

CHEMICAL POLLUTANTS IMPACT IN PLANTS: PHYSIOLOGICAL AND MOLECULAR STUDY - A REVIEW

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ABSTRACT

Huge studies on pollution monitoring have been realized to evaluate pollution impact on marine and terrestrial environments using physiological, biochemical and molecular aspects. Thereby, many plants species were used as a potent bioindicator for monitoring pollution with low cost. Employment of algae as bioindicator for detection of environmental pollution in marine ecosystems has recently attained much attention. Chemical pollutants impacts on living organism (bacteria, plants and animals) could be expressed as growth impairment, pigments content reduction, organism death and DNA damages and mutations. Their dangers occur in terrestrial and aquatic environmental ecosystems, in different ways. The current investigation mainly reported chemical pollutants impacts on plants due to their high potency in accumulating these pollutants in their tissues.

Keywords: Chemical pollutants, Marine Algae, Plants, Toxicity, Genotoxicity

No of Tables : 2

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INTRODUCTION

Heavy metal contamination forms the dangers that threaten the environmental ecosystems including seas, lakes and rivers worldwide. The interest in pollutants has increased worldwide because of their high toxicity on one hand and the fact that they do not break down naturally in the environment on the other hand. Therefore, many studies have focused on pollutants behavior investigation, their toxicity and ability to move on to the food chain and possible ways to remove it. This phenomenon may pose a direct threat to humans and public health (Phillips and Rainbow, 1993; Rainbow, 1995; Bing *et al.*, 2013; Khan *et al.*, 2013).

Pollution in marine ecosystems is a serious environmental issue. It considerably increased due to augmentation human industrial activities. Thereby, monitoring of chemical pollutants in marine ecosystems became one of the indispensable interests in environment. Even, use of living organisms for detection of marine pollution could be considered as a potent tool and provide early warning signals of water pollution trends with low cost.

Heavy metals impact has been widely investigated in many reports. In this regards, Jamers *et al.* (2013) reported that, cadmium (Cd), mercury (Hg) and lead (Pb) are considered by the US Environmental Protection Agency (EPA) the three contaminants of greatest threat to the environment. Their impacts on living organisms have been widely investigated

at physiological, biochemical and molecular levels. Many reports showed their toxicity in terrestrial (Shahrtash *et al.*, 2010; Liu *et al.*, 2012; Azimi *et al.*, 2013; Zhang *et al.*, 2015; Salarizadeh and Reza-Kavousi, 2015), aquatic (Gupta and Sarin, 2009) and marine (Wang *et al.*, 1998; Atienzar *et al.*, 2000; Zhou *et al.*, 2011; Bouzon *et al.*, 2012; Saleh, 2015a; 2016b; 2016e) environmental ecosystems.

Previously, it has been reported that heavy metals stress resulted in DNA changes manifested by loss or induction of new bands compared to the control. Thereby, DNA alteration patterns could be manifested by disappearance of control bands (DNA damage) or induction of new bands (DNA mutation) (Labra *et al.*, 2003; Atienzar and Jha, 2006; Saleh, 2015b, 2016a; 2016c & 2016d). In this respect, Labra *et al.* (2003) stated that RAPD or AFLP techniques were most potent than classic genotoxic assays, even RAPD marker could serve as an efficient tool for detecting temporary DNA changes that might not consequently manifest themselves as mutations. It has been demonstrated that PARD assay among available molecular markers, could be considered as a potent biomarker for monitoring DNA changes even DNA mutations induced by adverse environmental stresses in plants, animals, bacteria and algae (Wong *et al.*, 2000; Liu *et al.*, 2005; 2007; Aksakal *et al.*, 2013; Aksakal and Esim, 2015; Saleh, 2015b, 2016a, 2016c; 2016d).

Since 2014, Hori *et al.* reported that the terrestrial *Klebsormidium flaccidum* green algae shares some genes involved in light and drought tolerance with terrestrial plants; based on cell wall traits combined with the recently sequenced genome.

Thereby, the current review mainly focused on physiological and genetic DNA changes induced by heavy metals stress in plants and algae using molecular marker tools. Consequently, it will be somewhat give an overview image about the importance of some living organisms (plants and algae) as potent bioindicators for monitoring pollution in ecosystems.

Potential algae use

Algae display a multiuse role in different manner; e.g. as bioindicator for heavy metal pollution (Rainbow, 1995); heavy

metals biosorption (Vilar *et al.*, 2008); bioremediation of wastewater (Prabha *et al.*, 2016); as antimicrobial agent (Saleh and Al-Mariri, 2016).

Physiological approach

To date, toxicity of different chemical pollutants on living organisms at physiological level has been successfully documented in several researches (Table 1).

Cadmium (Cd) among chemical pollutants displayed an important role as a highly toxicant to bacteria, plants, algae, and fungi, where algae were the most sensitive (Trevors *et al.*, 1986; Saleh, 2016c).

Table 1. Chemical pollutants toxicity in living organisms at physiological approach.

Living organisms	Metal ion	Reference
Plants species		
Monocotyledons		
Wheat (<i>Triticum aestivum</i> L.)	Cd	Milone <i>et al.</i> (2003)
Wheat (<i>Triticum aestivum</i> L.)	Bo	Kekec <i>et al.</i> (2010)
Wheat (<i>Triticum aestivum</i> L.)	Pb	Pazoki <i>et al.</i> (2014)
		Verma and Dubey (2001)
Rice (<i>Oryza sativa</i> L.)	Cd	Shah <i>et al.</i> (2001)
Rice (<i>Oryza sativa</i> L.)	Cd	Maksimovic <i>et al.</i> (2007)
Maize (<i>Zea mays</i> L.)	Cd and Ni	Erturk <i>et al.</i> (2015)
Maize (<i>Zea mays</i> L.)	Bo and Zn	Gupta and Sarin (2009)
<i>Hydrilla verticillata</i>	Cd, Hg and Cu	
Dicotyledons		
<i>Iris pseudacours</i> L.	Cr and Zn	Caldelas <i>et al.</i> (2012)
Submerged aquatic plants	Hg, Pb, Cd and Cu	Jana and Choudhuri

Okra (<i>Abelmoschus esculantus</i> L.)	Cd	(1984) Aydın <i>et al.</i> (2013)
Chili peppers (<i>Capsicum annuum</i> L.)	Cd	Aslam <i>et al.</i> (2014) Ortega-Villasante <i>et al.</i> (2005)
Alfalfa (<i>Medicago sativa</i> L.)	Cd and Hg	Gichner <i>et al.</i> (2004)
Tobacco (<i>Nicotiana tabacum</i> L.)	Cd	Ghnaya <i>et al.</i> (2007)
<i>Sesuvium portulacastrum</i> and <i>Mesembryanthemum crystallinum</i>	Cd	Fusconi <i>et al.</i> (2007)
Pea (<i>Pisum sativum</i> L.)	Cd	Ozyigit <i>et al.</i> (2016a)
<i>Kalanchoe</i>	Cd	Zengin (2013)
Bean (<i>Phaseolus vulgaris</i> L.)	Ni, Co, Cr and Zn	Gupta and Sarin (2009)
<i>Ceratophyllum demersum</i>	Cd, Hg and Cu	Kaplan <i>et al.</i> (1995)
Algae		Amado Filho <i>et al.</i> (1996)
<i>Chlorella</i> sp (Chlorophyta)	Cd	Amado Filho <i>et al.</i> (1997)
<i>Padina gymnospora</i> (Phaeophyta)	Cu and Zn	Lewis <i>et al.</i> (1998)
Six examined seaweeds (C, R and Ph)	Zn	Malea <i>et al.</i> (2006)
<i>Enteromorpha intestinalis</i> (Chlorophyta)	Cu	Lee <i>et al.</i> (1998)
<i>Enteromorpha</i> spp. (Chlorophyta)	Cd, Zn and N	Unal <i>et al.</i> (2010)
<i>Potamocorbula amurensis</i> and <i>Macoma balthica</i>	Cd, Cr, and Zn	Saleh (2015a)
<i>Ulva lactuca</i> (Chlorophyta)	Cr(VI)	Saleh (2016e)
<i>Ulva lactuca</i> (Chlorophyta)	Cu, Pb, Cd and Zn	
<i>U. lactuca</i> (Chlorophyta)	Pb	
<i>U. lactuca</i> (Chlorophyta) and <i>Padina pavonica</i> (Phaeophyta)	Cd	Saleh (2016b)
<i>Ulva lactuca</i> (Chlorophyta)	Cd	Markham <i>et al.</i> (1980)
<i>Ulva lactuca</i> (Chlorophyta)	Pb, Cd and Co	Bulgariu and Bulgariu (2012).
<i>U. prolifera</i> and <i>U. linza</i> (Chlorophyta)	Cd	Jiang <i>et al.</i> (2013)
<i>Gelidium sesquipedale</i> (Rhodophyta)	Cu, Pb, Cd and Zn	Vilar <i>et al.</i> (2008)
<i>Gracilaria tenuistipitata</i> (Rhodophyta)	Cu	Collén <i>et al.</i> (2003)
<i>Gracilaria lemaneiformis</i> (Rhodophyta)	Cu and Cd	Xia <i>et al.</i> (2004)
<i>Gracilaria domingensis</i> (Rhodophyta)	Cd	dos Santos <i>et al.</i> (2012)
<i>Hypnea musciformis</i> (Rhodophyta)	Cd	Bouzon <i>et al.</i> (2012)
<i>Nitzschia closterium</i> (Bacillariaceae)	Cd, Ni, Pb, Zn, Fe and CrVI	Ova and Övez (2013)

It has been demonstrated that, some elements e.g. copper (Cu), zinc (Zn) and iron (Fe) in low concentrations are essential for catalyzing enzymatic reactions in living organisms. Other elements however, e.g. lead (Pb), mercury (Hg) and cadmium (Cd) could be associated as cofactors for activation of their enzymatic systems (Manoj and Padhy, 2013; Saleh, 2015a; 2016b; 2016e). Chemical pollutants can affect plant and algal growth and physiological and biochemical activities; e.g. Photosynthesis activity and chlorophyll content. These effects could be manifested by decline in specific growth rate (SGR%), pigmentations content (chlorophyll and carotenoids) and total protein content (Bouzon *et al.*, 2012; dos Santos *et al.*, 2012; Jiang *et al.*, 2013). It has been reported that Cd and Pb toxicity on chlorophyll content in plants could be attributed to their role in inhibition of key enzymes such as d-aminolevulinic acid dehydratase (ALA-dehydratase) and protochlorophyllide reductase associated with chlorophyll biosynthesis (Aslam *et al.*, 2014). Indeed these metals could impair the supply of Mg, Fe and Zn (Kupper *et al.*, 1996; Aslam *et al.*, 2014). More recently, Ozyigit *et al.* (2016b) investigated Pb (0, 100, 200 and 400 $\mu\text{mol/L}$) impact in rye (*Secale cereale* L.) after 2 weeks exposure period. The previous study showed a decline in physiological parameters e.g. chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids content by 6.68%, 6.08%, 2.89% and 8.57%, respectively at the highest Pb applied concentration

(400 $\mu\text{mol/L}$). Indeed, Erturk *et al.* (2015) reported boron (Bo) and zinc (Zn) impact on total soluble protein content in *Z. mays*. The previous study revealed that Zn ion reduced the previous parameter and this decline was more noticeable with Zn than Bo ions.

Moreover, Gupta and Sarin (2009) reported 10 $\mu\text{mol/L}$ Cd, 5 $\mu\text{mol/L}$ Hg, and 20 $\mu\text{mol/L}$ Cu impacts on *H. verticillata* and *C. demersum* aquatic plants for 96 h. The previous study revealed that exposure to these heavy metals reduced chlorophyll and protein contents in the two species. This reduction was more pronounced in *H. verticillata* compared to *C. demersum* one.

Whereas, Caldelas *et al.* (2012) reported physiological response of *Iris pseudacorus* L. plants to chromium ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) and zinc (ZnCl_2) (0, 10, 50, 100, and 200 $\mu\text{g/mL}$) for 5 weeks. The previous study revealed growth impairment by 65% and 31% (dry weight) with Cr and Zn treatment, respectively. Whereas, metal treatment has no effect on photosynthetic pigment content. While, Aslam *et al.* (2014) investigated Cd (20, 40, 60, 80 and 100 ppm) impact on *C. annuum* L. for 90 days exposure. The previous study showed that Cd stress caused significant reduction in shoot length, root length and fresh weight, protein, chlorophyll and carotenoid content.

More recently, Ozyigit *et al.* (2016a) reported toxicity of Cd in *Kalanchoe* plants *in vitro* for two months. The previous study

revealed that 400 μM Cd caused reduction in Cha, Chb, Total Chl and carotenoids pigments recorded by ~40.57, ~37.63, ~36.27 and ~37.66%, respectively compared to the control.

As for algae, Unal *et al.* (2010) reported a response of *U. lactuca* to chromium (VI) (0.2, 0.5, 1, and 5 mM K_2CrO_4) after 2 h Cr(VI) exposure. The previous study revealed morphological changes in thallus cells, decline in photochemical efficiency of PSII (Fv/Fm) ratio combined with an increase in necrotic cells along with an increase of Cr(VI) from 1 to 5 mM Cr(VI), than the control. Whereas, Jiang *et al.* (2013) reported an insignificant decline in both chlorophyll and carotenoid pigments observed under 1.8 mg/L Cd compared to the control in both *U. prolifera* and *U. linza* marine algae. While, with 3.7, 7.4 and 14.8 mg/L Cd, a decline in Chl content by 18, 25 and 45%, respectively in *U. prolifera* has been observed; and it was 16, 20 and 39%, respectively for *U. linza*. As for carotenoids, the decline was found to be 16, 29 and 54%, respectively in the case of *U. prolifera* and by 13, 16 and 44%, respectively below the control for *U. linza*.

Whereas, Saleh (2015a) reported Cu (5.8 mg/L), Pb (18.2 mg/L), Cd (10.5 mg/L) and Zn (9.9 mg/L) physiological impacts on *U. lactuca* after 5 days stress. The previous study showed that metals stress reduced SGR% in the following order: Cd > Pb > Zn & Cu. Whereas, decline in Chla by 66%, 64%, 53% and 50% with Pb, Cd, Cu and Zn treatment, respectively was recorded. While, Pb treatment caused the highest

reduction in Chlb reduced by 80% compared to other tested ions. More recently, Saleh (2016e) studied toxicity of Pb (0, 2, 4 and 8 mg/L) after a 2 days exposure period in *U. lactuca*. The previous investigation revealed that Chla decreased by 1.32, 22.7 and 40.4% at 2, 4 and 8 mg/L Pb, respectively. Whereas, this decline was recorded to be 15.8, 13.4 and 17.7% for Chlb below the control under the above mentioned Pb concentrations.

Moreover, Saleh (2016b) reported Cd (0, 2.5, 5 and 10 mg/L) toxicity in *U. lactuca* (Chlorophyta) green and *P. pavonica* (Phaeophyta) brown seaweeds for 4 days. The previous study could suggest that *U. lactuca* was most sensitive to Cd stress than *P. pavonica*; by showing a higher decline in the examined physiological indices than *P. pavonica*. The previous study showed that Cd stress caused a decline in Chla ranged from 56.123 to 66.865% in *U. lactuca* and from 16.183 to 21.365% in *P. pavonica* respectively, when Cd applied concentration increased from 2.5 to 10 mg/L. Whereas, carotenoids content decreased from 36.788 to 44.058% in *U. lactuca* when Cd applied concentration increased from 2.5 to 10 mg/L. While, the previous parameter increased from 0.687 to 80.308% in *P. pavonica* as Cd applied concentration increased from 2.5 to 10 mg/L. The observed increase in carotenoids content in *P. pavonica* with the increase in applied Cd concentration makes them more tolerant to Cd stress compared to *U. lactuca*. Carotenoids pigments act as antioxidant and thereby, serve as osmolytes involved in heavy metals

detoxification by minimize ROS induction generated by heavy metals stress (dos Santos *et al.*, 2012; Bouzon *et al.*, 2012).

Similarly, Zengin (2013) reported that carotenoids content significantly increased in leaves of bean (*P. vulgaris* L.) at various concentrations of Ni, Co, Cr and Zn heavy metals. Whereas, Pazoki *et al.* (2014) reported that Pb (0, 300, 600 and 900 mg/kg of soil) reduced chlorophyll *a* and *b* with increase in carotene content in wheat (*T. aestivum* L.) as Pb concentration increased from 300 to 900 mg/kg. Previously, Kaplan *et al.* (1995) reported the induction of phytochelatin in *Chlorella* sp. algae subjected to Cd. Moreover, Shariati and Yahyaabadi (2006) reported a decline in chlorophyll content and an increase in beta-carotene pigmentations in a two strains (Iranian and Australian) of *Dunaliella salina* green algae as Cd concentration increased from 0.005 to 0.5 mg/L, after 5 days exposure to Cd.

Overall, according to Saleh (2016d), marine algae differently responded to Cd metal than the higher plants. This observation could be related to the absence of root system in algae and its presence in higher plants on one hand, and to the completely different interaction between metal and these two living organisms on the other hand. The occurrence of algae in direct contact with metal in metal solution makes them respond differently to heavy metals stress.

Whereas, in terrestrial plants, Cd absorbed in different manner. Consequently, leading algae to adopt different protective mechanisms to minimize or detoxification unfavorable effect of applied metal.

Molecular approach

Previously, it has been documented that heavy metal 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), which arbitrary attack and damage DNA and important enzymatic proteins (Bal and Kasprzak, 2002). Lead (Pb) is one of fewer heavy metals beside Hg and Cd, classified as highly toxic element for living organisms in environmental ecosystems (Valavanidis and Vlachogianni, 2010).

Many investigations reported DNA changes induced by chemical pollutants in living organisms (Table 2).

Table 2. Genotoxicity effect of chemical pollutants in living organisms as revealed by different applied molecular markers.

Living organisms	Metal ion	Molecular marker	Reference
Plants species			
Monocotyledons			
Wheat (<i>Triticum aestivum</i> L.)	Bo	RAPD	Kekec <i>et al.</i> (2010)
Wheat (<i>Triticum aestivum</i> L.)	Cd	RAPD	Azimi <i>et al.</i> (2013)
Barley (<i>Hordium vulgare</i> L.)	Cd	RAPD	Liu <i>et al.</i> (2005)
Rice (<i>Oryza sativa</i> L.)	Cd	AFLP	Aina <i>et al.</i> (2007)
Rice (<i>Oryza sativa</i> L.)	AS (III)	RAPD	Ahmad <i>et al.</i> (2012)
Rice (<i>Oryza sativa</i> L.)	Cd	SRAP	Zhang <i>et al.</i> (2015)
Maize (<i>Zea mays</i> L.)	Cd	RAPD	Shahrtash <i>et al.</i> (2010)
Maize (<i>Zea mays</i> L.)	fungicide sportak	RAPD	Aksoy <i>et al.</i> (2015)
Maize (<i>Zea mays</i> L.)	Bo and Zn	RAPD	Erturk <i>et al.</i> (2015)
<i>Hydrilla verticillata</i>	Cd, Hg and Cu	RAPD and SCAR	Gupta and Sarin (2009)
Dicotyledons			
Okra (<i>Abelmoschus esculantus</i> L.)	Cd	RAPD	Aydin <i>et al.</i> (2013)
Cucumber (<i>Cucumis sativus</i> L.)	Cu and Zn	RAPD	Aydin <i>et al.</i> (2012)
Cumin (<i>Cuminum cyminum</i> L.)	Cd	RAPD	Salarizadeh and Reza-Kavousi (2015)
Eggplant (<i>Solanum melongena</i> L.)	Cu	RAPD	Körpe and Aras (2011)
Tomato (<i>Lycopersicon esculentum</i> L.)	Pb	RAPD	Aydin <i>et al.</i> (2015)
Rye (<i>Secale cereal</i> L.)	Pb	RAPD	Ozyigit <i>et al.</i> (2016b)
<i>Kalanchoe</i>	Cd	RAPD	Ozyigit <i>et al.</i> (2016a)
<i>Solanum nigrum</i> L. (wild relative for tomato)	Cd, Cu and Zn	ISSR	Al-Khateeb and Al-Qwasemeh (2014)
<i>Arabidopsis thaliana</i>	Cd	RAPD	Zhan <i>et al.</i> (2011)
<i>Arabidopsis thaliana</i>	Cd	RAPD	Liu <i>et al.</i> (2012)
White clover	Cd and AS	RAPD	Ghiani <i>et al.</i> (2014)
Egyptian clover and Sudan grass	Cd	RAPD	Aly (2012)
Common bean (<i>Phaseolus vulgaris</i> L.)	Cd, Pb, Mn	RAPD	Enan (2006)
Common bean (<i>Phaseolus vulgaris</i> L.)	Hg, Cr and Zn	RAPD	Cenkci <i>et al.</i> (2009)
Common bean (<i>Phaseolus vulgaris</i> L.)	Al and Ni	RAPD	Al-Qurainy (2009)

Common bean(<i>Phaseolus vulgaris</i> L.)	Bo	RAPD	Kekec <i>et al.</i> (2010)
Rucola (<i>Eruca sativa</i> L.)	Cd, Pb and Zn	ISSR	Al-Qurainy (2010)
Rucola (<i>Eruca sativa</i> L.)	Cd, Pb and Zn	RAPD	Al-Qurainy <i>et al.</i> (2010)
Artichoke (<i>Cynara scolymus</i> L.) and Runner bean (<i>Phaseolus coccineus</i> L.)	Pb	RAPD	Candan and Batir (2015)
<i>Ceratophyllum demersum</i>	Cd, Hg and Cu	RAPD and SCAR	Gupta and Sarin (2009)
Mung bean (<i>Vigna radiata</i> L.)	Tannery effluents (CETP)	RAPD	Raj <i>et al.</i> (2014)
Chili peppers (<i>Capsicum annum</i> L.)	Cd	RAPD	Aslam <i>et al.</i> (2014)
Water Lettuce(<i>Pistia stratiotes</i>)	Pb	ISSR	Neeratanaphan <i>et al.</i> (2014)
Algae			
<i>Ulva lactuca</i> (Chlorophyta)	Cu, Pb, Cd and Zn	RAMP	Saleh (2015b)
<i>Ulva lactuca</i> (Chlorophyta)	Cu, Pb, Cd and Zn	RAMP	Saleh (2016a)
<i>Ulva lactuca</i> (Chlorophyta)	Cd	RAPD	Saleh (2016c)
<i>Padina pavonica</i> (Phaeophyta)	Cd	RAPD	Saleh (2016d)

Heavy metals genotoxicity as expressed in DNA changes patterns could be manifested by estimated genomic template stability (GTS%) as a qualitative measurement of DNA damage induced by pollutants as reported in many investigations (Liu *et al.*, 2005; Cenkci *et al.*, 2010; Aydin *et al.*, 2012; 2013; Salarizadeh and Reza-Kavousi, 2015; Zhang *et al.*, 2015; Saleh, 2016c; 2016d).

More recently, Ozyigit *et al.* (2016b) reported Pb (0, 100, 200 and 400 $\mu\text{mol/L}$) genotoxicity in rye (*S. cereale* L.) after 2 weeks exposure, using RAPD marker. The previous study revealed RAPD profile alterations and that loss and gains bands were positively correlated with Pb applied

concentrations. In this regards, Pb treatment induced 3 newly bands appearance at 100 and 200 $\mu\text{mol/L}$ Pb combined with a loss of 2 bands at 200 and 400 $\mu\text{mol/L}$ compared to the control. Moreover, GTS% values decreased as Pb applied concentration increased.

Erturk *et al.* (2015) reported boron (Bo) and zinc (Zn) genotoxicity on *Z. mays* using 16 RAPD primers. The previous investigation showed that GTS% decreased as Zn and Bo applied concentrations increased.

Gupta and Sarin (2009) reported 10 mol/L Cd, 5 mol/L Hg, and 20 mol/L Cu impacts on *H. verticillata* and *C. demersum* aquatic plants for 96 h exposure, using RAPD and sequence characterized amplified region

(SCAR) markers. The previous investigation showed that heavy metals stress induced DNA profile alteration expressed as reduction in GTS%. This reduction was more noticeable in *H. verticillata* than *C. demersum* one.

Aslam *et al.* (2014) detected DNA changes induced by Cd stress in *C. annuum* L. using 10 RAPD primers. The previous study revealed 184 bands of which 62 bands were polymorphic, representing polymorphism level of 34%.

More recently, Saleh (2016a) applied Random amplified microsatellite polymorphism (RAMP) marker for detection of genetic DNA alteration in the green algae *U. lactuca* 5 days after Cu, Pb, Cd and Zn exposure. The previous investigation revealed that GTS% value was recorded to be 65.215, 64.630, 59.835 and 59.250% for Pb, Cu, Cd and Zn ions, respectively.

Moreover, Saleh (2016c) reported Cd-genotoxicity in *U. lactuca* green algae exposed to Cd stress for 4 days using 20 RAPD primers. The previous study showed that GTS% increased from 45.4 to 72.8% as Cd increased from 2.5 to 10 mg/L Cd. Similarly, more recently, Saleh (2016d) reported the same metal stress treatment in *P. pavonica* brown marine algae using 22 RAPD primers. The previous investigation revealed that GTS% value increased also from 30.7 to 42.7% as Cd applied concentration increased from 2.5 to 10 mg/L Cd.

It could be suggest from these observations that the interaction between RAPD pattern alterations and Cd applied concentrations was dose-dependent.

Similarly, Liu *et al.* (2005) reported similar trends in barley (*H. vulgare*) and Aydin *et al.* (2013) in okra (*A. esculantus* L.) under Cd stress using RAPD marker.

Indeed, Aydin *et al.* (2012) reported similar findings in Cu and Zn genotoxicity of cucumber (*C. sativus* L.) using RAPD marker. The previous investigation revealed that GTS% increased from 15.98 to 38.69% as ions concentration increased from 40 to 640 mg/L. Moreover, Aydin *et al.* (2013) studied Cd genotoxicity in okra (*A. esculantus* L.) after 21 Cd days exposure, using RAPD marker. The previous study showed that GTS% increased from 59 to 72.5% as Cd applied concentrations increased from 30 to 120 mg/L Cd. Whereas, Salarizadeh and Reza-Kavousi (2015) reported that the GTS% values decreased in *C. cyminum* as Cd applied concentration increased from 300 to 1050 μ M after 7 days Cd treatment using 10 RAPD primers. These findings could be explained by the fact; decreased GTS% does not related to high DNA damages. Increased GTS% as Cd applied concentrations increased, could serve as a defense mechanism leading to a better and effective DNA repair. This observation could be related to the fact, that DNA changes in RAPD profiles expressed as disappearance of normal control bands or/and appearance of new ones; appear to balance each other (Liu *et al.*, 2005; Aydin *et al.*, 2013; Saleh, 2016c; 2016d).

Meanwhile, other reports however mentioned an inverse tendency concerning GTS% under Cd treatment. Aly

(2012) reported Cd impact in Egyptian clover and Sudan grass seedlings for 21 days. The previous study showed that Cd caused increased P% and decreased GTS% as Cd applied concentration increased. Moreover, Zhang *et al.* (2015) stated Cd effect in two rice varieties by SRAP marker. The previous investigation revealed that GTS% decreased as Cd applied concentration increased. Similarly, Salarizadeh and Reza-Kavousi (2015) reported decrease in GTS% under Pb stress using RAPD marker in cumin (*C. cyminum*). Moreover, similar findings were reported in artichoke (*C. scolymus* L.) and runner bean (*P. coccineus* L.) (Candan and Batir, 2015) exposed to Pb stress using the same marker.

Whereas, Cenkci *et al.* (2010) stated that Pb exposure in fodder turnip (*Brassica rapa* L.) using 11 RAPD primers; caused decline in GTS% as Pb concentration increased from 0.5 to 5 mg/L Pb. However, Aydin *et al.* (2015) reported that GTS% values inversely increased from 78.1 to 90.1% as Pb concentration increased from 40 to 240 mg/L Pb in tomato (*L. esculentum*) after 21 days Pb exposure using RAPD marker.

CONCLUSION

It could be suggest from the data presented herein that, most investigated pollutants at physiological level were in the following order: Cd (71%) > Zn (26%) > Cu (24%) > Pb (19%). Moreover, it is worth noting that the given investigations mainly focused on genotoxicity induced by Cd (57%) followed by Pb (24%), Zn (21%) and Cu (14%). Overall, RAPD tool is the most

widely applied molecular markers in the field of pollutants genotoxicity. Thereby, RAPD marker could employ as a potent marker for detecting genotoxicity of pollutants in living organisms.

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