CADMIUM STRESS ASSESSMENT IN *ULVA LACTUCA* (CHLOROPHYTA) AND *PADINA PAVONICA* (PHAEOPHYTA) MARINE ALGAE

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(Received on Date: 18th August 2016 Date of Acceptance: 11th November 2016)

ABSTRACT

Ulva lactuca (Chlorophyta) green and *Padina pavonica* (Phaeophyta) brown marine algae species were evaluated under different cadmium (Cd) (0, 2.5, 5 and 10 mg/L) concentrations for 4 days. Our data revealed decrease in specific growth rate (SGR%), pigments (Chlorophyll *a* & *b*, total chlorophyll and total carotenoids) content and osmotic potential combined with increased electric conductivity (EC) under Cd applied concentrations in the both examined algae species. In this respect ,carotenoids pigment content increased in *P. pavonica* as Cd applied concentration increased, in contrary trends compared to *U. lactuca*. Based upon the above examined physiological parameters, the current investigation could suggest that *U. lactuca* was most sensitive to Cd stress than *P. pavonica;* by showing greatest reduction in the above physiological indices than *P. pavonica*. Physiological test successfully discriminate Cd toxicity between the two examined algae species.

Keywords: *Ulva lactuca*, *Padina pavonica*, cadmium stress, toxicity

No:of Tables: 1 **No:of Figures: 5** **No:of References: 20**

INTRODUCTION

Marine ecosystems pollution by different heavy metal pollutants became a huge problem and has been very interesting to researchers looking for potential and promising source for heavy metals detoxification. Chemical traditional methods have been employed to removing heavy metal. However, due to their high cost, side effects and their effectiveness only against some metals (Lamai *et al*., 2005) they were replaced by other tools. Thereby, big efforts have been done to employ a bio removal tools as a new technology based on living organisms such as algae. Their abundance worldwide, vast growth and their capacity to concentrate high amount of metals in their tissues with low cost, make them serve as a potential, effective and promising tool for metals detoxification in ecosystems.

Cadmium, mercury and lead are considered the three contaminants of greatest threat to the environment according to the US Environmental Protection Agency (EPA) (Jamers *et al*., 2013). Negative effects of different heavy metals in algae and other plants have been also demonstrated in many reports such as: Cd in *Hydrilla verticillata* (Garg *et al*., 1997) and *Microcystis aeruginosa* (Zhou *et al*., 2006); Chromium (VI) in *U. lactuca* (Unal *et al*., 2010); Nickel, Cobalt, Chromium and Zinc in *Phaseolus vulgaris* L. (Zengin 2013) and Pb in wheat (*Triticum aestivum* L.) (Pazoki *et al*., 2014). Many researchers reported Cd effects on algae; *e.g*. in *Chlorella sp*. green algae (Kaplan *et al*., 1995); *Cladophora fracta* green algae (Lamai *et al*., 2005); two strains (Iranian and Australian) of *Dunaliella salina* green algae (Shariati and Yahyaabadi 2006); *Hypnea musciformis* red (Bouzon *et al*., 2012); *Spirulina (Arthrospira) Indica* blue green algae (Siva Kiran *et al*., 2012); Agarophyte *Gracilaria domingensis* red algae (dos Santos *et al*., 2012), in *U. prolifera* and *U. linza* green algae (Jiang *et al*., 2013); and recently Pb, Cu, Cd and Zn in *U. lactuca* (Saleh 2015).

Extensive investigations mentioned toxicity of Cd at physiological level in plant and algae manifested by plant growth and photosynthetic activity inhibition (Bouzon *et al*., 2012; dos Santos *et al*., 2012; Jiang *et al*., 2013). Reactive oxygen species (ROS) induction is considered as one of fewer unfavorable effects of Cd stress (Collén *et al*., 2003; Bouzon *et al*., 2012). Chlorophyll (Chl) content decline in plant is a typical impact of Cd and Pb ions, particularly, Chl *a* is less affected than Chl *b*, due to the inhibition of chlorophyll synthesizing enzymes and the lack of Mg and Fe (Xia *et al*., 2004); or could be related to the interaction of Cd with –SH groups of various enzymes involved in chlorophyll biosynthesis (Griffiths 1975; Garg *et al*., 1997).

Toxicity of Cd in *U. lactuca* and *P. pavonica* algae has not investigated in detail. Thereby, the current investigation aimed to study different concentrations of Cd impacts in the two marine algae species, in comparative study. So, Cd toxicity in the two examined algae species will be allow somewhat to discriminate potent of algae species to tolerate the Cd toxicant element.

MATERIALS AND METHODS

Algae collection's: Algal samples for both *U. lactuca* and *P. pavonica* species were collected along the Syrian coast of the Mediterranean Sea. Collection of samples was carried out from 34°37′734″N longitude, 38°29′766″E latitude at 4 km North Lattakia - Syria. Only individuals with the similar size were harvested by hand with disposable gloves, biomass was washed with seawater where the algae were collected and then transported within a flask with 5 L seawater.

Algae cultivation and cadmium stress application: The *U. lactuca* and *P. pavonica* species were evaluated under different concentrations of Cd ions under Cd $(NO_3)_2$ forma salt [(Standard solution (1000 mg/L) from Fisher Scientific – UK)]. Algae were washed twice after their arrival to laboratory with autoclaved artificial seawater ASW (500 mM NaCl, 10 mM KCl, 30 mM $MgSO_4$, 10 mM $CaCl_2$ and 10 mM Tris-HCl at pH 7.8) medium as previously described by Unal *et al*. (2010). Then, they divided to a fresh flask with a fresh ASW previously described solution and placed under controlled laboratory conditions (Temperature of 20˚C, photoperiod of 12/12 h dark/light and illumination of 3195 Lux (~ 52.7 µmol photons $m^{-2}s^{-1}$) for 3 days before Cd stress application.

The mentioned ASW was considered as a control. Whereas, Cd stress was applied by adding Cd to achieved 0, 2.5, 5 and 10 mg/L as final concentration for each treatment with three replicates/treatment. Experiment was carried out in flask with 300 mL ASW with or without Cd metal. The same previous described controlled conditions were maintained during the experiment stress application. Four days later, algae were harvested for physiological study.

Investigated physiological parameters: The experiment was conducted in triplicates for 4 days. Algal specific growth rate (SGR%) was calculated in both control and stressed conditions according to Nielsen *et al*. (2012) as follows:

$$(SGR\%) = 100 \times (\ln (W_t/W_0))/t$$

Where W_0 was the initial biomass and W_t corresponded to the biomass after t days.

Chlorophyll (Chl) and carotenoids (Car) pigments were extracted in 80% acetone solvent. Hundred mg of thalli for each treatment were grind and 5 mL of acetone were added; then samples were kept in

dark conditions at 4˚C for 24 h. Samples were centrifuged at 1400 g/ 2 min. Then, the extracts were filtered with Whatman filter papers 0.3 µm; their absorbance was measured at 470, 645 and 662 nm. Chl *a* & *b*, total Chl, total Car and Car content was estimated as previously described by Lichtenthaler and Wellburn (1985).

$$Chla(mg/gFW) = 11.75A_{662} - 2.35A_{645}$$

$$Chlb(mg/gFW) = 18.61A_{645} - 3.960A_{662}$$

$$TotChl(mg/L) = 20.2A_{645} + 8.02A_{662}$$

$$Car(mg/gFW) = \frac{(1000A_{470} - 2.270Chla - 81.4Chlb)}{230}$$

$$TotCar(mg/L) = \frac{[(A\,max/\,0.25)x\,sup\,V]}{Sample\,W}$$

Where Amax = maximum absorbance; sup V = supernatant volume (mL) and sample W= sample weight (g).

Algal potential osmotic has been also measured, where; one hundred mg of thali were cut and transported immediately to 2 mL Eppendorff with 2 mL dH_2O. Algae samples tissues were ground and the tubes were centrifuged at 1400 g for 2 min, then 50 µL of supernatants were transferred to a new fresh 1.5 mL Eppendorff one. The osmotic potential was measured using a micro-osmometer (Osmomette) apparatus. Moreover, integrity of cell membranes was determined as previously described by Unal *et al*. (2010). One hundred mg of algal thalli for *U. lactuca* and *P. pavonica* were divided into small pieces using a sharp cutter; kept in 10 mL ddH_2O for 1 hour. Then the electric conductivity (EC) of solution was determined by an electric conductivity (Hanna HI 99301, Romania) instrument.

Statistical analysis

Statistical analyses were performed using Statview 4.5 (Abacus 1996) statistical package at the 5% significance level (P = 0.05). Data were subjected to analysis of variance (ANOVA) for the determination of differences in means between tested algae species via Cd applied concentrations. Differences between means were tested for significance by Fisher's least significant difference (PLSD) test. Data are expressed as mean of three replicates.

RESULTS AND DISCUSSION

Cadmium stress caused SGR% decline in the both two examined algae species (Fig. 1). Where, this reduction was significant ($p \leq 0.001$) and more pronounced in *U. lactuca* compared to *P. pavonica*. This reduction was not significant with Cd applied concentrations increased.

Pigmentation content involved chlorophyll Chl*a*, Chl*b*, total chlorophyll, carotenoids and total carotenoids was measured to evaluate Cd toxicity on the both examined algae species. Estimated Chl*a*, Chl*b* and

total chlorophyll pigments were inhibited with Cd treatment in different manner according to the applied Cd concentrations and examined algae species (Fig. 2 a, b & c). In this respect, total chlorophyll significantly ($p \leq 0.001$) decreased in *U. lactuca* with no significant decline in *P. pavonica* as Cd applied concentrations increased.

As for estimated carotenoids and total carotenoids, data presented herein demonstrated that Cd stress caused reduction in the above pigments content in *U. lactuca*. While, *P. pavonica* showed contrary trends at 5 and 10 mg/L Cd concentration (Fig. 3a). Whereas, a decline in total carotenoids content was observed in the two examined algae species (Fig. 3b). Osmotic potential as a direct response of living organism against given abiotic stress, was estimated (Fig. 4). Results obtained herein mentioned that, this index was significantly ($p \leq 0.001$) decreased as Cd applied concentrations increased in both examined algae species. Electric conductivity (EC) was also calculated as previously reported. Data presented indicated that this value increased as Cd applied concentrations increased in the both examined algae species (Fig. 5). In this regards, *U. lactuca* exhibited a higher EC values than *P. pavonica* at the above Cd concentrations. Overall, inhibitory rate (IR%) induced by Cd on the previous estimated parameters of algae after 4 days Cd treatment, was presented in Table 1.

Plant growth inhibition is often considered as environmental assay to determine heavy metals toxicity in environmental ecosystems. This inhibition due to cell division impair linked to different mechanisms including *e.g.* the direct metal-DNA interactions (Alex and Dupois 1989).

In plant cell, wall acts as barrier to prevent the transport of heavy metals into the cytoplasm under low ion concentration. While, at high concentration the cell wall becomes disable to capturing all the metal ions and thereby some enter the cell causing cell damages. In sensitive plants exposed to heavy metals, the cytoplasm becomes disorganized thereby heavy metals entry the cytoplasm (Jamers *et al*., 2013).

Bouzon *et al*. (2012) reported the negative effect of different Cd (9-55 mg/L) concentrations on *H. musciformis* algae for 7 days. The previous study mentioned that Cd treatment caused reduction in growth rate and Chl *a* content. Whereas, Zhou *et al*. (2006) reported a reduction in growth rate and Chl *a* in *M. aeruginosa* has been found after exposure to Cd at 0.73 mg/L in the medium with chelator addition (0.33 mg/L free Cd, MINEQL calculation).

Whereas, Siva Kiran *et al*. (2012) investigated Cd accumulation by *Spirulina*

(Arthrospira) Indica blue green algae exposed to different Cd concentrations (1-10 mg/L) for 8 days. The previous study revealed that the percentage decrease in the *S. indica* biomass was found to be 54.32% at 4 mg/L Cd after 7 days.

While, Jiang *et al*. (2013) reported the negative Cd effects on two *U. prolifera* and *U. linza* green marine algae after 7 days Cd exposure. The latter study revealed that SGR% significantly decreased by 93% and 39% in *U. prolifera;* while, this reduction was recorded to be 53% and 75% in *U. linza* at 1.8 and 3.7 mg/L Cd, respectively.

In our case, decline in different pigments content observed between the two examined algae species according to the tested Cd concentrations. Otherwise, this reduction increased as Cd applied concentration increased. In this respect, at 10 mg/L Cd, Ch *a* content deceased by 67% and 21% in *U. lactuca* and *P. pavonica* , respectively (Table 1). While for Ch *b*, this value was reduced by 60% and 48% in the previous species, respectively. Whereas, for total chlorophyll this reduction found to be 64% and 30% below their respective control in *U. lactuca* and *P. pavonica,* respectively at 10 mg/L Cd. As for total carotenoids, this decline was recorded to be 66% and 23% for the previous algae species, respectively.

Other study however has dealt with toxicity of different concentrations of each of Cd (0.5,1, 2, 4 or 8 mg/L) and Pb (5, 10, 20, 40 or 80 mg/L) into the green alga *C. fracta* after 2, 4, 6 and 8 days exposure to heavy metal stress (Lamai *et al*., 2005). The latter investigation revealed significant reduction in relative growth and total chlorophyll values. Indeed, significant increase in the above metals has been recorded as exposure time and metal concentrations increased. Moreover, based on the bioconcentration factor (BCF) the previous investigation suggested that *C. fracta* accumulation capacity for Cd was lower compared to Pb.

In our case study the two examined algae species showed an inverse behavior concerning carotenoids pigmentation as Cd concentration increased. In this respect, carotenoids value slightly decreased with Cd treatment from 37 to 44% in *U. lactuca* at 2.5 and 10 mg/L Cd, respectively. In contrast, it sharply increased from 1 to 80% in *P. pavonica* at 2.5 and 10 mg/L Cd, respectively.

Garg *et al*. (1997) reported the toxicity of six Cd concentrations (0.22-5.48 mg/L) for 24, 48, 72 and 168 h in *H. verticillata*. The latter study demonstrated that a reduction in chlorophyll and protein content was recorded of approximately 79 and 72% respectively at 0.25 mg/L Cd. Whereas, dos Santos *et al*. (2012) investigated different Cd concentrations (0, 0.1, 0.2 and 0.3 mg/L) toxicity on various physiological and biochemical parameters on Agarophyte

G. domingensis (Rhodophyta, Gracilariales) algae for 16 days. The previous study mentioned that Cd stress caused chloroplast alteration, decline in pigmentations *e.g*. chlorophyll *a* and phycobiliproteins.

Moreover, Jiang *et al*. (2013) reported none significant decrease in both chlorophyll and carotenoid pigments observed under 1.8 mg/L Cd than their respective control in both *U. prolifera* and *U. linza* marine algae. While, with 3.7, 7.4 and 14.8 mg/L Cd, a reduction in Chl content by 18, 25 and 45% in *U. prolifera*; and it was 16, 20 and 39% for *U. linza*. Whereas, for carotenoids this decline was found to be 16, 29 and 54%, respectively in the case of *U. prolifera* and by 13, 16 and 44% below the control for *U. linza*, respectively.

Collén *et al*. (2003) reported that, algae exposed to heavy metals tends to accumulate some antioxidant defenses *e.g*. flavonoids and carotenoids pigmentations as a defenses mechanism against ROS induced by heavy metal exposure. Whereas, dos Santos *et al*. (2012) reported that, algae act as Cd includer or excluder to minimize Cd toxicity and thereby, prevent cell damages after Cd exposure. In this regards, Bouzon *et al*. (2012) reported that *G. domingensis* algae tends to accumulate certain antioxidants compounds *e.g*. flavonoids, tocopherols and carotenoids as a strategy to prevent the negative effects of ROS formation by heavy metals. Whereas, Zengin (2013) reported that carotenoid content significantly increased in the bean (*P. vulgaris* L.) seedlings leaves at different concentrations of Ni, Co, Cr and Zn heavy metals. Moreover, Pazoki *et al*. (2014) reported that Pb (0, 300, 600 and 900 mg/kg of soil) caused chlorophyll *a* and *b* reduction with carotene content increasing in wheat (*T. aestivum* L.) as Pb concentration increased from 300 to 900 mg/kg.

Previously, Kaplan *et al*. (1995) stated the induction of phytochelatins in *Chlorella* sp. algae exposed to Cd. Moreover, Shariati and Yahyaabadi (2006) reported that increasing Cd concentration caused a decline in chlorophyll content and increase of beta-carotene pigmentations in the two strains (Iranian and Australian) of *D. salina* green algae after 5 days exposure to different Cd concentrations (0, 0.005, 0.05 and 0.5 mg/L).

Otherwise, osmotic potential declined with different Cd applied concentrations than their respective control. This reduction was significant ($p \leq 0.001$) between ,In our case study the two examined algae species. Recently, Saleh (2015) investigated the impact of 4 heavy metals (Cu, Pb, Zn and Cd ions) on *U. lactuca* after 5 days exposure. The latter study showed that heavy Cd metal stress significantly reduced chlorophyll (Chl *a* & *b*), total

chlorophyll and carotenoids values. In this respect, the reduction was recorded for Cd treatment with 64% (Chl *a*), 56% (Chl *b*), 61% (Total Chl) and 22% (Car). Moreover, heavy metal stress caused a decline in osmotic potential compared to the respective control, with no significant impact among the 4 tested ions.

Our data showed that estimated EC values increased as Cd concentration increased for the both tested algae species compared to their respective control. It was noticed that *U. lactuca* exhibited higher EC values than *P. pavonica*. It worth noting that at the highest Cd concentration (10 mg/L), the estimated EC increased by 43% and 83% over the control for *U. lactuca* and *P. pavonica*, respectively.

Unal *et al*. (2010) investigated the physiological response of *U. lactuca* exposed to different Cr (VI) (38.84, 97.09, 194.19 and 970.95 mg/L K_2CrO_4) concentrations. The previous study demonstrated that, activity of PSII photosynthesis decreased and EC values increased as Cr concentration increased. Indeed, Cr treatment caused morphological cells changes.

In conclusion, physiological discrimination between two marine algae *U. lactuca* and *P. pavonica* has been evaluated under different Cd concentrations for 4 days. Overall, Cd stress caused a decline in investigated physiological parameters. Both algae species have a similar behavior concerning a decline in Chl *a* & *b*, total chlorophyll and total carotenoids pigmentation as Cd concentration increased. While, *P. pavonica* showed an inverse trends in carotenoids content compared to *U. lactuca*. This finding could be explaining the observed difference between the two examined algae species against Cd stress. Where, carotenoids pigments act as antioxidant and thereby, serve as osmoprotector involved in heavy metal tolerance mechanism by minimize ROS induction under heavy metal stress. Based upon the examined physiological indices, the current study could be suggest that *P. pavonica* was more tolerant to Cd toxicity and could be serving as promising bioindicator for Cd pollution in marine ecosystems compared to *U. lactuca*. Further research on Cd molecular tolerance mechanisms at DNA level into the two examined algae species could support and give an overview about Cd tolerance in the two studied marine algae.

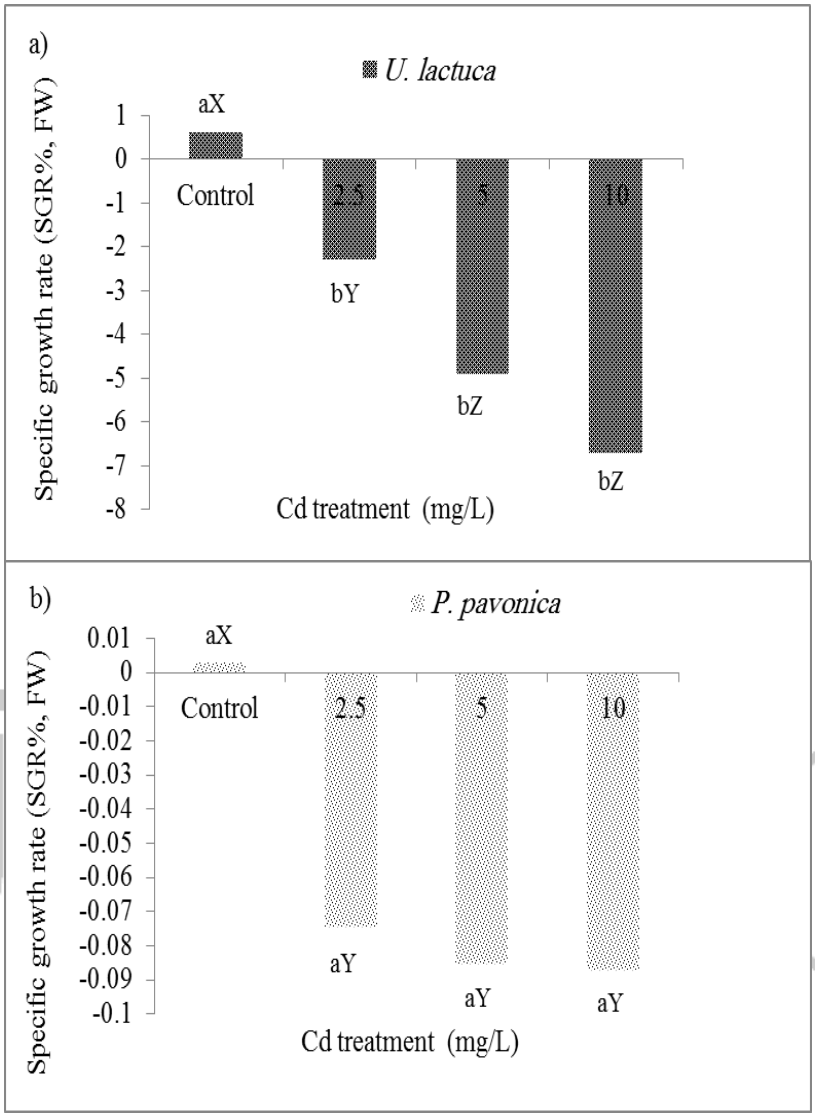

Figure 1. Algal specific growth rate (SGR%) 4 days after Cd stress in the two examined algae a): for *U. lactuca* and b): for *P. pavonica*. (n = 3).

Notes. Figures sharing the same lowercase letter are not significantly different at $p = 0.05$ probability by Fisher's PLSD test. $LSD_{0.05}$ algae species 1.606; Cd treatment: 2.271.

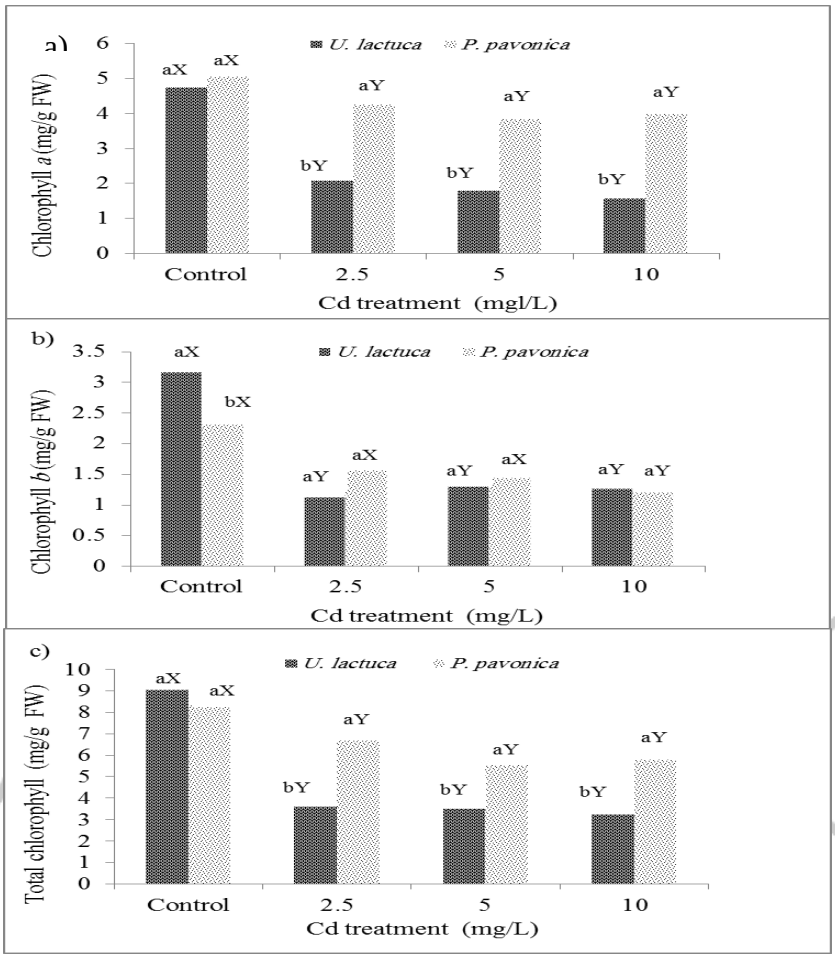

Figure 2. Chl *a* (a); Chl *b* (b) and total Chl (c) pigmentation content 4 days after Cd stress in the two examined algae (n = 3).

Notes. Figures sharing the same lowercase letter are not significantly different at p = 0.05 probability by Fisher's PLSD test. Chl *a*: algae species 0.505; Cd treatment: 0.708. Chl *b*: LSD0.05 algae species 0.731; Cd treatment: 1.034. Total Chl: $LSD_{0.05}$ algae species 1.145; Cd treatment:1.619.

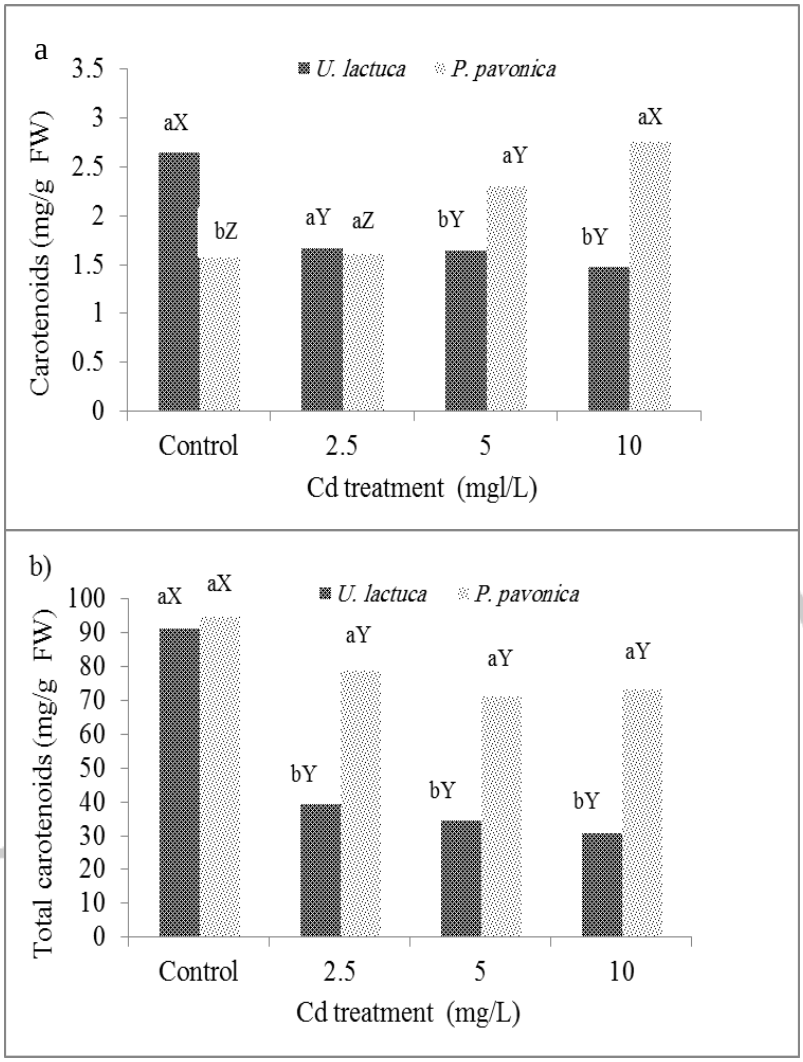

Figure 3. Carotenoids (a); and total carotenoids (b) pigmentation content 4 days after Cd stress in the two examined algae (n = 3).

Notes. Figures sharing the same lowercase letter are not significantly different at $p = 0.05$ probability by Fisher's PLSD test. Car: $LSD_{0.05}$ algae species 0.326; Cd treatment: 0.461. Total Car: $LSD_{0.05}$ algae species 9.385; Cd treatment: 13.273.

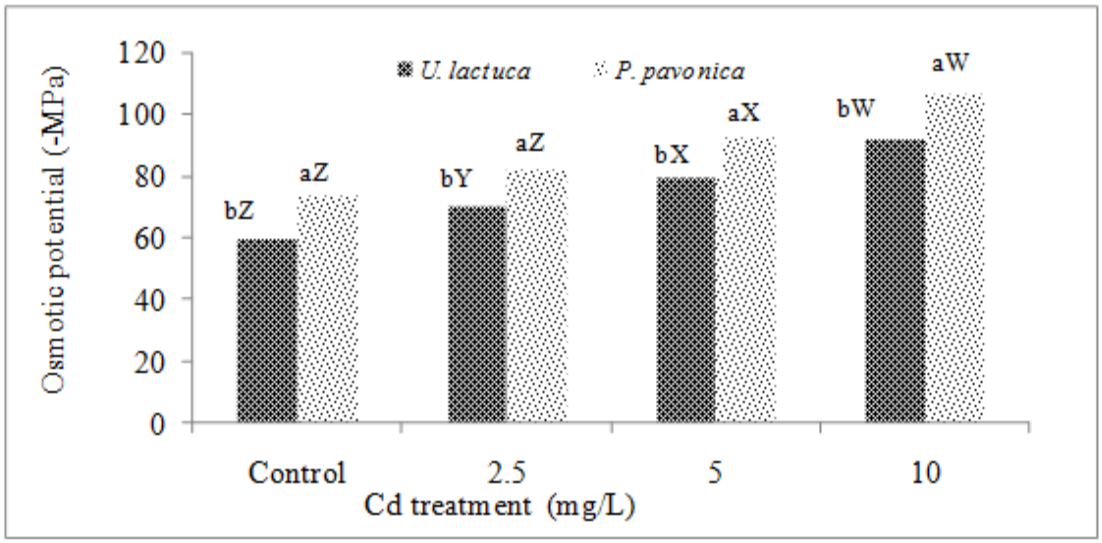

Figure 4. Osmotic potential 4 days after Cd stress in the two examined algae (n = 3).

Notes. Figures sharing the same lowercase letter are not significantly different at $p = 0.05$ probability by Fisher's PLSD test. $LSD_{0.05}$ algae species 6.752; Cd treatment: 9.549.

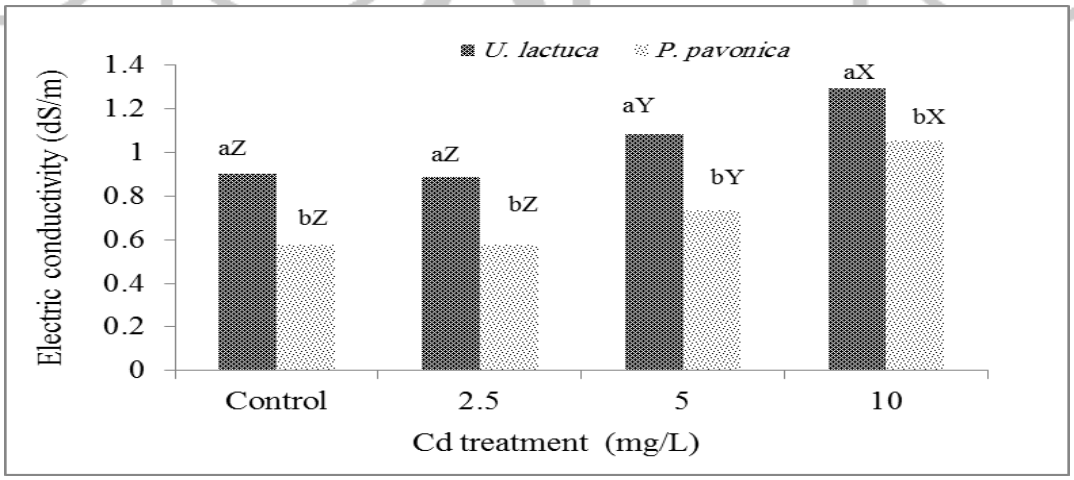

Figure 5. Electric conductivity (EC) 4 days after Cd stress in the two examined algae (n = 3).

Notes. Figures sharing the same lowercase letter are not significantly different at $p = 0.05$ probability by Fisher's PLSD test. $LSD_{0.05}$ algae species 0.086; Cd treatment: 0.122.

Table 1. Inhibitory rate (IR%) of applied Cd concentrations on studied physiological parameters of algae 4 days after treatment.

Algae species	Cd mg/L	SGR%	Chl a	Chl b	Total Chl	Car	Tot Car	Potential osmotic	EC
U. lactuca	2.5	-2.289	-56.134	-64.537	-59.727	-36.939	-56.788	-17.877	+7.407
	5	-4.905	-62.434	-59.298	-61.093	-37.954	-62.190	-35.196	+20.000
	10	-6.724	-66.865	-60.252	-64.037	-44.058	-66.350	-53.631	+43.333
P. pavonica	2.5	-0.075	-16.183	-25.903	-19.469	+0.687	-16.713	-3.797	+0.581
	5	-0.086	-24.251	-37.971	-28.890	+44.823	-25.000	-16.878	+27.907
	10	-0.088	-21.365	-48.078	-30.397	+80.308	-22.823	-34.599	+83.140

ACKNOWLEDGEMENTS.

I thank Professor Othman Ibrahim (director general of AECS) and Professor MirAli Nizar (head of Molecular Biology and Biotechnology Department in AECS) for their support, and also the Plant Biotechnology Group for technical assistance.

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