PHYSIOLOGICAL ACTIVITIES OF *ULVA LACTUCA* IN RESPONSE TO Cu AND Pb TREATMENTS.

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Abstract

A comparative study of copper (Cu) and lead (Pb) treatments to *Ulva lactuca* introduced from Damietta harbor, was conducted by examining dry weight and pigmentation, total soluble sugars, protein, ascorbate and superoxide dismutase. The lower concentrations of Cu and Pb (0.01 and 0.1 mg l^{-1} respectively) increased contents of chlorophylls, carotenoids, and total soluble sugars, and also induced a strong activation of antioxidant activity in *U. lactuca*. However, concentration (0.1 mg l^{-1}) of Pb exhibited a non significant change of dry weight and protein from untreated control. Whereas concentration (1 mg l^{-1}) of both Cu and Pb induced both of enzymatic (Superoxide dismutase) and non enzymatic (Ascorbate) antioxidants, but inhibited dry weight and contents of carotenoids, protein and total soluble sugars in *U. lactuca*. In the other hand, chlorophylls (*a* and *b*) were induced in case Cu treatment but were inhibited in case Pb treatment at the same concentration (1mg l^{-1}). Although, higher concentration (5 mg l^{-1}) of both Cu and Pb seemed to be toxic and inhibited most metabolic activities, it induced enzymatic antioxidant (Superoxide dismutase). However, Cu seemed advantageous to growth and physiological responses of *U. lactuca* than Pb. Both heavy metals particularly at the lowest concentrations are beneficial to *U. lactuca* growth and for production of antioxidants.

Keywords: Antioxidants; Carbohydrates; Growth; Heavy Metals; Protein; *Ulva lactuca.*

Introduction

Ulva appears as a valuable biosentinel of water quality in eutrophic littoral lagoons, or sheltered bays due to its massive developments and wide distribution (**Lazaridou *et al*., 1997**). *Ulva* species have shown to be particularly promising in monitoring trace metal contamination (**Villares *et al*., 2002**). Macroalgae can accumulate heavy metals, either essential or non–essential, from their living environments (**Salt *et al*., 1995**). When the extra-cellular concentration of metal ions is higher than that of intracellular, metal ions are adsorbed first to the surface of cells by the interactions between the metal ions and metal-functional groups such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thiol, etc., present in the cell wall and then they penetrate the cell membrane and enter the cells (**Wang and Chen**, **2006**), according to several possible mechanisms. Molecular mimicry is one of such mechanisms whereby metal ions either compete for binding to multivalent ion carriers or, after binding to low molecular weight thiols (such as cysteine), enter the cell by active transport. In another type of mechanism, metal ions bound to chelating proteins (such as metallothioneins) may enter the cell by endocytosis (**Van Ho *et al*.**, **2002**; **Zalups and Ahmad**, **2003**). Also, metal ions can enter the cells if the cell wall is disrupted (**Wang and Chen**, **2006**). After entering, the metal ions are

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compartmentalized into different subcellular organelles. The toxicity of algal cell primarily results from metal binding to sulphydryl groups in proteins or the disruption of protein structure or displacement of an essential element (**De Filippis and Pallaghy**, **1994**).

Beyond certain concentration thresholds, metals can induce the overproduction of reactive oxygen species, which typically result from the excitation of O_2 to form singlet Oxygen or from the transfer of one, two or three electrons to O_2 to form, respectively, superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) or hydroxyl radical ($HO^{\bullet}$). Reactive oxygen species are able to generate oxidative damage of lipids, proteins and nucleic acids (**Collen *et al*., 2003**). The amount of oxidized proteins and lipids in the algal cells indicates the severity of the stress (**Okamoto *et al*., 1996**). Reactive oxygen species are capable of unrestricted oxidation of various cellular components and can lead to oxidative destruction of the cell (**Hassan and Nemat Alla**, **2005**; **Nemat Alla and Hassan**, **2006**; **Garcia and Guil- Guerrero**, **2008**; **Pereira *et al*., 2009**). To counteract oxidative stress and protect the cells, an armory of endogenous antioxidants can be mobilized, therefore, changes in the expression of antioxidant enzymes or in the content of non-enzymatic reactive oxygen species scavengers have been used in several aquatic organisms as sensitive indicators of exposure to exogenous pro-oxidants, including metals (**Orbea *et al*., 2002**; **Aravind and Prasad**, **2005**; **Gravato *et al*., 2006**). Removal of oxygen species are regulated by antioxidants such as superoxide dismutase (SOD) and ascorbate (AsA) (**Nemat Alla**, **1995** and **2000**; **Foyer *et al*., 2001**; **Mittler**, **2002**; **Aravind and Prasad**, **2005**). Superoxide dismutase is responsible for the elimination of O^{-}_{2} generated in plant cells to H_2O_2.

The aim of this work was to study the responses of *U. lactuca* introduced from a coastal system (Damietta harbor) to treatments of Cu and Pb. Non – enzymatic and enzymatic antioxidants as well as chlorophylls, carotenoids, protein content and total soluble sugars were measured as defense mechanisms.

Materials and methods

Seaweed collection and growth conditions

Vegetative *U. lactuca* individuals were collected from Damietta harbor (longitude 31° 45′ E and latitude 31° 28′ N) in February 2009. The macroalga was transported to the laboratory, thoroughly cleaned and any epibiotics were carefully removed and then maintained in large tanks containing aerated natural sea water, under 14 hr. photoperiod at 80 -100 µmol m^{-2} s^{-1}. All experiments were conducted at 18 °C and salinity 35 gl^{-1}.

Algal central disks (5 cm diameter) were cut from the middle region of healthy thalli and placed in a 500 ml beaker with their filtered natural medium containing different concentrations of Cu and Pb (final concentrations ranged from

10-500 μgl^{-1} added as copper chloride and lead acetate in de-ionized water acidified with 1 N HCl).

Culture conditions for growth were established at 18 ^{o}C, continuous white florescent light of 80 -100 µmol photons m^{-2} s^{-1}, and salinity of 35‰. Control samples were grown in natural seawater medium without added Cu and Pb. After 3 days, the algal samples were harvested and dry weight was determined.

Assay of Superoxide dismutase (SOD; EC 1.15.1.1)

SOD activity was assayed using the photochemical nitroblue tetrazolium (NBT) method in terms of SOD's ability to inhibit reduction of NBT to form formazan by superoxide (**Beyer and Fridovich**, **1987**). Algal fresh tissue (about 2.5 g) was extracted in 50 mM phosphate buffer (pH 7.8) containing 0.1% (w/v) bovine serum albumin, 5.5 mM Ascorbate (AsA), and 8 mM ß-mercaptoethanol. SOD was assayed in 50 mM phosphate buffer (pH 7.8) containing 9.9 mM L- methionine, 0.057 mM nitroblue tetrazolium (NBT), 0.025 % (w/v) Triton x – 100, and 0.1 mM riboflavin. The photoreduction of NBT was measured at 560 nm. All extraction steps were carried out at 4^{o}C.

Assay of Ascorbate (AsA)

Ascorbate was measured according to **Mukherjee and Choudhuri** (**1983**). Algal tissue was extracted in 10 ml of 6 % Trichloroacetic (TCA). Extract (1-2 ml) was mixed with 2 ml of 2 % dinitrophenyl hydrazine (in acidic medium) and one drop of 10 % thiourea (in 70% ethanol). The mixture was boiled for 15 min and after cooling, 5 ml of 80 % H_2SO_4 was added at 0 ^{o}C (ice box). The absorbance was measured at 530 nm.

Determination of Chlorophylls and carotenoids

Contents of chlorophylls and carotenoids were determined in fresh tissue after extraction of disks in 85 % acetone according to the spectrophotometric method described by **Metzener *et al***. (**1965**).

Determination of total soluble sugars (TSS)

Total soluble sugars were extracted by overnight submersion of dry powders in 80 % (v/v) ethanol at 25 ^{o}C with periodic shaking. 0.1 ml aliquot of the alcoholic extract was heated with three ml of freshly prepared anthrone reagent for 10 minutes. The mixture was cooled and the absorbance was read at 625 nm according to **Yemm and Willis** (**1954**).

Determination of protein

Protein content was determined spectrophotometrically by reaction with Coomassie Brilliant Blue G according to **Bradford** (**1976**).

All values reported herein are means (±SE) of at least three biological replications from two independent experiments. The full data were statistically

analyzed using ANOVA test and subjected to the least significant difference (LSD) test at 5 % level (**Snedecor and Cochran**, **1980**).

Results

Figure (1) shows that the effects of Cu and Pb on dry weight content were strongly dependent on their concentrations. Dry weight of *U. lactuca* was significantly increased in presence 0.01 and 0.1 mg l^{-1} of Cu than control, but significantly reduced by 1-5 mg l^{-1}. However, Pb showed similar trend of effect with a non significant influence of 0.1 mg l^{-1}. Nevertheless, the magnitude of decrease was higher with Pb than with Cu.

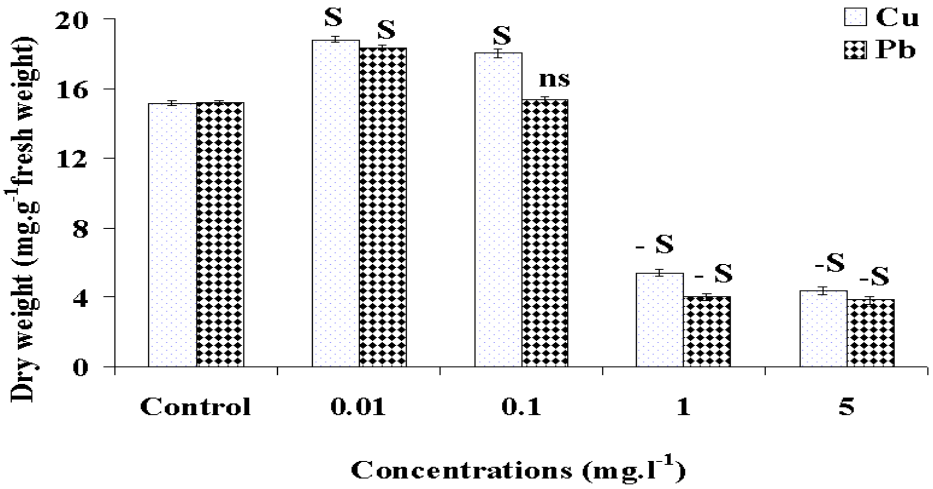

Figure (1): Changes in dry weight of *U. lactuca* after 3 days from exposure to Cu and Pb. Data are represented as means ± SE (n=3). S = positive significant difference from the untreated group ($p < 0.05$). -S= negative significant difference. ns= non significant difference. LSD (Least significant difference) values of Cu and Pb were 0.1 and 0.2 respectively

Growth is mainly controlled by protein and carbohydrate metabolism. In Figure (2) protein significantly increased in *U. lactuca* at low concentration of Cu (0.01 mg l^{-1}) but unchanged by the same concentration of Pb. Nevertheless, the highest concentrations of both elements (1 and 5 mg l^{-1}) induced significant reductions. The decrease in protein contents in *U. lacttuca* caused by enhanced protein degradation process as a result of increased protease activity.

In a similar manner, Cu and Pb at the lowest concentrations (0.01 mg l^{-1}) induced significant increase in TSS values (Figure. 3). Thereafter, 0.1 mg l^{-1} Cu had a non-significant change, whereas 0.1 mg l^{-1} Pb had a significant decreasing effect. However, both metals at the highest concentrations (1 and 5 mg l^{-1}) led to greatest decrease in TSS values. Higher concentrations of Cu (1 and 5 mg l^{-1}) decreased TSS

by 29% and 42% respectively, where Pb showed a decrease of 47 % and 41% respectively.

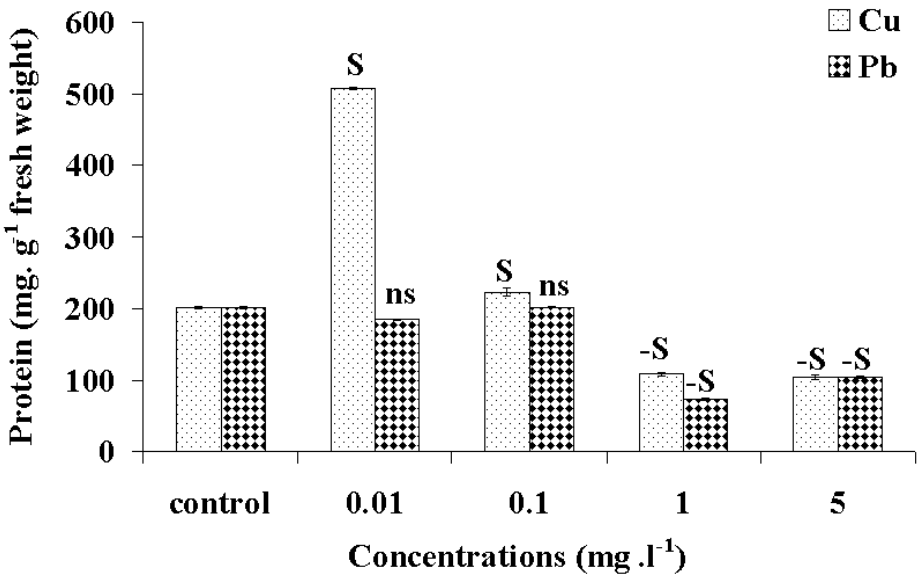

Figure (2): Changes in Protein content of *U. lactuca* after 3 days from exposure to Cu and Pb. Data are represented as means ± SE (n=3). S = positive significant difference from the untreated group (p< 0.05). -S= negative significant difference. ns= non significant difference. LSD (Least significant difference) values of Cu and Pb were 63.22 and 8.89 respectively

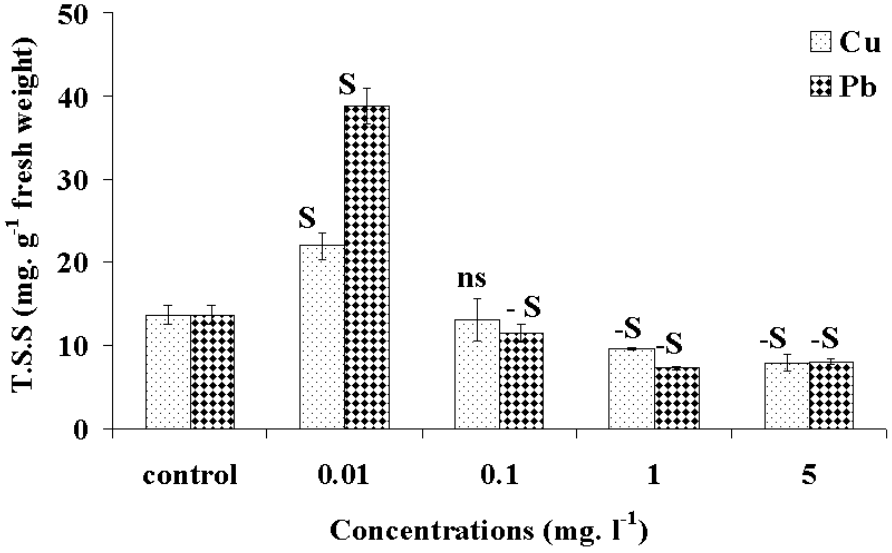

Figure (3): Changes in total soluble sugars (TSS) content of *U. lactuca* after 3 days from exposure to Cu and Pb. Data are represented as means ± SE (n=3). S = positive significant difference from the untreated group ($p < 0.05$). -S= negative significant difference. ns= non significant difference. LSD (Least significant difference) values of Cu and Pb were 0.889 and 1.979, respectively

Table (1) shows that there were changes in chlorophyll *a* and *b* contents as well as in carotenoids of *U. lactuca* with increasing of Cu and Pb concentrations. Cu at 0.01 mg l^{-1} and 0.1 mg l^{-1} increased chlorophyll *a* by 32 % and 35 % respectively, and chlorophyll *b* by 149 and 71% respectively. Similarly carotenoids increased by 121 and 97 % by Cu at both concentrations (0.01 and 0.1 mg l^{-1}). On the other hand, Pb at the same concentrations (0.01 and 0.1 mg l^{-1}) increased chlorophyll *a* by 60 % and 63 % respectively, chlorophyll *b* by 82 and 84 % respectively and carotenoids by 15 and 21 % respectively. At concentration of 1 mg l^{-1} of Cu there was significant increase in chl *a* and chl *b*, but not carotenoids. Pb concentrations of 1 and 5 mg l^{-1} had significant decrease on chlorophylls and carotenoids compared to control.

Table (1): Changes in Chlorophylls (a and b) and Carotenoids content of *U. lactuca* after 3 days from exposure to Cu and Pb. Data are represented as means ± SE (n=3). LSD (Least significant difference).

Conc. (mg l^{-1})	Chl *a*		Chl *b*		Carotenoids	
	Cu ±SE	Pb ± SE	Cu ±SE	Pb ± SE	Cu ± SE	Pb ± SE
Control	96 ± 3.03	96 ± 7.6	216 ± 3.1	216.2 ± 7.6	131.9 ± 3.1	131.9 ± 4.2
0.01	126.5± 1.67	153 ± 2.3	536.8 ± 7.1	393.3 ± 2.4	291 ± 3.5	151 ± 5.2
0.1	130 ± 2.03	156 ±1.8	369.9 ± 3.2	397.4 ± 2.6	260 ± 2.8	159.7 ± 5.9
1	96.3 ± 1.88	45 ± 0.54	274.6 ± 5.5	107.8 ± 7.9	108.9 ± 1.9	49.6 ± 1.3
5	51.6 ± 1.21	47.6 ± 1.5	79.4 ± 1.7	113.6 ± 1.5	98.7 ± 1.4	52.88 ± 1.8
LSD	2.2	1.3	12.7	4.9	13.3	13.3

The present results show that there was a significant reduction in total carotenoids by the higher concentrations of Cu and Pb (1 and 5 mg l^{-1}); the magnitude of reduction was greater with Pb relative to Cu indicating that *U. lactuca* was more tolerant to Cu than Pb.

Figures (4 and 5) show that Cu and Pb induced a significant increase in AsA and SOD contents. The magnitude of induction was greatest with the lowest concentrations. Cu at 0.01 and 0.1 mg l^{-1} increased AsA by 488 and 348 % respectively, and SOD by 336 and 129 % respectively. On the other hand, Pb at the same concentrations increased AsA by 133 and 77 % respectively, and SOD by 370 and 186 % respectively. Thereafter, this induction was being decreased with

increasing the concentrations of Cu and Pb. However, the highest concentrations of both metals (5 mg l^{-1}) exhibited a negative significant change of AsA content compared to controls. On the contrary, the activity of SOD was induced by both Cu and Pb at all the concentrations used.

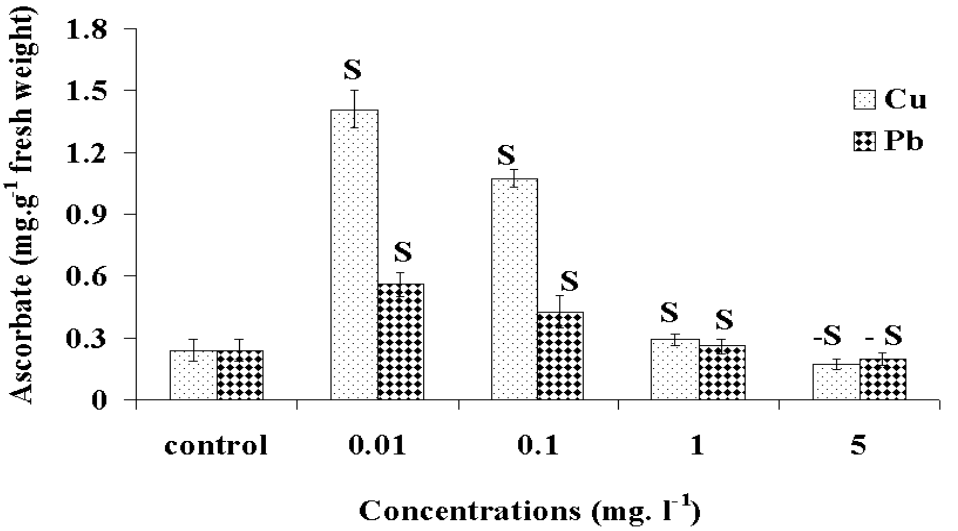

Figure (4): Changes in ascorbic acid of *U. lactuca* after 3 days of exposure to Cu and Pb. Data are represented as means ± SE (n=3). S = positive significant difference from those in the untreated group ($p < 0.05$). -S= negative significant difference LSD (Least significant difference) values of Cu and Pb were 0.021 and 0.02, respectively

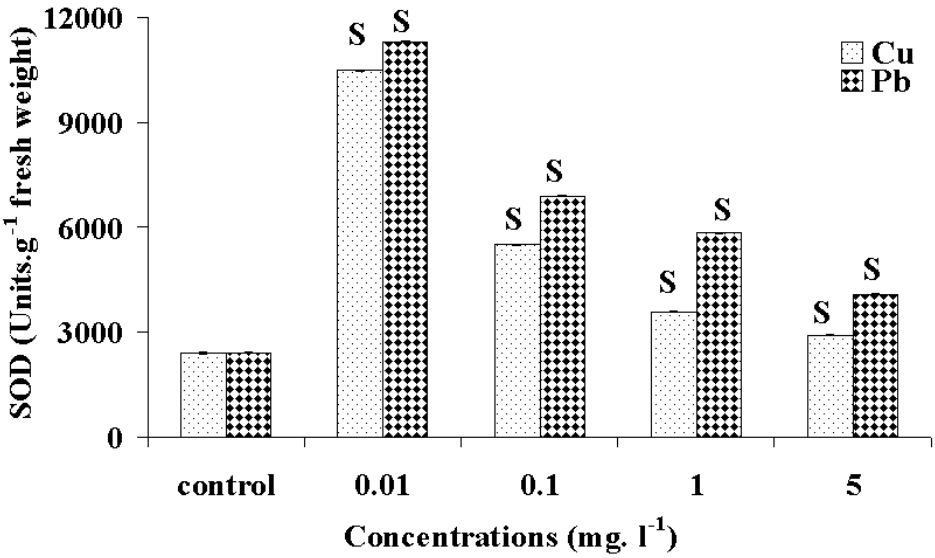

Figure (5): Changes in SOD of *U. lactuca* after 3 days of exposure to Cu and Pb. Data are represented as means ± SE (n=3). S = positive significant difference from those in the untreated group ($p < 0.05$). LSD (Least significant difference) values of Cu and Pb were 49.4 and 43.5, respectively

Discussion

The results of dry weight showed that, *U. lactuca* seemed to be more tolerant to Cu than Pb at each concentration. These findings are in accordance with other researches such as **Reed and Moffat** (**1983**), who revealed that, the threshold concentration that resulted in a significant reduction in dry weight was 1 mg l^{-1} of Cu and Pb. Other studies have also reported that significant reductions in *Ulva* growth occur at concentrations greater than 0.1 mg l^{-1} of copper (**Correa *et al*., 1996**). Copper induced interference with cell division and/or expansion has been proposed as possible reason for the observed reduction in growth (**Stauber and Florence, 1987**). Moreover, a decrease in turgor and/or a change in cell wall elasticity due to copper toxicity could lead to growth cessation (**Brown and Newman**, **2003**).

On the other hand, growth is mainly controlled by protein metabolism. The decrease in protein content in *U. lactuca* may be caused by enhanced protein degradation process as a result of increased protease activity (**Palma *et al.* 2002**) that is found to increase under stress conditions. **Gadd and Griffith** (**1978**) reported that heavy metals have the ability to denature proteins and stimulate the hydrolytic activity of protease. Toxicity of a metal seems to be related to cell surface interactions or to intracellular accumulation (**Morlon *et al*., 2005**). Toxicity primarily results from metal binding to sulphydryl groups in proteins or the disruption of protein structure or displacement of an essential element (**De Filippis and Pallaghy**, **1994**).

Metal detoxification by binding to peptides or proteins is another metal regulatory mechanism that could be active in organisms (**Mitchelmore *et al*., 2003**). The degradation and/or modification of bio-molecules, such as nucleic acids, lipids, proteins and other cellular constituents by ROS have been associated with ageing process (**Cooke *et al*., 2002**). For these reasons, many products from marine algae with antioxidants properties are widely used in order to minimize oxidative damage to living cells and to prevent oxidative deterioration of food.

Whereas in higher concentrations of Cu and Pb decreased TSS. The present results are in accordance with the findings of **Ahmed *et al*.** (**2006**) who found an increase in soluble sugars in *Pisum sativum* at low concentrations of salt stress and a decrease at higher concentrations. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase/oxygenase (**Stiborova *et al*., 1987**). These results may point to conclude that heavy metals possibly increase protein lysis and/or carbohydrate hydrolysis. Moreover, synthesis of protein and carbohydrates may by affected also by heavy metals. In this context, pollution of the environment with

excess of Pb retarded algal growth, decreased chlorophyll content and reduced chlorophyll stability to heat. Plants growing in Pb polluted soil accumulated much more free amino acids and less soluble sugars than the control plants (**John *et al*., 2008**).

There were changes in the content of chlorophyll *a* and *b* as well as in carotenoids of *U. lactuca* with increasing of Cu and Pb concentrations. Such a stimulatory effect of low concentrations of Cu on chlorophyll accumulation has previously been observed in copper–tolerant higher plants (**Maksymiec and Baszynski**, **1996**). More generally, significant increases in chlorophyll have been found to occur in response to range of environmental stresses and are associated with stress resistance (**Zhang *et al*., 2005**). Moreover, increases in chl *a* and *b* were considered to be an effective protective mechanisms of *U. rigida* against ultraviolet–B radiation (**Altamirano *et al*., 2000**) and of the aquatic plant, *Lemma gibba* (**Babu *et al*., 2003**).

On the contrary, a reduction in chlorophylls and carotenoids, due to higher concentration could be resulted from either a decrease in biosynthesis or an increase in the rate of degradation (**Gledhill *et al*., 1997**). **El-Baz *et al*.** (**2002**) reported that, the pigment contents in algae can be affected by several environmental factors among which hypersalinity stress.

The decline in chlorophyll content by Cu^{2+} and Pb^{2+} is believed to be due to inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase, impairment in the supply of Mg^{2+} required for the synthesis of chlorophylls and Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (**Van Assche and Clijsters**, **1990**).

The present results show that there was a significant reduction in total carotenoids with high concentrations of Cu and Pb; the magnitude of reduction was greater with Pb relative to Cu indicating that *U. lactuca* was more tolerant to Cu than Pb. Lead is a toxic non-essential heavy metal, once entering the cell, the ions may either be detoxified or adversely affect cell processes such as photosynthesis and cell division (**Patra *et al*., 2004**).

Abd El-Baky *et al*. (**2008**) reported that, chemical constituents of pigment produced by *U. lactuca* were differed quantitatively and qualitatively as a result of changing nutrient composition of growth medium. They concluded that, *U. lactuca* grown in artificial sea water has the ability to accumulate high amounts of β-carotene coupled with depletion in Chl *a* as compared with that in algae grown in natural sea water.

In addition to the role of photosynthetic pigments in primary metabolism, carotenoids have essential roles in protection of chlorophylls from photodestruction and as antioxidants active in scavenging superoxide and hydroxyl radicals, chelating ability, quenching singlet and triplet oxygen, and reducing power. Also, antioxidants

are essential for algae to counterbalance the stress imposed by several stimuli. Lots of algal and algae-derived compounds exhibited potent antioxidant such as carotenoids, phenolics, terpenoids and sulphated polysaccharides (**Ruberto *et al*., 2001**; **Athukorala *et al*., 2006**).

Cu and Pb induced a significant increase in ascorbate contents. The magnitude of induction was greatest with the lowest concentrations (0.01 mg l^{-1}). Thereafter, this induction was being decreased with increasing the concentrations of Cu and Pb. However, the highest concentrations of both metals (5.0 mgl^{-1}) exhibited a negative significant change from untreated controls of AsA content. AsA is the most abundant low molecular weight non-enzymatic antioxidants in plant cells participating in ROS scavenging through the AsA-GSH cycle (**Foyer *et al*., 2001**). AsA eliminates ROS through multiple mechanisms. It maintains the membrane –bound antioxidant α-tocopherol in the reduced state, eliminates H_2O_2 through the activity of AsA peroxidase and has a major role in photoprotection as a cofactor in the xanthophylls cycle (**Jimenez *et al*., 1997**). Therefore, the changes in AsA and GSH levels might explain the relative susceptibility of *U. lactuca* to heavy metals. The mechanism of defense of AsA and GSH (in AsA-GSH cycle) involves scavenging the potent reactive H_2O_2 (**Murgia *et al*., 2004**).

Similarly, the activity of SOD was induced by Cu and Pb at all the concentrations used. The increased activity was more pronounced with the lowest concentrations and retracted with increasing the concentrations. SOD is a key enzyme in protecting cells against oxidative stress. It catalyses the dismutation of O_2^- to H_2O_2 and O_2. Moreover, **Kurama *et al*.** (**2002**) concluded that overproduction of SOD in plant chloroplast leads to protection against some xenobiotics.

Similarly, algae have evolved adaptive ways to combat environmental stresses, adjusting the antioxidative enzyme being one such adaptive mechanism (**Tang *et al*., 2007**). It is likely that oxidative stress in cells depends mainly on maintaining a balance between ROS content and the antioxidative system – a strategy that may be an adaptive response that protects the alga from more extensive and irreversible oxidative damage (**Tang *et al*., 2007**). Ionic copper toxicity may result from an intracellular reaction between copper and reduced glutathione (GSH), leading to a lowering of the GSH:GSSG ratio and suppression of mitosis. In addition, copper inhibits the enzyme catalase and reduces cell defense mechanisms against H_2O_2 and oxygen-free radicals (**Stauber and Florence, 1987**).

Conclusion

The present results concluded that, *U. lactuca* seemed to be tolerant the low concentrations of Cu and Pb. Indeed, growth increase concomitant with induction of protein, carbohydrate and pigments as well as stimulation of AsA and SOD activity could confirm such tolerance.

However, Cu appeared more beneficial to *U. lactuca* than Pb. Anyway, water polluted with Cu and Pb particularly low concentrations could be used for growing *U lactuca*. Such process might lead to production of the macroalga which seemed beneficial as it used for phytoremediation of heavy metals and also as a source of natural and safe antioxidants instead of those synthetic toxic antioxidants, which are commonly used to inhibit lipid peroxidation and in the meantime are responsible for liver damage, promoters of carcinogenesis and alteration the enzyme activities.

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أنشطة فسيولوجية لطحلب *يولفا لاكتيوكا* استجابة للمعالجة بالنحاس والرصاص

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في تجربة معملية تمت دراسة التغيرات الفسيولوجية لطحلب *يولفا لاكتيوكا* الذى تم جمعه من ميناء دمياط نتيجة وتم معالجته بعنصري بتركيزات مختلفة من النحاس والرصاص 0.01 الي 5 مليجرام/لتر. وبمقارنة النتائج وجد أن التركيزات المنخفضة (0.01 مليجرام/لتر و 0.1 مليجرام) من العنصرين استحثت زيادة كل من الوزن الجاف و السكريات الكلية الذائبة و البروتين والكلورفيل أ,ب والكاروتينات كما نشطت Ascorbate Superoxide dismutase. بخلاف التركيز العالي (5 مليجرام/لتر) من العنصرين الذى ثبطت كل من الوزن الجاف و السكريات الكلية الذائبةو البروتين ,وكلورفيل أ و ب والكاروتينات و مضادات الأكسدة غير الإنزيمية الا أنه نشط مضادات الأكسدة الإنزيمية Superoxide dismutase. بينما التركيز 1 مليجرام/لتر من النحاس والرصاص نشط (Superoxide dismutase) و (Ascorbate), إلا أنه ثبط الوزن الجاف , السكريات الكلية الذائبة, البروتين و الكاروتينات . ولكن نفس التركيز ثبط الكلورفيل أ و ب في حالة الرصاص بينما نشط الكلورفيل أ ,ب في حالة النحاس. وعليه لوحظ أن قيمة الاستحثاث الخاصة بعنصر النحاس كانت أكبر من الناتجة من الرصاص مما يدل علي أن طحلب *يولفا لاكتيوكا* أكثر تحملاً لتركيزات المرتفعة من النحاس عن الرصاص. ويستنتج من ذلك أن تركيز متخفض من عنصر النحاس هام في نمو الطحلب للحصول علي نمو عالي من الطحلب لإنتاج مضادات الأكسدة.