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Oxidative Stress of Bioaccumulation of Cu, Cr and Ni in *Ulva Reticulate* in the Red Sea of Jeddah

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Abstract

We examined bioaccumulation of Cu, Cr and Ni and their effects on oxidative stress parameters in the green alga *Ulva lactuca* to determine at a Jeddah City, in order to determine their bioindicators potential.Concentrations of the selected elements (Cu, Ni and Cr) in algal tissues were 16, 24 and 11-fold higher than that in water.Algae collected from seawater had 40% lower total chlorophyll content when compared with controlled algae. On the other hand, lipid peroxidation and H_2O_2 content were increased in algae collected from seawater by 2-fold each when compared with controlled algae. Antioxidant enzymes showed difference in response, while SOD and POX were increased by 51 and 35%, respectively, CAT showed a decrease by 37% when compared with controlled algae. Our data prove that the toxicity levels of environmentally monitored or exposed heavy metals in algae can be simply and accurately estimated by measuring total Chl content, lipid peroxidation product MDA of samples, and non-enzymatic and enzymatic defense systems.

Keywords: Heavy metals bioaccumulation; Oxidative stress; Green algae; *Ulva reticulate*; Pollution

Introduction

The stability of aquatic ecosystems are negatively affected by accumulation of heavy metals and other pollutants due to intensive anthropogenic activity [1-3].

Higher plants can accumulate heavy metals in their tissues [4-6]. Contrary to them, lower plants (e.g. bryophytes and algae) have a relatively high surface/volume ratio and a differentiated epidermal layer without cuticle; thus, they absorb heavy metals with their whole surface in higher amounts [7-8].

Heavy metal-triggered generation of reactive oxygen species (ROS) in cells has been reported in higher plants [9,10]. Oxidative stress caused by heavy metals induces damage to lipids, proteins, and nucleic acids of cells. Trace metals such as Ni, Cu and Cr promote the formation of free radicals such as Hydroxyl radicals causing damage to DNA, proteins, lipids and carbohydrates. The Reactive oxygen species (ROS) are produced by the reaction causing oxidative stress. Fenton and Haber Weiss reaction plays a significant role in oxidative stress [11]. DNA bases can be modified by the Fenton gated oxidative stress and base substitutions G→C (in the presence of Ferrous), G→T and C→T (Copper and Nickel) by the reaction with ROS [12,13].

Plants evolved and developed antioxidant defense mechanisms to protect cells against oxidative stress. Enzymatic components of the antioxidant defense system include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and the ascorbate-glutathione pathway enzymes ascorbate peroxidase (APX) and glutathione reductase (GR). In addition, an increasing number of nonenzymatic antioxidant compounds are being defined, such as ascorbate, glutathione, proline, carotenoids, and phenol compounds, with roles in scavenging ROS.

Algae and lower plants in general provide important experimental models to explain complex biological processes [8]. They are also passive accumulators of heavy metals [14]. Green algae, especially, are generally considered as the best bioindicator of aquatic bodies contamination by nutrients as well as by heavy metals [15].

Algae of the Red sea have not been adequately explored for their potential as bioacuumlators of pollutants [3]. The green algae sea lettuce (*Ulva lactuca L.*) has long been used as food and as a traditional treatment for different diseases [16]. In a recent study [10] reported that Dunaliella Algae has the ability to remove pollutants from wastewater and accumulate them within its tissue. However, studies on oxidative stress level and antioxidant response mechanisms of mosses in relation to heavy metal bioaccumulation are very limited.

The aims of the present study were to evaluate bioaccumulation of selected trace metals (Cu, Cr and Ni) and their oxidative stress impacts in the green alga *Ulva lactuca* as well as its antioxidant defense response patterns.

Materials and Methods

Sampling

Algae and seawater were collected from the Red sea coast of Jeddah Saudi Arabia (0.2–2.5 m depth). 1000 ml of water was collected in acid-washed amber glass bottles [3], then they were filtered through binder-free glass fiber filters (Whatman GF/C). Filtered samples were stored in pre-cleaned (with deionized water) glass bottles at room temperature prior to analysis.

Algae (*Ulva lactuca*) were collected in plastic bags containing seawater and transported immediately in icebox to the laboratory for analysis. The samples were initially washed thoroughly with seawater to remove sand and any adhering substance and then washed thoroughly with distilled water to remove salts. Half of the algae were grown in liquid media and considered as a control [8].

Extraction of selected heavy metals

Algae were again washed with 5% ethanol to remove any epiphytes or any salts. One portion of the samples was kept under frozen condition and another small portion was kept in media containing seawater.

The remaining parts of the samples were subjected to dry air. After drying they were ground by an electrical mixer until they became a powder. Then the powdered samples were stored in a dark place, and subjected to different extraction methods.

Trace metal analysis

Air-dried algal samples were wet-digested in a mixture of HNO_3 and $HClO_4$ (4:1, v/v), and the concentrations of Cr, Cu, and Ni were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an IRIS Intrepid II XSP instrument. All chemicals used were of analytical reagent grade [4].

Determination of total chlorophyll content

Fresh weights of algae (0.15 g) were immersed in dimethyl sulfoxide (DMSO) for 60 min at 65°C in the dark. After cooling to room temperature, extracts were diluted with DMSO (1:1 v/v) and filtrated. The absorption spectra of the pigments were measured according to Aydoğan [8].

Lipid peroxidation

Lipid peroxidation was measured as the malondialdehyde (MDA) extinction coefficient [17].

H_2O_2

It was extracted from samples (0.5 g) with 5% trichloroacetic acid (TCA) and activated charcoal. Its content was calculated by using a standard.

Non enzymatic antioxidants

Reduced (GSH) and oxidized glutathione (GSSG) activities were assayed by monitoring the oxidation of NADPH [18]. Ascorbate was assessed spectrophotometrically [18].

Antioxidants enzyme activities

Ascorbate peroxidase "APX" (EC 1.11.1.11), Superoxide dismutase "SOD" (EC 1.15.1.1), Catalase "CAT" (EC 1.11.1.6) and Peroxidase "POX" (EC 1.11.1.7) were assayed [18,19].

Statistical analysis

Data were subjected to one way ANOVA using the SATATGRAPHICS statistical software package. Least Significant Difference (LSD) Test was applied to assess the significant differences among the mean values of different attributes. The values are means of 10 replications. The relationships between the accumulated of heavy metals and oxidative stress parameters

Results and Discussion

Table 1 shows the concentrations of the selected elements (CU, Ni and Cr) in water and algal tissues. Concentrations of these elements were 39.45, 28.37 and 23.83 ppm, respectively, while their concentrations in algal tissue were 673.28, 716.38 and 289.36, respectively. The magnitude of Cu, Ni and Cr metal bioaccumulation was found to be 16, 24 and 11 times increase, respectively.

Element	Seawater	Algal tissue
Cu	39.45	673.29***
Ni	28.37	716.38***
Cr	23.83	289.36***
***means are significantly different from each other at P<0.001		

Table 1: Concentrations of selected heavy metals (ppm).

Table 2 shows the effects of heavy metal accumulations on some selected biological parameters such as chlorophyll content, H_2O_2 and antioxidants in algae. Algae collected from seawater had 40% lower total chlorophyll content when compared with controlled algae. On the other hand, lipid peroxidation and H_2O_2 content were increased in algae collected from seawater by 2-fold each when compared with controlled algae.

Table 2 also show that Ascorbate was increased by 78 while ratio of GSH/GSSG was decreased by 54 in algae collected from seawater when compared with controlled ones.

Biomarker	Tissue	
	Control	Polluted
Total Chlorophyll (μg ml^{-1} FW)	16.38	9.85**
Lipid peroxidation (MDA, μmol Mg^{-1})	0.019	0.054**
H_2O_2 (nmol g^{-1})	39.27	141.27***
Ascorbate (μmol g^{-1})	17.86	31.28**
GSH/GSSG	1.93	0.89*
SOD (U mg^{-1} protein)	26.37	39.27**
POX (U mg^{-1} protein)	18.65	25.11**
APX (U mg^{-1} protein)	0.167	0.171
CAT (U mg^{-1} protein)	2.99	1.87*
***means are significantly different from each other at P<0.001.		

Table 2: Effects of heavy metal pollution on chlorophyll, H_2O_2 and Lipid contents and antioxidant enzymes.

Antioxidant enzymes showed difference in response, while SOD and POX were increased by 51 and 35%, respectively, CAT showed a decrease by 37% when compared with controlled algae. APX showed nonsignificant response (P>0.05).

Lower plants can tolerate heavy metal toxicity by binding uptaken metal cations to the cell wall anionic exchange sites created by generally negatively charged polyuronic acids [20].

Lower plants such as bryophytes (mosses) are known as bioindicators for heavy metal pollution because of their high accumulation capacity of heavy metals [21]. Chmielewská and Medved [15] have stated that the green algae have affinity for heavy metal cations.

Among heavy metals, accumulation of micronutrient Ni exhibited higher levels than micronutrient Cu and nonmicronutrient Cr (24-, 16 and 11-fold, respectively). The nonspecific cation binding was confirmed by the accumulation levels of metals irrespective of being essential for plant metabolism. The magnitude of heavy metal bioaccumulation was studied for mosses [22] but this is the first study to report such accumulation in algae in Saudi Arabia. Our study showed that the green alga *U. reticulate* has a high accumulation capacity to heavy metals.

Chl content is known to be a sensitive indicator for pollution. Cu-induced photosynthetic pigment loss was associated with loss in dry weight (data not shown). This could be attributed to the variability of the reactive potential of metals and species. Toxic effects of Cu and Cr on total chlorophyll are known for all plants [6,19]. Detrimental effects of heavy metals on the content of photosynthetic pigments are based on the inhibition of pigment synthesis [23] and/or the oxidative damage to pigments that results in changes of the total chlorophyll content. Therefore, our results confirmed that the total chlorophyll content can provide a specific and efficient definition in biomonitoring of heavy metals.

Cu and Cr and Ni induced H_2O_2 accumulation in *U. reticulate*, which causes an increase in lipid peroxidation (MDA), which indicates damage in membrane integrity and toxicity of the metal [24].

Ascorbate has an effective role in ROS quenching, α-tocopherol regeneration, and ascorbate-glutathione pathways of antioxidant metabolism [19]. The increase in ascorbate helps in alleviation of oxidative stress [25].

In contrast to the response of ascorbate, the GSH/GSSG ratio exhibits a reduction in response to heavy metal pollution which could indicate consumption GSH to scavenge ROS and/or metal binding activity in order to convert cellular redox status [26].

SOD is a first barrier against oxidative stress. Algae maintained their SOD activity well correlated with levels of H_2O_2 and lipid peroxidation. According to physicochemical properties, Cu, Cr and Ni are grouped among nonredox active metals, and they may indirectly cause oxidative stress in cells [27]. This fact could be the reason for the effects of heavy metals on SOD activity, as well as for limiting their interaction with metabolic redox status of cells.

The H_2O_2-scavenging enzyme POX is particularly located in cell walls. The induction of the POX enzyme was suggested as a potential biomarker in heavy metal toxicity. The POX activity operates in the elimination of H_2O_2 from cells.

APX plays a major role in H_2O_2 detoxification in the ascorbate-glutathione cycle by using ascorbate as an electron donor. In our study, heavy metals did not induce APX activity, thus contributes to the maintenance of cellular redox homeostasis.

CAT is an independent component of antioxidant metabolism for direct elimination of H_2O_2 without requiring any electron donor. Although heavy metal ions behave like competitive inhibitors of the CAT enzyme, results of this study showed that exposure to heavy metal pollution caused a decrease in CAT activity. Therefore, this may suggest that CAT activity is strongly based on species detoxification mechanisms and retention capacity of these metals in cell walls. However, Cu is a redox active metal and a noncompetitive inhibitor of catalase and therefore it is the most effective metal to suppress CAT activity, causing oxidative stress in cells [8]. Furthermore, our results are well correlated with high H_2O_2 content, along with lipid peroxidation.

A least-squares linear regression analysis was obtained for the accumulated metals and oxidative stress parameters (Table 3). The results show that the correlation coefficients (r) for these metals were significant at p<0.001excpet for APX. This in agreement with the previous results of Birben [13].

Stress parameter	r		
	Cu	Ni	Cr
Total Chlorophyll	0.598**	0.821***	0.506**
Lipid peroxidation	0.728***	0.901***	0.608**
H_2O_2	0.818***	0.927***	0.629**
Ascorbate	0.627**	0.839***	0.595**
GSH/GSSG	0.582*	0.769***	0.603**
SOD	0.602**	0.783***	0.611**
POX	0.629**	0.892***	0.598**
APX	0.317	0.421	0.392
CAT	0.501*	0.639**	0.608**
r-correlation coefficient, *p< 0.01, **p< 0.001, ***p < 0.001, n=10			

Table 3: Relationships between the accumulated of heavy metals and oxidative stress parameters.

Conclusion

The current point out that the Fenton metals Cu and Cr were accumulated more than non-Fenton metals Ni by the green alga *Ulva reticulate*. This accumulation indicates this alga can tolerate heavy metal pollution. Moreover, the extent of oxidative stress parameters allow us to hypothesize that this species is tolerant to the selected trace metals.

Heavy metal-induced oxidative stress triggered different components of antioxidant metabolism, most likely to activate antioxidant defense responses.

Our data prove that the toxicity levels of environmentally monitored or exposed heavy metals in algae can be simply and accurately estimated by measuring total Chl content, lipid peroxidation product MDA of samples, and non-enzymatic and enzymatic defense systems.

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