

## RESPONSE OF ANTIOXIDANT SYSTEM IN SOME MARINE ALGAE UNDER CADMIUM STRESS

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### ABSTRACT

*Ulva lactuca* and *Carolina mediterranean* were collected from Balteem Coast of Egypt. These two algae represented the most common species in two major divisions Chlorophycophyta and Rhodophycophyta which occur in Balteem coast. Treatment of both algae with CdCl<sub>2</sub> at various concentrations (50-250 µmol) resulted in an increase in the activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2). The increase in the activities of the four enzymes was proportional with the lower CdCl<sub>2</sub> concentrations. However, the higher concentrations (200 and 250 µmol) of CdCl<sub>2</sub> resulted in reduction of enzymes activities in both algae. In addition, CdCl<sub>2</sub> increased the reduced glutathione (GSH) and decreased the oxidized glutathione (GSSG) contents of both algae. Also, the contents of proline and glycine betaine (GB) as well as lipid peroxidation increased with the lower concentrations of CdCl<sub>2</sub> and decreased at the higher concentrations. However, lipid peroxidation increased continuously with increasing CdCl<sub>2</sub> concentrations.

**Keywords:** *Ulva lactuca*, *Carolina mediterranean*, Lipid peroxidation, Antioxidant enzymes, Reduced glutathione, Oxidized glutathione, Proline, Glycine betaine.

### INTRODUCTION

Macroalgal communities provide nutrition, reproduction, and an accommodating environment for other living organisms in marine ecosystems (McClanahan *et al.*, 2002). Because they contain proteins, carbohydrates and other nutritional elements. Macroalgae are one of the most important organisms.

Algae contain several enzymatic and nonenzymatic antioxidant defense systems to maintain the concentration of ROS (O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>) to protect cells from damage (Abd El-Baky *et al.*, 2004). The main cellular components susceptible to damage by these ROS are lipids, proteins, carbohydrates and nucleic acids (Susuki & Mittler, 2006). Damages in the polyunsaturated fatty acids of cell membrane under stress may result in a failure in the permeability of cell membrane and cause cell death (Gutteridge & Halliwell, 2000).

The primary scavenging enzymatic antioxidant defense system include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) (Mittler *et al.*, 2004).

GSH is an important non-enzymatic antioxidant in macroalgae. It has been documented that GSH can remove ROS, such as OH·, O<sub>2</sub><sup>-</sup>, <sup>1</sup>O<sub>2</sub> and alkyl peroxide, and also protect proteins from the oxidation of protein thiol groups (Noctor *et al.*, 2002).

Cd has so far unknown roles in living organisms, and are toxic even at very low concentrations (Nies, 1999). Heavy metals in general affect algae

through cell lysis, growth inhibition, reduced photosynthesis, disrupted calcification, disturbances in sexual reproduction, and changes in bioluminescence (Kupper *et al.*, 2002). In addition, heavy metals induce the production of reactive oxygen species (ROS) and cause a disbalance in the cellular oxidative status (Okamoto *et al.*, 2001a & 2001b).

Heavy metals block functional groups of proteins, displace and/or substitute essential metals, induce conformational changes, denature enzymes and disrupt cells and organelle integrity (Hall, 2002). Different heavy metals have been reported to affect macroalgae by interacting with enzymes and inhibiting their normal functions (Van Assche & Clijsters, 1990).

Cadmium is one of the most toxic water pollutants which penetrate into environment mainly through industrial processes and phosphate fertilizers. The bases of Cd toxicity are still not completely understood, but it might result from its high affinity for sulfhydryls (Schützendübel & Polle, 2002).

Thus, the aim of the present investigation is to study the effect of CdCl<sub>2</sub> at different concentrations (50-250 µmol) on the antioxidant enzymes and non-antioxidant compounds in *Ulva lactuca* and *Carolina mediterranean* collected from Balteem coast of Egypt.

## MATERIALS AND METHODS

### Location of collection

One station along Balteem coast of Egypt was chosen for collecting the algae under investigation, the algae were taken for analysis.

### Algal Material

The algae used in this study were *Ulva lactuca* and *Carolina mediterranean*. These algae represent the most common species in the two major divisions Chlorophycophyta and Rhodophycophyta which occur in this area. Some samples of *Ulva lactuca* and *Carolina mediterranean* were transported in ice-box directly to the laboratory. These samples were washed with distilled water several times and purified from sand and epiphytes before using in the extraction of antioxidant enzymes and non-antioxidant enzyme systems.

### Identification of algae

Algal taxa were identified according to Nasr (1947), Aleem (1978) and El-Nagger (1980).

### Treatment with CdCl<sub>2</sub>

The thalli were treated with 20 ml of CdCl<sub>2</sub> at various concentrations (50, 100, 150, 200, 250 µmol) or 20 ml of distilled water (control) for 24 h. After treatment, the thalli were used for preparation of the extract for measuring enzymic and nonenzymic antioxidants. All the treatments were carried out on orbital rotating plates (60 rpm) in light (150 µmol m<sup>-2</sup> s<sup>-1</sup>) at 28 °C

### Preparation of algal extract

Samples of *Ulva lactuca* and *Carolina mediterranean* were used for enzyme extraction as follow. The thallus was ground in 100 mM phosphate buffer (pH 7.0), 1.0 mM DTT and then centrifuged at 5000 g for 20 min. The supernatant was collected and used for determination of the contents of non-enzymic antioxidants as well as the activities of antioxidant enzymes.

**Determination of enzymes activities:**

**Superoxide dismutase (SOD, EC 1.15.1.1):**

SOD activity was assayed by measuring the inhibition of photochemical reduction of NBT (Giannopolitis & Reis, 1997).

**Catalase (CAT, EC 1.11.1.6):**

Catalase activity was measured according to Kato & Shimizu (1987).

**Ascorbate peroxidase (APX, EC 1.11.1.11):**

APX activity was determined according to the method of Nakano & Asada (1981).

**Glutathione reductase (GR, EC 1.6.4.2):**

Assay of GR was based on the method described by Carlberg and Mannervik (1985) and depends on the oxidation of NADPH at 340 nm.

**Determination of glutathione (GSH):**

The GSH content of algal cell extracts was measured by reaction with 5,5'-dithiobis-2-nitrobenzoic (DTNB) according to Silber *et al.* (1992).

**Estimation of proline content:**

The proline content was estimated by the method of Bates *et al.* (1973).

**Estimation of glycine betaine (GB) content:**

GB concentration in *Ulva lactuca* and *Carolina mediterranean* extract was determined according to the method of Wyn Jones & Storey (1977).

**Determination of lipid peroxidation**

The assay principle is that malondialdehyde (MDA), a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) in acidic medium and the absorbance was read at 532 nm (Heath & Packer, 1968).

**Statistical analysis**

All the data in the present study are expressed as mean  $\pm$  SE obtained from three measurements.

## RESULTS AND DISCUSSION

Studying the effect of various CdCl<sub>2</sub> concentrations (50-250  $\mu$ mol) on SOD activities (Fig. 1) in both *U. lactuca* and *C. mediterranean*, revealed that *U. lactuca* expressed lower activity than that expressed by *C. mediterranean*. The Cd induction of SOD activity has also been observed in the marine microalgae *Tetraselmis gracilis* (Okamoto *et al.*, 1996). In the marine dinoflagellate *Gonyaulax polyedra*, the activity of SOD was induced by exposure to acute Cd (Okamoto & Colepicolo, 1998).

The reduction in the level of SOD activity at high concentration of CdCl<sub>2</sub> in present investigation could be due to the inhibition of the enzyme through reaction with -SH group required for enzyme catalysis. Also, the reduction could be due to the increase of superoxide anion radical during its metabolism.

CdCl<sub>2</sub> induced catalase activity in *U. lactuca* and *C. mediterranean* at the lower concentrations (Fig 2). However, *C. mediterranean* expressed higher catalase activity than *U. lactuca*. The induction of CAT activity by Cd has been observed in the red macroalgae *Gracilaria tenuistipitata* (Collén *et al.*, 2003).

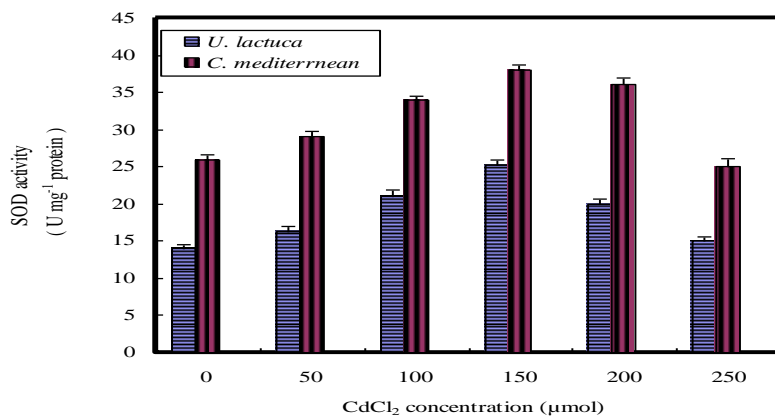


Fig.1: Effect of CdCl<sub>2</sub> on SOD activity.

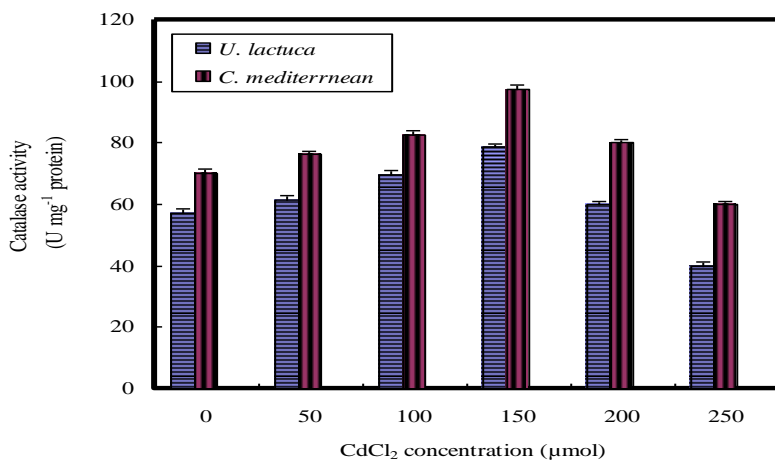


Fig.2: Effect of CdCl<sub>2</sub> on catalase activity.

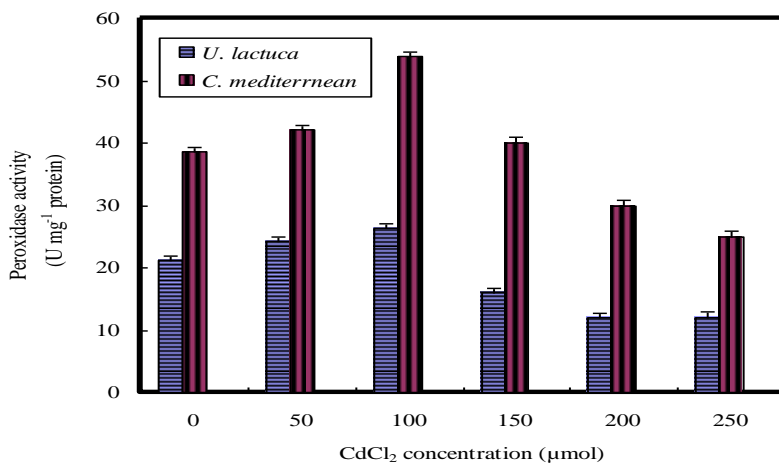
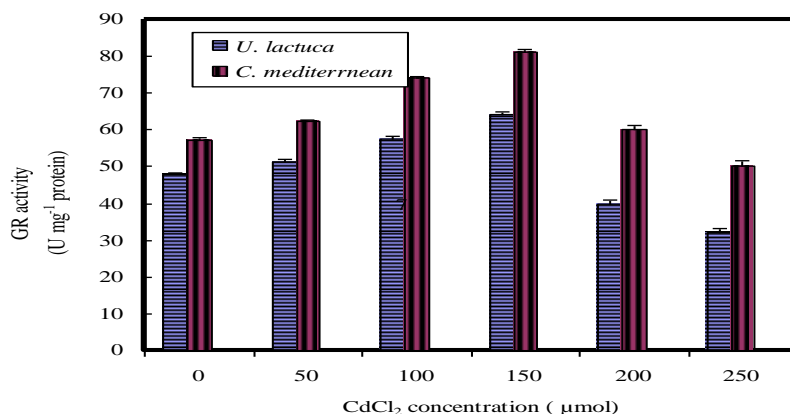


Fig.3: Effect of CdCl<sub>2</sub> on peroxidase activity

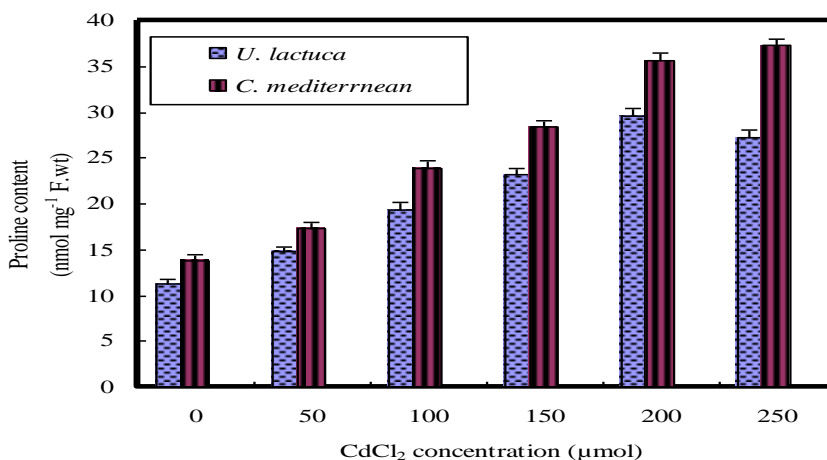
The observed reduction of catalase activity at the higher concentrations of CdCl<sub>2</sub> may be explained by insufficient supply of NADPH, which is required for the activation of catalase for its regeneration from its inactive form. Decline in the catalase activity under high stress condition may be due to increased accumulation of H<sub>2</sub>O<sub>2</sub> thereby leading to augmented lipid peroxidation

The activity of peroxidase was increased in both algae progressively with increasing of CdCl<sub>2</sub> concentrations (Fig. 3). *U. lactuca* expressed lower GR activity than *C. mediterranean* when treated with CdCl<sub>2</sub> at the various concentrations (Fig. 4).

The present data show that proline content was apparently increased at the lower concentrations of CdCl<sub>2</sub> but decreased at the higher ones. (Fig. 5). In addition, *C. mediterranean* showed higher activity than *U. lactuca*.



**Fig.4: Effect of CdCl<sub>2</sub> on GR activity.**



**Fig.5: Effect of CdCl<sub>2</sub> on proline content**

*C. mediterranean* expressed higher content of GB compared to that of *U. lactuca* (Fig. 6) and there was corresponding increase in GB content on treatment with the lower CdCl<sub>2</sub> concentrations. However, there was a decrease in its content at the higher concentrations.

The content of GSH as antioxidant was increased in both *U. lactuca* and *C. mediterranean* (Fig. 7) under treatment with various concentrations of CdCl<sub>2</sub>. On the other hand, GSSG content was reduced particularly with *C. mediterranean* (Fig. 8). It was remarkable that the reduction of GSSG content in both algae was dependent on CdCl<sub>2</sub> concentration. Under metal stress conditions seaweeds may use GSH primarily to counter oxidative stress from exposure to a mixture of toxic metals.

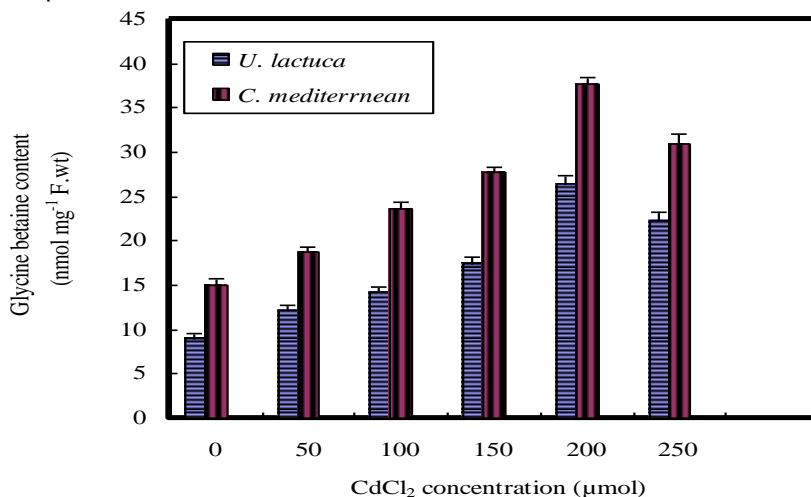


Fig.6: Effect of CdCl<sub>2</sub> on glycine betaine content.

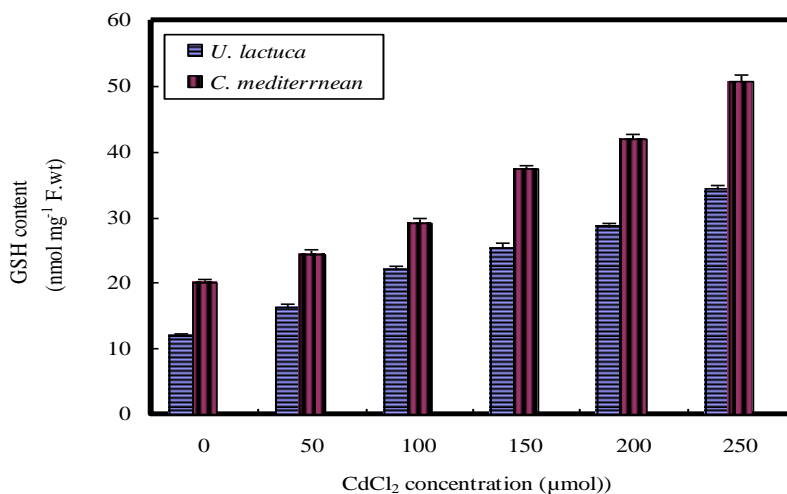
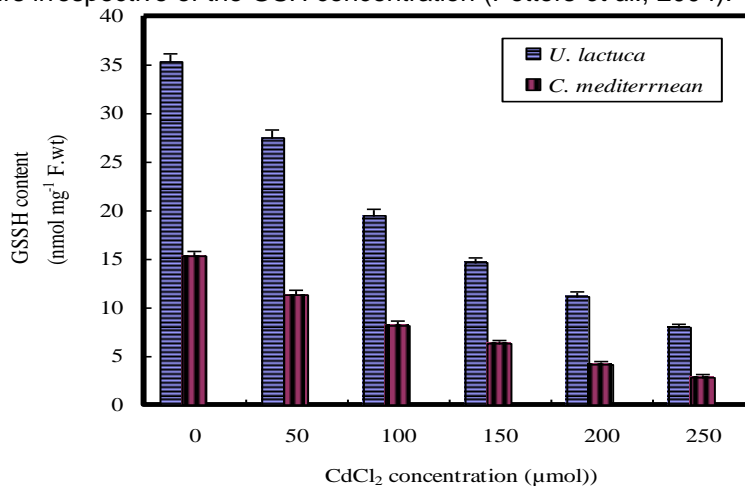


Fig. 7: Effect of CdCl<sub>2</sub> on GSH content.

Cd-induced increase of GSH was also observed in hyphomycetes (Miersch *et al.*, 1997). The increase in GSH content has been reported also for plant and algal cells subjected to stress by metals (Okamoto *et al.*, 2001 a; Lavoie *et al.*, 2009). Such increase in GSH content might be an important defense response to stresses as has been found in Cd-stressed plants (Pietrini *et al.*, 2003) although defense against stress situation sometimes occurs irrespective of the GSH concentration (Potters *et al.*, 2004).



**Fig. 8: Effect of CdCl<sub>2</sub> on GSSG content.**

Glutathione is used for synthesis of phytochelatins, which perform the intracellular sequestration of heavy metal ions in plants and algae (Ahner & Morel, 1995; Cobbett & Goldsbrough, 2002). Furthermore, the increase in GSH content under Cd stress was reported to be essential for the synthesis of phytochelatins, which tends to form stable complexes with Cd and minimize the toxicity of free metal ions (Srivastava *et al.*, 2004). GSH is able to modify metal toxicity by altering the rates of metal uptake and elimination (Kang, 1992) and by chelating metal ions in cells (Freedman *et al.*, 1989). In addition, the increase in GSH protects SH-containing enzymes from oxidation (Iannelli *et al.*, 2002) and GSH is essential as electron donor for the generation of ascorbic acid as another antioxidant in plant cells. Glutathione chelates Cd through sulfhydryl coordination (Rabenstein, 1989).

However, glutathione concentration in *Ulva lactuca* and *Carolina mediterranean* was declined at the higher concentrations of CdCl<sub>2</sub>. These results are in agreement with those reported for *Gracilaria tenuistipitata* (Collén *et al.*, 2003) and in the marine green macroalgae *Enteromorpha linza* (Malea *et al.*, 2006) under heavy metal stress.

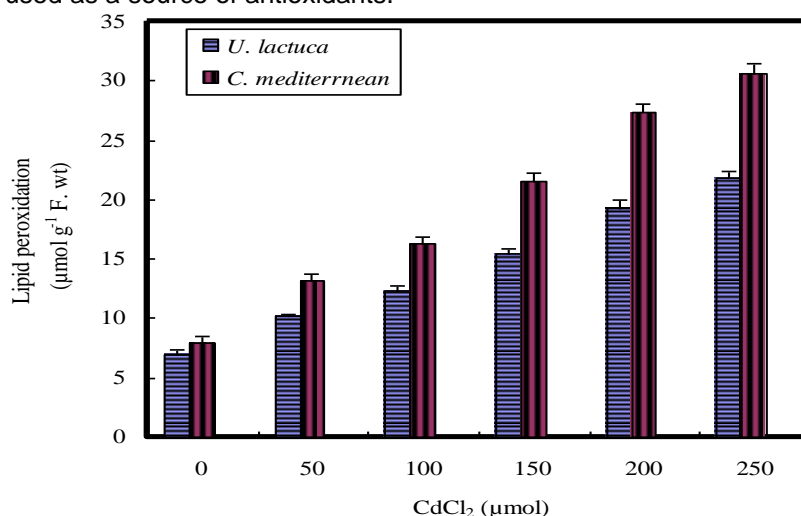
The lowered levels of GSH in algal extract under high metal stress conditions represent as excess free radical production, which may be due to the binding of heavy metal with various sulfhydryls that exist in the cell (Vijayavel *et al.*, 2006).

The effect of CdCl<sub>2</sub> on lipid peroxidation of *U. lactuca* and *C. mediterranean* at various concentrations (50-250 μmol) was investigated and

the results are illustrated in Fig. 9. The results indicate that there was a correlation between CdCl<sub>2</sub> concentrations and lipid peroxidation in both algae. The results also show that lipid peroxidation was higher in *C. mediterranean* than *U. lactuca*. It has been reported that Cd treatment resulted in an increase of membrane integrity loss (Schützend übel & Polle, 2002). Cd does not appear to generate free radicals, but it does elevate lipid peroxidation which results indirectly in ROS production (Gutteridge & Halliwell, 1988).

Marine phytoplankton are likely to be one of the important sources of low molecular weight thiols such as glutathione in surface seawater, since thiols have been shown to correlate well with Chl a levels in coastal seawater (Al-Farawati & Van Den Berg, 2001). Thus, it is important to understand possible controls on the intracellular concentrations of these compounds in marine algae.

In conclusion, both *U. lactuca* and *C. mediterranean* expressed appreciable activities of antioxidant enzymes as well as nonenzymic antioxidants under Cd-stress. These results suggest that both algae can be used as a source of antioxidants.



**Fig. 9: Effect of CdCl<sub>2</sub> on lipid peroxidation..**

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**استجابة النظام المضاد للأكسدة في بعض الطحالب البحرية تحت إجهاد الكادميوم**  
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تم جمع عينات من ألفا لاكتوكا و كارولينا مديترنيان من ساحل بلطيم بمصر وهذان الطحلبان ينتميان إلى الطحالب الخضراء الطحالب الحمراء بالتتابع. تم معاملة الطحالب بتركيزات مختلفة من محلول كلوريد الكادميوم (٥٠، ١٠٠، ١٥٠، ٢٠٠، ٢٥٠ ميكرومول) ثم تم تعيين أنشطة الإنزيمات المضادة للأكسدة مثل ديسميوتيز سوبر أكسيد، الكاتاليز، بيروكسيديز والاسكوريات ريدكتيز الجلوتاثيون بالإضافة إلى قياس محتوى كل من الجلوتاثيون المختزل والمؤكسد، البرولين، الجليسين بيتان. وتم كذلك تقدير أكسدة الدهون. ولوحظ أن التركيزات المنخفضة من محلول كلوريد الكادميوم أدت إلى زيادة نشاط الإنزيمات الأربعة السالف ذكرها بينما التركيزات المرتفعة أدت إلى انخفاض أنشطة الإنزيمات بمعدل مختلف. كذلك أدت المعاملة إلى زيادة محتوى كل من الجلوتاثيون المختزل والبرولين والجليسين بيتان بمعدلات مختلفة إلا أن التركيزات المرتفعة من محلول كلوريد الكادميوم أدت إلى انخفاض محتويات المركبات الثلاثة. أبدى محتوى الجلوتاثيون المؤكسد إنخفاضا مستمرا بزيادة تركيز محلول كلوريد الكادميوم وهذا على النقيض من الجلوتاثيون المختزل. كذلك أظهرت النتائج وجود علاقة طردية بين تركيز محلول كلوريد الكادميوم وزيادة أكسدة الدهون ومن هذه النتائج يستنتج أن الطحالب محل الدراسة يمثلان مصدرا هاما لمضادات الأكسدة.

**قام بتحكيم البحث**

**كلية الزراعة – جامعة المنصورة**  
**كلية العلوم – جامعة طنطا**

**أ. د/ محب طه محمد صقر**  
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