Toxin Reviews

Taylor & Francis
Taylor & Francis Group

ISSN: 1556-9543 (Print) 1556-9551 (Online) Journal homepage: https://www.tandfonline.com/loi/itxr20

Effect of heavy metal stress on antioxidant enzymes and DNA damage in *Nasturtium officinale* R.Br. (watercress)

Fahriye Sümer Ercan, Nuri Ercan & Dilek Demirezen Yilmaz

To cite this article: Fahriye Sümer Ercan, Nuri Ercan & Dilek Demirezen Yilmaz (2019) Effect of heavy metal stress on antioxidant enzymes and DNA damage in *Nasturtium officinale* R.Br. (watercress), Toxin Reviews, 38:4, 328-337, DOI: 10.1080/15569543.2018.1471091

To link to this article: https://doi.org/10.1080/15569543.2018.1471091

Published online: 18 May 2018.

Submit your article to this journal

Article views: 109

View related articles

View Crossmark data

Citing articles: 1 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=itxr20

TOXIN REVIEWS
2019, VOL. 38, NO. 4, 328–337
https://doi.org/10.1080/15569543.2018.1471091

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Effect of heavy metal stress on antioxidant enzymes and DNA damage in *Nasturtium officinale* R.Br. (watercress)

Fahriye Sümer Ercan[a], Nuri Ercan[b] and Dilek Demirezen Yilmaz[c]

[a]Department of Plant Protection, Faculty of Agriculture, Ahi Evran University, Kırşehir, Turkey; [b]Faculty of Agriculture, Ahi Evran University, Kırşehir, Turkey; [c]Department of Biology, Faculty of Science, Erciyes University, Kayseri, Turkey

ABSTRACT
The aim of the study was to investigate responses of *Nasturtium officinale* R.Br. (watercress) under heavy metal stress. RAPD-PCR was used to determine the banding pattern variation in the samples influenced by different doses of heavy metals (HMs). After treatment with HMs, catalase (CAT), superoxide dismutase (SOD), and the level of malondialdehyde (MDA) of samples have been determined. The maximum enzyme activities were observed at different exposure conditions. The level of MDA increased with increasing heavy metal concentration. Changes in RAPD profiles of heavy metal treated samples compared with control. Our results showed that exposed samples with Cr gave the minimum number of new fragments compared with control. These results suggest that increase in anti-oxidative enzyme activity may play important roles in alleviating the toxicity of Cr, Cu, and Cd in *N. officinale*.

ARTICLE HISTORY
Received 11 January 2018
Accepted 26 April 2018

KEYWORDS
DNA damage; antioxidant enzyme; RAPD-PCR; heavy metal; *Nasturtium officinale*

1. Introduction

Heavy metals (HMs) are one of the most important genotoxic environmental pollutants that cause serious threat to both human and environment. They can cause hazardous effects on plants by varying the major plant physiological and metabolic processes (DalCorso *et al*. 2008). HMs like lead (Pb), cadmium (Cd), chromium (Cr), and copper (Cu) may inhibit specific essential physiological activities (Censi *et al*. 2006). Cd has high toxicity on plants, animals, and humans. It can cause various phytotoxic symptoms that include chlorosis, growth inhibition, and accelerated senescence in plants (Mishra *et al*. 2006). Cr is one of the most abundant and important environmental contaminant released into the atmosphere because of its great industrial use (Nriagu and Nieboer 1988). This metal causes vigorous damages in plants and animals. Phytotoxic effects of Cr occurs inhibition of seed germination, pigment degradation, antioxidant enzymes, and induction of oxidative stress in plants.

Copper works as cofactor and activators of enzymatic reactions like Zn. Some HMs at certain concentrations are critically important in the functional activities of proteins that are important in grown and development of living organisms. However, at extreme concentrations, these ions can become destructive to living things with the inclusion of plants.

General result of HM toxicity is extreme production and accumulation of reactive oxygen species (ROS) that disrupt the cellular redox environment causing oxidative stress (Erdei *et al*. 2002). The damage that caused by heavy metal toxicity varies with plant species, metal type, concentration, and chemical form.

Antioxidant defense systems protect the living things from oxidative stress that caused by metals (Basha and Rani 2003). This system mainly contains antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nonenzymatic antioxidant compounds such as glutathione (GSH). SOD and CAT remove peroxides and superoxide radicals; SOD converts superoxide anion radical to hydrogen peroxide, CAT reduces hydrogen peroxide to water (Goswami and Das 2016).

Toxicants negatively affect the human health with a direct and indirect action. While the target of direct action is germ and somatic cells, indirect action cause physiological and ecological effects (Koh *et al*. 1997). The genetic effects of toxic compounds at the DNA level are called mutagenic effects. Germ cell mutations may change the genetic constitution of a population (Staton *et al*. 2001) at the same time, may decrease the viability of gametes, embryos and also affect the population genetic structure (Hebert and Luiker 1996). Despite that, somatic mutations are not effecting next

CONTACT Fahriye Sümer Ercan fahriyesumer@gmail.com Department of Plant Protection, Faculty of Agriculture, Ahi Evran University, Kırşehir, Turkey

© 2018 Informa UK Limited, trading as Taylor & Francis Group

generation, except in asexual or clonal organisms. But may give rise to cell death and malignant transformation of normal cell (Bickham *et al*. 2000).

Determining of population genetic effects of toxicant exposure, recently selective and sensitive assays developed in ecogenotoxicology. One of these DNA based techniques is random amplified polymorphic DNA (RAPD) and can be used effectively to determine the DNA fingerprints from individual exposed and/or non-exposed to genotoxic agents (Savva 1998).

This technique is used generally for detecting species classification, genetic mapping, and phylogeny, but, ecotoxicological studies showed that, if RAPD conditions standardized strictly, this technique can be used effectively to determine toxicological population genetic effects. At the same time, this technique is relatively inexpensive and can give information large number of loci without sequence data for primer design (De Wolf *et al*. 2004).

RAPD may potentially uses as a biomarker assay for detection of DNA damages like rearrangements, point mutation, small insert, or deletions of DNA in bacteria, plants, invertebrate, and vertebrate animal cells (Savva 1998, Atienzar *et al*. 2000). Also, this technique has proposed as a very reliable, sensitive, and simple method for genotoxicity studies (Kumar *et al*. 2015).

DNA banding patterns are showed after agarose gel electrophoresis by ethidium bromide staining, and missing bands or appearance of new bands can be detected by the comparison of profiles generated from control and treated DNA.

Watercress (*Nasturtium officinale*), an edible aquatic herb belonging to the Brassicaceae (cabbage) family, is a "luxury feeder" that can grow rapidly and take up nitrogen in excess of its growth requirements NIWA (2008). This plant can use phytoremediation of some pollutants from ecosystem. The properties of *N. officinale* that entail metal accumulation were extensively studied in the past. However, even then the available knowledge regarding the Cu, Cd, and Cr effects on DNA damage of watercress exposed to these HMs is not sufficient.

The aim of this study was to detect the DNA damage induced by Cd, Cu, and Cr by using RAPD-PCR technique in watercress and to determine changes in the antioxidant enzyme activity including SOD and CAT the level of malondialdehyde (MDA) after treatments in laboratory conditions.

2. Materials and methods

2.1. Plant material, cultivation, and heavy metal estimation

Fresh samples of *Nasturtium officinale* R.Br. were obtained from ponds in Kayseri, Turkey. Plants were grown in a growth chamber at 25 ± 1 °C during 16/8 light/dark cycle with 350 μmol m^2 s^{-1} of irradiance by cold fluorescent lamps. The plants were growth in Hoagland nutrient solution, and the solution was renewed every four days. In this study, cadmium nitrate was used for treatments and were exposed to various concentrations of cadmium nitrate: 0, 2, 5, 10, 15, and 20 ppb. Plants were exposed to various concentrations of $CuCl_2{\cdot}2H_2O$: 200, 500, 1000, 2500 ppb and $K_2Cr_2O_7$: 1, 3, 10, 25, and 50 ppb. The solution pH was maintained to 5.8 ± 0.1 by titration with NaOH or HCl solutions (0.1 M) when required. On the seventh day, heavy metal treated plants were harvested from containers. The plants were rinsed twice with distilled water and subsequently, biochemical parameters were determined. Dried samples of plant were digested with 10 ml of concentrated HNO_3, using a CEM microwave digestion system. After digestion, the volume of each sample was adjusted to 25 ml using double deionized water (Demirezen 2007). Determination of the cadmium, chromium and copper concentrations in all samples was carried out by inductively coupled plasma optical emission spectrometry (Varian). The samples were analyzed in triplicate.

2.2. Estimation of lipid peroxidation (MDA)

Lipid peroxidation level was estimated according to the method of Razinger *et al*. (2008) with modifications (weight of used tissue, time etc.). Fresh tissues (0.5 g) were homogenized in 3 ml of 0.3 M trichloroacetic acid (TCA) and centrifuged at 14,000 *g* for 15 min. For every 1 ml of the supernatant, 1 ml of 0.3 mM TCA comprised of 42 mM 2-thiobarbituric acid (TBA) was added. The mixture was incubated at 96 °C for 20 min and then transferred into an ice bath to stop the reaction. The tubes were centrifuged at 10,000 *g* for 10 min and the absorbance of the resulting supernatant was measured at 532 and 600 nm wavelengths. The amount of MDA (extinction coefficient of 155 mM^{-1} cm^{-1}) was calculated by subtracting the nonspecific absorbance at 600 nm from the absorbance at 532 nm (Heath and Packer 1968).

2.3. Antioxidative enzymes

2.3.1. Enzyme extraction

Fresh tissue (0.2 g) was homogenized with 5 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone in an ice-cooled mortar. Homogenate was centrifuged at

15,000 *g* for 15 min at 4 °C (Hou *et al*. 2007). The supernatant was used for enzyme determination.

2.3.2. Superoxide dismutase (EC 1.15.1.11)

The SOD activity (EC 1.15.1.11) was analyzed by measuring the inhibition of the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp and Fridovich 1971). The assay mixture contained 20 mM phosphate buffer (pH 7.5), 10 mM methionine, 0.1 mM NBT, 0.1 mM EDTA, 0.005 riboflavin, 50 μg mL^{-1} of enzyme extract and 0.25 ml of deionized H_2O in a total volume of 3 ml. Riboflavin was added at the end, and the tubes were shaken and then illuminated for 15 min. The absorbance was recorded at 560 nm and the absorbance of the non-irradiated reaction mixture served as a control. Inhibition of 50% of the reaction was defined as one unit of enzyme.

2.3.3. Catalase (EC 1.11.1.6)

CAT (EC 1.11.1.6) activity was measured spectrophotometrically by following the consumption of H_2O_2 at 240 nm, according to Sheldon and Pelt (2013), in potassium phosphate buffer (150 mM, pH 7) containing 15 mM H_2O_2 and enzyme extract (exactly 50 mg of protein) in a final volume of 1 ml. Addition of H_2O_2 started the reaction.

2.3.4. Total protein content

The protein content was determined by the method of Lowry *et al*. (1951) using bovine serum albumin as the standard protein.

2.4. DNA extraction and RAPD-PCR procedure

DNA isolation was performed by using CTAB method (Rogers and Bendich 1985) and the concentration of DNA was estimated with a spectrophotometer at 260 nm. RAPD-PCR was performed in a standard 15 μl reaction mixture that contains PCR buffer 1.5 μl (10× buffer with $(NH_4)_2$ SO_4, Fermentas), $MgCl_2$ (2.5 mM, Fermentas) 1.2 μl, dNTP (10 mM stock solution) 0.5 μl, BSA (10 mg/ml) 0.6 μl, primers (10 μM, Opc) 1.0 μl, Taq Polymerase (5 u/μl, Fermentas) 0.25 μl and filled up with sterile deionized water to the final volume. 100 ng of genomic DNA was also added to each PCR tube.

The RAPD protocol consisted of an initial denaturating at 94 °C for 2.5 min, followed by 36 cycles at 94 °C for 45 s (denaturation), 35 °C for 45 s (annealing) and 72 °C for 2 min (extension), with final extension at 72 °C for 10 min. After amplification process, the products were electrophoresed in Tris-Acetic Acid-EDTA (TAE) buffer on a base of 2% agarose gel at 80 V and stained with ethidium bromide. The resultant gels were visualized under UV on a UV transilluminator and photographed.

Table 1. Random primers that gave polymorphic bands in RAPD-PCR.

RAPD primer	Sequences of primers (5′→3′)	GC%
Opc 2	GTG AGG CGT	70
Opc 5	GAT GAC CGC	70
Opc 8	TGG ACC GGT	70
Opc 9	CTC ACC GTC	70
Opc 11	AAA GCT GCG	60
Opc 15	GAC GGA TCA	60

100 bp DNA ladder was used as a standard marker. Ten oligomers that contain 10 nucleotides were used for amplification reactions. The sequences of oligomers that are gave polymorphic bands shown in Table 1. The amplification reactions were repeated at least three times to get reproducible results. RAPD pattern modifications as disappearance of bands and appearance of new bands were given the arbitrary score of +1.

The primers that showed clear variations were calculated, despite that RAPD profiles of primers with no specific changes were not considered in the final calculation of genomic template stability (GTS) which was a qualitative measurement of RAPD banding pattern changes.

2.5. Estimation of genomic template stability

Genomic template stability (GTS; %) was calculated as following:

$$GTS = (1 - a/n) \times 100 \quad (1)$$

where a was the average number of RAPD polymorphic profiles detected in each samples treated and the number of total bands found in the control. The polymorphism that observed in RAPD banding profiles included the disappearance of a normal band and appearances of a new band in comparison to banding patterns of control. The average polymorphism was calculated for each experimental group exposed to different doses of different HMs (Cd, Cu, and Cr). For comparing the sensitivity of each parameter, changes occurring in these values were calculated as a percentage of its control (set to 100%).

2.6. Statistical analysis

In this study, all experiments were repeated in triplicates. Values shown in the figures represent the

average values ± standard deviation (SD) for each heavy metal concentration. Data were subjected to a two-way analysis of variance (ANOVA) to confirm the variability of data and validity of results, and Tukey test was performed to determine the significant differences between treatments. In figures, the values are marked as * (asterisk) for the significance level ($p \leq 0.05$) as compared to control.

3. Results

3.1. Heavy metal contents

The results related to the contents of Cd, Cr, and Cu in *N. officinale* R.Br. are presented in Figure 1. The maximum Cd content was found to be 4.7 ppb DW at 15 ppb Cd. On prolonged exposure to higher concentrations of Cd (25 ppb), there was a significant decline in the Cd rate. According to Figure 1(b), the higher Cr content was obtained from 4.9 ppb Cr application (25 ppb) and the differences in all treatments were significant for *N. officinale* R.Br. Similarly, the maximum Cu content was found to be 680 ppb at 2500 ppb

The analysis of one-way ANOVA showed that the differences in all the treatments were significant ($p < 0.05$).

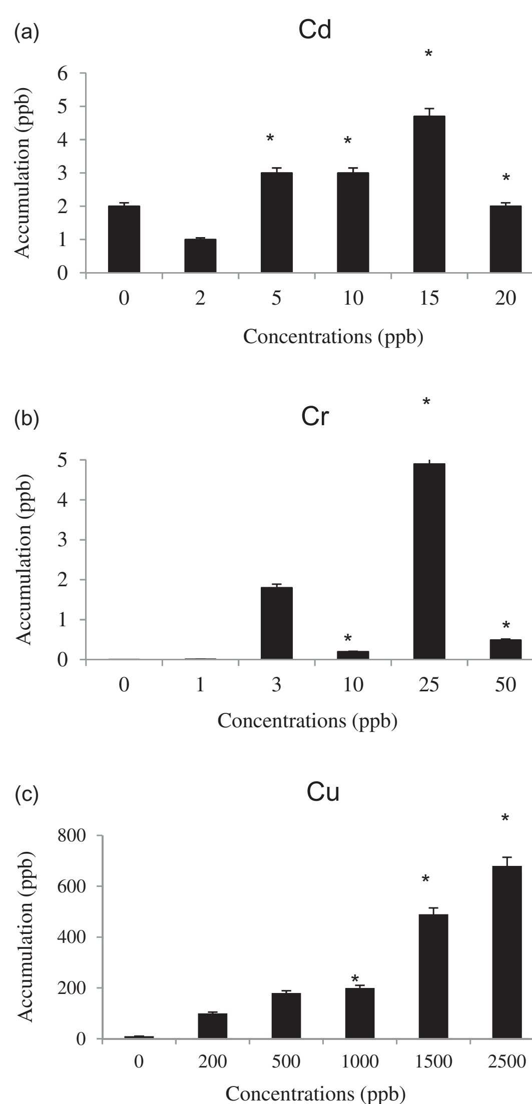

Figure 1. Heavy metal accumulation in *N. officinale*. Values represent mean ± SE ($n = 3$). Asterisks indicate significant differences at $p < 0.05$.

3.2. Effect of heavy metals on the level of MDA

The effect of Cd, Cu, and Cr on MDA concentration is presented in Figure 2. In the experiments, increasing in MDA concentration in *N. officinale* R.Br. was observed. However, exposure of highest concentrations of HMs, MDA level was decreased. The analysis of one way ANOVA showed that the differences of all treatments were significant ($p < 0.05$) for plant.

3.3. Effects of heavy metals on SOD and CAT activity

In this study, the decline in CAT activity was observed with the highest concentration (25 ppb) of Cd in *N. officinale* R.Br. The highest concentration of Cd (25 ppb) proved to be extremely toxic declining CAT activity (Figure 2). The analysis of one way ANOVA showed that the differences of all treatments were significant ($p < 0.05$) for plant. Similarly, the highest concentration of Cr (25 and 50 pbb) and Cu (1500–2500 ppb) proved to be toxic declining CAT activity (Figure 2).

Figure 2 indicated that SOD activity increased linearly with increasing heavy metal levels. The maximum SOD activity was recorded at 15 ppb Cd (1.18 ± 0.0085 units g^{-1} protein). Results showed, at the highest concentration of Cd, the SOD accumulation decreased. Additionally, the significant differences were found in SOD activity among the treatments. At 1500 ppb Cu (2.14 ± 0.0075 units g^{-1} protein), the maximum SOD activity was recorded. The analysis of one way ANOVA showed that the differences of all treatments were not significant ($p < 0.05$) for plant SOD contents. Similarly, SOD activity increased linearly with increasing Cr levels in *N. officinale* R.Br. The maximum SOD activity was recorded at 25 ppb Cr (2.086 ± 0.0085 units g^{-1} protein).

3.4. Total protein content

Protein levels indicated a significant negative correlation with heavy metal concentrations in plant tissues

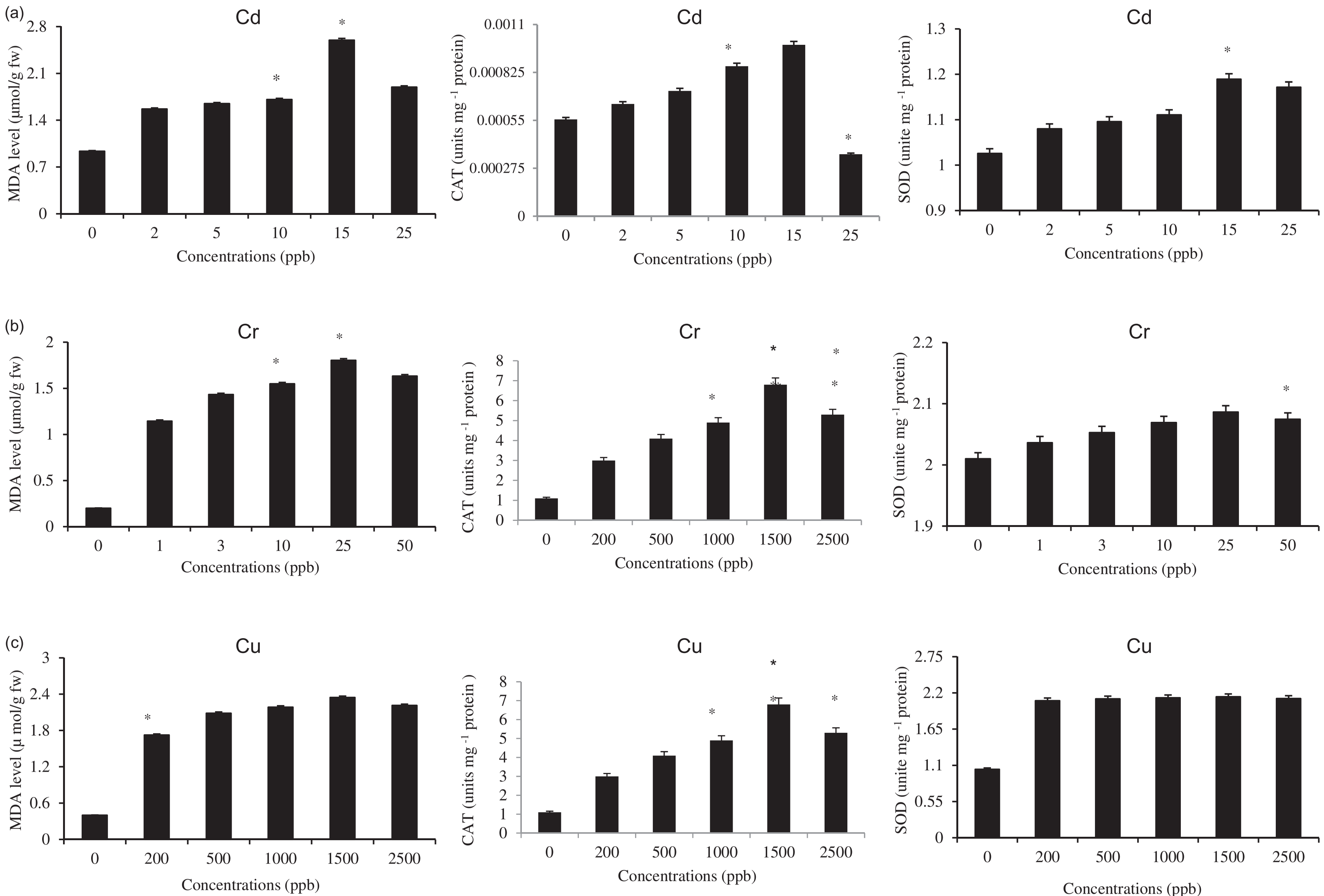

Figure 2. Lipid peroxidation, CAT and SOD activities in *N. officinale* upon Cd (a), Cr (b) and Cu (c) exposures. Values represent mean ± SE ($n = 3$). Asterisks indicate significant differences at $p < 0.05$.

($p \leq 0.05$). At Cd applications of 2 and 5 ppb, the protein content of plant tissues was found to be significantly high, in comparison with the control (Figure 3(a)). Furthermore, the effect of a Cd application of 5 ppb on the content of protein was noted to be significantly higher than the effect of 2 ppb of Cd. Additionally, the cadmium applications of 10 and 15 ppb did not produce any statistically important differences with the control sample. However, at a Cd application of 25 ppb, a decrease was observed to appear in the protein content in comparison with the control. Similarly, at Cr applications of 1 and 3 ppb, the protein content was observed high in comparison with the control (Figure 3(b)). At a Cr application of 25 and 50 ppb, a decrease was observed to appear in the protein content in comparison with the control.

Observed protein levels in plant tissues treated with copper was found to be high in compare to the control (Figure 3(c)). At the copper applications of 200 and 500 ppb, the protein content was observed high in comparison to other treatments. The analysis of one way ANOVA indicated that the differences among treatments were not significant ($p < 0.05$) for plant protein contents.

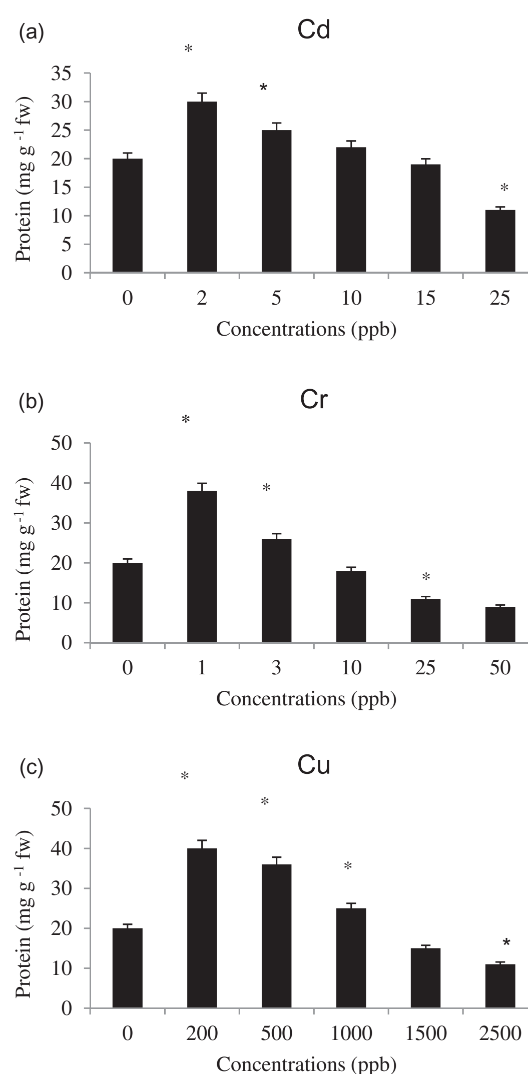

Figure 3. Protein level in *N. officinale* upon Cd (a), Cr (b) and Cu (c) exposures. Values represent mean ± SE ($n = 3$). Asterisks indicate significant differences at $p < 0.05$.

3.5. Effects of heavy metals on RAPD profiles

Of the 10 decamer oligonucleotide primers evaluated, 6 of them gave specific and stable results (Table 1). RAPD fingerprints showed the occurrence of substantial differences among unexposed and exposed plant materials, with obvious changes in the number, size, and the intensity of amplified DNA fragments. The RAPD banding patterns generated by the HMs exposed plants were evidently different from the control. Changes in RAPD profiles obtained from treated plant samples with HMs included variation in band intensity, appearance of new bands and loss of normal bands compared with control plants. GTS did not decrease gradually.

The decrease in band intensity was only apparent for watercress exposed to 15 ppb Cd for primers Opc 9 and Opc 11, 1500 ppb Cu for primers Opc2 and Opc 9 and 10 ppm Cr for primer Opc 15 (Tables 2–4). However, the increase in band intensity was clearly obvious for all doses of Cd and Cu treatments except Opc 8 primer. The disappearance of normal bands was found at all concentrations of HMs treatments, especially for primer Opc 8 (Tables 2–4).

Finally, it was defined that maximum number of extra bands appeared with Opc 8 at 5 ppb Cd (four new bands), Opc 15 at 1000 ppb Cu (four new bands) and Opc 5 at 25 ppb Cr (three new bands). The extra bands that appeared were determined to be of approximately 800–1100 bp molecular sizes (Figure 4). The occurrence of polymorphism was due to the loss and/or gain of the bands in the treated plant with HMs in comparison with the control. Value of polymorphism was found above 35% for all doses of each heavy metal applications.

4. Discussion

The HMs cause oxidative damage and some reactive oxygen species (ROS) can affect the signal transduction pathways. For analyzing of the DNA damages in eco-genotoxicity studies, PCR-based techniques such as RAPD, AFLP, SSR, have provided very informative results (Savva 1996). In general, RAPD reactions are performed with a single 10 bp primer and after

Table 2. Changes of total bands in control, and of polymorphic bands and varied bands in leaves of *N. officinale* exposed to different concentrations of Cd.

	Cd concentration (ppb)																				
		2				5				10				15				25			
Primers	0	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Opc 2	6	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	1	0	0	0	1
Opc 5	4	2	0	0	3	2	0	0	3	2	0	0	3	0	0	0	0	0	0	0	2
Opc 8	4	3	4	0	0	4	4	0	0	2	4	0	0	2	4	0	0	2	4	0	0
Opc 9	2	1	0	0	1	2	0	0	1	1	0	0	1	0	1	1	0	1	0	0	1
Opc 11	2	0	0	0	1	0	0	0	2	0	0	0	2	0	1	1	0	0	0	0	2
Opc 15	3	2	0	0	2	3	0	0	3	3	0	0	3	0	0	0	3	2	0	0	3
Total bands	21	8	4	0	8	11	4	0	10	8	4	0	11	2	6	2	4	5	4	0	9
a + b		12				15				12				8				9			
a + b + c + d		20				25				23				14				18			

a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities, a + b denote polymorphic bands, and a + b + c + d, varied band.

Table 3. Changes of total bands in control, and of polymorphic bands and varied bands in leaves of *N. officinale* exposed to different concentrations of Cu.

	Cu concentration (ppb)																				
		200				500				1000				1500				2500			
Primers	0	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Opc 2	6	0	0	0	2	0	0	0	2	0	0	0	2	0	0	2	0	0	0	0	2
Opc 5	4	1	0	0	3	2	0	0	3	2	0	0	3	0	0	0	1	2	0	0	3
Opc 8	4	2	4	0	0	2	4	0	0	3	4	0	0	2	4	0	0	3	4	0	0
Opc 9	2	1	0	0	1	2	0	0	1	2	0	0	1	0	1	1	0	0	0	0	1
Opc 11	2	0	0	0	2	0	0	0	2	0	0	0	2	0	1	0	1	0	0	0	2
Opc 15	3	2	0	0	3	2	0	0	3	4	0	0	3	3	0	0	3	3	0	0	3
Total bands	21	6	4	0	11	8	4	0	11	11	4	0	11	5	6	3	5	8	4	0	11
a + b		10				12				15				11				12			
a + b + c + d		21				23				26				19				23			

a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities, a + b denote polymorphic bands, and a + b + c + d, varied band.

Table 4. Changes of total bands in control, and of polymorphic bands and varied bands in leaves of *N. officinale* exposed to different concentrations of Cr.

	Cr concentration (ppm)																				
		1				3				10				25				50			
Primers	0	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Opc 2	6	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	5	0	0	0	2
Opc 5	4	0	1	0	0	2	0	0	3	0	0	0	2	3	0	0	3	0	0	0	2
Opc 8	4	2	4	0	0	2	4	0	0	2	4	0	0	2	4	0	0	2	4	0	0
Opc 9	2	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0
Opc 11	2	0	1	0	1	1	0	0	2	1	0	0	2	1	0	0	2	1	0	0	2
Opc 15	3	2	0	0	2	1	0	0	3	2	0	1	2	2	0	0	2	2	0	0	2
Total bands	21	4	7	0	3	6	5	0	9	5	5	1	7	8	5	0	12	5	5	0	8
a + b		11				11				10				13				10			
a + b + c + d		14				20				18				25				18			

a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities, a + b denote polymorphic bands, and a + b + c + d, varied band.

amplification the fragments are demonstrated by agarose gel electrophoresis. In genotoxicitiy studies, RAPD bands are lost or gained when the point mutations, inversions or deletions affect the presence/absence of priming sites, their complementary to primers or the distance between priming sites (Fritsch and Rieseberg 1996). RAPD is a qualitative method that clearly used in the detection of pollutant-induced DNA effects (Atienzar *et al*. 2001). In the present study, we detected the genotoxicity induced by different concentrations of HMs using RAPD technique.

DNA damages and mutations may alter a primer binding site and thus genomic template stability changes and polymorphism occurs within dosedependent treatments and untreated organisms

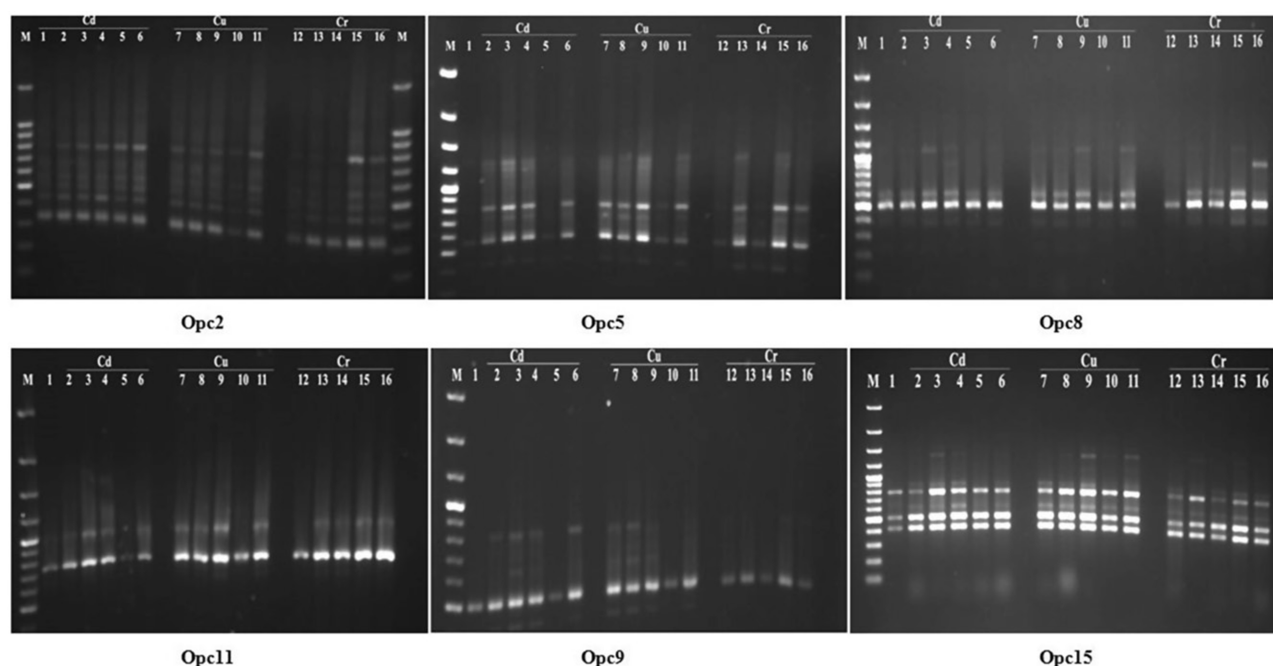

Figure 4. RAPD profiles of genomic DNA from watercress exposed to varying heavy metal concentrations with different primers. (M: DNA marker, 1: Control, 2–16 increasing doses of heavy metal treatments).

Changes in the RAPD banding profiles like loss of bands and the band intensity modifications may be occurred by the changes of priming sites and polymorphism may occur between treated and control organisms (Pal 2016). Appearance of new bands could be occurred due to the mutations, large deletions or homolog recombination in DNA sequence (Atienzar *et al*. 1999). Our results showed that largest number of new bands obtained from Cu and Cr with 1000 ppb and 5 ppb doses, respectively. Increase in band intensities was observed at all doses of all exposed HMs, whereas decrease in band intensities was obtained only at one dose application with each heavy metal.

Atienzar *et al*. (1999) suggested that genotoxic application induced alterations in RAPD profiles can be evaluated as changes in GTS. Abdelmigid (2010) reported that GTS of exposed plants decreased gradually according to control group. Author also reported that differences in GTS variation could be attributed to multiple changes in RAPD profiles. In our study, GTS did not decreased gradually. This can be explained as the GTS is not only directly related to the extent of DNA damage but also related to the performance of DNA repair and replication. For instance, a high level of DNA damage does not necessarily decrease the GTS (in comparison to a low level of DNA alterations) because DNA repair and replication may be inhibited due to excessive, lethal actions of the pollutant-induced adducts. The genomic template stability cannot be completely affected because the induction of DNA damage may not increase linearly (plateau effect). Besides, GTS may be related to different DNA damages like mutations, rearrangements and it would be really difficult to guess dose-response relationship (Rocco *et al*. 2014).

Our results showed that DNA polymorphisms detected by RAPD can be used as a biomarker system for detection of the genotoxic effects of environmental pollutants like HMs. The effects of DNA damage on the RAPD profiles can only be speculated when the amplicons are analyzed and specific methods are needed to obtain quantitative data (Abdelmigid 2010).

Changes in the RAPD profile revealed the effect of HMs on DNA integrity. The RAPD-PCR can be applicable to study the effect of genetic contamination on plants and this technique is fast, reliable, and easy to detect the genotoxicity induced the HMs.

According to obtained results, an increase in the total protein content was found to occur at lower heavy metal concentrations; however, at high concentrations of selected HMs a significant decrease occurred in the protein content. This ultimate decrease in the protein content may be dependent on an increase of ROS. In the present study, *N. officinale* R.Br. were grown in hydroponic culture in the presence of increasing Cd, Cu and Cr concentrations to evaluate its possible defense mechanisms. The results obtained indicated that at highest levels Cd, Cu and Cr were toxic to *N. officinale* R.Br.

According to Hou *et al*. (2007), MDA is the decomposition product of polysaturated fatty acids (PUFA) of biomembranes and its increase shows plants under high level antioxidative stress. MDA concentration obtained from this study was linearly with increased heavy metal levels in the solution. The main site of attack by any redox active metal in a plant cell is usually the cell membrane. Similar results were found with *P. crinitum* by Hou *et al*. (2018).

Metal toxicity is often driven by ROS generation, directly via the catalytic production of superoxide ($O_2^{\bullet -}$) by the Haber–Weiss and Fenton reactions, or indirectly by other mechanisms (Nehnevajova *et al*. 2012). ROS can damage membrane lipids, proteins, pigments, and nucleic acids and plant evolves the antioxidant defense system to prevent the adverse effects of oxidative stress to cells (Kasperczyk *et al*. 2015). Inadequate activities of antioxidant defense systems cause oxidative damage in plants exposed to HMs (Mittler 2002). The antioxidative enzyme system is one of the protective mechanisms in a plant. The increased activity of antioxidative enzymes in a plant indicated the formation of ROS and the present study indicated the generation of oxidative stress in *L. gibba* since all studied enzymes activity increased at high Cd added levels (Demirezen Yılmaz and Uruç Parlak 2011). In other words, *L. gibba* analyzed in this work contains high Cd concentrations sufficient to activate ROS production and then oxidative stress.

According to Sharma and Dietz (2009), antioxidant enzyme activities in metal-stressed plants are highly variable, depending on the plant species, metal ion, concentration and exposure duration, but these processes reflect the modified redox status of the stressed cells. Since the primary reduced product of oxygen is the superoxide anion radical, SOD represents the first defense against oxidative stress.

Superoxide dismutase, the first enzyme in the detoxifying process, converts superoxide radicals to H_2O_2 at a very fast rate. The enhanced SOD activity observed in the present study is consistent with studies in which other plant species were treated with HMs (Sharma and Dubey 2007). The effect of heavy metal stress on SOD expression is likely to be governed by the tissue and sub cellular sites at which oxidative stress is generated as supported by the higher activity of SOD in roots than in leaves of metal stressed plants (Hou *et al*. 2007).

Our data showed significant increases in CAT activity were observed between the treatments. The results obtained indicated that CAT activity decreased linearly with increasing Cd, Cu and Cr levels. Contrary to our results, a decline in the specific activity of CAT with increase in Cr concentration (20–80 ppm, 0.5 mM) has been reported by Shankar *et al*. (2005). According to Bashir *et al*. (2015), it is likely that excess production of ROS by HMs can inactivate CAT probably by inactivating enzyme-bound heme group. These inconsistent results regarding CAT activity might be due to differences in the plant organs studied, the durations and concentrations of metals utilized, and the plant species under investigation (Radić *et al*. 2010).

5. Conclusion

Considering these results, we strongly suggest that *N. officinale* R.Br. may not suffer from oxidative stress in spite of the high levels of Cd, Cu and Cr present in medium, as would be expected for a species that is successfully adapted to polluted environments. Therefore, the data shown here can be considered to illustrate how *N. officinale* R.Br. responds to its stressful environment. *N. officinale* has the capacity to overcome the heavy metal-induced stress, especially at a moderate degree of exposure. Successful adaptation to the metal stressing environment of aquatic macrophyte species is the basis for the proposals of several authors to use this singular species for the re-vegetation and recovery of contaminated metal areas.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Abdelmigid, H.M. 2010. Qualitative assessment of cadmium stress using genome template stability in *Hordeum vulgare*. *Egyptian journal of genetics and cytology*, 39, 291–303.

Atienzar, F.A., *et al*., 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. *Environmental toxicology and chemistry*, 18, 2275–2282.

Atienzar, F.A., *et al*., 2001. Fitness parameters and DNA effects are sensitive indicators of copper-induced toxicity in *Daphnia magna*. *Toxicological sciences*, 59, 241–250.

Atienzar, F.A., Cordi, B., and Donkin, M.E., 2000. Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palnaria palnata*. *Aquatic toxicology*, 50, 1–12.

Basha, P.S. and Rani, A.U., 2003. Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicology and environmental safety*, 56, 218–221.

Bashir, H., *et al*., 2015. Chloroplast and photosystems: impact of cadmium and iron deficiency. *Photosynthetica*, 53 (3), 321–335.

Beauchamp, C. and Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, 44, 276–287.

Bickham, J.W., *et al*., 2000. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation research*, 463, 33–51.

Censi, P., *et al*., 2006. Heavy metals in coastal water system. A case study from the North Western Gulf of Thailand. *Chemosphere*, 64 (7), 1167–1176.

DalCorso, G., *et al*., 2008. How plants cope with cadmium: staking all on metabolism and gene expression. *Journal of integrative plant biology*, 50 (10), 1268–1280.

De Wolf, H., Blust, R., and Backeljau, T., 2004. The use of RAPD in ecotoxicology. *Mutation research*, 566, 249–262.

Demirezen Yılmaz, D. and Uruç Parlak, K., 2011. Changes in proline accumulation and antioxidative enzyme activities in *Grolendia densa* under cadmium stres. *Ecological indicators*, 11, 417–423.

Demirezen, Y.D., 2007. Effects of salinity on growth and nickel accumulation capacity of *Lemna gibba* (Lemnaceae). *Journal of hazardous materials*, 147, 74–77.

Erdei, S., *et al*., 2002. Heavy metal induced physiological changes in the antioxidative response system. *Acta biologica szegediensis*, 46, 89–90.

Fritsch, P. and Rieseberg, L.H., 1996. The use of random amplified polymorphic DNA (RAPD) in conservation genetics. *In*: T.B. Smith and R.K. Wayne, eds. *Molecular genetic approaches in conservation*. London: Oxford University Press, 54–73.

Goswami, S. and Das, S., 2016. Copper phytoremediation potential of *Calandula officinalis* L. and the role of antioxidant enzymes in metal tolerance. *Ecotoxicology and environmental safety*, 126, 211–218.

Hebert, P.D.N. and Luiker, M.M., 1996. Genetic effects of contaminant exposure—towards an assessment of impacts on animal populations. *Science of the total environment*, 191, 23–58.

Hou, W., *et al*., 2007. Effect of copper and cadmium on heavy metal polluted water body restoration by duckweed (*Lemna minor*). *Plant physiology and biochemistry*, 45, 62–69.

Hou, X., *et al*., 2018. Pb stress effects on leaf chlorophyll fluorescence, antioxidative enzyme activities, and organic acid contents of *Pogonatherum crinitum* seedlings. *Flora*, 240, 82–88.

Kasperczyk, A., *et al*., 2015. Environmental exposure to lead induces oxidative stress and modulates the function of the antioxidant defense system and the immune system in the semen of males with normal semen profile. *Toxicology and applied pharmacology*, 284, 339–344.

Koh, H.L., Hallam, T.G., and Lee, H.L., 1997. Combined effects of environmental and chemical stressors on a model Daphnia population. *Ecological modelling*, 103, 19–32.

Kumar, P., *et al*., 2015. In vivo assessment of DNA damage in *Cyprinus carpio* after exposure to potassium dichromate using RAPD. *Turkish journal of veterinary and animal sciences*, 39, 121–127.

Lowry, O.H., *et al*., 1951. Protein measurement with the Folin phenol reagent. *The journal of biological chemistry*, 193, 265–275.

Mishra, S., *et al*., 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant physiology and biochemistry*, 44, 25–37.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405–410.

Nehnevajova, E., *et al*., 2012. Metal accumulation and response of antioxidant enzymes in seedlings and adult sunflower mutants with improved metal removal traits on a metal-contaminated soil. *Environmental & Experimental botany*, 76, 39–48.

NIWA (National Institute of Water & Atmospheric Research), 2008. Watercress: one step to cleaner waterways? Available from: http://www.niwa.co.nz/our-science/freshwater/publications/all/wru/2008-28/watercress [Accessed 23 August 2009].

Nriagu, J.O. and Nieboer, E., 1988. *Chromium in the natural and human environments*. New York: Wiley.

Pal, S., 2016. Detection of environmental contaminants by RAPD method. *International journal of current microbiology and applied sciences*, 5 (8), 553–557.

Radić , S., *et al*., 2010. Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L. *Ecotoxicology and environmental safety*, 73, 336–342.

Razinger, J., *et al*., 2008. Oxidative stress in duckweed (*Lemna minor* L.) caused by short term cadmium exposure. *Environmental pollution*, 153, 687–694.

Rocco, L., *et al*., 2014. RAPD-PCR analysis for molecular characterization and genotoxic studies of a new marine fish cell line derived from *Dicentrarchus labrax*. *Cytotechnology*, 66 (3), 383–393.

Rogers, S.O. and Bendich, A.J., 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant molecular biology*, 5 (2), 69–76.

Savva, D., 1996. DNA fingerprinting as a biomarker assay in ecotoxicology. *Toxicology and ecotoxicology news*, 3, 110–114.

Savva, D., 1998. Use of DNA fingerprinting to detect genotoxic effects. *Ecotoxicology and environmental safety*, 41 (1), 103–106.

Shankar, A.K., *et al*., 2005. Chromium toxicity in plants. *Environment international*, 1, 739–753.

Sharma, S.S. and Dietz, K.J., 2009. The relationship between metal toxicity and cellular redox imbalance. *Trends in plant science*, 14, 43–50.

Sharma, P. and Dubey, R.S., 2007. Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. *Plant cell reports*, 26, 2027–2038.

Sheldon, R.A. and Pelt, S., 2013. Enzyme immobilisation in biocatalysis: why, what and how. *Chemical society reviews*, 42, 6223–6235.

Staton, J.L., *et al*., 2001. Ecotoxicology and population genetics: the emergence of "phylogeographic and evolutionary ecotoxicology". *Ecotoxicology (London, England)*, 10, 217–222.