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PHYSIOLOGICAL RESPONSES OF *DUNALIELLA TERTIOLECTA* TO Hg^{2+}-INDUCED OXIDATIVE STRESS*

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Abstract – Some species of the genus *Dunaliella* are the major primary producers in Maharlu salt lake, which is polluted with different heavy metals including Hg^{2+}. Short-term (48 h) and prolonged exposure (28 d) of *D. tertiolecta* to 5, 10, 20, and 50 μM $HgCl_2$ decreased Chlorophyll "a" content, but caused enhanced carotenoid production and increased ascorbate peroxidase (APX) activity, both of which function as antioxidants, buffering oxidative stress by scavenging reactive oxygen species (ROS). Increase in total carotenoids in terms of pictogram per cell (pg $cell^{-1}$) was higher in prolonged exposure, whereas APX activity was greater in short-term exposure to $HgCl_2$. In both experiments, despite the increase in antioxidant capacity, algal growth in terms of cell number per ml of culture decreased relative to the control. It seems that generation of ROS in the presence of $HgCl_2$ exceeds the total antioxidant capacity of the cells, which is evident from the increase in cell malondialdehyde (MDA) content and, as a result, reduced cell number. The SDS-PAGE profile of the extracted proteins showed enhanced synthesis of two protein bands of about 29 and 38 kD.

Keywords – Dunaliella tertiolecta; Hg^{2+}; oxidative stress; antioxidant

1. INTRODUCTION

One of the major environmental problems caused by industrialization is the increase in the levels of heavy metals concentrations in the air, land and water [1]. Growth inhibition is the most pronounced effect of heavy metals on plants and micro-organisms [2]. The mechanism underlying heavy metals toxicity is in part related to the increased production and accumulation of reactive oxygen species (ROS) such as $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$ [3]. ROS have strong oxidizing activities, attacking many types of biomolecules such as proteins, lipids and nucleic acids [4]. Mercury, as one of the most hazardous heavy metals, is detrimental to all organisms including algae, plants and animals [5]. During the past century, industrial and agricultural activities have released several hundred thousand tons of mercury into the biosphere [6]. Since mercury is a redox-inactive toxic metal [7], it is not directly involved in ROS production via the Fenton type reaction. The biochemical toxicity of mercuric ions is mainly due to their reaction with thiol groups of proteins and other important biological molecules such as glutathione [8, 9]. Since reduced glutathione is crucial for the enzymatic systems that scavenge ROS [10], depletion of reduced glutathione and inactivation of antioxidant enzymes play a significant role in ROS accumulation in the presence of mercuric ions [8]. In addition, Hg^{2+} can disrupt the photosynthetic electron transport chain leading to the production of singlet O_2 ($^1O_2$) and $O_2^{\cdot-}$, which is dismutated to O_2 and H_2O_2 in the chloroplast [11]. Organisms have developed several protective mechanisms to scavenge ROS [12]. These mechanisms are divided into low molecular weight antioxidants such as glutathione, ascorbate, tocopherol and carotenoids,

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and antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase [13].

The unicellular green algae *Dunaliella* is widely distributed throughout the world and its presence in Maharlu salt lake in Shiraz, Iran was first reported by Ginzburg and Ginzburg [14]. Analysis of water samples collected from Maharlu salt lake indicated the presence of relatively high concentrations of several heavy metals including Cu, Cd and Hg. Since the effects of heavy metals on ROS metabolism in algae are varied [15, 16] and information on the response of *Dunaliella* to Hg^{2+} is scarce, to assess the sensitivity of *Dunaliella tertiolecta* to Hg^{2+}, the effects of $HgCl_2$ on the growth, pigmentation, lipid peroxidation and ascorbate peroxidase activity are investigated and compared with reports on other algae and plants.

2. MATERIALS AND METHODS

a) Algal isolation, growth and $HgCl_2$ treatment

D. tertiolecta was isolated from water samples collected from Maharlu salt lake located 30 Km southeast of Shiraz, Iran. Single colonies were derived from individual cells on agar plates [17, 18] and *D. tertiolecta* was identified as described by Preisig [19]. Purified algae were grown in sterilized liquid nutrient media, as described previously [20]. Cells were exposed to 0, 5, 10, 20 and 50 μM $HgCl_2$ according to two models of treatment. In long-term exposure (Exp. 1), $HgCl_2$ was supplied to algae initiating a fresh culture as follows: To 500-ml flasks each containing 250 ml nutrient media were added 1 ml algal suspension containing 2.5×10^5 cells and 1 ml proper concentration of $HgCl_2$. Flasks were kept in a growth chamber set at 22 ± 2 ˚C under continuous light provided by cool white fluorescent lamps with 4000 lux illumination. Growth measurements were made on samples taken at weekly intervals for 28 days and other measurements are reported only on samples taken three weeks (21 days) after $HgCl_2$ exposure. In short-term exposure (Exp.2), $HgCl_2$ was added to the algal cultures at the late exponential phase of growth, and after 48 h samples were taken for various measurements. All experiments were carried out in triplicate and each value represents mean ± standard error.

b) Growth measurement

Algal growth was measured by cell count using a haemocytometer or a coulter counter model ZBI with a 100μM orifice.

c) Pigments determination

From each flask, 3 ml of algal suspension was centrifuged at 1000g for 10 minutes and pigments were extracted from the algal pellet with 100% acetone. After centrifugation, chlorophyll "a" (Chl. a) and total carotenoids were determined as described by Eijckehoff and Dekker [21].

d) Lipid peroxidation

An appropriate volume of algal suspension was pelleted and homogenized for 30 s with a sonicator model W-220 F in 5 ml of 0.1% TCA in ice bath. Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) by the thiobarbituric acid (TBA) reaction described by Dhindsa and Matowe ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$) [22].

e) Ascorbate peroxidase (APX) activity

APX was extracted from *D. tertiolecta* as described previously [20] and its activity was measured by following the decrease in absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) [23].

f) Hg^{2+} content of the growth medium

10 ml of the algal suspension subjected to different concentrations of $HgCl_2$ for 48 h was centrifuged at 5000g for 10 minutes. The supernatant was analyzed for Hg^{2+} using Inductive Coupled Plasma (ICP) model Vista-pro.

g) Protein extraction and electrophoresis

Appropriate volumes of control $HgCl_2$ treated algal cultures each containing 10^8 cells were centrifuged at 1000g for 10 min, then 0.5 ml of the extraction buffer containing 0.1 M Tris-HCl pH=8.0, 0.5 M NaCl, 5mM DTT, 5 mM EDTA and10 mM phenylmethansulfonyl fluoride (PMSF) were added to the pellets and sonicated at 4^0C for 30 s. Equal volumes of sample buffer were added to each homogenate and proteins were separated by SDS-PAGE using 6% stacking and 12% running gel and stained overnight with Coomassie Blue R-250 [24].

h) Statistical analysis

All results are the mean of three replicates ± SE. Data analysis were performed using SPSS 11.5.

3. RESULTS

a) Effects on growth

Figure 1 shows algal growth, expressed as number of cells ml^{-1}, as affected by different concentrations of $HgCl_2$ added to the growth culture at the start of the inoculation of media with algae (long-term exposure, Exp. 1). During 28 days of algal growth, the number of algal cells increased in nearly all treatments, being highest in the absence of $HgCl_2$ (control) and lowest in the presence of 50 µM $HgCl_2$. When algal suspensions were grown to late exponential phase and subjected to $HgCl_2$ for 48 h (short-term exposure, Exp. 2), the number of cells decreased with the increase in $HgCl_2$ concentrations (Fig. 2). At 50 µM $HgCl_2$, the number of cells decreased from 3.8×10^6 in control to 1.7×10^6 cells, which is about a 55% decrease in cell number in 48 h.

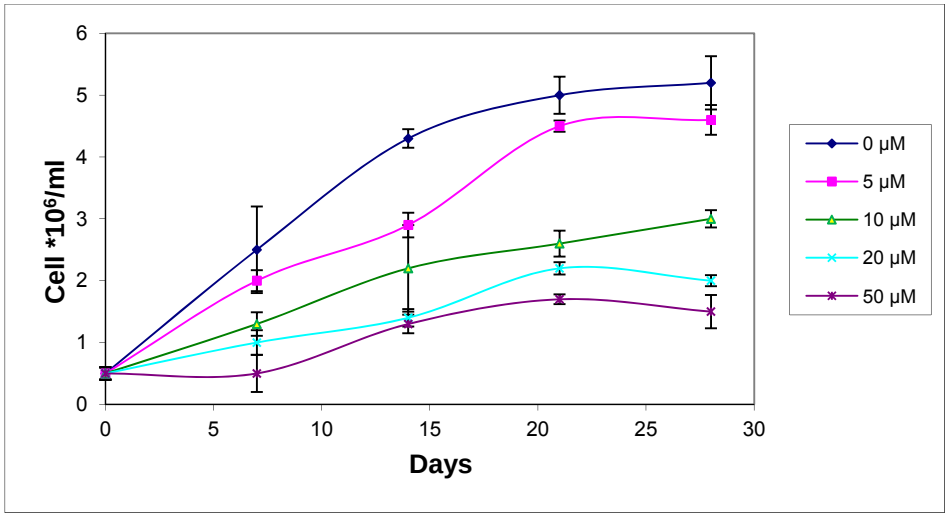

Fig. 1. Cell number ml^{-1} of algal cultures × 10^6 as affected by $HgCl_2$. Cells were exposed to $HgCl_2$ for 28 days and number of cells were determined at weekly intervals (Exp. 1).

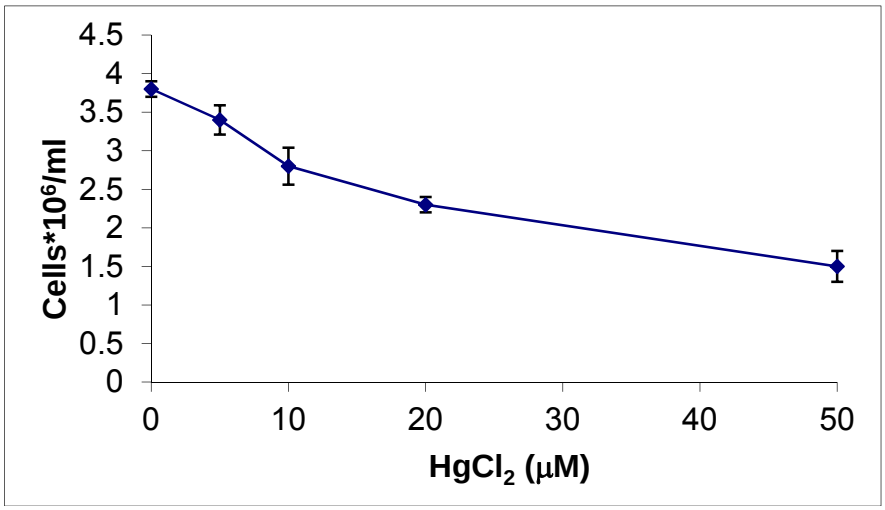

Fig. 2. Cell number ml^{-1} of algal culture × 10^6 as affected by $HgCl_2$. Cells were exposed to $HgCl_2$ at late exponential phase of growth and number of cells were determined after 48 hrs of exposure (Exp. 2).

b) Pigments content

Chl. "a" content, expressed as μg ml^{-1} of algal suspension, decreased with the increase in $HgCl_2$ concentrations in both types of treatments (Table 1). The decrease was more pronounced in long-term than in short-term exposures. When expressed in terms of pg $cell^{-1}$, in long-term exposure a nearly 12% increase in Chl. "a" content was observed at 5 μM $HgCl_2$ relative to control. At 10, 20 and 50 μM $HgCl_2$, Chl. "a" content decreased by 4, 12 and 44%, respectively, while in short-term exposure , Chl. "a" content in terms of pg $cell^{-1}$ was relatively unaffected by $HgCl_2$. Compared to that in the control, there was only a 4% decrease in Chl. "a" content observed at 50 μM $HgCl_2$..

Table 1. Effects of $HgCl_2$ on Chl. "a" content of *Dunaliella tertiolecta* expressed as μg ml^{-1} of algal culture and pg $cell^{-1}$. Exp.1: long-term exposure to $HgCl_2$, Exp. 2: short-term exposure to $HgCl_2$

Chl. "a" content		$HgCl_2$ (μM)				
		0	5	10	20	50
Exp. 1	μg ml^{-1}	1.9 ± 0.11 (100)	1.4 ± 0.08 (74)	0.62 ± 0.07 (33)	0.4 ± 0.08 (21)	0.15 ± 0.08 (8)
	Pg $cell^{-1}$	0.50 ± 0.04 (100)	0.56 ± 0.01 (112)	0.48 ± 0.04 (96)	0.44 ± 0.04 (88)	0.28 ± 0.04 (56)
Exp. 2	μg ml^{-1}	2.166 ± 0.08 (100)	2.040 ± 0.06 (94)	1.796 ± 0.08 (82)	1.500 ± 0.12 (70)	0.935 ± 0.07 (43)
	Pg $cell^{-1}$	0.57 ± 0.024 (100)	0.60 ± 0.037 (105)	0.61 ± 0.022 (107)	0.60 ± 0.02 (105)	0.55 ± 0.037 (96)

Each value is mean ± SE. In each row figures in parentheses show percent Chl. "a" content relative to control

Total carotenoids as affected by $HgCl_2$ are shown in Table 2. In long-term exposure, total carotenoids per ml of algal culture decreased with an increase in $HgCl_2$ concentrations, while total carotenoids per cell increased steadily. At 50 μM $HgCl_2$ total carotenoids increased by more than twofold relative to control. In short-term exposure, total carotenoids per ml of culture remained relatively unaffected up to 10 μM $HgCl_2$. When expressed in terms of pg $cell^{-1}$, the total carotenoid increased with an increase in $HgCl_2$ concentration, but the percent increase was less than that in long-term exposure. At 50 μM $HgCl_2$, a 57% increase was observed compared to a 104% increase in long-term exposure.

Table 2. Effects of $HgCl_2$ on total carotenoids of *Dunaliella tertiolecta* expressed as μg ml^{-1} of algal culture and pg $cell^{-1}$

Total carotenoids		**$HgCl_2$ (μM)**				
		0	5	10	20	50
Exp. 1	μg ml^{-1}	2.80 ± 0.16 (100)	2.30 ± 0.11 (82)	1.50 ± 0.09 (54)	1.17 ± 0.06 (42)	0.85 ± 0.06 (30)
	Pg $cell^{-1}$	0.74 ± 0.064 (100)	0.92 ± 0.06 (124)	1.15 ± 0.06 (155)	1.30 ± 0.05 (176)	1.51 ± 0.06 (204)
Exp. 2	μg ml^{-1}	2.660 ± 0.06 (100)	2.550 ± 0.12 (96)	2.639 ± 0.11 (99)	2.425 ± 0.13 (91)	1.870 ± 0.10 (70)
	Pg $cell^{-1}$	0.70 ± 0.050 (100)	0.75 ± 0.037 (107)	0.91 ± 0.049 (130)	0.97 ± 0.025 (139)	1.10 ± 0.035 (157)

Each value is mean ± SE. In each row figures in parentheses show percent total carotenoids relative to control

c) Effects on APX

In long-term exposure, ascorbate peroxidase (APX) showed 52, 60, 76 and 36% increase in activity at 5, 10, 20 and 50 μM $HgCl_2$, respectively (Table 3). Similar results were obtained when cells were treated with $HgCl_2$ for 48 h at the late exponential phase of growth (Exp. 2). The APX activity increased with an increase in $HgCl_2$ up to 20 μM $HgCl_2$ and then declined. At 5, 10, 20 and 50 μM $HgCl_2$, the APX activities were 43, 74, 94 and 71 percent of that in the control, respectively. At 10, 20, and 50 μM $HgCl_2$, APX activity was higher in Exp. 2 than in Exp. 1.

Table 3. Ascorbate peroxidase (APX) activity as affected by different $HgCl_2$ concentrations expressed as Δ O.D. $min.^{-1}$ 10^6 $cells^{-1}$

	$HgCl_2$ (μM)				
	0	5	10	20	50
Exp. 1	0.042 ± 0.002 (100)	0.064 ± 0.002 (152)	0.067 ± 0.003 (160)	0.074 ± 0.001 (176)	0.057 ± 0.003 (136)
Exp. 2	0.035 ± 0.002 (100)	0.050 ± 0.003 (143)	0.061 ± 0.004 (174)	0.068 ± 0.002 (194)	0.060 ± 0.005 (171)

Each value is mean ± SE. In each row figures in parentheses show percent APX activity relative to control

d) Effects on MDA content

The lipid peroxidation, expressed as the main thiobarbituric acid reactive metabolite malondialdehyde (MDA), increased in the presence of $HgCl_2$ (Table 4). In Exp. 1, MDA concentration increased from 48×10^{-4} nmole $cell^{-1}$ in the control, to 108×10^{-4} in the presence of 50 μM $HgCl_2$. In Exp. 2, the MDA level was 52×10^{-4} nmole $cell^{-1}$ in the control, which rose to 76×10^{-4} at the highest level of $HgCl_2$.

Table 4. Lipid peroxidation in the presence of different $HgCl_2$ concentrations expressed as nmole MDA 10^9 $cell^{-1}$

	$HgCl_2$ (µM)				
	0	5	10	20	50
Exp. 1	0.048 ± 0.04 (100)	0.53 ± 0.02 (110)	0.71 ± 0.03 (148)	0.91 ± 0.05 (190)	1.08 ± 0.01 (225)
Exp. 2	0.52 ± 0.02 (100)	0.56 ± 0.05 (108)	0.67 ± 0.02 (129)	0.68 ± 0.03 (130)	0.76 ± 0.06 (146)

e) SDS-PAGE analysis of proteins

The SDS-PAGE profile of the extracted proteins from the control and $HgCl_2$ treated algal culture is shown in Fig. 3. Treatment with $HgCl_2$ for 48 h enhanced the synthesis of two protein bands about 29 and 38 kD. The intensities of the protein bands generally increased with an increase in $HgCl_2$ concentration.

f) Hg^{2+} depletion from growth media

Compared to the cell-free control, more than 90% of Hg^{2+} in the growth media was depleted by algal cells in 24 h at all $HgCl_2$ levels. Fairly rapid Hg^{2+} depletion is partly due to the large number of cells present at the late exponential phase of growth, during which $HgCl_2$ was added to the media.

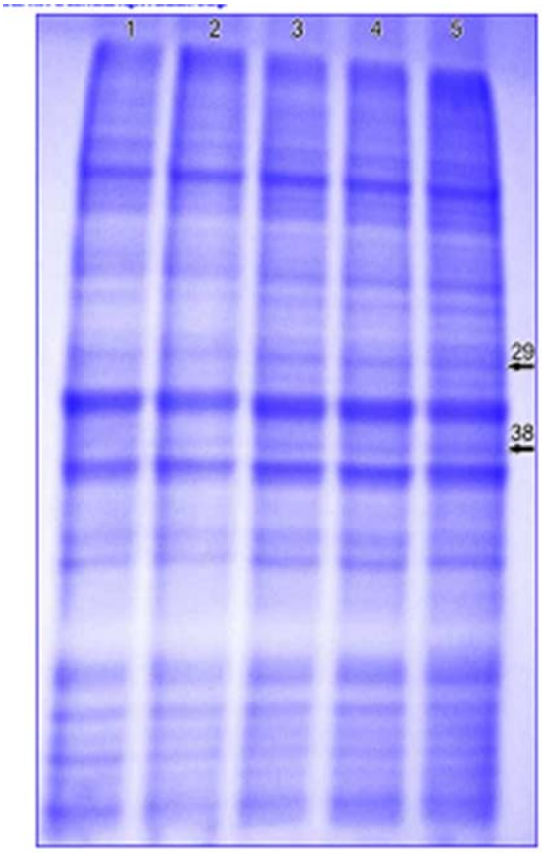

Fig. 3. The SDS-PAGE (12%) of proteins extracted from *D. tertiolecta* treated with $HgCl_2$ for 48 h. Lanes 1, 2, 3, 4 and 5 represent control, 5, 10, 20 and 50 µM $HgCl_2$, respectively. Arrows indicate the locations of 29 and 38 kD protein bands.

4. DISCUSSION

Growth reduction in *D. tertiolecta* exposed to different $HgCl_2$ concentrations has been observed in other organisms including plants and algae [20, 25]. Since Hg^{2+} has a high affinity for the sulfhydryl (-SH) group, it can disturb almost any function where proteins are involved [8, 26]. Cell division is one of the first algal processes affected by Hg^{2+}. The inhibition of cell multiplication was suggested to be a sensitive index of heavy metals toxicity [9, 27]. In addition, cell membrane is reported to be the primary site of mercury toxicity resulting in cell lysis, as observed in the present study (Fig. 2). Rapid adsorption of Hg^{2+} by *Anabaena inaequalis* and induction of cell lysis is reported by Stratton et al., [28].Within 5 min. about 96% of Hg^{2+} in the growth culture was removed by *A. inaequalis*, and a linear relationship existed between the number of cells and the amount of Hg^{2+} required to induce culture lysis. As shown in Fig. 1, in the absence of Hg^{2+} there was a rapid initial increase in cell number before day 7, although with the lower rate a rapid increase in cell number also occurred in the presence of 5 μM Hg^{2+}. Due to the batch culture of the algae, the growth stoppage in the control cells after day 21 is mainly due to a reduction in mineral nutrition.

Decrease in Chl."a" content in terms of μg ml^{-1} algal culture is partly due to the decrease in cell number ml^{-1} of the culture. Decrease in Chl. "a" content of each cell in Exp. 1 may be due to both inhibition of Chl. "a" biosynthesis and enhanced degradation of this pigment. Heavy metals often interfere with chlorophyll biosynthesis and cause stress-induced chlorosis, which influences the structure and function of the photosynthetic apparatus and thus photosynthesis, globally [29]. Mercury inhibits several enzymes involved in the chlorophyll biosynthesis pathway, including delta aminolaevulinic acid (ALA) synthase, ALA dehydratase and NADPH: protochlorophyllide oxidoreductase [29, 30]. This last enzyme has three conserved cysteine (-SH) residues which are potential target sites for mercuric ions.

Total carotenoids, in terms of μg ml^{-1} of algal culture, decreased in both Exp.1 and Exp. 2, which is mainly due to the decrease in cell number ml^{-1} of the culture. When expressed in terms of pg $cell^{-1}$ total carotenoids increased with an increase in $HgCl_2$ concentration (Table 2). Due to the long period of exposure to $HgCl_2$, the increase in total carotenoids $cell^{-1}$ was higher in Exp. 1 compared to Exp. 2. The rise in total carotenoids content of *D. tertiolecta* cells has been reported in the presence of other heavy metals such as $CuCl_2$ [20]. Since Hg^{2+} disrupts the photosynthetic electron transport chain which leads to the production of singlet O_2 ($^1O_2$) and superoxide anion ($O_2^{\cdot-}$), carotenoids protect the cell against the damage caused by singlet O_2 and other ROS [11]. Increase in cell carotenoids content is also reported when *Dunaliella* is subjected to nitrogen deficiency. Under this condition, due to limited amino acid biosynthesis, carotenoids may act as the carbon sink during photosynthesis [31]. The increase in carotenoids in the presence of Hg^{2+} may be due to up-regulation of rate limiting genes in the carotenoids biosynthetic pathway. Mercuric ion-induced gene expression in *Arabidopsis thaliana* has been reported by Heindenreich et al., [32]. Functional classification of induced genes showed that the transcripts were induced by Hg^{2+} encoded proteins for the photosynthetic apparatus and for some antioxidant enzymes such as catalase. Induction of carotenoids biosynthetic pathway genes by heavy metals in *Dunaliella* await clarification.

Increase in APX activity in response to heavy metals has been reported by several investigators [2, 33]. Although APX activity was higher in Exp. 1, the increase in activity with increased $HgCl_2$ concentrations was higher in Exp. 2 compared to Exp. 1. APX reduces H_2O_2 to H_2O using ascorbate as the reductant and as a result, together with carotenoids, they decrease the damage caused to biomolecules by ROS.

Despite the protective role of antioxidant systems, such as APX and carotenoids, lipid peroxidation, which is considered as a biomarker of cellular damage, increased with an increase in Hg^{2+} concentration

(Table 4). This indicates that the antioxidative mechanisms were overtaxed and could not completely prevent enhancement of membrane lipid peroxidation. This is evident from the decrease in cell number and increased cell lysis in the presence of Hg^{2+} (Figs. 1 and 2). Increased lipid peroxidation is reported in animals and plants exposed to various metals [34, 35, 36]. Tomato suppression of seedling growth by Hg^{2+} was suggested to be the result of enhanced H_2O_2 production and the subsequent increase in the MDA level [25].

Induced or enhanced synthesis of distinct sets of proteins upon exposure of organisms to environmental stresses has been suggested by Sachs and Ho [37]. In our study, *Dunaliella tertiolecta* subjected to $HgCl_2$ revealed the intensity of two protein bands on SDS-PAGE (Fig. 3). Treatment of sorghum seedlings with $HgCl_2$ resulted in enhanced synthesis of a 16 KD protein band, whereas $CdCl_2$ resulted in increased synthesis of several proteins over a broad molecular weight range [38]. In general, higher plants and algae subjected to biotic and abiotic stresses display numerous biochemical, physiological and developmental responses such as changes in the pigments content, activation of antioxidant enzymes, induction or enhanced synthesis of distinct sets of proteins, and reduced growth and reproduction.

Although higher APX activity was observed at 50 μM $HgCl_2$ in Exp. 2, the antioxidant carotenoids in terms of pg $cell^{-1}$ were higher in Exp. 1. Fairly rapid decrease in cell number in Exp. 2 shows that abrupt generation of high levels of ROS over a short period of stress will usually exceed the total antioxidant capacity of cells. The same is true for prolonged exposure to high levels of $HgCl_2$ (Exp. 1). In contrast, prolonged exposure to low levels of $HgCl_2$ allows cells to acclimatize to increased levels of ROS by elevation of total antioxidant capacity. Since some species of *Dunaliella* including *D. tertiolecta* are the major primary producers in salt lakes and also large quantities of economically important pigments such as carotenoids are produced by *Dunaliella* spp., investigation on responses of *Dunaliella* to different environmental and biological stresses helps to optimize both biomass and carotenoids production.

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REFERENCES

1. Nriagu, J. O. (1990). Global metal pollution. *Environment*, 32, 7-33.
2. Rellan-Alvarez, R., Ortega-Villasante, C., Alvarez-Fernandez, A., Campo, F. F. & Hernandez, L. E. (2006). Stress responses of *Zea mays* to cadmium and mercury. *Plant Soil*, *279*, 41-50.
3. Stohs, S. J. & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med*., *18*, 321-336.
4. Hall, J. L. (2000). Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.*, *53*, 1-11.
5. Bizily, S. P., Rugh, C. L., Summers, A. O. & Meagher, R. B. (1999). Phytoremediation of ethylmercury pollution: mer B expression in *Arabidopsis thaliana* confers resistance to organomercurials. *Ecology*, *96*, 6808-6813.
6. Andren, A. W. & Nriagu, J. O. (1979). *The global cycle of mercury.* Amsterdam. Elsevier Biomedical.
7. Sigaud-Kutner, T. C. S., Leitao, M. S., Okamoto, O. K., Morse, D. & Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *J. Phycol*., *39*, 1008-1018.
8. Ercal, N., Gurer-Orhan, H. & Aykin-Burns, N. (2001). Toxic metals and oxidative stress. Part I: mechanisms involved in metal induced oxidative damage. *Curr. Topics Med. Chem*., *1*, 529-539.
9. Patra, M., Bhowmik, N., Bandopadhyay, B. & Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Bot*., *52*, 199-223.

10. May, M. J., Vernoux, T., Leaver, C., Montagu, M. V. & Inze, D. (1998). Glutathione homeostasis in plants: Implications for environmental sensing and plant development. *J. Exp. Bot.*, *49*, 649-667.
11. Asada, K. & Takahashi, M. (1987). *Production and scavenging of active oxygen in photosynthesis*. In: Kyle, D. J., Osmond, C., Arntzen, C. J. (Eds.). Photoinhibition. NewYork, Elsevier.
12. Ratkevicius, N., Correa, J. A. & Moenne, A. (2003). Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (chlorophyta) from heavy metal-enriched environment in northern Chile. *Plant Cell Environ*., *26*, 1599-1608.
13. Okamoto, O. K., Pinto, E., Latorre, L. R., Bechara, E. J. H. & Colepicolo, P. (2001). Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts. *Arch. Environ. Contam. Toxicol*., *40*, 18-24.
14. Ginzburg, M. & Ginzburg, B. Z. (1984). Ion and glycerol concentrations in 12 isolates of *Dunaleilla*. *J. Exp. Bot*., *36*, 1064-1074.
15. Adonaylo, V. N. & Oteiza, P.I. (1999). Lead intoxication: antioxidation defenses and oxidative damage in rat brain. *Toxicology*, *135*, 77-85.
16. Pinto, E., Sigaud-Katner, T. C. S., Leitao, M. A. S., Okamoto, O. K. & Morse, D. (2003). Heavy metal-induced oxidative stress in algae. *J. Phycol.*, *39*, 1008-1018.
17. Lers, A., Biener, Y. & Zamir, A. (1990). Photoinduction of massive ß-carotene accumulation by the alga *Dunaliella barawil*. *Plant Physiol*., *7*, 75-90.
18. Powtongsook, S., Kitakoop, P., Menasveta, P. & Wisessange, S. (1995). Isolation and characterization of *Dunaliella salina* from Thailand. *J. Appl. Phycol.*, *7*, 75-90.
19. Preisig, H. R. (1992). *Identification of Dunaliella*. In: Avron, M., Ben-Amotz, A., (Eds.). *Dunaliella*: physiology, biochemistry and biotechnology. Boca Raton, CRC Press, PP. 1-15.
20. Nikookar, K., Moradshahi, A. & Kharati, M. (2004). Influence of salinity on the growth, pigmentation and ascorbate peroxidase activity of *Dunaliella salina* isolated from Maharlu salt lake in Shiraz. *IJST-Trans. A*, *28*, 117-125.
21. Eijckelhoff, C. & Dekker, J. P. (1997). A routine method to determine the chlorophyll "a", pheophytin a, and ß-carotene contents of isolated photosystem II reaction center complexes. *Photosynth. Res*., *52*, 69-73.
22. Dhindsa, R. S. & Matowe, W. (1981). Drought tolerance in two mosses: Correlated with enzymatic defense against lipid peroxidation. *J. Exp. Bot*., *32*, 79-91.
23. Amako, K., Chen, G. X. & Asada, K. (1994). Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant Cell Physiol*., *35*, 497-504.
24. Laemmeli, U. K. (1970). Cleavage of structure proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680-685.
25. Cho, U. H. & Park, J. O. (1999). Changes in hydrogen peroxide content and activities of antioxidant enzymes in tomato seedlings exposed to mercury. *J. plant Biol*., *42*, 41-48.
26. Patra, M. (2000). Comparative analysis of mercurials in higher plants in vivo. *J. Indian Bot. Soc.,* 79, 133-138.
27. Berland, B. R., Bonin, D. J., Guerin-Ancey, O. J., Kapkov, V. I. & Ailhac, D. P. (1977). Action of sublethal doses of heavy metals on the growth characteristics of the diatom *Skeletonema costatum. Marine Biol*., *42*, 17-30.
28. Stratton, G. W., Huber, A. L. & Corke, C. T. (1979). Effect of mercuric ion on the growth, photosynthesis and nitrogenase activity of *Anabaen inaequalis*. *Appl. Environ. Micro.*, *38*, 537-543.
29. Solymosi, K., Lenti, K., Kurdziel, B. M., Fidy, J., Strzalka, K. & Boddi, B. (2004). Hg^{2+} reacts with different components of the NADPH: protochlorophyllide Oxidoreductase macro domains. *Plant Biol*., *6*, 358-368.
30. Lenti, K., Fodor, F. & Boddi, B. (2002). Mercury inhibits the activity of the NADPH: Protochlorophyllide oxidoreductase (POR). *Photosynthetica, 40*, 145-151.
31. Marin, N., Morales, F., Loderios, C. & Tamigneaux, E. (1998). Effects of nitrate concentration on growth and

pigment synthesis of *D. Salin*a cultivated under low illumination and pre-adapted to different salinities. *J Appl. Phycol*., *10*, 405-411.

32. Heidenreich, B., Mayer, K., Sandermann, H. & Ernst, D. (2001). Mercury-induced genes in *Arabidopsis thaliana*: identification of induced genes upon long-term mercuric ion exposure. *Plant Cell Environ*., *24*, 1227-1234.
33. Sinha, S. & Saxena, R. (2005). Effects of iron on lipid peroxidation, enzymatic and non-ezymatic antioxidants and bacoside-A content in medicinal plant *Bacopa monnieri* L. *Chemosphere*, *62*, 1340-1350.
34. Huang, Y. L., Cheng, S. L. & Lin, T. H. (1996). Lipid peroxidation in rat administrated with mercuric chloride. *Biol. Trace Elem. Res., 52*, 193-206.
35. Ali, M. B, Vajpayee, P., Tripathi, R. D., Rai, U. N., Kumar, A., Singh, N., Behl, H. M. & Singh, S. P. (2000). Mercury bioaccumulation induced oxidative stress and toxicity to submerged marcophyte *Potamogeton crispus* L. *Bull. Environ. Tox*., *65*, 573-582.
36. Dong, B., Sang, W. L., Jiang, X., Zhou, J. M., Kong, F. X. Hu, W. & Wang, L. S. (2002). Effects of aluminum on physiological metabolism and antioxidant system of wheat (*Triticum aestivum* L.). *Chemosphere*, *47*, 87-92.
37. Sachs, M. M. & Ho, T. D. (1986). Alterational gene expression during environmental stress in plants. *Annu. Rev. Plant Physiol*, *37*, 363-376.
38. Traylor, E. A., Shore, S. H., Ransom, R. F. & Donkle, L. D. (1987). Pathotoxin effects in sorghum are also produced by mercuric chloride treatment. *Plant Physiol*., *84*, 975-978.