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Arsenic accumulation and biological responses of watercress (*Nasturtium officinale* R. Br.) exposed to arsenite

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ABSTRACT

The objective of the present study was to investigate biological responses of watercress (*Nasturtium officinale* R. Br.) under arsenic stress. Watercress samples were exposed to 1, 3, 5, 10, and 50 μM of arsenite (As(III)) for 7 days. Arsenic accumulation in the leaves of watercress was investigated, and its influence on the rates of lipid peroxidation, ion leakage, photosynthetic pigmentation, proline content, enzymatic antioxidant performance, and DNA damage was examined. Watercress was capable of accumulating large amounts of arsenic in the leaves. The highest accumulation of As (1012 $\mu g\,g^{-1}$ dw) was found in the leaves of the plants exposed to 50 μM of As(III). Plant growth was stimulated at 1 μM As(III) application, while higher concentrations unfavorably affected plant growth. It was observed that exposure to As(III) significantly increased the ion leakage and lipid peroxidation compared to the control. An increase in protein and proline content was observed, followed by a gradual decline at higher concentrations. Stress conditions caused up-regulation of the antioxidant enzyme activity in dose dependent manner. The results indicated that the changes that occur in the random amplified polymorphic DNA (RAPD) profiles after an As(III) treatment, include the presence of certain modifications in the band intensity and the gain or loss of bands. The results of the present study confirmed that *N. officinale* is capable of overcoming the occurrence of As(III)-induced stress, especially, due to conditions of moderate exposure.

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1. Introduction

Inorganic arsenic compounds have been employed in industrial and agricultural activities and also in the control of aquatic weed. The phytotoxicity of arsenic depends on its oxidation state. Arsenite (As(III)) and arsenate (As(V)) are inorganic, phytoavailable forms of arsenic and are highly toxic to plants (Mkandawire et al., 2004). These inorganic forms are interconvertible, depending on the redox condition of the aquatic ecosystem.

Arsenic exists predominantly as As(V) in natural waters. However, Zhao et al. (2009) have stated that under aerobic conditions, As(III) may also exist in the rhizosphere due to activity of microbes and root exudates. Consequently, plants growing in aerobic environment may encounter a mixed pool of As(III) and As(V) (Wang et al., 2010). Zhao et al. (2009) determined that arsenic exists in the plant predominantly as As(III), even though plants had been exposed to As(V). Therefore, it can be concluded that, following uptake, As(V) is reduced efficiently to As(III) in plant cells. As(V) and phosphate are chemically similar, allowing arsenate to act as a phosphate analogue, thereby permitting transport into the cell (Meharg and Macnair, 1990). In contrast, As(III) is transported in its natural form, $As(OH)_3$. However, Wang et al. (2010) determined that *Pteris vittata* was more efficient in taking up As(III) than As(V) in the presence of phosphate.

As(III) is considered as phytoavailable and the most phytotoxic arsenic species (Mkandawire et al., 2004). As(III) is powerful inhibitors of the sulfhydryl groups found in some enzymes and tissue proteins. They attack plant cell membranes, causing an inhibition of cellular function and death (Sizova et al., 2002). Relative growth rate (RGR) is an important parameter in evaluating the physiological effects of toxic chemicals on plants (Cedergreen, 2008). Stress condition may enhance protective processes such as accumulation of compatible solutes and increase in the activities of detoxifying enzymes. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Mishra and Dubey (2006) determined that when rice seedlings are subjected to arsenic stress, the normal rate of activity of their proteolytic enzymes and the level of proteins are change.

Another noteworthy reaction induced in the plants that are exposed to arsenic stress is the accumulation of certain specific metabolites, such as, free proline. Proline accumulates violently in several plants under stress, procuring the plants defense against damage by ROS. Proline plays important roles in osmoregulation,

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protection of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity (Choudhary et al., 2007).

Some authors previously reported that at increasing rates of As concentrations, certain plants produce reactive oxygen species (ROS) which damage cell membranes, DNA, protein, lipid and chloroplast pigments (Cao et al., 2004; Patra et al., 2004). To minimize the harmful effects of ROS, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes (Meharg, 1994). The major enzymatic antioxidant pathways of plants include superoxide dismutase (SOD), found in almost all cellular compartments. SOD is a metalloenzyme that catalyzes dismutation of superoxide anion into oxygen and hydrogen peroxide. Such enzymes provide a defense system for the survival of aerobic organisms (Beyer et al., 1991).

Several studies have used the comet, micronucleus or chromosome aberration assays to measure genotoxic effects of toxic chemicals on plants. Recently, the development of molecular marker technology has provided new tools for detection of genetic alteration in response to toxic chemicals tolerance by looking directly at the level of DNA sequence and structure. Over the last years the random amplified polymorphic DNA (RAPD) analysis has been used also for determination of genome rearragements caused by genotoxic factors including toxic chemicals (Atienzar et al., 1999). RAPD assay is a PCR-based technique that amplifies random DNA fragments of genomic DNA with single short primers of arbitrary nucleotide sequence under low annealing conditions (Williams et al., 1990). The assay was successfully applied to detect genomic DNA alterations induced by several DNA damaging agents, such as Cd, Pb and UV radiation in plants (Liu et al., 2009; Cenkci et al., 2010; Atienzar et al., 2000). It is suggested that alterations to RAPD profiles due to genotoxic exposure can be regarded as changes in genomic template stability (GTS, a qualitative measure of genotoxic effect) (Atienzar et al., 1999).

It is known that As accumulation potential of aquatic macrophytes is very high (Alvarado et al., 2008). As(V) uptake and toxicity have been well documented in plants (Smith et al., 2008; Mrak et al., 2008), whereas As(III) uptake and toxicity have been less studied (Mkandawire et al., 2004). Watercress, *Nasturtium officinale* R. Br., is an edible aquatic plant. As a medicinal plant, watercress has been traditionally considered a diuretic, purgative and tonic, and consumed as a salad green. The properties of *N. officinale* that entail metal accumulation were extensively studied in the past. However, even then the available knowledge regarding the As accumulation characteristics of watercress exposed to As(III) and the resultant effect of accumulation is not sufficient.

First, the present study focused on the accumulation properties of As. Second, this study aimed to demonstrate the biological responses of watercress against the As, based on the varied concentrations of As(III) application. The results of the investigations may help to explain the effects of arsenite on aquatic plants, and the consequent biologic responses of these plants. Furthermore, the findings may be useful when this plant is used as a phytoremediator in polluted water.

2. Materials and methods

2.1. Sample collection and cultivation

N. officinale seedlings were collected in April of 2008 from the Karasu Stream in Kayseri, Turkey. Prior to the experiment, containers were disinfected by immersion in 1% (v/v) NaClO for 3–5 min. Containers were then rinsed three times with distilled water (Hou et al., 2007). Collected samples were washed in tap water and acclimatized for 3 days in a climate chamber with a water temperature of 15 °C, a relative humidity of 70% and light/dark photoperiod of 16 h light/8 h dark. Containers were mildly aerated.

2.2. Experimental design

The experiments conducted in the present study were set-up in triplicate, wherein each replicate constituted approximately 4 g of the evaluated plants. The As(III) solutions utilized in the present study were prepared from $NaAsO_2$. Each plant sample of watercress were exposed to four varied test concentrations (1, 3, 5, 10, and 50 μM) of As(III) maintained in 10% Hoagland's solution in separate 400 mL conical flasks (Srivastava et al., 2006). The plants that were not exposed to As(III) served as the control groups of this experiment. The flasks that comprise the plant and As concentrates were placed in a climate chamber under the aforementioned conditions for 7 days. Flasks were not aerated during experiment. The change that occurred in the volume of the solution within the flasks due to evapotranspiration was compensated for by the addition of double distilled water. At the end of the exposure experiment, the resultant plant samples were collected and sieved with a plastic griddle. Each plant was rinsed with deionized water, drained, and then blotted on paper towels for 2 min.

2.3. Quantification of arsenic and determination of RGRs

The relative growth rate (RGR) of *N. officinale* was calculated in each treatment using the equation:

$$\mathrm{RGR}(\%/\mathrm{day}) = \frac{[\ln(W_2) - \ln(W_1)]}{t} \times 100\% \quad (1)$$

W_1 and W_2 are the initial and final fresh weights (g), respectively, and t is the length of the experimental period.

Leaves of plants sample was dried at 70 °C. Each sample was then digested with 10 mL of pure HNO_3 using a CEM Mars 5 (CEM Corporation Mathews, NC, USA) microwave digestion system. The digestion conditions were as follows: the maximum power was 1200 W, the power was at 100%, the ramp was set for 20 min, the pressure was 180 psi, the temperature was 210 °C and the hold time was 10 min. After digestion, the solution was evaporated to near dryness in a beaker. The volume of each sample was adjusted to 10 mL using 0.1 M HNO_3. The total concentration of arsenic was determined using an inductively coupled plasma mass spectrometer (Agilent, 7500a). The stability of the device was evaluated every ten samples by examining an internal standard. Reagent blanks were also prepared to detect any potential contamination during the digestion and analytical procedure. Peach leaves (NIST, SRM-1547) were used as the reference material for all of the performed analytical procedures. The samples were analysed in triplicate. All chemicals used in this study were analytical reagent grade (Merck, Darmstadt, Germany).

2.4. Determination of ion leakage and lipid peroxidation

The ion leakage induced by the As(III) was estimated by measuring the electrical conductivity (EC) (Devi and Prasad, 1998). Leaves of arsenic-exposed plants were washed with double-deionized water. Then, 500 mg of plant material was transferred to 100 mL of deionized water for 24 h to facilitate maximum ion leakage. The EC of the water was then recorded with WTW model conductivity meter. For determination of lipid peroxidation, leaf material (500 mg) was homogenized with 3 mL of 0.5% TBA in 20% TCA (w/v). The homogenate was incubated at 95 °C for 30 min, and ice was used to stop the reaction. The samples were centrifuged at 10,000 × g for 10 min, and the absorbance of the resulting supernatant was recorded at 532 and 600 nm. The amount of malondialdehyde (MDA) (extinction coefficient of 155 mM^{-1} cm^{-1}) was calculated by subtracting the non-specific absorbance at 600 nm from the absorbance at 532 nm (Heath and Packer, 1968).

2.5. Determination of photosynthetic pigment contents

Photosynthetic pigments of leaves of treated and untreated plants (100 mg) were extracted in 10 mL of chilled acetone solution in the dark. After centrifugation at $4000 \times g$ for 10 min, the absorbance of the supernatant was taken at 480, 510, 645 and 663 nm. The chlorophyll content was estimated by the method of Arnon (1949), and the carotenoid content was determined by the formula given in Duxbury and Yentsch (1956).

2.6. Determination of total protein, proline and SOD activity

Leaves of plant samples (500 mg) were homogenized in 1 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP, (w/v)) at 4 °C. The homogenate was centrifuged at $15{,}000 \times g$ for 15 min at 4 °C. The supernatant was extracted and used to measure the activities of SOD. The protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard protein.

The amount of proline was determined according to a modified method of Bates (1973). Free proline content was extracted from 0.25 g of samples in 3% (w/v) aqueous sulphosalicylic acid and estimated by ninhydrin reagent. Absorbance of the upper phase was recorded at 520 nm against toluene blank.

SOD was assayed by the photochemical method described by Giannopolitis and Ries (1977) with some modifications. The reaction mixture contained 20 mM sodium phosphate (pH 7.5), 10 mM methionine, 0.1 mM EDTA, 0.1 mM nitro-blue-tetrazolium (NBT), 5 μM riboflavin and 50 $\mu g\,mL^{-1}$ crude enzyme extract in a total volume of 3 mL. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT (ρ-nitro-blue-tetrazolium chloride) reduction measured at 560 nm (Liang et al., 2003).

2.7. DNA isolation and RAPD-PCR and estimate of the genomic template stability

The process of DNA isolation was performed according to the CTAB method (Rogers and Bendich, 1985) and the DNA concentration was estimated with a spectrophotometer at 260 nm. Moreover, the RAPD-PCR experiments were conducted according to the method of Williams et al. (1990) and the 12 oligonucleotide pimers (Alpha DNA, Canada) were used for the purpose of carrying out the RAPD-PCR analysis.

Further, a standard 25 μL reaction mixture that constitutes 100 ng of genomic DNA, 1.5 U of *Tag* polymerase, 0.4 μM of primer, 0.2 mM of each dNTP, 2.0 mM of $MgCl_2$, and 2.5 μL of 10× reaction buffer. The process of DNA amplification was performed in a Thermo Thermal Cycler. The initial 2 min denaturation at 94 °C was followed by 44 cycles of 1 min at 94 °C, 1 min 36 °C, 2 min 72 °C, and 1 cycle of 5 min at 72 °C. After the completion of the amplification process, the obtained products were separated on a base of 2% agarose by means of electrophoresis carried out in the Tris–Borate–EDTA (TBE) buffer and stained with ethidium bromide. The resultant gels were then photographed on a UV-transilluminator.

The process of amplification of each DNA sample was repeated at least three times in order to ensure reproducible results. Each obvious modification observed in the RAPD patterns (disappearance of bands and appearance of new bands) was given the arbitrary score of +1, and for each experiment group of rice seedlings the average was calculated by taking into account 12 primers, which showed clear variations in their RAPD profiles. However, the primers that did not reveal any specific changes in their RAPD profiles or were too difficult to be scored were not considered in the final calculation of the genomic template stability (GTS), a qualitative measure that reflects the changes that occur in the RAPD profiles.

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

$$GTS = (1 - a/n) \times 100 \quad (2)$$

where a is the average number of the polymorphic bands detected in each treated sample and n the total number of bands found in the control. The polymorphism arising in the RAPD profiles included the disappearance of a normal band and appearances of a new band in comparison with the control. The average polymorphism arising in each experimental group exposed to different As(III) treatments was calculated. To compare the degree of sensitivity of each parameter, the changes occurring in this value was calculated as a percentage of its control (set to 100%).

2.8. Statistics

Data were expressed as means with standard errors (SE). The Kolmogorov–Smirnov test and Levene's test were used to ensure the normality assumption and the homogeneity of variances, respectively. Where heterogeneity of variance were recognized, data were log transformed ln $(x+1)$ and re-evaluated. Analysis of variance (ANOVA) was performed to confirm significant differences among treatments. All pairwise mean comparisons were made using post-hoc analyses. Duncan's test was used to determine the significant difference between treatments. Correlations between accumulated As and studied parameters were also performed. We used 0.05 as the statistical significance threshold. All statistical analyses were performed with the SPSS 15.0 software package.

3. Results

3.1. Effects of As(III) on growth of watercress

The results of the present study revealed that an initial exposure to up to 1 μM of As(III) induced an increase in the RGR values (Fig. 1a) and in producing leaves of a darker color. The plants that were exposed to 3 and 5 μM of As(III) revealed a significant effect on their growth parameters as is evident by the visual changes, such as, chlorosis of leaves and the weakening of the stem. It was determined that at 3, 5, and 10 μM of As(III) applications, the RGR values were measured to be lower than those of the control ($P \le 0.05$). Further, 50 μM of arsenite exposure revealed a negative effect on plant growth.

3.2. As accumulation in leaves of watercress treated with As(III)

The rate of accumulation of arsenic in the leaves of *N. officinale* was found to depend on the varied concentrations of exposure (Fig. 1b). The highest accumulation of As (1012 $\mu g\,g^{-1}$ dw) was found in the leaves of the plants exposed to 50 μM of As(III). Significant negative correlation was determined between RGR values and accumulated As ($R = -0.946$, $P \le 0.01$).

3.3. Effects of As(III) on the level of EC and MDA

It was noted that when the plants were subjected to 5 μM of As exposure, their EC values did not show any alterations (Fig. 2a). However, an exposure above this concentration resulted in increasing the EC values significantly ($P \le 0.05$). In comparison with the control, the highest increase in the value was observed to be 307% application of 50 μM. As accumulation and EC were significantly positively correlated with each other ($R = 0.950$, $P \le 0.01$).

Further, at an As(III) application of 1 μM, the MDA content was not found to be significantly high, in comparison with that of the

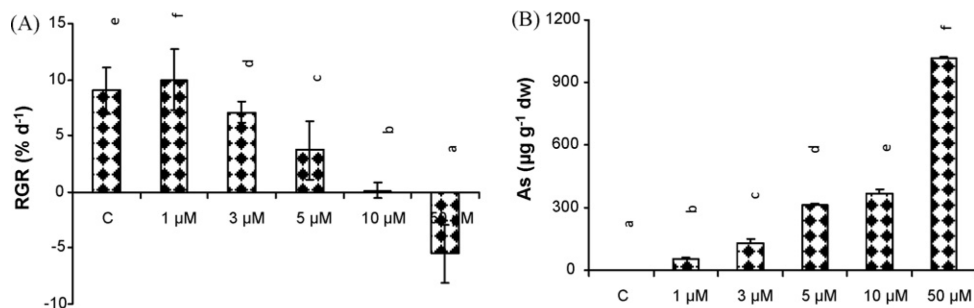

Fig. 1. (A) RGR values of *N. officinale* exposed to different concentrations of As(III) (B) As concentrations ($\mu g\,g^{-1}$) in leaves of *N. officinale* exposed to different concentrations of As(III) during 7 d. Values represent means ± SD ($N = 3$); C – control. Means with common letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's test.

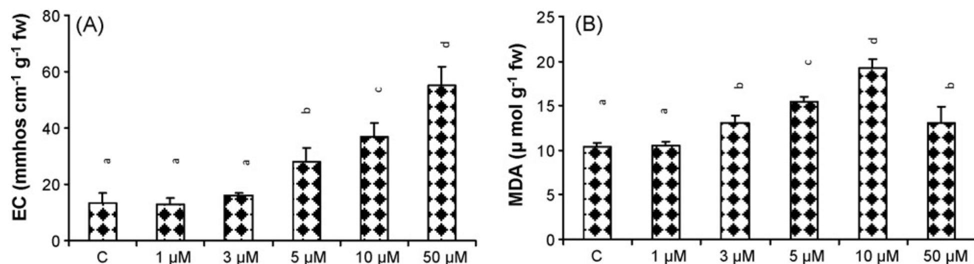

Fig. 2. Effects of As(III) treatment on (A)ion leakage and (B) lipid peroxidation in leaves of *N. officinale*. Values represent means ± SD ($N = 3$); C – control. Means with common letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's test.

control. However, at an As(III) application of 3–10 μM, the MDA content increased with an in increase in the As(III) concentration up to 10 μM, beyond that the MDA content revealed a tendency to decrease in value (Fig. 2b), even though it never reached a level that was significantly lower than that of the control ($P \leq 0.05$). Further, the highest increase in the value of MDA content in comparison with the control was observed to be 86% at an As(III) application of 10 μM. Significant correlation between MDA content and As accumulation was observed ($R = 0.606$, $P \leq 0.01$).

3.4. Effects of As(III) on photosynthetic pigments

Both chlorophyll a and chlorophyll b negatively correlated with accumulated As ($R = -0.724$, $P \leq 0.01$; $R = -0.769$, $P \leq 0.01$, respectively). The photosynthetic pigment content of the plants that were treated with As(III) increased slightly up to 3 μM, in comparison with the control. However, when the As(III) concentrations were increased, the content of chlorophyll a and b were found to be lower than those of the control (Fig. 3a and b). The maximum decrease in the content of chlorophyll a and b contents was observed to be 58% and 52%, respectively, at an As(III) application of 50 μM. No significant correlation was observed between accumulated As and carotenoid content ($R = 0.140$, $P \geq 0.05$). Under all exposure conditions, the carotenoid content of the plants that were exposed to As(III) revealed higher values than the control sample (Fig. 3c). Moreover, in comparison with the control, the maximum increase in the carotenoid content was observed as 46% at an As(III) application of 3 μM.

3.5. Effects of As(III) on the protein and proline content, and SOD activity

Protein levels showed significant negative correlation with As accumulation ($R = -0.933$, $P \leq 0.01$). At As(III) applications of 1 and 3 μM, the protein content was found to be significantly high, in comparison with the control (Fig. 4a). Further, the effect of an As(III)

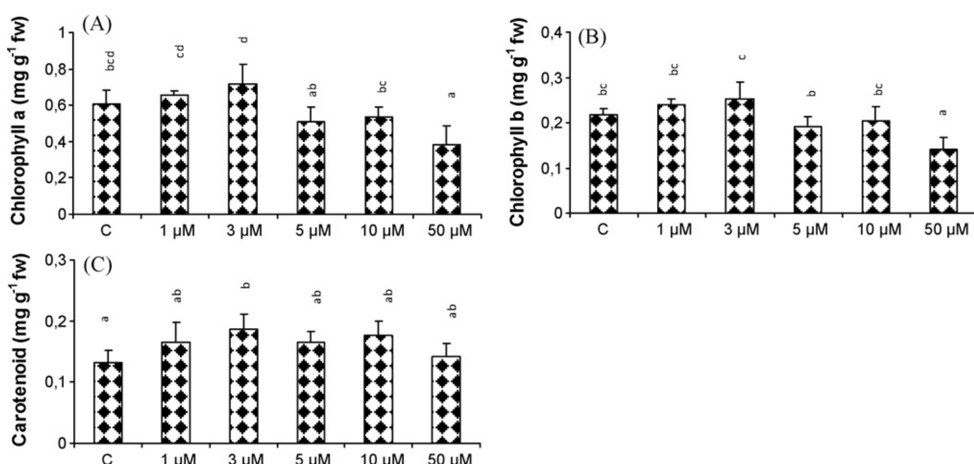

Fig. 3. Effects of As(III) treatment on (A)chlorophyll a, (B) chlorophyll b and (C) carotenoid content in leaves of *N. officinale*. Values represent means ± SD ($N = 3$); C – control. Means with common letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's test.

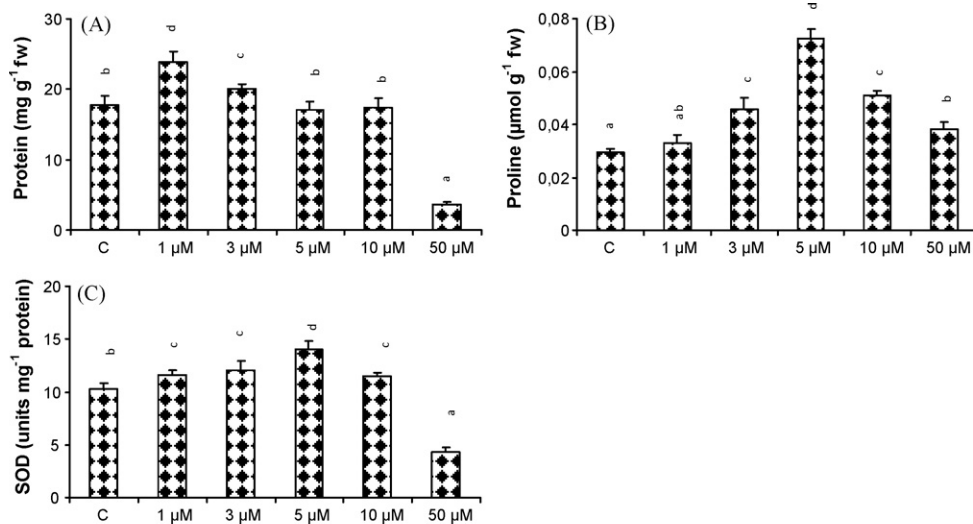

Fig. 4. Effects of As(III) treatment on (A) protein content, (B) proline content and (C) SOD activity in leaves of *N. officinale*. Values represent means ± SD (N = 3); C – control. Means with common letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's test.

Table 1
RAPD primers used for PCR amplification.

RAPD primer	Sequences (5′–3′)	GC%
M13 (Primer 1)	GAGGGTGGCGGTTCT	66,7
B18 (Primer 2)	CCACAGCAGT	60
OPW10 (Primer 3)	TCGCATCCCT	60
OPB05 (Primer 4)	TGCGCCCTTC	70
OPB10 (Primer 5)	CTGCTGGGAC	70
OPB08 (Primer 6)	GTCCACACGG	70
OPU16 (Primer 7)	CTGCGCTGGA	70
OPD12 (Primer 8)	CACCGTATCC	60
OPI18 (Primer 9)	TGCCCAGCCT	70

application of 1 μM on the content of protein was noted to be significantly higher than the effect of 3 μM of As(III). Additionally, the As(III) applications of 5 and 10 μM did not produce any statistically important differences with the control sample. However, at an As(III) application of 50 μM, a decrease (383%) was noted to appear in the protein content in comparison with the control.

No significant correlation was observed between accumulated As and proline content (R = 0.119, $P \geq 0.05$). Hence, it can be ascertained that under all exposure concentrations, the proline levels were found to be significantly higher in comparison with the control (Fig. 4b). Additionally, it was also noted that the proline content increased with an increase in the concentration of As(III) up to 5 μM, beyond which the proline content revealed a tendency to decrease in value, even though it never reached a level that was significantly lower than that of the control.

At As(III) applications of 1–10 μM, the rate of SOD activity was recorded to be significantly higher than that of the control (Fig. 4c). Additionally, it was seen that in comparison with the control, the maximum increase in the rate of SOD activity was observed to be 37% for an As(III) application of 5 μM. However, once the value of the SOD activity reached its maximum levels it revealed a tendency to decline in accordance with increasing concentrations. Similar

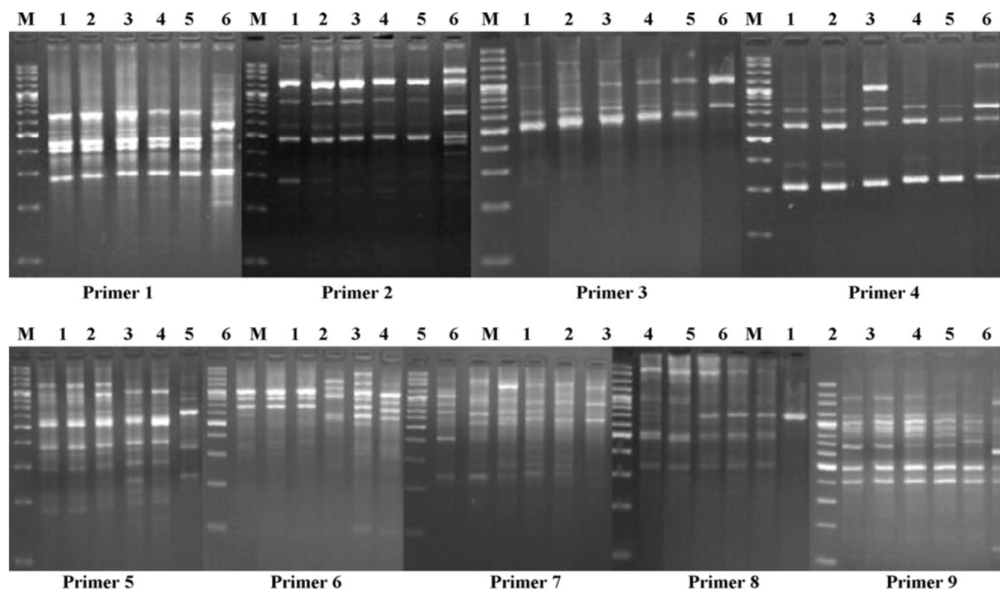

Fig. 5. RAPD profiles of genomic DNA from leaves of *N. officinale* exposed to different concentrations of As(III). Lane 1: control, Lane 2: 1 μM As(III), Lane 3: 3 μM As(III), Lane 4: 5 μM As(III), Lane 5: 10 μM As(III), Lane 6: 50 μM As(III) respectively. M. DNA molecular size marker (3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp from top to bottom).

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 As(III).

Primers	AsIII concentration (μM)																				
	0	1				3				5				10				50			
		a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
Primer 1	7	0	0	0	1	0	0	0	3	0	0	2	1	0	0	2	1	7	2	3	1
Primer 2	5	0	0	1	1	1	1	0	1	1	1	0	0	0	0	3	0	7	2	2	0
Primer 3	5	0	0	0	3	0	0	0	3	0	1	3	0	0	2	2	0	0	3	0	1
Primer 4	6	0	0	0	0	1	1	2	0	0	0	0	1	0	3	2	0	2	3	2	1
Primer 5	11	0	0	0	3	0	0	0	3	1	0	3	0	0	0	2	2	1	4	1	0
Primer 6	5	0	1	0	0	0	1	0	0	3	2	1	1	4	1	1	1	0	0	0	1
Primer 7	10	2	0	3	4	2	2	3	2	2	2	0	0	3	3	0	0	2	1	0	2
Primer 8	9	0	0	2	1	1	1	2	1	0	3	3	1	0	3	3	1	0	6	0	1
Primer 9	8	0	0	0	3	0	0	2	5	0	0	2	1	1	1	4	0	5	2	8	0
Total bands	63	2	1	6	16	5	6	9	18	7	9	14	5	8	13	19	5	24	23	16	7
a + *b*		3				11				16				21				47			
a + *b* + *c* + *d*		25				38				35				48				70			

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.

to the effect on protein content, an As(III) application of 50 μM caused a decrease (142% in comparison with the control) in the SOD activity.

3.6. Effects of As(III) on the RAPD profiles

Among the 12 decamer oligonucleotide primers evaluated, only 9 furnished specific and stable results (Table 1). The consequent RAPD fingerprints revealed the occurrence of substantial differences among the unexposed and the exposed plants, demonstrating apparent changes in the number, the size, and the intensity of the amplified DNA fragments. Further, it was noted that the RAPD patterns generated by the As(III)-exposed plants in all cases were clearly different from those obtained by evaluating the DNA of the controls.

The principal events that were observed to occur subsequent to an As(III) exposure were a variation in band intensity, loss of normal bands, and appearance of new bands in comparison with the normal control plants. The decrease in the band intensity was particularly obvious for watercress exposed to 5, 10, and 50 μM of As(III) for primers, numbered 1, 2, 3, 4, 5, 8, 9 (Fig. 5). Whereas, the increase in the band intensity was particularly obvious with an increase of 50 μM in the As(III) concentration for primers 3 and 8.

The maximum number of disappearing RAPD bands was found to be at two higher (10 and 50 μM) As(III) concentration for primers 3, 4, 8, 9, and the bands of molecular size approximately 200–1200 bp were noted to have disappeared (Fig. 5). Finally, it was noted that extra bands appeared with primer 1 (eight new PCR amplification products), primer 4 (one to two new bands), primer 6 (seven new bands), primer 5 (one new band), primer 8 (one new band), primer 9 (one to five new band), and primer 2 (one to seven bands) (Table 2). The extra bands that appeared were noted to be of molecular size approximately 100 to 2000 bp.

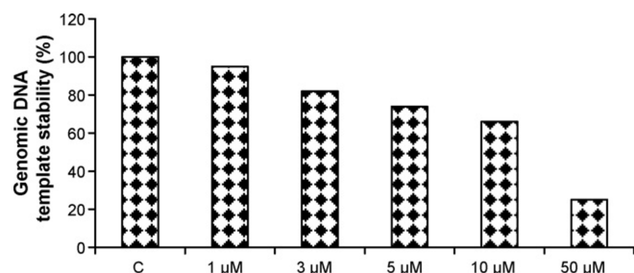

Fig. 6. Genomic template stability in watercress exposed to different As(III) concentration.

Nine primers revealed the presence of 63 bands ranging from 200 to 2100 bp in molecular size, whereas in the control group different polymorphic bands were detected at each concentration of As(III) for different primers. The value of the polymorphic was *P*(%) = 4%, 17%, 25%, 33%, and 74% for 1, 3, 5, 10, and 50 μM of As(III) concentration, respectively. In all cases, the occurrence of polymorphism was due to the loss and/or gain of the PCR fragments in the treated plant in comparison with the control. Table 2 also indicates that the number of changed bands observed in RAPD profiles in the As(III)-contaminated watercress plant increased dramatically subsequent to an exposure to As(III). In this experiment, the genomic template stability was used to compare the changes of RAPD profiles with control group (Fig. 6). According control group, GTS of exposed plant with As(III) reduced gradually.

4. Discussion

4.1. As accumulation and growth characteristics

Similar to the results obtained by the earlier studies (Cao et al., 2004; Abbas and Meharg, 2008), the present study also found that the uptake of As(III) was concentration-dependent. Earlier studies confirmed the occurrence of a high accumulation of arsenic in aquatic plants. In the present study, however, experimental conditions were designed to represent a “worst case scenario” condition; additionally, this study draws attention to the tendency of *N. officinale* to accumulate significant concentrations of As(III) under favorable growth conditions. Srivastava et al. (2007) who evaluated the accumulation properties of the As species from an aquatic plant, *Hydrilla verticillata*, and confirmed that the Hydrilla plant tolerated higher concentrations of As(III) than the amount normally present in the contaminated areas. The results of the present study are in accordance with these evaluations. Therefore, it can stated that this plant may be used as an As phytoaccumulator for arsenic-polluted waters.

Toxic chemicals stimulate the growth of plants at lower doses and have a toxic effect at higher doses (Cedergreen, 2008). Similarly, the present study ascertained that a lower dose of arsenite (1 μM) stimulates growth; however, higher doses negatively affect plant growth. Southam and Erlich (1943) called the effect of toxicants to stimulate growth at lower doses as “hormesis effect”. Additionally, it was recorded that the RGR values may tend to decrease when a high dose of toxicants are applied based on the fact that the cell wall and the cell membrane may be destroyed due to the reactive oxygen species formed as result of the stress depending on an increased concentration of exposure (Srivastava et al., 2009).

4.2. Ion leakage and lipid peroxidation

An increase in the MDA level depending on the increasing toxicant concentration and exposure period indicates the existence of an oxidative stress. In this context, the present study observed that an As(III) application of 1 μM did not significantly harm the plasma membrane of leaves of *N. officinale*. However, accordance with the literature (Shri et al., 2009), increase in the exposure concentration caused increase in the MDA content. Therefore, it can be stated that the application of a high concentration of As(III) to plants may cause harm to their membrane integrity. As(III) may modify the activities of the enzyme lipoxygenase (Chakrabarty et al., 2009), which could result in altered membrane permeability and, consequently, increased ion leakage. However, the MDA content was recorded to decrease at an As(III) application of 50 μM. Similar to the results obtained in the present study, Wang and Zhou (2006) also determined that the MDA level of wheat decreases when an herbicide is applied to it in high doses.

4.3. Photosynthetic pigmentation

Further, it was also revealed that an excess amount of As(III) affected the content of the photosynthetic pigments present in *N. officinale*. In addition, the amount of photosynthetic pigments was recorded to increase whereas the metal concentration was found to become low. Subsequently, as the metal concentration increased, there was a decrease in the amount of photosynthetic pigments produced. The results of the present study are also in accordance with the report generated by Seth et al. (2007). This situation may be construed as an adaptive response produced against a xenobiotic present in the media. The decrease in the amount of photosynthetic pigment content may be due to various factors: the per oxidative breakdown of the pigments and the chloroplast membrane lipids by the reactive oxygen species (Li et al., 2006; Singh et al., 2006), an impaired uptake of nutrients (such as, Mn, Cu, Fe, and P) (Srivastava et al., 2007), or the degradation of chlorophyll through an increase in the rate of chlorophyllase activity (Sharma and Dubey, 2005).

4.4. Protein and proline content

However, it is also known that the plants that are exposed to arsenic stress try to survive by producing stress proteins as a response to the changing environmental conditions in the early stages of application (Seth et al., 2007). In this study, an increase in the total protein content was found to occur at lower As(III) concentrations; however, at high concentrations (50 μM) of As(III) a significant decrease occurred in the protein content. This eventual decrease in the protein content may be dependent on an increase of ROS.

Proline is known as an important organic solute accumulates in many plant species under conditions of abiotic stresses (Ozden et al., 2009). In the experiments conducted in the present study, the proline level increased significantly with an increase in the As(III) concentration up to 5 μM. An almost similar effect was earlier observed in *Oryza sativa*, under a condition of As_2O_3 stress (Mishra and Dubey, 2006). Along with the results obtained by Mishra and Dubey (2006), the present study revealed that beyond a value of As(III) concentration of 5 μM, the proline content decreased in a manner that depend on the varied concentrations of exposure. However, it never reached a level that was significantly lower than the one noted in the control. The increase of the proline may regulate the toxicity of As(III) in reducing its uptake (Wu et al., 1998).

4.5. Enzymatic antioxidant performance

SOD is considered to be the key enzyme of the cellular defense system that is formed against ROS. The activity of SOD reduces the formation of hydroxide. The present study revealed that the rate of activity of SOD increased at an As(III) concentration up to 5 μM. Similar to the results obtained in the present study, Srivastava et al. (2007) studied the arsenite-induced oxidative stress caused on *Hydrilla verticillata,* which is dependent on the concentration of the toxicant and the duration of the application; their investigations concluded that the highest rate of SOD activity occurs at 5 μM for 4 days. The increase in the rate of SOD activity at lower concentrations indicated that *N. officinale* is able to tolerate moderate exposures to arsenite and to efficiently combat oxidative stress. However, the rate of activity of SOD significantly decreased at the maximum arsenic concentration of 50 μM. This situation may be caused by the potential damages that occur in the antioxidant defense system. This result suggests that the plant cannot cope with very high arsenite concentrations.

4.6. The change of RAPD profiles

The changes in DNA caused by genotoxic chemicals may be monitored using different biomarker assays both at biochemical and molecular level (Savva, 1998). RAPD profiles detect alterations in genomic DNA with the use of arbitrarily primed PCR reactions and clearly show promise in the detection of pollutant-induced DNA effects (Conte et al., 1998; Atienzar et al., 2001). However, it is only a qualitative method through which nature and amount of DNA can only be speculated.

The present study shows the first report on As(III) induced RAPD profiling in aquatic plants. Changes observed in the DNA profiles such as modifications in band intensity and loss of bands may be due to the changes in oligonucleotide priming sites mainly due to genomic rearrangements, and less likely to point mutations or DNA damage in the primer binding sites or the presence of DNA photo products which can block or reduce the polymerization of DNA in the PCR reaction (Nelson et al., 1996). Appearance of new PCR products or appearance of bands could be attributed to the presence of oligonucleotide priming sites which become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations or large deletions or homologous recombination (Atienzar et al., 1999).

In earlier studies, the RAPD profiles generated from the barley and rice seedling treatment cadmium revealed different patterns of the appearance and disappearance of bands to the control RAPD pattern (Liu et al., 2007; Liu et al., 2009). Similarly, in the present study, different polymorphic bands were determined to be present at each As(III) concentration in the nine primers (Fig. 5). According to the results, the polymorphisms were considered to be caused by the loss or gain of the amplified bands in the treated group in comparison with the control group. Table 2 describes the changes observed in the RAPD profiles, such as, the appearance/disappearance of the bands and the rate of decrease/increase in the band intensities. It was found that the new bands were found to be in a greater number in a higher As(III) concentration (10–50 μM), with molecular sizes of 200–2000. The appearance of new bands showed that the effect can be involved in DNA repair and replication mechanism or it may also be the result of genomic template instability connected with the level of DNA damage and the effectivity of DNA repair and replication (Atienzar et al., 1999).

Finally, a comparison between the untreated and the treated genomes revealed that the RAPD analysis can be employed to evaluate the manner in which the environmental pollutants modify the structure of the DNA present in *N. officinale.* The effects of each category of DNA damage (e.g., strand breakage, modified bases, abasic sites, oxidized bases, and bulky adduct) on the RAPD profiles can only be speculated when the amplicons are analysed (e.g., sequencing) and more specific methods, such as, the comet assay

and ^{32}P-postlabelling assay are needed to obtain a quantitative data (De Wolf et al., 2004).

5. Conclusions

The conclusions of the research conducted in the present study are: (1) large amount of arsenic can be accumulated by leaves of *N. officinale* (2) the photosynthetic pigment most sensitive to As(III) exposure is chlorophyll a; (3) increasing the concentration of the As(III) exposure affects the genomic template stability and may induce modifications in the band intensity and a gain or loss in the number of bands; (4) *N. officinale* has the capacity to overcome the arsenite-induced stress, especially at a moderate degree of exposure.

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