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Research article/Araştırma makalesi

Assesment of genotoxicity induced by lead pollution in tomato (*Lycopersicum esculentum*) by molecular and population markers

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Abstract

Heavy metal contamination is an important environmental problem that may lead to alterations in vital growth processes, mineral nutrition, transpiration, photosynthesis, enzyme activity and nucleic acids. The effects of environmental pollutants on plants can be monitored using various techniques at different levels. Random amplified polymorphic DNA (RAPD) is a semi-quantitative technique that has been used for genetic mapping, taxonomy, phylogeny and the detection of various kinds of DNA damage and mutations that result from genotoxic agents such as heavy metal contamination. In this study, tomato (*Lycopersicum esculentum* L.) seeds that had germinated in various concentrations of $Pb(NO_3)_2$ solutions were used for measuring population parameters such as dry weight, total soluble protein content, root length and, ultimately, inhibitory rate (IR) values. The seeds were also used to determine the genotoxic effect of the lead, reflected as the appearance or disappearance of bands in RAPD profiles. Inhibition or activation of root elongation was found to be the first effect of metal toxicity to show up in the plants that were tested. Also, total soluble protein content was significantly affected by increased Pb^{2+} concentrations. The data obtained from RAPD band profiles and genomic template stability (GTS) revealed results that were consistent with the population parameters.

Key words: Tomato (*Lycopersicum esculentum* L.), RAPD, lead (Pb^{2+}), population parameters

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Domates Bitkisinde (*Lycopersicum esculentum* L.) Kurşun Kirliliği ile İndüklenen Genotoksisitenin Moleküler ve Populasyon Markörleri ile Değerlendirilmesi

Özet

Ağır metal kirliliği bitkilerin gelişim süreci, mineral beslenme, terleme, fotosentez, enzim aktivitesi ve nükleik asit sentezi gibi bir çok alanda farklılaşmalara sebep olan çevresel bir problemdir. Çevresel kirleticilerin bitkiler üzerindeki etkileri çeşitli teknikler kullanılarak belirlenebilir. Rastgele çoğaltılmış polimorfik DNA (RAPD) yöntemi genetik haritalama, taksonomi, filogeni ve ağır metal kontaminasyonu gibi genotoksik ajanların neden olduğu çeşitli DNA hasarları veya mutasyonlarının belirlenmesinde kullanılan yarı-nicel bir tekniktir. Bu çalışmada, domates (*Lycopersicum esculentum* L.) tohumları farklı konsantrasyonlardaki $Pb(NO_3)_2$ solüsyonu içerisinde çimlendirilmiş ve bu tohumların kuru ağırlık, toplam çözünebilir protein içeriği, kök uzunluğu ve sonuçta engelleyici oranı (IR) belirlenmiştir. Tohumlar aynı zamanda kurşun solüsyonunun RAPD profilinde yeni bir band oluşumu veya kayboluşu olarak akseden genotoksik etkisini belirlemek amacıyla kullanılmıştır. Kök uzamasının inhibisyonu veya aktivasyonu bitkilerde metal toksisitesinin ilk belirtisi olarak gözlenmiştir. Aynı zamanda toplam çözünebilir protein içeriği artan Pb^{2+} konsantrasyonundan önemi oranda etkilenmiştir. RAPD band profillerinden ve genomik kalıp stabilitesinden (GTS) elde edilen veriler populasyon parametreleri ile uyumludur.

Anahtar kelimeler: Domates (*Lycopersicum esculentum* L.), RAPD, kurşun (Pb^{2+}), populasyon parametreleri

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1. Introduction

Heavy metals are considered primarily to be beneficial micronutrients and play an important role as cofactors in plant growth, enzymes and the structure of proteins. However, in excess concentrations of these metals can also be toxic to organisms (Nedelkoska and Doran, 2000). The major causes of heavy metal contamination in the environment relate to antropogenic activities such as industrial activities, the development of new technologies, exhaust gases from motor vehicles, agricultural fertilizers and pesticides. General responses to heavy metal toxicity include the inhibition of growth parameters, the reduction of biomass production (dry weight biomass etc.) and alterations in mineral nutrition, transpiration, photosynthesis, enzyme activity and DNA (Munzuroğlu and Geçkil, 2002; Chojnacka et al., 2005; Aksoy-Körpe and Aras, 2011).

In recent years several organisms, such as lichens, mosses, fungi and plants, have started to be used widely for biomonitoring the effects of environmental pollution (Cansaran-Duman et al., 2011). Many plant species, such as *Solanum melongena, Oryza sativa, Silene paradoxa, Hordeum vulgare, Hydrilla verticillata, Allium cepa,* have recently been used as effective bio-indicators of genetic toxicity resulting from environmental pollutants (Liu et al., 2007; Gupta and Sarin, 2009; Soudek et al., 2009; Aksoy-Körpe and Aras, 2011).Although the plant genome is very stable, its DNA could be damaged through exposure to heavy metals. DNA damage or mutations (e.g., rearrangements, point mutations, small insertions or deletions of DNA and ploidy changes) cause many physiological and cytological effects that, in turn, affect the growth and development of the entire organism (Atienzar et al., 2000). Comet assay, micronucleus assay or chromosome aberration assay techniques were used to measure the genotoxic effect of metals on plants (Angelis et al., 2000; Liu et al., 2005). The changes that genotoxic chemicals cause in DNA may be monitored using different biomarker assays, at both population and molecular levels (Savva, 1998). Advances in molecular biology have led to the development of selective and sensitive assays for DNA damage that could also be applied to the field of genotoxicology. RAPD analysis has also been able to detect DNA damage or mutations due to genotoxicity (Williams et al., 1990). Heavy metals acting as genotoxic agents could lead to visible alterations in oligonucleotide priming sites and could cause variations in the activity of the *Taq* DNA polymerase, which turns as change occur in the electrophoretic profiles of RAPD reaction products. These changes include the appearance of extra-amplified bands and the disappearance of amplified bands, or alternations in amplified band fluorescence (Atienzar et al., 1999).

The aim of this study is to describe DNA alterations in the exposed tomato (*Lycopersicum esculentum* L.) seedlings through RAPD analysis, to reveal the pattern of genetic variation influenced by the various concentrations of Pb^{2+} (40, 80, 120, 160 and 240 mg l^{-1}) contaminations. The study also compares the changes in RAPD profiles with respect to population parameters such as root length, dry weight and total soluble protein content.

2. Materials and methods

Plant material, growth conditions and total soluble protein level

Tomato (*Lycopersicum esculentum* L., Falcon type) seeds were surface-sterilized with 70% alcohol and 30% sodium hypochlorite solution and then washed with distilled water and germinated to primary roots of 2 mm long in petri dishes containing 15 ml test solutions (40, 80, 120, 160 and 240 mg l^{-1} $Pb(NO_3)_2$. Each treatment was replicated three times. After 21 days of incubation, the root lengths, dry weights and total soluble protein levels of tomato seedlings were measured. Inhibitory rate (IR, %) was calculated by the following formula: IR = (1- *x*/*y*)x 100, where *x* and *y* are the average values detected in the control and each sample treated, respectively. After 21 days of incubation, root dry weights were measured; following the incubation period at 70ºC for 48 h. Roots of tomato seedlings were homogenized with (1:1, w/v) 0.2 M phosphate buffer (pH 7.0) with a cold mortar and pestle. The homogenate was centrifuged at 27,000 *g* for 20 min. The supernatant was used for assays of total soluble protein content according to Bradford method using bovine serum albumin (BSA) as a standard (Bradford, 1976).

DNA extraction and RAPD procedure

After 21 days of growth, seedlings were collected, ground in liquid nitrogen, and total genomic DNA was extracted according to the protocol defined by Aras et al., (2003). DNA concentration of each sample was quantified by NanoDrop ND-1000 Spectrophotometer.

PCR conditions were optimized according to ref. 10, with some minor modifications. PCR was performed in a reaction mixture of 25 µl containing approximately 200 ng of genomic DNA, 0.2 µM primer, 20 µM dNTPs, 2.5 mM $MgCl_2$, 0.5 U of *Taq* DNA polymerase (Promega) and 1X reaction buffer. 12 decamer primers were tested and 6 of them displayed clear and reproducible results. The sequences of 6 primers were given in Table 1. The PCR program had an initial denaturing step of 2 min at 94ºC, followed by 35 cycles of; 94ºC for 30s (denaturation), 36ºC for 60s (annealing) and 72ºC for 90s (extension) and final extension period of 8 min at 72 ºC. A negative control of PCR mix without any template DNA was also used to test any other kinds of contamination. The amplifications were carried out in duplicate. To test the reproducibility of the bands in biological replicates, RAPD assay was performed from each of the treatments prepared in triplicate. PCR products and 100 bp DNA ladder (Fermentas) were resolved

electrophoretically in a 1.6 % agarose gels containing 0.05µl/ml ethidium bromide, and run at 60 V for about 4h. Samples were visualized and analyzed under UV light using the system Gene Genius, Syngene.

Table 1. The sequence of the primers used in the study

Code of primers	Sequences of primers (5' →3')
Tube A09	**GGGTAACGCC**
Tube A11	**CAATCGCCGT**
Tube A13	**CAGCACCCAC**
Tube A17	**GACCGCTTGT**
Tube A19	**CAAACGTCGG**
Tube A20	**GTTGCGATCC**

Data Analysis

Polymorphisms in RAPD profiles included disappearance and appearance of bands in comparison to control and the average was calculated for each test group exposed to different concentrations of $Pb(NO_3)_2$ treatment (Table 2). To compare the sensitivity of each parameter (root length, root dry weight, root total soluble protein content), changes in these values were calculated as a percentage of their control (set to 100%).

Estimation of genomic template stability

Genomic template stability (GTS) values were also calculated according to the results of RAPD analysis. GTS % was calculated as GTS %= (1- a/n)* 100, where 'a' indicates the RAPD polymorphic profiles in each sample, and 'n' is the number of total bands in the control. Changes in the RAPD patterns were expressed as a decrease in GTS, a qualitative measure showing the obvious changes to the number of RAPD profiles generated by the tomato samples exposed to $Pb(NO_3)_2$, in relation to profiles obtained from the control samples (Table 3).

Statistical analysis

The SPSS statistical package software (Windows 15.0) was used to analyze the changes in root length, dry weight, and total soluble protein content. Data were tested by performing variance analysis (ANOVA). The Duncan's test was used to reveal the statistical differences of distinct groups.

3. Results

Effect of $Pb(NO_3)_2$ on root growth (length, dry weight) and total soluble protein level

This study evaluates the inhibition of seed germination in response to lead pollution by treating tomato seeds with different concentrations of Pb^{2+} (40, 80,120, 160, 240 mg l^{-1}) solutions. It has found that seed germination and root length were substantially reduced after 21 days of exposure to all of the increased Pb^{2+} concentrations (*$p < 0.05$) except for the 40 mg l^{-1} concentration. The maximum inhibitory rate (IR) of the Pb^{2+} on root length was observed at a concentration of 240 mg l^{-1}, with a 77% value (Table 2).

The increased concentrations of Pb^{2+} also lowered the dry weight as root biomass production in comparison to the weights of the tomato seedlings used as controls. All concentrations of lead caused a decrease in the development of root and dry weights. The maximum IR (95.8%) of dry weight was determined to be with treatment at 160 mg l^{-1} $Pb(NO_3)_2$ (Table 2).

The IR percentage of the lead on total soluble protein levels is shown in Table 2. Significantly decreased levels were determined in the samples exposed to Pb^{2+} contaminations of 40 mg l^{-1}, 80 mg l^{-1} and 240 mg l^{-1}. However, Pb^{2+} concentrations of 120 mg l^{-1} and 160 mg l^{-1} tended to increase the total soluble protein levels in tomato seedlings. All of the changes detected in total protein levels, due to Pb^{2+} contaminations, were found to be statistically important.

Table 2. Effect of Pb on dry weight, root lenght and total soluble protein level of root tips in tomato seedlings after 21 d of treatment

Concentration (ppm)	Total Protein Average	% IR	Dry Weight (mg)	% IR	Radikula Length (mm)	% IR
Control	1,029	0	0,017	0	2,76	0
40 ppm	0,895	13,02	0,003	82,35	3,75	35,86
80 ppm	0,89	13,5	0,001	94,11	1,6	42,02
120 ppm	1,136	10,39	0,002	88,23	1,6	42,02
160 ppm	1,195	16,13	0,0007	95,88	1,46	47,1
240 ppm	0,842	18,17	0,0009	94,7	0,63	77,17

Effect of Pb $(NO_3)_2$ stress on RAPD profile

In order to verify the genotoxic effect of Pb^{2+} on tomato seedlings, DNA was isolated from the samples that had been exposed to 21 days of incubation in test solutions. 12 decamer RAPD primers were tested and 6 of them displayed clear and reproducible results. The sequences of primers used in the study are given in Table 1. All three replications were tested twice, in order to verify the reproducibility of all polymorphic bands. A representative example of the results obtained by RAPD analysis is shown in Fig. 1. A total of 81 bands were observed; 22 of them were polymorphic (Table 3). RAPD patterns for samples exposed to Pb^{2+} concentrations were clearly different from the non-stressed samples, with the appearance of new bands and the disappearance of control bands.

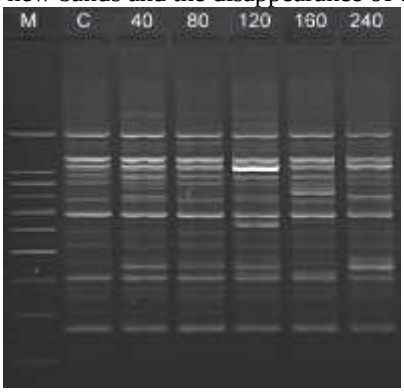

Figure 1. RAPD profiles of genomic DNA from root tips tomato seedlings exposed to Pb^{2+} for 21 days generated by the primer Tube A 11. **(M:** Marker**, C:** Control**, 40, 80, 120, 160 and 240:** mg l^{-1} Pb^{2+}**)**

Table 3. The polymorphism ratios of the primers

Primer	Total Band	Polymorphic Band	Ratio %
Tube A 09	15	2	13,33
Tube A 11	13	8	61,53
Tube A 13	19	1	5,26
Tube A 17	14	3	21,42
Tube A 19	9	4	44,44
Tube A 20	11	4	36,36
TOTAL	81	22	

The highest number of new band appearances was observed in the samples treated with the 40 mgl^{-1} Pb^{2+} solution and band disappearances were determined in the samples treated with the 80 mgl^{-1} and 240 mgl^{-1} Pb^{2+} solutions. The DNA isolated from seedlings germinated in the 40 mgl^{-1} and 120 mgl^{-1} Pb^{2+} concentrations displayed the maximum polymorphic bands in RAPD assays (Table 4). In addition, the genomic template stability ratios (GTS), which imply a qualitative measure to reflect the changes in RAPD profiles, are shown in Table 5. The lowest GTS value, of 78.14%, was obtained in the seedlings that were exposed to the 40 mgl^{-1} Pb^{2+} solution. Pb^{2+} treatments of 80 mgl^{-1} and 240 mgl^{-1} yielded the highest GTS values, at 90.62% and 90.08%, respectively (Table 5).

Table 4 . Changes of total bands in control and of polymorphic bands in exposed samples in tomato seedlings

Primer		Pb 40 ppm		Pb 80 ppm		Pb 120 ppm		Pb 160 ppm		Pb 240 ppm	
	C	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Tube A 09	15	2	0	2	0	2	0	2	0	1	0
Tube A 11	13	7	0	2	0	6	1	4	1	3	2
Tube A 13	19	0	0	0	0	1	0	0	0	0	0
Tube A 17	14	2	0	0	1	2	0	2	0	2	0
Tube A 19	9	2	0	1	0	2	0	1	0	0	0
Tube A 20	11	3	0	0	1	0	0	0	0	0	0
Total	81	16	0	5	2	13	1	9	1	6	2
a+b		**16**		**7**		**14**		**10**		**8**	

C: Control sample, a: Appearance of new bands, b: Disapperance of control bands, a+b: Indicates polymorphic bands

Table 5. GTS values for all primers

Pb Concentration	Tube A09 % GTS	Tube A11 % GTS	Tube A13 % GTS	Tube A17 % GTS	Tube A19 % GTS	Tube A20 % GTS	Averange % GTS
40 mg/lt	86,6	46,15	100	85,71	77,7	72,72	**78,14**
80 mg/lt	86,6	84,61	100	92,85	88,8	90,9	**90,62**
120 mg/lt	86,6	46,15	94,7	85,71	77,7	100	**81,81**
160 mg/lt	86,6	61,5	100	85,71	88,8	100	**87,10**
240 mg/lt	93,3	61,5	100	85,71	100	100	**90,08**

% GTS: (1- a/n)* 100 **a**: polimorphic band **n**: total bands in the control

Comparison of RAPD profiles, root growth and total soluble protein content

GTS is a qualitative measurement reflecting the changes in RAPD profiles. The average GTS values of the samples exposed to different concentrations of Pb^{2+} are presented in Table 5. Minimum and maximum GTS values were determined as 78.14% and 90.62%, at 40 mg l^{-1} and 80 mg l^{-1}, respectively. Modifications to IR values for total soluble protein levels, root lengths and dry weights were also calculated (Table 2). The minimum IR value of total soluble protein levels was recorded as 10.39% for tomato seedlings exposed to Pb^{2+} concentrations at 120 mgl^{-1}, while the maximum was 18.17%, at 240 mg l^{-1}. The minimum and maximum IR values were 82.35% and 95.88%, at 40 mg l^{-1} and 160 mg l^{-1}, respectively, for dry weights and 35.86% and 77.17% for 40 mg l^{-1} and 240 mg l^{-1}, respectively, for radicula lengths. A positive correlation between total soluble protein levels and root lengths was also recorded for samples with Pb^{2+} concentrations at 240 mg l^{-1}. Ultimately, the maximum polymorphic band pattern was observed in the RAPD assay for tomato seedlings with Pb^{2+} concentrations of 40 mg l^{-1} (Table 4).

4. Conclusions

In order to fully understand the effects of heavy metals on plants, it is essential to accumulate the data at different levels of the biological organizations. Measuring various parameters of the population level can facilitate the interpretation of the data at the molecular level. Simultaneous application of more than one biomarker can enhance the detection of toxic effects, since different biomarker responses vary at different stages of plant development. In the current research, the population biomarkers, such as root length, dry weight and total soluble protein content, were compared to the assessment of genotoxicity results achieved by RAPD assay, to evaluate the effects of Pb^{2+}. Other studies have reported similar clear advantages to using population and molecular markers together (Theodorakis et al., 2006; Aksoy-Körpe and Aras, 2011).

The roots are expected to be the parts of the plants that are most affected by metal accumulation, with impacts reflected through changes in germination, morphology and anatomy. Indeed, the inhibition of root elongation is considered to be the first sign of metal toxicity in plants. Cell division at the root tips and cell elongation in the extension zones, which are two different mechanisms in root growth, are both affected by the presence of heavy metals (Arduini et al., 1994). In this study, the increased Pb^{2+} concentrations significantly reduced all the root lengths (*$p < 0.05$) except for the Pb^{2+} treatments at 40 mg l^{-1}. However, germination and root elongation showed an increase with the treatment of 40 mg l^{-1} Pb^{2+} concentrations in the tomatoes. (Table 2). The highest level of inhibition in the germination was observed with treatment at 240 mg l^{-1} Pb^{2+} concentrations. Also, DNA damage or mutations in the root cells, which are reflected as changes in the electrophoretic profiles of RAPD reaction products, might be related to the inhibition or alteration in root elongation.

The IR percentage rates of dry weights were significantly higher than the IR percentage rates of root elongations and total soluble protein contents. The highest level of IR percentage rate for dry weight was observed in the 160 mg l^{-1} Pb^{2+} treatment (Table 2). These results are consistent with the data indicated in the references (Sinha et al., 1996).

Alteration in the total soluble protein content is one of the important biomarkers of heavy metal toxicity in plants (Mollema and Cole, 1996). Also, in this study, all concentrations of Pb^{2+} tended to alter the total soluble protein content in tomato seedlings. However, the 40 mg l^{-1}, 80 mg l^{-1} and 240 mg l^{-1} concentrations of Pb^{2+} decreased the total soluble protein level, while the 120 mg l^{-1} and 160 mg l^{-1} concentrations of Pb^{2+} increased the level of protein (Table 2). This research confirmed the potential application of soluble protein levels as a valuable biomarker, which was also reported in (Singh and Tewari, 2003).

The RAPD assay is able to reveal the genetic variation that is due to heavy metal toxicity (Atienzar et al., 1999; Aksoy-Körpe and Aras, 2011). The variation in band appearances or disappearances may be attributed to the presence of DNA photoproducts (e.g. pyrimidine dimers, 6-4 photoproducts), which can block or reduce (bypass event) the polymerization of DNA in the PCR reaction (Nelson et al., 1996). In the current research the changes in bands, both the disappearance of bands and the appearance of new PCR products in the RAPD profiles in comparison to controls, are shown for the primer Tube A 11 (Fig. 1) and summarized in Table 4. The maximum appearance of new bands was observed in the 40 mg l^{-1} Pb^{2+} contamination. The results of this research suggest that higher concentrations of heavy metal contamination may induce less DNA damage than lower concentrations, in germinating tomato seedlings. Other

experiments conducted in our laboratory obtained similar results with the seedlings of other higher plants, such as cucumber (Soydam-Aydın et al. 2012) and okra (Soydam-Aydın et al. 2013). However, this situation seems not to be the case for lichens, a non-vascular cryptogam, in which band differences were observed with higher metal concentrations (Aras et al., 2010). Higher GTS values obtained at higher concentrations than 40 mg l^{-1} Pb^{2+} might be an indication of introduction of an effective DNA repair system or any other kind of cellular adaptation and/or defense sytem (Table 5). According to Liu et al., (2005) this situation was explained by the plateau effect, ascribed to multiple changes in RAPD profiles (appearance of new bands, disappearance of the existing bands) that tend to counterbalance each other. In addition, the 40 mg l^{-1} Pb^{2+} concentration is remarkable for stimulating root elongation (Table 2). Genomic template stability and total soluble protein levels were both increased with the 80 mg l^{-1} Pb^{2+} contamination, compared to Pb^{2+} treatment at 40 mg l^{-1} (Table 2, Table 5).

The RAPD assay has potantial to detect point structural modifications and the temporary DNA changes such as DNA methylation and histone deacetylation that could not finally manifest themselves as mutations (Castano and Becerril, 2004). Linking molecular/cellular effects to changes such as variations in population parameters can further enhance the value of RAPD procedures (Theodorakis et al., 2006). In addition, the multiple biomarker approach presented in the current study can lead to better identification of DNA damage, temporary changes in DNA and structural variations. The higher sensitivity of RAPD, combined with total soluble protein levels, root lengths and dry weights, can show clearer impacts of Pb^{2+} contaminations on tomato seedlings. This research evaluates the suitability of combining RAPD assays with population biomarkers, to provide a better understanding of the effect of Pb^{2+} contamination on tomato seedlings. The simple and straightforward method that this study presents for identifying the effects of heavy metals on germination parameters, total soluble protein level and DNA may also prove useful in the future for both assessment of the risks of environmental contamination and remediation.

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References

Aksoy Körpe, D., Aras, S. 2011. Evaluation of copper-induced stress on eggplant (Solanum melongena L.) seedlings at the molecular and population levels by use of various biomarkers. Mutation Research. 719: 29–34.

Angelis, J., McGuffie, M., MenKe, M., Schubert, I. 2000. Adaptation to Alkylation Damage in DNA Measured by the Comet Assay. Environmental and Molecular Mutagenesis. 36:146–150.

Aras, S., Duran, A., Yenilmez, G. 2003. Isolation of DNA for RAPD Analysis From Dry Leaf Material of Some Hesperis L. Specimens. Plant Molecular Biology Reporter. 21: 461-461.

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

Arduini, I., Godbold, D.L., Onnis, A. 1994. Cadmium and copper change root growth and morphology of Pinus pinea and Pinus pinaster seedlings. Physiologia Plantarum. 92: 675-680.

Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N., Depledge, M.H. 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., Cordi, B., Donkin, M.B., Evenden, A.J., Jha, A.N., Depledge, M.H. 2000. Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, Palmaria palmate. Aquatic Toxicology. 50: 1–12.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72: 254-284.

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

Castano, A., Becerril, C. 2004. In vitro assessment of DNA damage after short-and long-term exposure to benzo (a) pyrene using RAPD and the RTG-2 fish cell line. Mutation Research. 552: 141-151.

Chojnacka, K., Chojnacki, A., Gorecka, H., Gorecki, H. 2005. Bioavailability of heavy metals from polluted soils to plants. Science of the Total Environment. 337: 175-182.

Gupta, M., Sarin, N.B. 2009. Heavy metal induced DNA changes in aquatic macrophytes: Random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker. Journal of Environmental Sciences. 21: 686-690.

Liu, W., Li, P.J., Qi, X.M., Zhou, Q.X., Zheng, L., Sun, T.H. 2005. DNA changes in barley (Hordeum vulgare) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere. 61: 158–167.

Liu, W., Yang, Y., Zhou, Q., Xie, L., Li, P., Sun, T. 2007. Impact assessment of cadmium contamination on rice (Oryza sativa L.) seedlings at molecular and population levels using multiple biomarkers. Chemosphere. 67: 1155–1163.

Mollema, C., Cole, R.A. 1996. Low aromatic amino acid concentrations in leaf proteins determine resistance to Frankliniella occidentalis in four vegetable crops. Entomologia Experimentalis et Applicata. 78:325-333.

Munzuroğlu, Ö., Geçkil, H. 2002. Heavy metal effect on seed germination, root elongation, coleoptile and hypocotyl growth in Triticum aestivum and Cucumis sativus. Archives of Environmental Contamination and Toxicology. 43: 203-213.

Nedelkoska, T.V., Doran, P.M. 2000. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Minerals Engineering. 13: 549-561.

Nelson, J.R., Lawrence, C.W., Hinkle, D.C. 1996. Thymine-Thymine Dimer Bypass by Yeast DNA Polymerase ζ. Science. 272: 1646-1649.

Singh, P.K., Tewari, R.K. 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. Journal of Environmental Biology. 24: 107-112.

Sinha, S., Gupta, M., Chandra, P. 1996. Bioaccumulation and biochemical effects of mercury in the plant *Bacopa monnieri* (L). Environmental Toxicology and Water Quality. 11: 105-116.

Soudek, P., Kotyza, J., Lenikusova´, I., Petrova´, S., Benesˇova´, D., Vaneˇk, T. 2009. Accumulation of heavy metals in hydroponically cultivated garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leek (*Allium porrum* L.) and chive (*Allium schoenoprasum* L.). Journal of Food, Agriculture and Environment. 7: 761–769.

Soydam Aydın, S., Gökçe, E., Büyük, İ., Aras, S. 2012. Characterization of Copper and Zinc induced stress on Cucumber (*Cucumis sativus* L.) by molecular and population parameters. Mutation Research. 746(1): 49-55.

Soydam Aydın, S., Başaran , E., Cansaran-Duman, D., Aras, S. 2013. Genotoxic effect of cadmium in okra seedlings: Comperative investigation with population parameters and molecular markers. Journal of Environmental Biology. 34: 985-990.

Theodorakis, M.J., Carlson, O., Michopoulos, S., Doyle, M.E., Juhaszova, M., Petraki, K., Egan, J.M. 2006. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. American Journal of Physiology - Endocrinology and Metabolism. 290: 550–559.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18: 6531– 6535.

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