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Physiological and Molecular Response of *Padina pavonica* (*Phaeophyta*) brown Alga Towards Cadmium Heavy Metal

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Physiological and molecular response of *Padina pavonica* (phaeophyta) marine alga exposed to different cadmium (0, 2.5, 5 and 10 mg/L) concentrations after 4 days of exposure has been investigated. Physiological data revealed decrease in specific growth rate (SGR%), pigments (Chlorophyll *a* & *b*, total chlorophyll and total carotendois) content, osmotic potential with increased electric conductivity (EC) under Cd applied concentrations in the studied alga species. Whereas, carotendois pigments content increased in *P. pavonica* alga as Cd applied concentrations increased. As for molecular test, RAPD marker has been applied, a decrease in polymorphic bands (PB) from 95 to 87 has been recorded when applied Cd concentration increased from 2.5 to 10 mg/L. Whereas, genomic template stability (GTS%) as a qualitative measurement reflect DNA changes induced by Cd treatment was displayed by RAPD marker. Molecular study revealed increased GTS% value from 30.7 to 42.7% when applied Cd concentration increased from 2.5 to 10 mg/L. Based upon observed physiological (significant increase in carotendois content) and molecular data (decrease in the new induced bands number and increase in disappeared bands as Cd concentration increased from 2.5 to 10 mg/L) in *P. pavonica* alga, the current investigation could be assumed that *P. pavonica* alga adopted certain mechanism to minimize Cd stress damages.

Key words: Padina pavonica, Cd heavy metal, Physiological parameters, RAPD, Genotoxicity, DNA variation

From very long time up till now, environmental pollution by heavy metals (HM) was considered as a global public health concern and has been dramatically increased due to their exponentially increased used for various purposes like *e.g*. industrial, agricultural, domestic and technological applications. Heavy metals (HM) as an abiotic stress caused perturbation of biological activity in living organisms, reflecting in changes at anatomical, morphological, physiological, biochemical and molecular aspects. Cadmium (Cd) among HM was considered as one of the most toxicants in ecosystems; and that their harmful effects on plant could be manifested by membrane distortion, reactive oxygen species (ROS) production, photosynthesis impair, DNA and RNA damages and cell death (Farid *et al.*, 2013).

Many studies reported HM effect in living organisms on physiological parameters (relative growth, pigments including total chlorophyll, chlorophyll *a* & *b*, carotenoids, total carotenoids, osmotic potential and electric conductivity); *e.g*. Cd & Pb in the green alga *Cladophora fracta* (Lami *et al.*, 2005); Cd in *Hypnea musciformis* (Rhodophyta) alga (Bouzon *et al.*, 2012); Cd with chelator addition in cyanobacterium *Microcystis aeruginosa* (Zhou *et al.*, 2006); Cd in *Ulva prolifera* and *Ulva linza* green algae (Jiang *et al.*, 2013); Cd in Agarophyte *Gracilaria domingensis* (Rhodophyta) alga (dos Santos *et al.*, 2012); Cd in *Chlorella* sp. algae (Kaplan *et al.*, 1995); Cd in the two strains (Iranian and Australian) of *Dunaliella salina* green algae (Shariati and Yahyaabadi, 2006); Cd in *Thalassia hemprichi* (Tuapattinaya *et al.*, 2016) and in *Brassica juncea* species (Kapoor *et al.*, 2014); Ni and Zn in bean (*Phaseolus vulgaris* L.) (Zengin 2013). Cu, Pb, Zn and Cd metals in *Ulva lactuca* green alga (Saleh, 2015); Cd in two lichen species of *Parmotrema tinctorum* and *Usnea barbata* (Santos *et al.*, 2022). Recently, Nowicka (2022) reviewed heavy metals toxicity in algae and mechanisms involved in their detoxification. More recently, Ejaz *et al.* (2023) reviewed different strategies developed by plants for heavy metals detoxification.

PCR-based DNA marker systems have been extensively employed for monitoring different effects of HM in living organisms on genetic stability through genetic variations in DNA pattern including the appearance of new bands induced or/and disappearance of control bands ones. For example, random amplified polymorphic DNA (RAPD) analysis for monitoring genomic stability induced by Cd stress in barley (*Hordium vulgare*) (Liu *et al.*, 2005); RAPD for Cd stress in maize (*Zea mays* L.) (Mohsenzadeh *et al.*, 2010); RAPD for Cu and Zn stresses in Cucumber (*Cucumis sativus* L.) (Aydın *et al.*, 2012); RAPD for Cd stress in Okra (*A. esculantus* L.) (Aydın *et al.*, 2013); random amplified microsatellite polymorphism (RAMP) marker for heavy metals (Cu, Pb, Cd and Zn) stress in *U. lactuca* green alga (Saleh, 2016) and RAPD for Cd and Pb stresses in *Ramalina farinaceae* lichen (Hamutoğlu, 2021).

Thereby, the current study has been designed for investigation of physiological and molecular response of *P. pavonica* (phaeophyta) marine alga exposed to different cadmium (0, 2.5, 5 and 10 mg/L) concentrations after 4 days of exposure.

MATERIALS AND METHODS

Sampling

Samples of *P. pavonica* alga species were collected along the Syrian coast of the Mediterranean Sea. Sampling was carried out from 34°37734'N longitude, 38°29766'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 alga were collected and then transported within a flask with 5 L seawater.

Cultivation of algae and cadmium stress

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 *P. pavonica* alga species was evaluated under different Cd concentrations under Cd $(NO_3)_2$ forma salt [(Standard solution (1000 mg/L) from Fisher Scientific – UK)].

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 and molecular studies.

Physiological study

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). Whereas, chlorophyll (Chl) and carotendois (Car) pigments were extracted in 80% acetone solvent and estimated as previously described by Lichtenthaler and Wellburn (1985). While, the osmotic potential was measured using a micro-osmometer (Osmomette) apparatus. Moreover, the electric conductivity (EC) was determined by an electric conductivity (Hanna HI 99301, Romania) instrument.

Statistical analysis

Statistical analyses for physiological study, 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 among 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.

Molecular study

Genomic DNA extraction

Total genomic algal DNA was isolated from (bulk of 3 replicates/ treatment) tissues for both of the control and stressed alga by a CTAB (cetyltrimethylammonium bromide) protocol as previously described by Doyle and Doyle (1987) with minor modifications. DNA concentration was quantified by DNA Fluorimeter at 260/280 nm and adjusted to final concentration of 10 ng/µL. DNA was stored at -80 °C until needed.

RAPD marker and data analysis

To evaluate Cd-genotoxicity in the studied *P. pavonica* alga species, 22 RAPD primers were tested (Table 1). RAPD assay was performed as previously described by Williams *et al.* (1990). Then PCR products were separated on a 1.5% ethidium bromide- stained agarose gel (Bio-Rad) in 0.5X TBE buffer. Electrophoresis was performed for 1.5 h at 85 V and visualized with a UV transilluminator. A VC 100bp Plus DNA Ladder (Vivantis) ladder standard was used to estimate molecular weight of amplification products.

RAPD data analysis was performed by comparing the PCR products patterns for control with treated Cd algae at the mentioned Cd applied concentrations.

Estimation of genomic template stability (GTS%)

Genomic template stability value was estimated as previously described by Atienzar *et al.* (2002).

RESULTS AND DISCUSSION

Effect of different cadmium (0, 2.5, 5 and 10 mg/L) concentrations has been investigated into *P. pavonica* (phaeophyta) marine alga after 4 days of exposure to Cd stress at physiological and molecular levels.

Physiological test revealed that cadmium stress caused significant SGR% ($p \leq 0.001$) reduction into the studied alga species (Table 2). Moreover, estimated pigmentation content including Chl*a*, Chl*b* and total chlorophyll was reduced following Cd stress in different manners according to the applied Cd concentrations (Table 2). In this regards, total chlorophyll decreased with no significant decline in *P. pavonica* alga as Cd applied concentrations increased.

As for estimated carotene and total carotendois, data showed that Cd stress caused decline in total carotendois in *P. pavonica* alga as Cd applied concentrations increased (Table 2). Whereas, carotendois pigments content significantly ($p \leq 0.001$) increased in the studied alga as Cd applied concentrations increased (Table 2).

Osmotic potential as a direct response of living organism against given abiotic stress, was significantly

($p \leq 0.001$) decreased as Cd applied concentrations increased in the studied alga species (Table 2). While, Electric conductivity (EC) value increased as Cd applied concentrations increased in the studied alga species (Table 2).

In plant cells, 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 become 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 became disorganized even heavy metals entry the cytoplasm (Jamers *et al.*, 2013).

Physiological test revealed decline in different pigments content in different manner according to the tested Cd concentrations. This reduction increased as Cd applied concentrations increased. In this regards, at 10 mg/L Cd, Ch *a* content deceased by 21% in *P. pavonica* alga (Table 2). Whereas, for Ch *b*, this index was reduced by 48% in *P. pavonica* alga. While, for total chlorophyll this reduction found to be 30% below their respective control in *P. pavonica* alga*,* at 10 mg/L Cd.

Similarly, significant decline in relative growth and total chlorophyll values as applied Cd & Pb concentrations increased has been recorded in the green alga *C. fracta* (Lami *et al.*, 2005); decline in growth rate and Chl *a* content in *H. musciformis* alga exposed to different Cd (9-55 mg/L) concentrations (Bouzon *et al.*, 2012); decline in growth rate and Chl *a* in *M. aeruginosa* has been observed following exposure to 0.73 mg/L Cd with chelator addition (0.33 mg/L free Cd, MINEQL calculation) (Zhou *et al.*, 2006); SGR% significantly decreased by 93% and 39% in *U. prolifera* alga*;* whereas, this reduction was recorded to be 53% and 75% in *U. linza* alga at 1.8 and 3.7 mg/L Cd, respectively; combined with decline in Chl content by 18, 25 and 45% at 3.7, 7.4 and 14.8 mg/L Cd in *U. prolifera* alga, respectively and by 16, 20 and 39% for *U. linza* alga*,* respectively under the above applied Cd concentrations, respectively. Indeed, reduction in carotenoids by 16, 29 and 54%, respectively in the case of *U. prolifera* alga and by 13, 16 and 44% below the control for *U. linza* alga, respectively under the above applied Cd concentrations, respectively (Jiang *et al.*, 2013); reduction in pigmentations *e.g*. Chl *a* and phycobiliproteins has been observed in Agarophyte *G. domingensis* (Rhodophyta, Gracilariales) alga exposed to different Cd (0, 0.1, 0.2 and 0.3 mg/L) concentrations (dos Santos *et al.*, 2012). However, in the current study, this parameter it sharply increased in *P. pavonica* alga by 1 and 80% at 2.5 and 10 mg/L Cd, respectively.

Researches demonstrated how living organisms | (plants and algae) developed different mechanisms defense systems against adverse effects caused by heavy metals stress through induction of different antioxidants compounds types. In this regards, Collén *et al.* (2003) reported that, algae exposed to heavy metals tend 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 respect, Bouzon *et al.* (2012) reported that *G. domingensis* alga 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 leaves bean (*P. vulgaris* L.) seedlings 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 Chl *a* and *b* reduction with carotene content increasing in wheat (*T. aestivum* L.) as Pb concentrations 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 concentrations 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).

Similarly, Zengin (2013) reported increase in carotenoids content by 8%, 13.5% and 17% at 1.5, 2.0 and 2.5 mM Zn & by 15%, 23.1% and 27.3% at 0.1, 0.3

and 0.5 mM Ni, respectively in bean (*Phaseolus vulgaris* L.). Our data were in coherent with previous reports; *e.g*. Increase in carotenoids content in *Thalassia hemprichii*-Cd treated (Tuapattinaya *et al.*, 2016) and in *Brassica juncea*-Cd treated species (Kapoor *et al.*, 2014); in bean (*Phaseolus vulgaris* L.)-Ni and Zn treated (Zengin 2013) and recently in two lichen species of *Parmotrema tinctorum* and *Usnea barbata* when Cd concentrations increased from 10 to 500 µM (Santos *et al.*, 2022).

Heavy metals in particularly Pb and Cd generated an oxidative stress, so some of plants species developed some mechanisms defense systems to combat their adverse negative effect through enzymatic systems (CAT, SOD, POX….) or non- enzymatic systems by producing phenolics, carotenoids, proline and some vitamins (Michalak, 2006; Ahmad *et al.*, 2020). In has been demonstrated that carotenoids pigments serve as antioxidants against free radicals and photochemical damage (Sengar *et al.*, 2008).

Carotenoids could be considered as the first defense line against singlet oxygen. They have special chemical structure that is able to neutralize or extinguish the singlet oxygen reactivity and to provide energy to the entire carotenoid molecule (Ramel *et al.*, 2012). To be able to extinguish the singlet oxygen, carotenoids must have at least 9 double bonds with single bonds between the double bonds. This chemical composition is called a double bond conjugation. The energy of singlet oxygen was transferred to the carotenoid and returned to its original energy level. At the time, singlet oxygen has been converted into normal oxygen. In addition to singlet oxygen, the other kinds of ROS molecules can be neutralized by the carotenoids via electron transfer reactions, the formation of radical cluster formation or by transferring hydrogen atom. Thus, the carotenoids will increase concurrently with the increase of the cadmium heavy metal to prevent the damaging effects that can be caused by metal cadmium (Ramel *et al.*, 2012). Increased of carotenoids content in response to heavy metals could play a significant role in photosystem protection as an antioxidant agent (Alsherif *et al.*, 2022). Otherwise, the current study revealed decline in osmotic potential following different Cd applied concentrations. Similarly, Saleh (2015) reported decline in osmotic potential compared to their respective control in *U. lactuca* after 5 days exposure to 4 heavy metals (Cu, Pb, Zn and Cd ions). Indeed, physiological data revealed that the estimated EC values increased as Cd concentrations in the studied alga compared to its respective control. In this regards, this index was increased by 83% over the control for *P. pavonica* alga.

As for molecular study, cadmium stress caused alteration of DNA profile expressed as polymorphic bands (PB) including induction of new bands in comparison to the control (a) and disappearance of a normal control bands (b). DNA changes profiles induced by Cd stress as yielded by OPE15, OPK17 and RPi-C8 RAPD primers in *P. pavonica* alga have been illustrated in Figure 1.

In this regards, decrease in (a) value from from 51 to 34 bands, has been recorded in *P. pavonica* (Figure 1) alga when applied Cd concentrations increased from 2.5 to 10 mg/L. Whereas, decrease in (b) value increased from 44 to 53 bands when applied Cd concentrations increased from 2.5 to 10 mg/L. Overall, decline in total PB value from 95 to 87 bands has been recorded in the studied alga when applied Cd concentrations increased from 2.5 to 10 mg/L.

Similar data have been reported by Mohsenzadeh *et al.* (2010), who reported genotoxicity of Cd (40 and 80 mg/L) in maize (*Z. mays* L.) seedlings after 7 days Cd exposure, using 11 RAPD primers. The previous study revealed that disappear bands increased from 33 to 45 bands combined with increased new bands also from 3 to 5 compared to their respective control, as Cd applied concentrations increased from 40 to 80 mg/L. Moreover, Aydin *et al.* (2013) reported Cd genotoxicity in Okra (*A. esculantus* L.) after 21 days of Cd (30, 60 and 120 mg/L) exposure, using 9 RAPD primers. The previous study revealed that polymorphic bands number decreased from 25 to 22 as Cd applied concentrations increased from 30 and 120 mg/L Cd.

Moreover, GTS% value as a qualitative measurement reflect DNA changes induced by Cd treatment was displayed by RAPD marker (Figure 3). In this regards, it increased from 30.7 to 42.7% in the studied *P. pavonica* alga when applied Cd concentrations increased from 2.5 to 10 mg/L.

Our data were coherent with results supported by Hamutoğlu (2021), who reported that GTS% value increased from 69.04 to 80.95% and from 67.85 to 71.42% when applied both of Cd and Pb concentrations increased from 30 to 120 ppm after 72 h exposure using RAPD marker in *Ramalina farinaceae* lichen. Our data were also coherent with Soydam Aydın *et al.* (2012) who reported increase in GTS% values from 15.98 to 38.69% as ions (Cu and Zn) concentrations increased from 40 to 640 mg/L in Cucumber (*Cucumis sativus* L.) using RAPD marker. Similarly, Aydin *et al.* (2013) reported increase in GTS% from 59 to 72.5% as Cd applied concentrations increased from 30 to 120 mg/L Cd in Okra (*A. esculantus* L.) using RAPD marker.

The current investigation proved that increased GTS % as Cd applied concentrations increased, may be act as a protective mechanism leading to best and effective DNA repairing. This phenomenon could be attributed to the fact, that the DNA alterations in RAPD patterns (appearance of new or/and disappearance of normal control bands); tendency to balance each other (Liu *et al.*, 2005, Aydin *et al.*, 2013).

Based upon observed physiological (significant increase in carotenoids content) and molecular data (decrease in the new induced bands number and increase in disappeared bands as Cd concentrations increased from 2.5 to 10 mg/L) in the studied *P. pavonica* alga, the current investigation could be assumed that *P. pavonica* alga adopted certain mechanism to minimize DNA damages induced by Cd stress.

Table 1: Selected RAPD primers for assessment of Cd stress in P. pavonica alga.

Primer	Sequence 5' - 3'	Primer	Sequence 5' - 3'
RPi-C2	AACGCGTCGG	OPD20	GGTCTACACC
RPi-C3	AAGCGACCTG	OPE15	ACGCACAACC
RPi-C4	AATCGCGCTG	OPG05	CTGAGACGGA
RPi-C5	AATCGGGCTG	OPK17	CCCAGCTGTG
RPi-C6	ACACACGCTG	OPR09	TGAGCACGAG
RPi-C7	ACATCGCCCA	OPT18	GATGCCAGAC
RPi-C8	ACCACCCACC	OPV03	CTCCCTGCAA
RPi-C9	ACCGCCTATG	OPY10	CAAACGTGGG
RPi-C10	ACGATGAGCG	OPW17	GTCCTGGGTT
OPA02	TGCCGAGCTG	UBC159	GAGCCCGTAG
OPB17	AGGGAACGAG	UBC702	GGGAGAAGGG

Table 2: Physiological parameters investigated in *P. pavonica* alga exposed to cadmium stress.

Parameter	C	T1	T2	T3
SGR%	0.003 X	-0.075 Y	-0.086 Y	-0.088 Y
Chl a	5.049 X	4.232 Y	3.824 Y	3.970 Y
Chl *b*	2.308 X	1.710 X	1.431 X	1.198 Y
Total *Chl*	8.352 X	6.726 Y	5.939 Y	5.813 Y
Carotenoids	1.589 Z	1.599 Z	2.301 Y	2.864 X
Total Carotenoids	94.933 X	79.067 Y	71.200 Y	73.267 Y
Potential osmotic	79.000 Z	82.000 Z	92.333 X	106.333 W
EC	0.573 Z	0.577 Z	0.733 Y	1.050 X

C: control; T1: 2.5 mg/L Cd; T2: 5 mg/L & T3: 10 mg/L.
Figures sharing the same case letter are not significantly different at $p = 0.05$ probability by Fisher's PLSD test.
SGR%: $LSD_{0.05}$ Cd treatment: 2.271.
Chl *a*: $LSD_{0.05}$ Cd treatment: 0.708.
Chl *b*: LSD0.05 Cd treatment: 1.034.
Total Chl: $LSD_{0.05}$Cd treatment:1.619.
Car: $LSD_{0.05}$ Cd treatment: 0.461.
Total Car: $LSD_{0.05}$ Cd treatment: 13.273.
Potential osmotic : $LSD_{0.05}$ Cd treatment: 9.549.
EC: $LSD_{0.05}$ Cd treatment: 0.122.

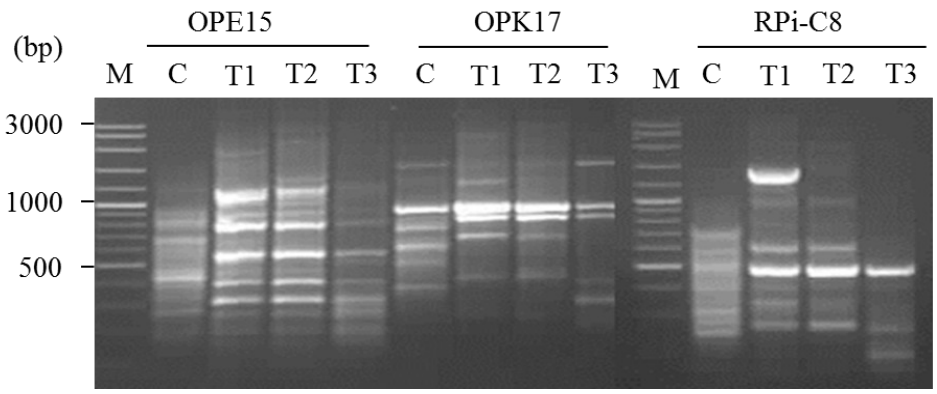

Figure 1. RAPD pattern yielded by OPE15, OPK17 and RPi-C8 RAPD primers into *P. pavonica* alga species revealed DNA changes induced by different Cd concentrations. M: A VC 100bp Plus DNA Ladder (Vivantis) ladder standard.
C: control; T1: 2.5 mg/L Cd; T2: 5 mg/L & T3: 10 mg/L.

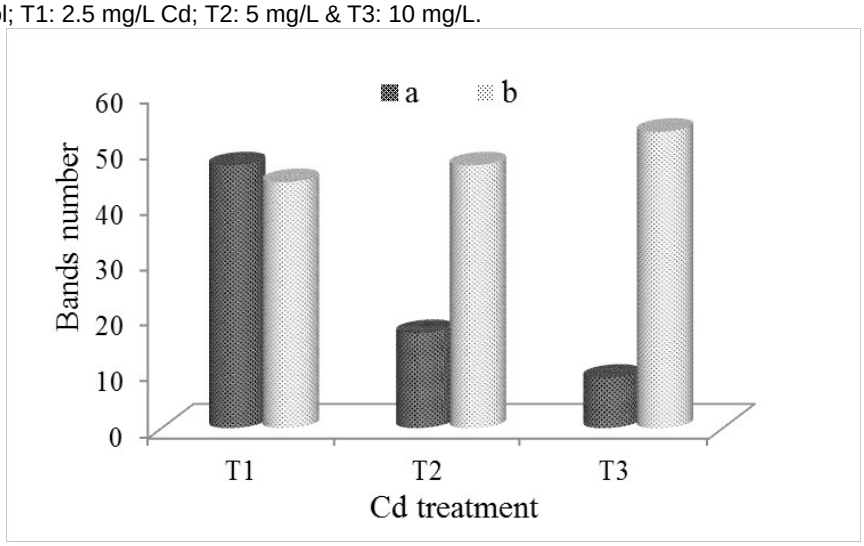

Figure 2. Polymorphic bands (PB) including new induced bands number (a) and disappeared of control bands number (b) using RAPD marker, into *P. pavonica* alga species revealed DNA changes induced by different Cd concentrations.
T1: 2.5 mg/L Cd; T2: 5 mg/L & T3: 10 mg/L.

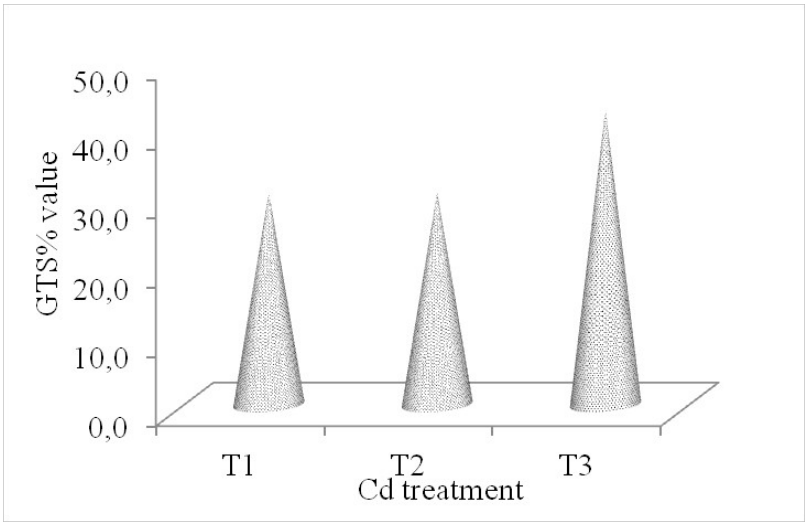

Figure 3. Genomic template stability (GTS%) value induced by different Cd concentrations yielded by RAPD marker into *P. pavonica* alga species.
T1: 2.5 mg/L Cd; T2: 5 mg/L & T3: 10 mg/L.

CONCLUSION

Physiological and molecular response of *P. pavonica* marine alga has been evaluated under different Cd concentrations for 4 days. For physiological test, Cd stress caused a decline in investigated physiological parameters (SGR%, Chl *a* & *b*, total chlorophyll and total carotenoids content and osmotic potential). Whereas, molecular test revealed increased GTS% from 30.7 to 42.7% when applied Cd concentrations increased from 2.5 to 10 mg/L, in the studied *P. pavonica* alga. Overall, data presented herein based upon observed physiological (significant increase in carotenoids content) and molecular data (decrease in the new induced bands number and increase in disappeared bands as Cd concentrations increased from 2.5 to 10 mg/L) in the studied *P. pavonica* alga, the current investigation could be assumed that *P. pavonica* alga was Cd tolerant. This observation could be related to the fact that *P. pavonica* alga adopted certain mechanism to minimize Cd toxicity at physiological level (significant increase of carotenoids pigments content) as Cd applied concentrations increased; where, carotenoids pigment acts as antioxidant and thereby, serves as osmoprotector involved in heavy metal tolerance mechanism by minimize ROS induction under heavy metal stress. Overall, *P. pavonica* alga could serve as a promising bioindicator for Cd pollution in marine ecosystems.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Ahmad I, Tahir M, Daraz U, Ditta A, Hussain MB, Khan ZUH (2020). Responses and tolerance of cereal crops to metal and metalloid toxicity. *Agronomic Crops*; Hassanuzzaman, M., Ed.; Springer: Singapore, 2020; pp. 235–264

Alsherif EA, Al-Shaikh TM, Almaghrabi O, AbdElgawad H (2022). High redox status as the basis for heavy metal tolerance of *Sesuvium portulacastrum* L. inhabiting contaminated soil in Jeddah, Saudi Arabia. *Antioxidants* (Basel). 22; 11(1):19.

Atienzar FA, Venier P, Jha AN, Depledge MH (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutation Research* 521(1-2): 151–163.

Aydin SS, Gökçe E, Büyük I, Aras S (2012). Characterization of copper and zinc induced stress on cucumber (*Cucumis sativus* L.) by molecular and population parameters. *Mutation Research* 746: 49-55.

Aydin SS, Başaran E, Cansaran-Duman D, Aras S (2013). Genotoxic effect of cadmium in okra (*Abelmoschus esculantus* L.) seedlings: comparative investigation with population parameter and molecular marker. *Journal of Environmental Biology* 34: 985-990.

Bouzon ZL, Ferreira EC, Dos-Santos R, Scherner F, Horta PA, Maraschin M, Schmidt, EC (2012). Influences of cadmium on fine structure and metabolism of *Hypnea musciformis* (Rhodophyta, Gigartinales) cultivated *in vitro*. *Protoplasma* 249(3): 637–650.

dos-Santos RW, Schmid ÉC, Martins RP, Latini A, Maraschin M, Horta PA, Bouzon ZL (2012). Effects of cadmium on growth, photosynthetic pigments, photosynthetic performance, biochemical parameters and structure of chloroplasts in the Agarophyte *Gracilaria domingensis* (Rhodophyta, Gracilariales). *American Journal of Plant Science* 3(8): 1077-1084.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin* 19: 11–15.

Ejaz U, Khan SM, Khalid N, Ahmad Z, Jehangir S, Fatima Rizvi Z, Lho LH, Han H, Raposo A (2023). Detoxifying the heavy metals: a multipronged study

of tolerance strategies against heavy metals toxicity in plants. *Frontiers in Plant Science* 14:1154571.

Farid M, Shakoor MB, Ehsan S, Ali S, Zubair M, Hanif MA (2013). Morphological, physiological and biochemical responses of different plant species to Cd stress. *International Journal of Chemical and Biochemical Sciences* 3: 53-60.

Hamutoğlu R, Cansaran-Duman D, Derici MK, Aras ES, Aslan A. 2021. Accumulation of Cr^{6+}, Pb^{2+} and Cd^{2+} and ultraviolet radiation alter methylation and genomic DNA status in *Ramalina farinaceae*. *Communications Faculty of Sciences University of Ankara Series C-Biology* 30(2): 98-118.

Jamers A, Blust R, De-Coena W, Griffinab JL, Jonesc OAH (2013). An omics based assessment of cadmium toxicity in the green alga *Chlamydomonas reinhardtii. Aquatic Toxicology* 126: 355– 364.

Jiang H, Gao B, Li W, Zhu M, Zheng C, Zheng Q, Wang C (2013). Physiological and biochemical responses of *Ulva prolifera* and *Ulva linza* to cadmium stress. *The Scientific World Journal*: 2013: 1-11.

Kaplan D, Heimer EYM, Abeliovich A, Goldsbroug B (1995). Cadmium toxicity and resistance in *Chlorella sp. Plant Science* 109(2): 129-137.

Kapoor D, Kaur S, Bhardwaj R. (2014). Physiological and biochemical changes in *Brassica juncea* plants under Cd-induced stress. *BioMed Research International* 2014: 726070.

Lami C, Kruatrachu M, Pokethitiyook P, Upatham ES, Soonthornsarathool V (2005). Toxicity and accumulation of lead and cadmium in filamentous green alga *Cladophora fracta* (O.F. Müller ex Vahl): A laboratory study. *ScienceAsia* 31: 121-127.

Lichtenthaler HK, Wellburn AR (1983). Determination of total carotenoids and chlorophylls *a* and *b* of leaf in different solvents. *Biochemical Society Transactions* 11(5): 591-592.

Liu W, Li P, Qi XM, Zhou Q, Zheng L, Sun T, Yang Y (2005). DNA changes in barley (*Hordium vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemophere* 61: 158-167.

Michalak A (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* 15: 523–530.

Mohsenzadeh S, Shahrtash M, Mohabatkar H (2010). Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots. *Journal of Cell and Molecular Research* 2 (1): 42-48.

Nielsen MM, Bruhn A, Rasmussen MB, Olesen B, Larsen MM, Moller HB (2012). Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *The Journal of Applied Phycology* 24(3): 449–458.

Nowicka B (2022). Heavy metal-induced stress in eukaryotic algae-mechanisms of heavy metal toxicity and tolerance with particular emphasis on oxidative stress in exposed cells and the role of antioxidant response. *Environmental Science* and *Pollution Research* 29(12): 16860-16911.

Pazoki A, Ilkaee MN, Far MD, Golzardi F (2014). Impact of bio-fertilizers and different levels of lead on pigment content of wheat (*Triticum aestivum* L.). *Journal of Biodiversity and Environmental Sciences* 5: 348-355.

Ramel F, Birtic S, Cuine S, Triantaphylides C, Ravanat J, Havaux M (2012). Chemical quenching of singlet oxygenb carotenoids in plants. *Plant Physiology* 158: 1267-1278.

Saleh B (2015). Physiological response of the green algae *Ulva lactuca* (Chlorophyta) to heavy metals stress. *Journal of Stress Physiology and Biochemistry* 11(3): 38-51.

Saleh B. (2016). Genomic DNA changes in *Ulva lactuca* (Chlorophyta) under heavy metal stress. *International Journal of Environmental Sciences* 7(3): 245-255.

Santos AMd, Vitorino LC, Cruvinel BG, Ávila RG, Vasconcelos Filho SdC, Batista PF, Bessa LA (2022). Impacts of Cd pollution on the vitality, anatomy and physiology of two morphologically different lichen species of the genera *Parmotrema* and *Usnea*, evaluated under experimental conditions. *Diversity* 14(11):926.

Sengar RK, Gautam M, Sengar RK, Grag SK, Sengar K, Chaudhary R (2008). Lead stress effects on

physiobiochemical activities of higher plants. *Reviews of Environmental Contamination and Toxicology* 196: 73-93.

Shariati M, Yahyaabadi S (2006). The effect of different concentrations of cadmium on the growth rate and beta-carotene synthesis in unicellular green algae *Dunaliella salina. Iranian Journal of Science and Technology* 30(A1): 57-63.

Tuapattinaya PMJ, Rumahlatu D, Tulalessy S (2016). Bioaccumulation of cadmium heavy metal and its effect on the level of chlorophyll and carotenoids of *Thalassia hemprichii* in the waters of Ambon island. *Research Inventy: International Journal of Engineering And Science* 6(5): 28-33.

Unal D, Isik NO, Sukatar A (2010). Effects of chromium VI stress on green alga *Ulva lactuca* (L.). *Turkish Journal of Biology* 34: 119-124.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 653l-6535.

Zengin F (2013). Physiological behavior of bean *(Phaseolus vulgaris* L.) seedlings under metal stress. *Biological Research* 46(1): 79-85.

Zhou W, Juneau P, Qiu B (2006). Growth and photosynthetic responses of the bloom-forming cyanobacterium *Microcystis aeruginosa* to elevated levels of cadmium. *Chemosphere* 65(10): 1738-1746.