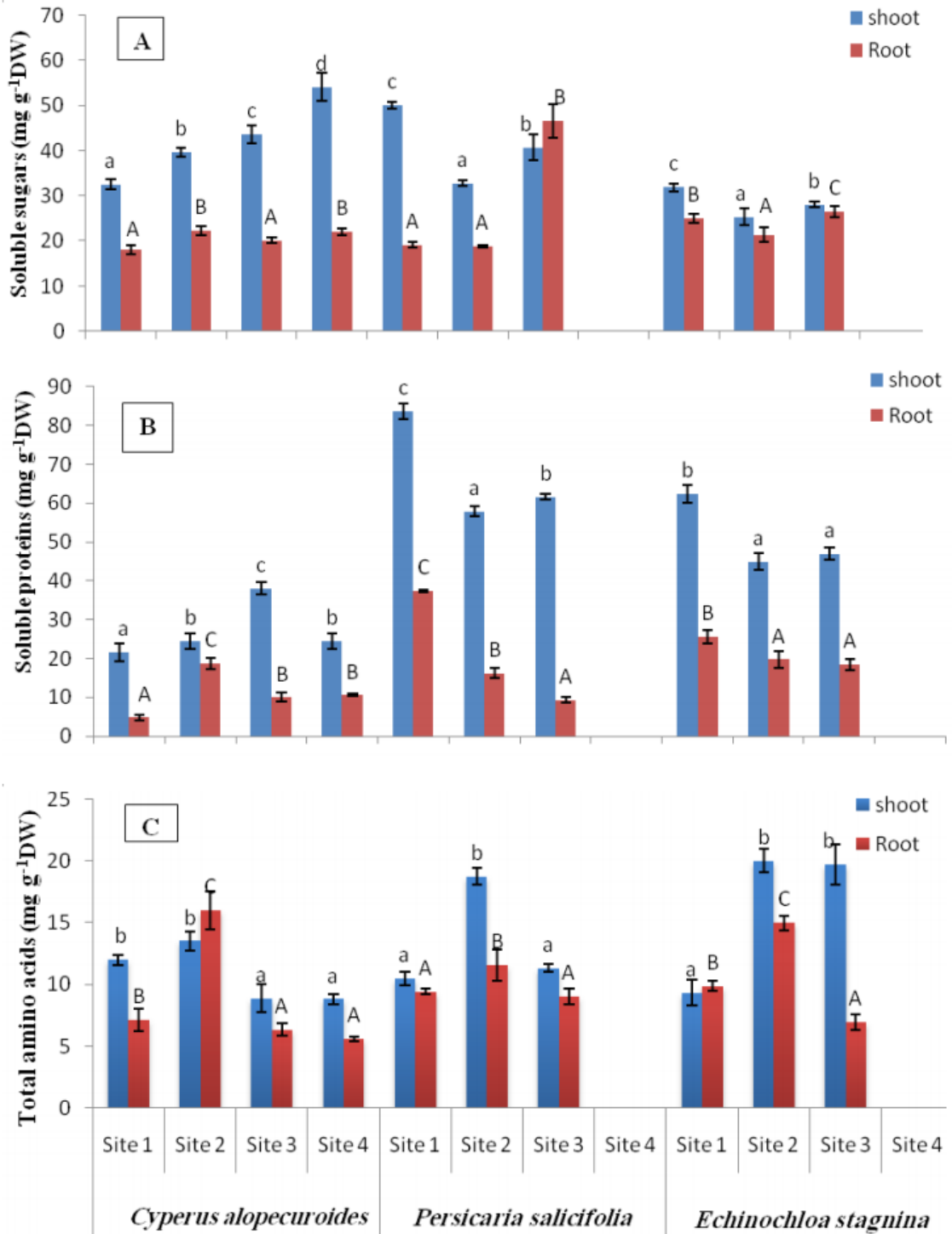


Figure 3. Changes in (A) soluble sugar (SS), (B) soluble protein (SP) and (C) total free amino acid (TAA) contents (mg g⁻¹ DW) of shoots and roots of *Cyperus alopecuroides*, *Persicaria salicifolia*, *Echinochloa stagnina* plants sampled from different polluted sites. Vertical bars represent standard deviation. Different letters denote significant differences between different treatments at p ≤ 0.05 (DMRT). Site1: Control Nile site, Site 2: Sewage effluents, Site 3: Oils and detergents factory, Site 4: Agricultural runoff.

Table 1 Physico-chemical properties and their F-values for water samples from different polluted sites. Data are means of three replicates (\pm standard error).

Parameter	Site 1	Site 2	Site 3	Site 4	F-values
PH	7.52 \pm 0.265	7.17 \pm 0.395	8.88 \pm 0.099	7.22 \pm 0.087	1.80 ^{ns}
E.C [mScm ⁻¹]	107.3 \pm 31.5	836.6 \pm 26.36	459.3 \pm 8.45	1032 \pm 53.56	72.54 ^{**}
Cl ⁻	0.11 \pm 0.01	0.18 \pm 0.01	0.143 \pm 0.02	0.26 \pm 0.01	24.87 ^{**}
Ca ²⁺	30.67 \pm 2.33	18.33 \pm 0.88	13.33 \pm 0.33	20.67 \pm 1.86	21.71 ^{**}
Mg ²⁺	0.11 \pm 0.021	0.20 \pm 0.013	0.19 \pm 0.006	0.40 \pm 0.036	5.81 ^{ns}
Na ⁺	39.67 \pm 3.53	49.03 \pm 1.93	27.67 \pm 0.92	43.63 \pm 0.38	69.96 ^{**}
K ⁺	0.34 \pm 0.018	0.31 \pm 0.003	0.13 \pm 0.003	0.31 \pm 0.003	111.17 ^{**}

Explanations: EC - electric conductivity, Site1: Control Nile site, Site 2: Sewage effluents, Site 3: Oils and detergents factory, Site 4: Agricultural runoff, ns - nonsignificant, * - significant at 5% confidence level; ** - significant at 1% confidence level. Contents of ions were expressed in mg L⁻¹.

DISCUSSION

Various a biotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Gill and Tuteja, 2010). Measurement of H₂O₂ in plant tissue under water pollution is important because it can be precursor of highly reactive oxygen species. Surface water pollution with wastes coming from sewage effluents, oils and detergents factory discharge and agricultural drainages resulted in an increase in hydrogen peroxide (H₂O₂) and lipid peroxidation (MDA) concentrations in shoots and roots of the three studied plants as compared with those sampled from unpolluted River Nile site (Site 1). Currently, research data show that H₂O₂ can play a dual role in cells. During oxidative stress, H₂O₂ is a strong toxic oxidant causing cell damage or even cell death. At the same time it serves conversely as a signaling molecule to activate a rescue/defense system for restoring the redox homeostasis in plant cells (Yang and Poovalah,

2002; Desikan *et al.*, 2003). Numerous studies have shown that the application of H₂O₂ at low concentrations could improve plant tolerance to salt stress (De Azevedo Neto *et al.*, 2005 ; Li *et al.* 2011) and heavy metal stress (Lin *et al.*, 2004; Hu *et al.*, 2009; Xu *et al.*, 2011; Guzeli and Terzi, 2013). According to Hu *et al.* (2009) the increased metal stress tolerance was attributed to induced antioxidant defense system after pretreatment with H₂O₂ in rice seedlings.

Hydrogen peroxide has been commonly used as an oxygen source because of the limited concentrations of oxygen that can be transferred into the groundwater using above-ground aeration (Zappi *et al.*, 2000). Therefore it has been used for pollution control, municipal wastewater treatment and industrial waste/wastewater applications. It may be needed to oxidize the more resistant organic and inorganic pollutants which contribute to BOD and COD - catalytic and may also affect BOD/COD removal and to improve supply and oxidation rate of suspended and dissolved particles that cause pollution in such

water effluent (Chen *et al.*, 1996 and Badmus *et al.*, 2007). In this study the overproduction of H₂O₂ under water pollution especially in roots of the studied aquatic macrophytes could be used as oxygen source and supply oxygen to oxidize the more resistant organic and inorganic pollutants and could be promotes aerenchyma formation in these plants where aerenchyma formation is mediated by reactive oxygen species (Steffens *et al.*, 2011).

Under water pollution conditions, ascorbic acid concentration decreased in shoots and roots of the studied plants. Ascorbic acid is the most abundant, powerful and water soluble antioxidants acts to prevent or minimizing the damage caused by ROS in plants (Smirnoff, 2005; Ather *et al.*, 2008). It serves as a cofactor for enzymes involved in photosynthesis, hormone biosynthesis, and the regeneration of antioxidants such as α -tocopherol. Ascorbic acid was also found to enhance the efficiency of antioxidant defense system in plants and their tolerance to oxidative stress (Galli, 2013)

Leaf membrane damage by either heat (51 °C) or dehydration (40% PEG) was generally higher in plants exposed to liquid wastes from the three pollution sources compared to membrane injury in plants growing at unpolluted River Nile site (control). Increasing membrane injury under water pollution could be due to lower ascorbic acid concentration in polluted plants (Fig. 1) which can provide protection to membrane by directly scavenge the O₂• and OH• (Gill and Tuteja, 2010). Enhancement accumulation of H₂O₂ in polluted plants (Fig. 1) which may affect membrane properties could be another alternative

probability to increase membrane damage (high injury %) under water pollution condition.

Monitoring total chlorophyll concentration can be used as early warning for toxic effect of water pollution on plants (Calheiros *et al.*, 2009). In this study, *P salicifolia* plants collected from polluted sites had lower chlorophyll contents than those sampled from control site (Site 1). Reduction in Chl content under the influence of water pollution has been reported (Gadallah, 1994). Chlorophyll reduction induced by waste water could be associated with high concentration of mineral and increased alkalinity of water (e.g. site 3 polluted with oils and detergents factory wastes). Some of possible reason for any decreases in Chl content may be due to: 1- formation of chlorophyllase which is responsible for chlorophyll degradation (Drazkiewice, 1994). 2- retardation of chlorophyll synthesis and 3- enhancement of chlorophyll loss due to increases endogenous abscisic acid (Cizkova, 1990). On the contrary, contents of chlorophyll in *E stagnina* and *C alopecuroides* plants growing at polluted sites were higher than in those of control site. Our results confirming the finding of Calheiros *et al.* (2009) who reported an increase in *Phragmites australis* chlorophyll content under the industrial water pollution. Increased Chl content in polluted plants especially those exposed to agricultural drainage could be due to the high Mg²⁺ (Tab. 1).

Soluble sugars accumulated in shoots and roots of the studied plants showed irregular response to changes in water habitats and presence of pollution sources. *E stagnina* and *P salicifolia* plants collected

from polluted sites were lower in soluble sugars than those at control site. This reduction in SS under water pollution might be result of decrease photosynthesis (Warrier *and* Saroja, 2008) through chlorophyll reduction (Fig. 2). On the other hand, *C alopecuroides* plants growing at polluted sites had higher contents of chlorophyll than the control plants. The higher accumulation of SS in polluted plants with corresponding higher chlorophyll content (Fig. 2) indicates that increases in SS was the result of higher photosynthesis and increasing metabolic activities of the tissues under water pollution (Steinbachová-Vojtišová *et al.*, 2006). Accumulation of SS could be an adaptive mechanism of water pollution where SS are able to protect the structural integrity of cell membrane which has been as a measure of adverse environmental stresses. Water pollution with wastes coming from different sources decreased the content of soluble proteins in shoots and roots of *P salicifolia*. Lower SP contents under water pollution might be due to oxidative damage to proteins through over production of H₂O₂ (Figure 3) which may inactivate enzymes (Gill *and* Tuteja, 2010), enhancement of proteolysis or inhibition of protein synthesis. On the contrary, the same pollution sources increased SP content in *C alopecuroides* could be probably due to the high rate of nitrogen metabolism. Our results were in consist results of Warrier *and* Sayoja (2008).

Regardless of pollution sources, total free amino acids were generally higher in plants growing at polluted sites than in those at control site. These results are in agreement with the result of Steinbachová-Vojtišová *et al.*, (2006). Accumulation

of TAA in polluted plants in comparison with unpolluted control plants could be an adaptive response to help plants to ovoid toxic concentration of metals in polluted water (Tomsett *and* Thurman, 1988). The total free amino acids (especially proline) accumulation could be used as an indicator to exposure to stress such as water pollution (Megateli *et al.*, 2009).

In conclusion, water pollution with different wastes coming from different sources adversely affected the metabolic activities and stability of leaf membrane of the investigated plants. The three waste water reduced the antioxidant defense ability through: 1- reduction of ascorbic acid concentration which considered as most powerful ROS scavenger. 2- enhancement H₂O₂ which has been established that excess of H₂O₂ in plant cells leads to the occurrence of oxidative stress and 3- increasing lipid peroxidation intensity. The over production of hydrogen peroxide by the studied aquatic plants under water pollution could be used as an oxygen source and oxidize the more resistant organic and inorganic pollutants which contribute to BOD and COD catalytic. The effect of water pollution was not the same for the three plants studied. The same pollution source could give antagonistic effect on the same parameter of the different species (e.g. Chl, SS and SP contents). The investigated macrophytes play vital role in oxidizing more resistant organic and inorganic pollutants, and provide a cheap and easy way to monitor the different source of water pollution.

REFERENCES

Ather,H.R., Khan. A., Ashraf, M. (2008) Exogenously

- applied ascorbic acid alleviates salt- induced oxidative stress in wheat. *Environmental and Experimental Botany* **63**, 224-231.
- Badmus, M.A.O., Audu , T.O.K., Anyata, B.U.(2007) Removal of heavy metal from industrial wastewater using hydrogen peroxide. *African Journal of Biotechnology* **6**, 238-242.
- Blum, A., Ebercon, A. (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science* **21**, 43-47.
- Buysse, J., Merckx, R. (1993) An improved colorimetric method to quantify sugar content of plant tissue. *Journal of Experimental Botany* **44**, 1627-1629.
- Calheiros, C.S., Rangel, A.O., Castro, P.M. (2007) Constructed wetland system vegetated with different plants applied to the treatment of tannery waste water. *Water Research* **41**, 1790-1798
- Calheiros, C.S., Rangel, A.O., Castro, P.M. (2009) Treatment of industrial waste water with two-age constructed wetland planted with *Typha latifolia* and *Phragmites australis*. *Bioresource technology* **100**, 3205- 3212.
- Chen, W., Tan, S.K., Tay, J.K. (1996) Distribution, Fractional Composition and Release of Sediment-Bound Heavy metals in Tropical Reservoirs. *Water Air and Soil Pollution* **92**, 472.
- Cizkova, R. (1990) Acidification stress of root environment as related to endogenous cytolinins and gibberellins in oak seedlings. *Biologia Plantarum* **32**, 97-103.
- Dantas, M., Locligiani, P. (2003) Trace element concentration in fresh water mussels and macrophytes as related to these in their environment. *Journal of Limenology* **62**, 61-70.
- De Azevedo Neto, A.D., Prisco, J.T., Eneas-Filho, J., Medeiros, J.V.R., Gomes-Filho, E. (2005) Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *J Plant Physiology* **162**, 1114-1122.
- Desikan, R., Hancock, J.T., Neill, S.J. (2003) Oxidative stress signaling. In H. Hirt and K. Shinozaki (eds.), *Plant responses to a biotic stress: topic in current genetics*. Springer-Verlag, Berlin, Heidelberg, New York, pp. 121-148.
- Drazkiewicz, M. (1994) Chlorophyll - occurrence, function and mechanism of action. Effects of external and internal factors. *Photosynthetica* **50**, 321-331.
- Gadallah, M.A.A. (1994) Effects of industrial and sewage waste water on the concentration of soluble carbon, nitrogen and some mineral elements in sunflower plants. *Journal of Plant Nutrition* **17**, 1369-1384.
- Galli, D.R. (2013) The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. *Journal of Experimental Botany* **64**: 433-443.
- Gill, S.S., Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in a biotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**, 909-930.

- Gürlük, S. (2009) Economic growth, industrial pollution and human development in the Mediterranean region. *Ecological Economics* **68**, 2327-2335.
- Guzel, S., Rabiye, T. (2013) Exogenous hydrogen peroxide increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. *Botanical Studies* **54**, 26:2-10.
- Hu, Y., Ge, Y., Zhang, C., Ju, T., Cheng, W. (2009) Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regulation* **59**, 51-61.
- Jagota, S. K., Dani, H. M. (1982) A new colorimetric technique for estimation of vitamin C using folin phenol reagent. *Analytical Biochemistry* **127**, 178-182.
- Jackson, M.L. (1973) Soil Chemical Analysis. Prentice-hall of India Private Limited, New Delhi.
- Karatas, F., Obek, L., Kamisli, F. (2009) Antioxidant capacity of *Lemna gibba* L exposed to waste water treatment. *Ecological Engineering* **35**, 1225-1230.
- Khan, S., Ahmad, I., Shah, M.T., Rehman, S., Abdul Khaliq, A. (2009) Use of constructed wetland for removal of heavy metals from industrial waste water. *Journal of Environmental pollution* **90**, 3451-3457.
- Lee, Y.P., Takahashi, T. (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. *Analytical Biochemistry* **14**, 71 - 77.
- Li, J.T., Qiu, Z.B., Zhang, X.W., Wang, L.S. (2011) Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. *Acta Physiologia Plantarum* **33**, 835-842.
- Lin, Q., Chen, Y., Wang, Z., Wang, Y. (2004) Study on the possibility of hydrogen peroxide pretreatment and plant system to remediate soil pollution. *Chemosphere* **57**, 1439-1447.
- Lowry, C. H., Rosebrough, N. J., Farr, A. L., Bandall, H. J. (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- Maddison, M., Muring, T., Remm, K., Lesta, M., Mander, U. (2009) Dynamics of *Typha latifolia* populations in treatment wetlands in Estonia. *Ecological Engineering* **35**, 285-264.
- Madhava, Rao, K.V., Sresty, T.V.S. (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Science* **57**, 113-128.
- Megateli, S., Semsari, S., Couderchet, M. (2009) Toxicity and removal of heavy metals (cadmium, copper and zinc) by *Lemna gibba*. *Ecotoxicology and Environmental Safety* **72**, 1774-1780.
- Oketola, A.A., Osibanjo, O. (2007). Estimating sectoral pollution load in Lagos by Industrial Pollution Projection System (IPPS). *Science of the Total Environment* **377**, 125-141.

- Pérez-Ruiz, J.M., Spínola, M.C., Kirchsteiger, K., Moreno, J., Sahrawy, M., Cejudo, F. G. (2006) Rice NTRC is a high-efficiency redox system for chloroplast protection against oxidative damage. *Plant and Cell* **18**, 2356–2368.
- Smirnov, M. (2005) Ascorbate, tocopherol and carotenoids metabolism pathway engineering and functions. In Smirnov N (Ed). *Antioxidants and reactive oxygen species in plants*. Blackwell Publishing Ltd Oxford UK. Pp: 53-86.
- Schwarzenbach, G., Biederman, W. (1948) Komplexe X. Erdalkalikomplexe von o, o'-Dioxyazofarbstoffen. *Helv. Chemistry Acta*, **31**: 678-687.
- Steinbachová, V., Tylová, E., Soukup, A., Novická, H., Votrubová, O., Lipavská, H., Cizková, H. (2006) Influence of nutrient supply on growth, carbohydrates and nitrogen metabolic relations in *Typha angustifolia*. *Environmental and Experimental Botany* **57**, 246-257.
- Steffens, B., Geske, T., Sauter, M. (2011) Aerenchyma formation in the rice stem and its promotion by H₂O₂. *New Phytologist* **190**, 369–378.
- Tomsett, A.B., Thurman, D.S. (1988) Molecular biology of metal tolerances of plant. *Plant Cell and Environment* **11**, 383-394.
- Velikova, V., Yordavov, I., Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science* **151**, 59-66.
- Ward, F.A. (2006) Economic impacts of in stream flow protection for the Rio Grande silvery minnow in the Rio Grande basin. *Environmental and Natural Resource Economics*, Prentice-Hall.
- Warrier, R.R., Saroja, S. (2008) Histochemical studies on water hyacinth with particular reference to water pollution. *International Journal of Integrative Biology* **3**, 96-99.
- Wellburn, A. R. (1994) The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvent with spectrophotometers of different solution. *Journal of Plant Physiology* **144**, 307-313.
- Wood, Z.A., Poole, L.B., Karplus, P.A. (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* **300**, 650–653.
- Xu, F.J., Jin, C.W., Liu, W.J., Zhang, Y.S., Lin, X.Y. (2011) Pretreatment with H₂O₂ alleviates aluminum-induced oxidative stress in wheat seedlings. *Journal of Integrative Plant Biology* **53**, 44–53.
- Yang, T., Poovaiah, B.W. (2002) Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. *Proceeding of the National Academy of Sciences of the United States of America* **99**, 4097-4102.
- Zappi, M., White, K.H., Wang, H.M., Bajpai, R., Qasim, M. (2000) The fate of hydrogen peroxide as an oxygen source for bioremediation activities within saturated aquifer systems. *Journal of Air Waste Management Association* **50**, 1818-1830.