LEAD GENOTOXICITY IN *ULVA LACTUCA* (CHLOROPHYCEAE) SEAWEED AS REVEALED BY RAPD MARKER

Basel Saleh*

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria

ABSTRACT

The current study was conducted to monitoring Pb-genotoxic effect on *Ulva lactuca* seaweed, 2 days after exposure to different Pb (0, 2, 4 and 8 mg/l) concentrations using 15 RAPD primers. Data presented herein showed that Pb treatment induced DNA change patterns in the treated algae compare to their respective control. RAPD marker produced 322 bands as a total bands number of which 230 (71.429%) were polymorphic. Data showed that polymorphic bands number increased from 60 to 101 bands when applied Pb concentration increased from 2 to 8 mg/l. Whereas, genomic template stability (GTS%) values decreased from 87.274% to 70.667 % as applied Pb concentrations increased from 2 to 8 mg/l. Furthermore, band sharing index (BSI) values followed similar tendency as GTS%. In this respect, BSI decreased from 0.654 to 0.480 as Pb increased from 2 to 8 mg/l.Thereby, the current study could be suggested that RAPD marker successfully highlighted Pb-genotoxicityreflected inDNA change patterns yielded by Pbpollutant. Moreover, *U. lactuca* could be used as a potential bioindicator for monitoring Pb-genotoxicity in environmental ecosystems.

Keywords: *Ulva lactuca* Seaweed**,** Pb-genotoxicity, DNA change, RAPD marker

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INTRODUCTION

It has been demonstrated that heavy metals especially cadmium (Cd) and lead (Pb) formed part of fewer oldest environmental problems and remains a serious health concern today. Where, the general human exposure to these metals comes from ambient air, drinking water, food, industrial materials and consumer products(Goyer and Clarkson2001; Nordberg *et al*., 2011).

Heavy metals are toxic to the living organisms, as they affect negatively many essential biological processes such as respiration, photosynthesis, reproduction and metabolism, which cause a partial or total damage to the living organisms (Anderson 2003).

Previously, it has been documented that heavy metals pollutants caused oxidative and carcinogenic effects to living organisms in ecosystems. Their toxicity comes from its binding with nucleic acids through various reactions (direct or/and indirect)with DNA sites as well as affecting DNA replication. These phenomena lead consequently to DNA damages and mutations (Valavanidis and Vlachogianni 2010). These chemical pollutants provoked free radicals and reactive oxygen species (ROS), whicharbitrary attack and damage DNA and important enzymatic proteins (Chang *et al*., 1996; Bal and Kasprzak2002).Lead (Pb) is one of fewer heavy metals beside Hg and Cd, classified as high toxic element for living organisms in environmental ecosystems (Valavanidis and Vlachogianni 2010).

Heavy metal genotoxicity has been successfully screened by molecular markersin terrestrial ecosystems. Out available molecular systems, RAPDtechnique has found widespread acceptance in heavy metal genotoxicity living organisms researches.

RAPD marker is a sensitive, simple, cheaper technique, their ability to detect even the lowest DNA changes manifested as point mutation makes them a potential tool for monitoring mutational effects of organic and inorganic genotoxic agents in different organisms (Cansaran-Duman *et al*., 2011). In this respect, RAPD technique has been extensively employed for monitoring Pb-induced DNA changes *e.g*. in cumin (*Cuminum cyminum*) (Salarizadeh and Kavousi 2015), artichoke (*Cynara scolymus* L.) and runner bean (*Phaseolus coccineus* L.) (Candan and Batir 2015),tomato (*Lycopersicum esculentum*)(Aydin *et al*., 2015) and more recently in rye (*Secale cereal*) (Ozyigit *et al*., 2016).Whereas, Neeratanaphan *et al*. (2014) reported Pb-genotoxicity in *Pistia stratiotes*using ISSR marker.

Whereas, in algae, most heavy metal stress investigation has so far been focused on the physiological response with little value on biochemical aspect. While, molecular mechanisms involved in heavy metal pollution has not however delineated in details. In this respect, for algae, Pb-genotoxicity in *U. lactuca* seaweed has not been reported so far to our knowledge. In this regards, Saleh (2015) recently reported Pb, Cu, Cd and Zn

genotoxicity in comparative study in the same algae species using random amplified microsatellite polymorphism (RAMP) marker. More recently, Saleh (2016a) reported Cd- genotoxicity in *U. lactuca* algae using RAPD marker. Moreover, Saleh (2016b) reported Cd-genotoxicity in *Padina pavonica* (Phaeophyta) brown marine algae using also the same marker (RAPD).

Otherwise, Aras *et al*. (2011) used *Pseudevernia furfuracea* (L.) Zopf lichen as potential bioindicator for environmental pollution in Turkey by RAPD marker.

DNA changes induced by lead (Pb) heavy metal in marine *U. lactuca* macroalgae did not receive more attention. Thereby, the current investigation focused on generated DNA alterations due to Pb exposure using RAPD marker system.

MATERIALS AND METHODS

Algal Sampling

*U. lactuca*algae samples were collected along the Syrian coast of the Mediterranean Sea. Samples collection was carried out from 34°37′734″N longitude, 38°29′766″E latitude at 5 km North Lattakia - Syria.Only individual with the similar size was harvested by hand with disposable gloves, algae thali was washed twice with seawater where the algae were collected and then transported within a flask with 5 lfresh seawater.

Cultivation of algae and Pb stress application

Genotoxicity of different Pb concentrations in *U. lactuca* species has been evaluated under Pb $(NO_3)_2$ forma salt[(Standard solution (1000 mg/l) from Fisher Scientific – UK].Algae biomass were washed twice 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 kept 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 Pb stress application.

The mentioned ASW was considered as a control, whereas,Pb stress was applied by adding Pb to achieved 0, 2, 4 and 8 mg/l as final concentration for each treatment with three replicates/treatment. Experiment was carried out in flask with 300 mlASW with or without Pb metal. The same previous described controlled conditions were maintained during the experiment stress application. Algae were harvested 48 h after Pb treatment for molecular study.

DNA extraction

Algal genomic DNA was isolated from (bulk of 3 replicates/ treatment) tissues for both of the control and stressed algae by a CTAB (cetyltrimethylammonium bromide) protocolas previously described by Doyle and Doyle (1987).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 assay and data analysis

RAPD technique was carried out as previously reported byWilliams*et al*. (1990). Where, ten primers RPi-C01 to RPi-C10, cat number 610692700101730; designed by

Merck, India, Three RAPD primers belonging to Operon Technologies Inc., USA, and two primers from the University of British Columbia were employed for detecting Pb genotoxicity in *U. lactuca* (Table 1).

PCR amplification reaction was performed in 25 µl reaction volume containing 1X PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 25 pmol primer, 1.5 U *Taq* DNA polymerase and 50 ng template DNA. PCR amplification was performed in a *T-gradient* thermal cycler (Bio-Rad; T-Gradient). It was programmed to 42 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step for 1 min at 94°C, an annealing step for 2 min at 35°C, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle. 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 visualised 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 profile for control with treated Pb algae at the mentioned Pb applied concentrations.

Genomic template stability (GTS%) estimation

Genomic template stability value (GTS%) was calculated as previously described by Atienzar *et al*. (2002)according to the following formulate:

$$GTS\% = (1 - a/n) * 100$$

Where (a) was RAPD polymorphic profiles detected in each samples treated and (n) the number of total bands in the control. Polymorphism observed in RAPD profiles included disappearance of a normal band and induction of a new band in comparison to the control RAPD profiles.

Band sharing index (BSI) estimation

Band sharing index (BSI) values between Pb-treated algae and their respective control, have been calculated as described by Savva (2000) and Salarizadeh and Kavousi (2015) as following:

$$BSI = 2s / (a + b)$$

Where *s* is the shared bands between two samples, *a* is the presented bands in the first sample and *b* is the presentedbands in the second sample. Where, BSI value of 1 refers that two samples are identical, whereas, a BSI value of zero refers that two samples are totally different.

RESULTS AND DISCUSSION

Fifteen RAPD primers were employed to monitoring DNA genetic variation induced by Pb exposure into *U. lactuca* macroalgae. As shown in Fig. 1, DNA change profiles into *U. lactuca* under different Pb (0, 2, 4 and 8 mg/l) concentrations as yielded by RPi-C04, RPi-C06 and UBC159 RAPD primers. Estimated polymorphic bands [(appearance of new bands (a) + disappearance of control bands (b)] were recorded to be 60, 69 and 101 bands at 2, 4 and 8 mg/l Pb, respectively (Table 2). Similarly, estimated changes bands [(appearance of new bands (a) + disappearance of control bands (b) + decreased bands intensity (c) and increased bands intensity (d)]

increased from 95 to 155 bands as Pb applied concentration increased from 2 to 8 mg/l (Table 2). Whereas, GTS% values decreased from 87.274 to 70.667% as Pb applied concentration increased from 2 to 8 mg/l (Table 3). Furthermore, BSI values were also estimated under Pb stress, data revealed that this parameter followed similar tendency of GTS% (Table 4). In this respect, their values decreased from 0.654 to 0.480 as Pb applied concentration increased from 2 to 8 mg/l (Table 4).

Molecular toxicity generated by Pb has been discussed under different Pb concentrations in *U. lactuca* algae using RAPD marker. Data presented herein revealed that the high Pb concentrations (4 and 8 mg/l) clearly caused toxic effects. Similarly,Jamerset al. (2009)reported similar trends in *Chlamydomonas reinhardtii*, a unicellular freshwater chlorophyte after exposure to $CdCl_2$ for 72 h, using microarrays system.

Our data showed that Pb treatment induced a total bands number of 322 bands as revealed by RAPD marker, of which 60 (18.633%), 69 (21.429%) and 101 (31.366%) were polymorphic under 2, 4 and 8 mg/l Pb, respectively. Data proved that polymorphic bands (PB) number increased thereby from 60 to 101 PB as Pb concentration increased from 2to 8 mg/l.

Saleh (2015) reported four pollutants (Pb, Cu, Cd and Zn) genotoxicity after 5 days exposure in the same algae species using RAMP marker. The previous study revealed that DNA polymorphic level was recorded to be 34.8, 35.4, 40.2 and 40.7% with Pb, Cu, Cd and Zn ions, respectively. Moreover, Saleh (2016a) reported Cd-genotoxicity (0, 2.5, 5 and 10 mg/l) in *U. lactuca* algae after 4 days Cd exposure, using 20 RAPD primers. The previous study showed that estimated PB decreased from 99 to 32 bands as Cd concentration increased from 2.5 to 10 mg/l Cd. Indeed, Saleh (2016b) reported Cd-genotoxicity (0, 2.5, 5 and 10 mg/l) in *P. pavonica*brown algae after 4 days Cd exposure using 22 RAPD primers. The previous investigation revealed that estimated PB decreased from 95 to 87 bands when as Cd concentration increased from 2.5 to 10 mg/l.

Previously, Aras *et al*. (2010) investigated Pb genotoxicity (different Pb concentrations during different time interval) on *P. furfuracea* lichen collected from Turkey, using 4 RAPD primers. The previous study revealed that 2 out of 4 RAPD primers gave clear bands under Pb stress. Otherwise, Tube A01 RAPD primer indicated that the lichen subjected to Pb for 18, 24 and 48 h showed different manner of P% compared to the other samples. Moreover, Aras *et al*. (2011) reported the potential use of *P. furfuracea* (L.) Zopf lichen collected from 10 different *Pinus* species from Turkey, as a useful bioindicator for environmental pollution using 25 RAPD marker. The previous study showed that 10 out 25 applied RAPD primers gave clear and reproducible bands. Indeed, the PB ranged between 3-14 bands.

Candan and Batir (2015) reported Pb-genotoxicity (0.020, 0.040, 0.080, 0.160, 0.320, 0.640 and 1.280 mg/l) in artichoke (*C. scolymus* L.) and runner bean (*P. coccineus* L.) after 14 days exposure using

23 RAPD primers. The previous study revealed that 6 and 7 out of tested 23 RAPD primers gave monomorphic RAPD pattern for *C. scolymus* and *P. coccineus*, respectively. Indeed, P% values were recorded to be 98.03%, 96.71%, 94.74%, 94.41%, 93.42%, 92.76% and 91.79% for *C. scolymus*. Whereas, it was found to be 95.68%, 95.68%, 94.96%, 95.68%, 95.68%, 94.96% and 93.5% for *P. coccineus* at 0.020, 0.040, 0.080, 0.160, 0.320, 0.640 and 1.280 mg/l Pb, respectively.

Whereas, Aydin *et al*. (2015) reported DNA damages in tomato (*L. esculentum*) after 21 days exposure to Pb (40, 80, 120, 160 and 240 mg/l) using RAPD marker. The previous study revealed that 6 out of examined 12 RAPD primers gave polymorphic pattern. RAPD marker gave 81 total bands of which 22 (27.2 %) were polymorphic. Moreover, variant bands as expressed in disappearance of normal bands combined with newly induced bands were recorded to be 16, 7, 14, 10 and 8 bands at 40, 80, 120, 160 and 240 mg/l Pb, respectively.

As well as, Salarizadeh and Kavousi (2015) reported DNA-induced changes by Cd (0.300–1.050 mg/l) for 7 days in two cumin (*C. cyminum*) ecotypes using 10 RAPD primers. The previous study showed that PB increased from 11 to 22 in Isfahan and from 10 to 12 in Khorasan ecotype as Cd applied concentration increased from 0.300 to 1.050 mg/l.

Indeed, Ozyigit *et al*. (2016) investigated Pb genotoxicity two weeks exposure to Pb (0, 33120, 66240 and 132480 mg/l) in rye (*S. cereal*) using RAPD marker. The previous study showed that polymorphic bands (a and b) were positively correlated with Pb applied concentrations.

In the current study, estimated GTS% decreased from 87.274 to 70.667% as Pb applied concentration increased from 2 to 8 mg/l Pb. Saleh (2015) reported that estimated GTS% yielded by heavy metals stress as revealed by RAMP marker, was recorded to be 65.215, 64.630, 59.835 and 59.250% for Pb, Cu, Cd and Zn metals, respectively. Other investigations however, revealed an inverse tendency regarding Cd-genotoxicity in marine algae. For example, more recently, Saleh (2016a) reported Cd-genotoxicity in *U. lactuca* algae after 4 days Cd exposure, using 20 RAPD primers. The previousinvestigatioin revealed thatGTS% increased from 45.4 to 72.8% as Cd concentration increased from 2.5 to 10 mg/l. furthermore, Saleh (2016b) reported Cd-genotoxicity in *P. pavonica*brown algae after 4 days Cd exposure using 22 RAPD primers. The previousstudy demonstrated that GTS% increased from 30.7 to 42.7% as Cd increased from 2.5 to 10 mg/l.

Whereas, Cenkci *et al*. (2010) evaluated DNA variation induced by Pb (0.5–5 mg/l) exposurein fodder turnip (*Brassica rapa* L.) using 11 RAPD primers. The previous study mentioned significantly decreased in GTS% values as increased Pb concentration.

Aydin *et al*. (2015) however, reported DNA alteration induced by Pb stress in tomato (*L. esculentum*) after 21 days exposure using RAPD marker. The previous investigation revealed that GTS% values were found to be 78.1, 90.6, 81.8, 87.1 and 90.1% at 40, 80, 120, 160 and 240 mg/l Pb, respectively.

Whereas, Ozyigit *et al*. (2016) reported Pb-genotoxicity two weeks exposure to Pb in rye (*S. cereal*) using RAPD marker. The previous study showed that estimated GTS% values decreased as Pb applied concentration increased. Similar data have been reported by Erturk et al. (2015) in *Zea mays* exposed to boron and zinc using RAPD marker.

Moreover, Al-Qurainy (2010) reported DNA damages after 8 days exposure to heavy metals (Cd , Pb and Zn) in *Eruca sativa* L. using 20 ISSR primers. The previous study showed that 4 out of 20 tested ISSR primers failed in DNA amplification and 3 gave one band. Consequently, 13 ISSR primers were used for monitoring heavy metal genotoxicity and gave an average of 4 bands/primer. Moreover, medium concentrations of each metal (100 mg/l) exhibited the highest DNA mutations than the lowest ones. Indeed, Neeratanaphan et al. (2014) stated Pb-genotoxicity in *P. stratiotes* induced by Pb (0.2, 0.3, 0.5 mg/l Pb) after 4 days exposure using ISSR marker. The previous investigation revealed that *P. stratiotes* can be used as a bio-indicator for Pb wastewater monitoring.

Whereas, Aras *et al*. (2011) reported the potential use of *P. furfuracea* (L.) Zopf lichen collected from 10 different *Pinus* species from Turkey, using 25 RAPD marker. The previous study revealed that GTS% ranged between 81.3-96.0% according to the examined site.

Overall, based on changed bands (a+b+c+d), GTS% and BSI values, our findings were in accordance with Candan and Batir (2015) and Ozyigit *et al*. (2016) in the case of Pb gentoxicity and with Salarizadeh and Kavousi (2015) in the case of Cd genotoxicity. Where, chemical pollutants caused changed bands augmentation combined with decline in both of GTS% and BSI values.

According to previous findings of Labra *et al*. (2003) and Atienzar and Jha (2006), heavy metals pollution lead to DNA changes patterns including missing of control normal bands or/and newly induced bands. The previous researchers stated that loss bands lead to DNA damages; whereas, gains bands refer to DNA mutations. In this regards, in our case study, gains bands increased from 25 to 70 bands as Pb applied concentration increased from 2 to 8 mg/l, indicating that increasing Pb levels provoked increase in DNA mutations level. While, loss bands inversely decreased from 35 to 31 bands as Pb applied concentration increased from 2 to 8 mg/lcompare to the gains patterns; referring that increasing Pb levels caused relative decline in DNA damages.

Saleh (2016c) reported physiological response of the same seaweed species via different Pb (0, 2, 4 and 8 mg/l) concentrations for 2 days. The previous study revealed that the reduction in osmotic potential was significantly ($p \leq 0.001$) different. Whereas, electric conductivity (EC) values significantly increased ($p \leq 0.001$) as applied Pb concentration increased, as a protective mechanism for Pb detoxification.

Whereas, Salarizadeh and Kavousi (2015) reported DNA-induced changes by Cd (0.300–1.050 mg/l) for 7 days in two cumin (*C. cyminum*) ecotypes using 10 RAPD primers The previous study revealed that

changed bands (gains or loss bands and bands intensity) has been observed with Cd treatment compare to their respective reference. in this respect, a+b+c+d values increased from 42 to 57 in Isfahan and from 34 to 47 in Khorasan ecotype as Cd applied concentration increased from 0.300 to 1.050 mg/l. Indeed, GTS% value gradually decreased in both ecotypes as Cd concentrations increased. Moreover, BSI values decreased except at 0.450 mg/l concentration in the both examined ecotypes.

Our data presented herein were comparable with other investigations. These differences could be related to the fact that in terrestrial and aquatic plants (developed plants) develop different mechanisms towards unfavorable heavy metals effect due to the presence of root system. Moreover, the interaction between metal ions and plants grown in soil is completely differing from algae (undeveloped) where their biomass was in directly contact with metal solutions. These data make algae differently respond to Cd stress at DNA level compared to the higher plants.

Table 1. Selected RAPD primers tested in the current study.

Primer name	Sequence 5' - 3'	G+C content (%)
RPi-C1	AAAGCTGCGG	60
RPi-C2	AACGCGTCGG	70
RPi-C3	AAGCGACCTG	60
RPi-C4	AATCGCGCTG	60
RPi-C5	AATCGGGCTG	60
RPi-C6	ACACACGCTG	60
RPi-C7	ACATCGCCCA	60
RPi-C8	ACCACCCACC	70
RPi-C9	ACCGCCTATG	60
RPi-C10	ACGATGAGCG	60
OPJ07	CCTCTCGACA	60
OPK17	CCCAGCTGTG	70
OPR12	ACAGGTGCGT	60
UBC132	AGGGATCTCC	60
UBC159	GAGCCCGTAG	70

Table 2. RAPD pattern changes induced by different Pb applied concentrations as revealed by RAPD marker.

		T1				T2				T3			
Primer	Control	a	b	c	d	a	b	c	d	a	b	c	d
RPi-C1	8	3	4	1	3	5	3	1	3	6	3	1	3
RPi-C2	8	2	4	1	1	7	3	0	4	6	2	0	4
RPi-C3	6	1	3	2	2	6	4	0	4	8	3	0	5
RPi-C4	9	2	5	2	1	4	2	1	5	5	1	0	4
RPi-C5	3	1	0	1	0	1	1	0	2	4	1	0	3
RPi-C6	13	3	6	4	5	1	0	0	4	6	6	2	5
RPi-C7	8	0	1	0	0	1	2	0	0	0	1	0	0
RPi-C8	4	1	2	0	2	1	2	0	2	3	2	0	3
RPi-C9	3	3	1	0	1	1	1	0	1	6	1	0	5
RPi-C10	4	0	1	0	0	0	0	0	1	3	2	0	4
OPJ07	3	1	0	1	2	2	0	1	2	4	0	0	4
OPK12	3	1	1	1	0	2	2	0	1	5	2	0	2
OPR12	6	0	2	0	1	2	1	0	3	5	3	0	2
UBC132	7	3	3	1	1	6	1	0	5	3	2	0	3
UBC159	7	4	2	0	2	6	2	1	4	6	2	0	4
Total bands	92	25	35	14	21	45	24	4	41	70	31	3	51
a+b			60				69				101		
a+b+c+d			95				114				155		

Notes: a: appearance; b: disappearance; c: decreased intensity; d: increased intensityT1: 2 mg/l; T2: 4 mg/l and T3: 8 mg/l.

Table 3. Estimated genomic template stability (GTS%) yielded by RAPD marker.

Primer	C	T1	T2	T3
RPi-C1	100.000	89.063	87.500	85.938
RPi-C2	100.000	90.625	84.375	87.500
RPi-C3	100.000	88.889	72.222	69.444
RPi-C4	100.000	91.358	92.593	92.593
RPi-C5	100.000	88.889	77.778	44.444
RPi-C6	100.000	94.675	99.408	92.899
RPi-C7	100.000	98.438	95.313	98.438
RPi-C8	100.000	81.250	81.250	68.750
RPi-C9	100.000	55.556	77.778	22.222
RPi-C10	100.000	93.750	100.000	68.750
OPJ07	100.000	88.889	77.778	55.556
OPK12	100.000	77.778	55.556	22.222
OPR12	100.000	94.444	91.667	77.778
UBC132	100.000	87.755	85.714	89.796
UBC159	100.000	87.755	83.673	83.673
Average	100.000	87.274	84.174	70.667

Notes:C: Control; T1: 2 mg/l; T2: 4 mg/l and T3: 8 mg/l.

Table 4. Estimated band sharing index (BSI) yielded by RAPD marker.

Primer	T1	T2	T3
RPi-C1	0.533	0.471	0.333
RPi-C2	0.571	0.444	0.636
RPi-C3	0.400	0.267	0.125
RPi-C4	0.533	0.667	0.632
RPi-C5	0.857	0.857	0.667
RPi-C6	0.545	0.880	0.400
RPi-C7	0.933	0.800	0.933
RPi-C8	0.750	0.889	0.600
RPi-C9	0.667	0.667	0.182
RPi-C10	0.857	0.750	0.444
OPJ07	0.667	0.333	0.222
OPK17	0.857	0.750	0.600
OPR12	0.800	0.615	0.400
UBC132	0.308	0.706	0.667
UBC159	0.533	0.556	0.353
Average	0.654	0.643	0.480

Notes: T1: 2 mg/l; T2: 4 mg/l and T3: 8 mg/l.

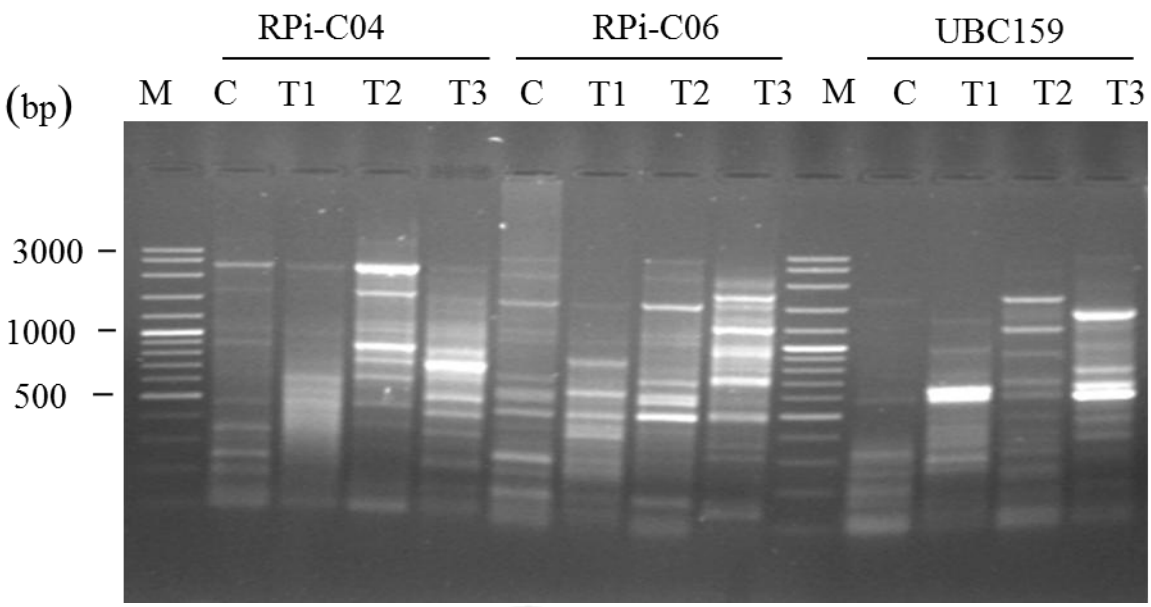

Figure 1.

Figure Legends

Figure 1. RAPD pattern yielded by RPi-C04, RPi-C06 and UBC159 RAPD primers into *U. lactuca* revealed DNA change profiles under different Pb concentrations. M: A VC 100bp Plus DNA Ladder (Vivantis) ladder standard.
C: control; T1: 2 mg/l; T2: 4 mg/l and T3: 8 mg/l.

CONCLUSION

DNA alterations induced by Pb heavy metal in *U. lactuca* seaweed have been evaluated using RAPD marker. The current study suggested that thepolymorphic bands (a+b) and changes bands (a+b+c+d) were positively correlated with applied Pb concentrations. Whereas, both of the GTS% and BSI values decreased as Pb applied concentration increased. Based upon this observation, higher Pb concentration was more toxic as manifested by highest DNA mutations. Thereby, *U. lactuca* macroalgae could be used as a potential reliable bioindicator for Pb pollution detection in aqueous solution. Moreover, RAPD marker seems to be a useful tool for detection genotoxic effects of Pb in the studied algae.

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REFERENCES

Al-Qurainy, F. 2010. Application of inter simple sequence repeat (ISSR marker) to detect genotoxic effect of heavy metals on *Eruca sativa* (L.). African Journal of Biotechnology 9(4): 467-474.

Anderson, D. 2003. Introduction to heavy metal monitoring. European Environment

Agency (EEA), Environmental Assessment Report No. 10, "Europe's Environment: The 3rd Assessment", published on the Web by EEA.

Aras, S., Ç. Kanlitepe, D. Cansaran-Duman, M.G. Halici, and T. Beyaztaş. 2010. Assessment of air pollution genotoxicity by molecular markers in the exposed samples of *Pseudevernia furfuracea* (L.) Zopf in the province of Kayseri (Central Anatolia). Journal of Environmental Monitoring. 12(2): 536-543.

Aras, S., T. Beyaztaş, D. Cansaran-Duman,and E. Gökçe-Gündüzer.2011.Evaluation of genotoxicity of *Pseudevernia furfuracea* (L.) Zopf by RAPD analysis. Genetics and Molecular Research 10 (4): 3760-3770.

Atienzar, F.A., and A.N. Jha. 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutation Research 613(2-3): 76-102.

Aydin, S.S., Z. Kilic, C. Donmez, E. Altunkaynak, and S. Aras. 2015. Assesment of genotoxicity induced by lead pollution in tomato (*Lycopersicum esculentum*) by molecular and population markers. Biological Diversity and Conservation 8(1): 83-89.

Bal, W., and K.S. Kasprzak. 2002. Induction of oxidative DNA damage by carcinogenic metals.Toxicology127:55-62.

Candan, F., and M.B. Batir. 2015. The comparison of physiological, biochemical and molecular parameters in seedlings of artichoke (*Cynara scolymus* L.) and runner bean (*Phaseolus coccineus* L.) seeds exposed to lead (Pb) heavy metal stress in the point of ecological pollution. International Conference on Agricultural, Ecological and Medical Sciences (AEMS-2015) April 7-8, 2015 Phuket (Thailand) pp. 29-33.

Cansaran-Duman, D., O. Atakol, and S. Aras. 2011. Assessment of air pollution genotoxicity by RAPD in *Evernia prunastri* L. Ach. from around iron-steel factory in Karabuk, Turkey. Journal of Environmental Sciences 23(7): 1171–1178.

Cansaran-Duman, D., E. Altunkaynak, A. Aslan, İ. Büyük, and S. Aras.2015. Application of molecular markers to detect DNA damage caused by environmental pollutants in lichen species.Genetics and Molecular Research 14 (2): 4637-4650.

Chang, L., L. Magos, and T. Suzuki (Eds.). Toxicology of Metals. CRC Press, Boca Raton, FL, 1996.

Erturk, F.A., G. Nardemir, H. Ay, E. Arslan, and G. Agar. 2015. Determination of genotoxic effects of boron and zinc on Zea mays using protein and random amplification of polymorphic DNA analyses. Toxicology and Industrial Health 31(11):1015-1023.

Goyer, R.A., and T.W. Clarkson. 2001. Toxic effects of metals. In: Klaassen C.,

editor. Casarett and Doull's Toxicology: The Basic Science of Poisons. 6th ed. McGraw-Hill Health Professions Division; New York, NY, USA.

Jamers. A., R. Blust, and W.D. Coen. 2009.*Omics* in algae: Paving the way for a systems biological understanding of algal stress phenomena? Aquatic Toxicology 92: 114–121.

Labra, M., T. Di Fabio, F. Grassi, S.M. Regondi, M. Bracale, C. Vannini, and E. Agradi. 2003. AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants. Chemosphere 52(7): 1183-1188.

Neeratanaphan, L., R. Sudmoon, and A. Chaveerach. 2014. Assessment of genotoxicity through ISSR marker in *Pistia stratiotes* induced by lead. EnvironmentAsia 7(2): 99-107.

Nordberg, G.F., K. Nogawa, M. Nordberg, and Foreword, L.T. F. 2011. Metals—A new old environmental problem and Chapter 23: Cadmium. In: Nordberg G.F., Fowler B.A., Nordberg M., Friberg L.T., editors. Handbook on the Toxicology of Metals. 3rd ed. Academic Press; Burlington, MA, USA: 2011. pp. vii, 446–451, 463–470, 600–609.

Ozyigit, I.I., I. Dogan, S. Igdelioglu, E. Filiz, S. Karadeniz, and Z. Uzunova. 2016. Screening of damage induced by lead (Pb) in rye (*Secale cereale* L.) - a genetic and physiological approach. Biotechnology and Biotechnological Equipment 1-8

Salarizadeh, S., and H.R. Kavousi. 2015. Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of cadmium on tow Iranian ecotypes of cumin (*Cuminum cyminum*). Journal of Cell and Molecular Research 7 (1): 38-46.

Saleh, B. 2015. Detection of genetic variations in marine algae *Ulva lactuca* (Chlorophyta) induced by heavy metal pollutants. Journal of Stress Physiology and Biochemistry 11 (3): 26-37.

Saleh, B. 2016a. Genetic variation in *Ulva lactuca* (Chlorophyceae) marine algae under cadmium stress. Journal of Bioinnovation 5 (5): 673-685.

Saleh, B. 2016b. DNA alteration induced by cadmium (Cd) metal in *Padina pavonica* (Phaeophyta) marine brown algae. Plant Cell Biotechnology and Molecular Biology (In press).

Saleh, B. 2016c. Lead (Pb) heavy metal impacts in the green *Ulva lactuca* (Chlorophyceae) marine algae. Journal of Stress Physiology and Biochemistry 12 (2): 62-71.

Savva, D. 2000. The use of arbitrarily primed PCR (AP-PCR) fingerprinting to detect exposure to genotoxic chemicals. Ecotoxicology 9(5): 341-353.

Valavanidis, A., and T. Vlachogianni, 2010. Metal pollution in Ecosystems. Ecotoxicology studies and risk assessment in the marine environment. Science advances on Environment, Toxicology and Ecotoxicology 1-14.