Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

September 2010, 31(5) 661-666 (2010)
For personal use only
Commercial distribution of this copy is illegal

Detection of lipid peroxidation and cytotoxicity induced by aluminium (Al) and cobalt (Co) ions in barbunia root tip cells

Kultigin Cavusoglu* and Emine Yalcin

Department of Biology, Faculty of Science and Art, Giresun University, 28049, Debboy Location, Giresun, Turkey

(Received: August 29, 2008; Revised received: January 20, 2009; Accepted: March 02, 2009)

Abstract: *The aim of this study was to investigate the cytotoxic effects of different concentrations of Aluminium (Al) and Cobalt (Co) heavy metal ions on Phaseolus vulgaris L. cv. Barbunia (Fabaceae) root tips. We used the germination percentage (GP), root length (RL), weight gain (WG) and micronucleus (MN) frequency as indicators of cytotoxicity, and correlated these data with statistical parameters. Additionally to the cytogenetic analysis, lipid peroxidation and DNA analyses were performed in root tips of barbunia seeds treated with Al and Co metals. The seeds were divided into five groups as control, Al and Co treatment groups. They were treated with 25 and 50 ppm doses of Al and Co during 7 days. The results indicated that there was an alteration in the GP, RL, WG and MN frequency depending on the treatment dose in the seeds exposed to Al and Co metal ions when compared with the controls. Al and Co metal ions at both the doses significantly reduced the GP, RL and WG in seeds of all the treatment groups. The highest GP was observed in seeds of the control group (in proportion as 96%). 25 and 50 ppm doses of Co and Al caused 30 , 50 and 42, 64% decrease of seed germination, respectively. In the control group, the final weights of all the seeds increased about 1.31 g when compared to initial weight. The mean RL of control seeds were measured as 3.71 cm at the end of experimental period. In Co and Al groups, the final weights of seeds increased about 0.34 g and 0.19 g according to initial weight at 50 ppm dose, respectively. But, Al and Co ions caused a dose-dependent increase in the frequency of MN. The highest frequency of MN was observed at 50 ppm dose of Al and least frequency of MN was observed at 25 ppm dose of Co. Besides, 25 and 50 ppm concentrations of Al and Co significantly enhanced the lipid peroxidation and caused an increase in malondialdehyde (MDA) levels at both the doses. In roots treated with 25 and 50 ppm doses of Al, the increase of MDA was about 62 and 136% according to control, respectively. In Co-treated roots, the increase of MDA was about 31 and 91% according to the control at 25 and 50 ppm doses, respectively. The investigated parameters (except MN and MDA) were higher in the seeds exposed to Co than the seeds treated with Al. Moreover, it was observed that the yields of DNA in the seeds treated with Al and Co metals were lower than recorded in the controls. Hence, DNA yields exposed to Al and Co were run ahead on agarose gel according to the control group. The results of the present study indicate that Al and Co metal ions have toxic effects on barbunia root tip cells, and the selected parameters such as the GP, RL, WG, MN and MDA are very sensitive and useful biomarkers for biomonitoring these effects.*

Key words: *Aluminium and Cobalt, Toxicity, Barbunia bean, Micronucleus, Seed physiology*
PDF of full length paper is available online

Introduction

The pollution of environment by heavy metal ions as a result of human, agricultural and industrial activities is now-a-days fairly widespread. Among the heavy metals, especially lead (Pb), aluminium (Al) and cobalt (Co) are the most abundant pollutants in the environment (Sharma and Dubey, 2005; Gupta *et al.,* 2008; Kamaruzzaman *et al.,* 2009).

Aluminium (Al) is a fairly toxic metal for plants. Al toxicity is one of the most important factors limiting crop production in acid soils (Symeonidis *et al*., 2004). Approximately 40% of the world's cultivable soils are acid and may be mentioned from the negative side-effects of Al toxicity in these soils (Alvarez *et al*., 2005). Although Al is not an essential element for plant cells and found very low-rate in biological systems, it has been recognized as a toxic agent for a large part of terrestrial ecosystems (Rosseland *et al*., 1990; Rodushkin *et al*., 1995).

Cobalt (Co) is an important trace element in all living organisms. It has long been applied to plants for raise crop manufacture or animals in order to prevent *diseases* caused by Co d*eficiency* in certain regions of the world such as, Britain, South Australia, New Zealand, Kenya and the United States. At the same time, it is necessaried by *Rhizobia* in root nodules of leguminous plants and by some species of nitrogen-fixing blue green algae for symbiotic nitrogen fixation. However, like other trace metals when Co is presented in excess amounts in plant tissues, it causes significant alterations in tissues (Liu *et al*., 2000). Al and Co easily are absorbed by soil and accumulate in different parts as root, stem and leaf. Thus, they can get into the food chain and may cause negative effects in plant tissues (Zengin and Munzuroglu, 2005). For example, they cause various anomalies such as decrease of chlorophyll content and mitotic activity, inhibition of root and plant growth, reduce of macro- or micro-element uptake, increase of cell toxicity, chlorosis of leaves, formation of reactive oxygen species (ROS) and lipid peroxidation (Symeonidis *et al*., 2004; Liu *et al*., 2000). Hence, when the soils are contaminated with heavy metals, it may reduce in harvest amount, a serious problem for agricultural economies (Johnson and Eaton, 1980; Sahu *et al*., 2007). Despite regulatory measures carried out in many countries, heavy metals continue to increase in the environment.

* **Corresponding author:** kultigincavusoglu@mynet.com

Consequently, Al and Co are environmental pollutants that inhibit plant growth. Although many cytological studies have been carried out to detect the effects of Al and Co metals on plants in recent years, the mechanisms of Al and Co toxicity in plants are still poorly understood. The aim of the present study was to evaluate the toxic effects of different concentrations of Al and Co metal ions on barbunia root tip cells.

Materials and Methods

Preparation of root tips: In the present study, 25 and 50 ppm concentrations of Al and Co metal ions were used. Aluminium Sulfate (Merck, 1.01102) and Cobalt II Chloride (Merck, 1.02539) were dissolved in water to prepare treatment solutions. All the solutions were freshly prepared in distilled water before use (pH 6.7). Healthy and proximate equal-sized barbunia seeds were selected. The seeds were washed in ultra-distilled water for 24 hr. Controls were placed in tap water only. The treatment group seeds were placed on two sheets of filter paper (Whatman No. 1) in 11 cm diameter Petri plates. 50 seeds were planted in each Petri dish and were treated with test solutions for 7 consecutive days at 23°C in incubator. Petri dishes were controlled and treated with 2 ml of Al and Co solutions once daily (a 24 hr period) during 7 days. Each solution contained 25 and 50 ppm of Al and Co, respectively. For the cytogenetic analysis, when the roots attained a length of approximately 1.5-2.0 cm, they were treated with distilled water, and temporary squash preparations were made.

Determination of root length, weight gain and germination percentage: The RL of germinated seeds were measured by a milimetric ruler. The RL was determined by radicula formation bases of barbunia seeds non-exposed and exposed to different doses of Al and Co metals. The WG of the seeds were determined by measuring the weights of seeds before and after treatment with a sensitive balance. The GP of seeds exposed to Al and Co metal ions were calculated.

Germination (%) = Germinated seeds/total seeds x 100

Quantification of lipid peroxidation: Lipid peroxidation was determined by measuring the amount of MDA according to Unyayar *et al*. (2006). About 0.5 g of root tissues from control and treated groups were cut into small pieces and homogenized by the addition of 5 ml of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12,000 rpm for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution were added into a new tube and boiled at 96 °C for 25 min. The tubes were transferred into ice-bath and then centrifuged at 10,000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, 0.5% TBA in 20% TCA solution was used as the blank. MDA contents were calculated using the extinction coefficient of 155 M^{-1} cm^{-1}. Values of MDA contents were taken from measurements of three independent samples, and SD of the means were calculated.

Micronucleus (MN) assay: The root tips were fixed for 6 hr in a Clarke's fixator (3:1 *i.e.* acetic acid glacial and distilled water), washed for 15 min in 96% ethanol and stored in 70% ethanol in the fridge at 4°C until making the microscopic slides. The root tips were hydrolyzed roots in 1N HCl at 60 °C for 17 min, treated with 45% CH_3COOH for 30 min. For microscopic observation the root tips were stained for 24 hr in Acetocarmine. After staining, the root meristems were separated and squashed in 45% CH_3COOH (Staykova *et al*., 2005; Wei, 2004).

For the analysis of MN, 1000 cells were scored in each slide to calculate the frequency of MN. Micronucleated cells were evaluated under a binocular light microscope (Japan, Olympus BX51) at 500X magnification. For the scoring of MN the following criteria were adopted from Fenech *et al*. (2003). (i) the diameter of the MN should be tenth of the main nucleus, (ii) MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary, (iii) MN should have similar staining as the main nucleus.

DNA isolation: Modified DNA isolation protocol was applied according to Sharma *et al*. (2002). For DNA isolation the plant material was grinded in liquid nitrogen (2 g of fresh tissue). The grinded material was transferred to a solution containing 1M Tris-HCl, 0.5 M EDTA, 5M NaCl, 1M b-merkaptoethanol, distilled H_2O and incubated at 65°C for 30 min. Centrifugation at 6800 rpm at room temperature for 15 min was applied and the supernatant carefully transferred into a fresh polypropylene tube and 5M potassium acetate was added. The solution was incubated in ice-bath and centrifugated at 15000 rpm at room temperature for 15 min. An equal volume of chloroform-isoamylalcohol (24:1) was added onto supernatant and mixed by inversion for about 1 min. After centrifugation at 15000 rpm the aqueous phase transferred into a new polypropylene tube and ethanol: sodium acetate (2:1) was added. The mixture was incubated for 40 min at -20°C and centrifugated at 15000 rpm. The pellet was washed with 80% ethanol, air-dried for 30 min and dissolve in 0.5 ml Tris-EDTA buffer. The DNA solutions were runned on a 0.8% agarose gel, and molecular imaging and DNA concentration were achieved by "Biovision+100/26MX" analyzer.

Statistical analysis: For the statistical analysis, differences between the groups were tested by analysis of variance (ANOVA) and Duncan test using SPSS for Windows version 10.0 computer program. The data were displayed as means ± standard deviation (SD) and p values less than 0.05 were considered significant.

Results and Discussion

Effects of Al and Co ions on germination percentage (GP): As shown in Table 1, the GP of seeds treated with Al and Co were rather different from the control group. The highest GP was observed in seeds of the control group (in proportion as 96%). Al and Co metals caused a decrease in the GP at all doses. Besides, the GP of Co-treated groups was higher than that of Al. 25 and 50 ppm doses of Co and Al caused 30, 50, 42 and 64%

Table - 1: Effect of Al and Co metals on germination percentage of barbunia seeds (treatment time 7 days)

Group	Concentr-ations (ppm)	Number of germinated seeds	Number of not germinated seeds
Group I	–	48 (96%)	2
Group II	25	35 (70%)	15
Group III	50	25 (50%)	25
Group IV	25	29 (58%)	21
Group V	50	18 (36%)	32

* Each group contains 50 seeds. The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al

Table - 2: Effect of Al and Co metals on root length of barbunia seeds (treatment time 7 days)

Groups	Concentrations (ppm)	Average ±SD	Percent root growth
Group I	–	3.71±0.08	100
Group II	25	3.11±0.06	84
Group III	50	2.30±0.18	62
Group IV	25	2.68±0.09	72
Group V	50	1.65±0.09	44

* All values the mean ± SD (Each group contains 50 seeds). The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al

Table - 3: Mean weight gain of Al and Co metals-treated barbunia seeds at the end of 7th day (treatment time 7 days)

Groups (ppm)	Concentr-ations of seeds	Initial weight of seeds	Final weight gain	Weight increase	Per-cent
Group I	–	0.31 ± 0.03	1.62 ± 0.06	1.31	423
Group II	25	0.31 ± 0.02	1.05 ± 0.03	0.74	239
Group III	50	0.31 ± 0.02	0.65 ± 0.03	0.34	110
Group IV	25	0.31 ± 0.02	0.85 ± 0.03	0.54	174
Group V	50	0.31 ± 0.01	0.50 ± 0.03	0.19	61

*All values the mean ± SD (Each group contains 50 seeds). The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al

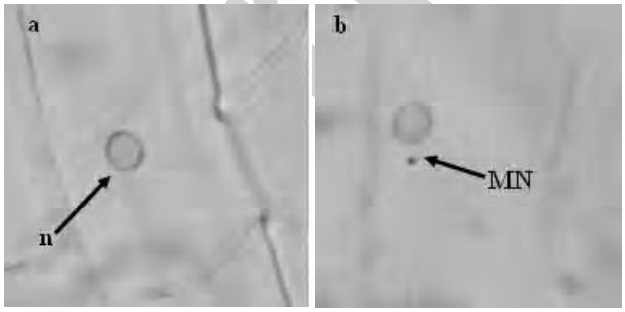

Fig. 1: (a) showed absence of micronucleus (MN) formation, (b) formation of MN in seeds of Al and Co treatment groups. Acetocarmine staining (X 500)

Fig. 2: Plate showing isolated DNA from barbunia root tip cells resolved on 0.8% agarose gel. (a) Control group, (b) Treatment group exposed to 50 ppm Co, (c) Treatment group exposed to 50 ppm Al

decrease of seed germination, respectively. These results showed that the effects of Al and Co on the GP depending on their doses.

Effects of Al and Co ions on root length (RL) and weight gain (WG): The results related with the WG and RL were given in Table 2, 3. These data showed that Al and Co treatments significantly prevented the RL and WG of seeds. A correlation was determined among heavy metal doses with the RL and WG. The highest RL and WG were observed in seeds of the control group at the end of experimental period. The least RL and WG were observed at 50 ppm doses in seeds of Al and Co treatment group. In the control group, the final weight of all the seeds increased about 1.31 g when compared to initial weight. The mean RL of control seeds were measured as 3.71±0.08 cm at the end of experimental period. In Co and Al groups, the final weight of seeds increased about 0.34 g and 0.19 g according to initial weight at 50 ppm dose, respectively. The Al-treated seeds showed a lower the RL and WG than Co-treated group, and differences were statistically significant ($p<0.05$). Besides, there were significant differences between the control and heavy metal treatment groups, and differences were statistically significant (Table 6, $p<0.05$).

Effects of Al and Co ions on lipid peroxidation: Lipid peroxidation in roots of barbunia plant, measured as the content MDA, is given in Table 4. MDA concentration in the roots of barbunia

Table - 4: Effects of Al and Co metals on MDA content (µmol g^{-1} freshweight) of barbunia seeds (treatment time 7 days)

Groups	MDA content (average ± SD)	Percent increase
Group I	13.05±2.91	
Group II	17.15±3.22	31
Group III	24.90±2.22	91
Group IV	21.10±3.42	62
Group V	30.85±3.27	136

*All values the mean ± SD (Each group contains 50 seeds). The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al

Table 5. Effects of Al and Co metals on MN frequency of barbunia seeds (treatment time 7 days)

Groups	Number of scored cell	Average ± SD
Group I	1000	00.00±0.00
Group II	1000	15.30±1.34
Group III	1000	24.90±1.66
Group IV	1000	19.40±1.17
Group V	1000	37.50±1.43

**All values the mean ± SD (Each group contains 50 seeds). The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al

exposed to 25 and 50 ppm doses of Al and Co metals was significantly higher than in the control ($p<0.05$). Al and Co metals significantly affected the MDA production indicating altered lipid peroxidation. The increased MDA content was observed in roots at both concentrations of Al and Co. In roots treated with 25 and 50 ppm doses of Al, the increase of MDA was about 62 and 136% according to control, respectively. In co-treated groups, the increase of MDA was significantly lower when compared with Al-treated groups, and differences were statistically significant ($p<0.05$). In Co-treated roots, the increase of MDA was about 31 and 91% according to the control at 25 and 50 ppm doses, respectively. Besides, it is observed that the increase in MDA concentration is dose-dependent.

Effects of Al and Co ions on MN frequency: Microscopic examination of the squashes of barbunia root tip meristem cells showed that absence of the MN formation was seen in the control group (Fig.1a). However, a significant increase in MN formation was observed in all the seeds exposed to Al and Co metal ions (Fig.1b). The MN frequency increased with rising of the Al and Co doses. There was a certain dose-effect relationship between the MN frequency and Al and Co metal ions. The MN frequency was indicated in Table 5. Al-treated seeds showed a higher frequency of MN than Co-treated seeds. The highest frequency of MN was observed at 50 ppm dose of Al and least frequency of MN was observed at 25 ppm dose of Co. There is a statistically significant difference between treatment and control groups and among treatment groups for the MN frequency ($p<0.05$).

Effects of Al and Co ions on DNA content: It has been observed that the yield of DNA in seeds treated with Al and Co were lower than recorded in the control. The DNA yield of Al and Co was run ahead on agarose gel comparison to control group (Fig. 2).

A negative correlation was observed between heavy metal doses and the GP of seeds. The results showed that the GP can be considered as a sensitive indicator for Al and Co toxicity. This information is parallel with other genotoxic data available so far. In many studies, results indicated that, the test substances as heavy metal ions can lead to decrease of the GP in different plant seeds. For example, Munzuroglu and Geckil (2002) reported reduce with increasing concentrations of Hg, Co, Cu, Pb, Cd and Zn of the GP in *Triticum aes*tivum and *Cucumis sativus* plants. Verma and Dubey (2003) showed about twofold decrease of the GP in rice seeds exposed to high concentrations of Pb. Dubey and Dwivedi (1987) determined that Co inhibits seed germination and seedling growth in plants. Similarly, Neogy *et al*. (2002) observed significantly decrease of the GP in *Vigna radiata* seeds exposed to different concentrations of aluminum sulphate. Besides, there were various studies the indicatived similar effects of heavy metal ions on the GP of *Phaseolus vulgaris*, *Pisum sativum, Brassica napus* (Wierzbicka and Obidzinska, 1998), *Triticum aestivum* seeds (Aybeke and Olgun, 2004).

The visual non-specific symptoms of heavy metal toxicity are inhibition of root growth and seed weight (Burton *et al*., 1984). In 50 ppm Al dose, the root growth was about 2.25 times lower than in the controls. The decrease in root growth was pronounced with the increase in Al and Co concentrations. Aluminium (Al) and cobalt (Co) metals at their both concentrations (25 and 50 ppm) significantly decreased root growth. The root growth about 16, 38% decreased at 25-50 ppm dose of Co and 28, 56% decreased at 25-50 ppm dose of Al, when compared with the controls, respectively. The root growth was higher in Co-treated seeds when compared with those Al-treated. In previous studies were reported that high concentrations of Zn, Cd, Pb, Hg and Cu may lead to inhibition of vegetative organ growth in some plant species (Dimitrova and Ivanova, 2003). For example, Shafig and Igbal (2006) determined decrease according to the control group at 25-100 ppm concentrations of Pb and Cd of the RL in *Cassia siamea*. In a similar study, Zengin and Munzuroglu (2002) investigated the effect on the root growth of bean seedling exposed to Cd and Hg. As a result, they showed inhibition of root growth in bean seedling treated with Cd and Hg. In another study, Kochian (1995) reported that the major symptom of Al toxicity is a rapid inhibition of root growth. Besides, Godbald and Kettner (1991) observed a significant decrease in primary, secondary and tertiary root growth of *Picea abies* seedlings treated with different Pb solutions. Similarly, Obroucheva *et al*. (1998) demonstrated inhibition of primary root growth by heavy metals.

The findings obtained from this experiment showed that the Al and Co metals affected the WG depending on dose. Namely, the

Table - 6: Statistically comparison of RL, WG, MN and MDA data determined in treatment groups at the end of 7th day

Parameters	Group I	Group II	Group III	Group IV	Group V
Root length	3.71±0.08[a]	3.11±0.06[b]	2.30±0.18[d]	2.68±0.09[c]	1.65±0.09[e]
Weight gain	1.62±0.06[a]	1.05±0.03[b]	0.65±0.03[d]	0.85±0.03[c]	0.50±0.03[e]
MN frequency	0.00±0.00[e]	15.30±1.34[d]	24.90±1.66[b]	19.40±1.17[c]	37.50±1.43[a]
MDA content	13.05±2.91[e]	17.15±3.22[d]	24.90±2.22[b]	21.10±3.42[c]	30.85±3.27[a]

The Group I (control group) seeds were treated with tap water; Group II seeds were treated with 25 ppm Co; Group III seeds were treated with 50 ppm Co; Group IV seeds were treated with 25 ppm Al; Group V seeds were treated with 50 ppm Al. Values presented as mean±SD. Means denoted with different superscripts are within the same column are statistically significant ($p<0.05$)

control group seeds showed an increase of 423% while Co and Al treated seeds showed an increase of 239-110% and 174-61% according to initial at the end of experimental period, respectively. The results indicated that Al and Co metals depressed and decreased the WG of plant materials. Besides, the Al-treated seeds showed a lower WG than Co-treated seeds. Although the mechanism of Al and Co toxicity on the WG is not yet clearly known, it seems plausible that these metal acts as a blocking agent by interaction with the cell components. Sharma and Dubey (2005) reported that lead blocks the entry of cations and anions into plant tissues. They also determined that lead causes a decline in transpiration rate and water content of tissues. In similar studies, prevented root respiration, decreased deposition of cell wall polysaccharides, modified structure and function of plasma membranes, reduced uptake of water, and inhibited the uptake, transport and metabolism of several essential nutrients of Al compounds in plants has been reported (Pietraszewska, 2001; Rana and Aery, 2005). Besides, it has been reported reduce of plant fresh weight and inhibition the uptake of macro- and microelements in the presence of 5 mM Co (Liu *et al*., 2000). All these conditions may cause significant alterations in nutrient contents of tissues. As a result, may be the reason for reduce WG of plant and seeds.

It is known that heavy metal ions in cells cause molecular damage to plants either directly or indirectly through the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical and superoxide radicals. Harmful ROS can damage biological molecules such as lipids, which are altered by peroxidation (Meng *et al*., 2007). Measurement of MDA levels is routinely used as an indicator of lipid peroxidation under stress conditions (Wu *et al*., 2003). As a result, we found that MDA levels increased significantly with Al and Co metals in root cells. We know that free radicals are formed during metal stress, as measured by an increase in the MDA production. These radicals can interact with many cellular constituents, including DNA, proteins, enzymes and lipids, leading to radical chain processes, crosslinks, peroxidation, membrane leakage, and the production of toxic compounds (Hong *et al*., 2000; Choudhury and Panda, 2004; Unyayar *et al*., 2006; Meng *et al*., 2007; Pandey *et al*., 2007).

In our present study, the frequency of MN was also recorded. The results showed that there was a dose-related increase in the frequency of MN in heavy metals-treated seeds. The highest frequency of MN was observed at 50 ppm dose of Al, and least frequency of MN was observed at 25 ppm dose of Co. Moreover, MN frequency was clearly higher than in Al-treated seeds when compared with Co, and difference was statistically significant. The results are related to be higher in of Al when compared to Co. All these findings suggested that Al and Co had cytotoxic activity induced MN formation in seeds of barbunia. These observations are in agreement with those cytotoxicity data reported by other authors so far. In many studies, results indicated that, the test substances as heavy metals can produce chromosomal or spindle damage and mitotic apparatus damage leading to the formation of MN (Inceer *et al*., 2003). Al and Co metal ions interact with biomolecules and bind them via reactive groups such as hydroxyl, carboxyl and sulphydryl. As a result, may be alterated conformations of biomolecules (protein or nucleic acid) or the metabolic reactions may be broken (Kark, 1979). In our opinion about this matter, heavy metals may enter into the cell nucleus and bind to purine and pyrimidine bases or spindle. These interactions may denature spindles and may cause a delay in the formation of chromosome-spindle complex, and this condition may causes to *MN* formation. For example, Staykova *et al*. (2005) reported a significant increase in the MN frequency induced by the lagging of whole chromosomes or the immobility of large acentric fragments in *Allium cepa*. In a similar study, it was showed a systematically increase in MN rate and chromosome aberrations with increased concentration of CrO_3 in *V. faba* (Wei, 2004). Similarly, Rosa *et al*. (2003) determined a significant increase in MN rate at 20 μM Cd^{++} dose. Consequently, it was concluded that the *Phaseolus vulgaris* L. cv. barbunia MN assay may be used as an endpoint biomarker acceptable in biomonitoring environmental pollutives such as Al and Co.

This is the first study about a correlation between MN formation and the concentration of DNA. We extracted obtained DNA from nuclei of meristematic root tips of barbunia seeds and used agarose gel electrophoresis to measure the concentration of DNA. As a result, it was found that DNA was rather sensitive to 50 ppm doses of Al and Co heavy metals. We suggest that the length of DNA bands in Al and Co treated seeds were longer than recorded in the control. And also, the DNA yields in the control group were higher than observed in Al and Co treatment groups. The DNA yield order obtained in this study was control>Co>Al. This diversity may be associated with a loss of genetic material.

From all these results, we concluded that Al and Co metals have serious toxic effects on the root tip cells of *P. vulgaris* L. cv.

Barbunia, and the parameters such as GP, RL, WG, MN and MDA are suitable indicators for biomonitoring the effects.

References

Alvarez, E., M.L. Fernandez-Marcos, C. Monterroso and M.J. Fernandez-Sanjurjo: Application of Aluminum toxicity indices to soils under various forest species. *For. Ecol. Manage.,* **211**, 227-239 (2005).

Aybeke, M. and G. Olgun: The effect of olive oil mill effluent on the mitotic cell division and total protein amount of the root tips of *Triticum aestivum* L. *Turk. J. Biol.,* **24**, 127-140 (2004).

Burton, K.W., E. Morgan and A. Roig: The influence of heavy metals on the growth of sitka-spruce in South wales forests. II green house experiments. *Plant Soil,* **78**, 271-282 (1984).

Choudhury, S. and S.K. Panda: Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. *Bulg. J. Plant. Physiol.,* **30**, 95-110 (2004).

Dimitrova, I. and E. Ivanova: Effect of heavy metal soil pollution on some morphological and cytogenetical characteristics of flax (*Linum usitatissum* L.). *J. Balkan. Ecol.,* **4**, 212-218 (2003).

Dubey, R.C. and R.S. Dwivedi: Effect of heavy metals on seed germination and seedling growth of soybean. *Proc. Natl. Acad. Sci. India Sci. Lett.,* **10**, 121-123 (1987).

Fenech, M., W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi and E. Zeiger: Human MicronNucleus project. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat. Res.,* **534**, 65-75 (2003).

Godbold, D.L. and C. Kettner: Lead infuluences root growth and mineral nutrition of *Picea abies* seedlings. *J. Plant Physiol.,* **139**, 95-99 (1991).

Gupta, D.K., A. Srivastava and V.P. Singh: EDTA enhances lead uptake and facilitates phytoremediation by vetiver grass. *J. Environ. Biol.,* **29**, 903-906 (2008).

Hong, Z., K. Lakkineni, Z. Zhang and D.P.S. Verma: Removal of Feedback Inhibition of A^1- Pyrroline-5-Carboxylate Synthetase Results in Increased Proline Accumulation and Protection of Plants from Osmotic Stres. *Plant Physiol.,* **122**, 1129-1136 (2000).

Inceer, H., S. Ayaz, O. Beyazoglu and E. Senturk: Cytogenetic effects of copper chloride on the root tip cells of *Helianthus annuus* L. *Turk. J. Biol.,* **27**, 43-46 (2003).

Johnson, M.S. and J.W. Eaton: Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. *J. Environ. Qual.,* **9**, 175-179 (1980).

Kamaruzzaman, B.Y., M.C. Ong, K.C.A. Jalal, S. Shahbudin and O. Mohd Nor: Accumulation of lead and copper in *Rhizophora apiculata* from Setiu mangrove forest, Terengganu, Malaysia. *J. Environ. Biol.,* **30**, 821-824 (2009).

Kark, P.: Clinical and neurochemical aspects of inorganic mercury intoxication. In Handbook of Clinical Neurology. Elsevier, Amsterdam (1979).

Kochian, L.V.: Cellular Mechanisms of Aluminium Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **46**, 237-260 (1995).

Liu, J., R.J. Reid and F.A. Smith: The mechanism of cobalt toxicity in mung beans. *Physiol. Plantarum.,* **110**, 104-110 (2000).

Meng, Q., J. Zou, J. Zou, W. Jiang and D. Liu: Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*allium sativum* L.). *Acta Biol. Cracov. Bot.,* **49**, 95-101 (2007).

Munzuroglu, O.H. Geckil: Heavy metal effect on seed germination, root elongation, coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Arch. Environ. Con. Tox.,* **43**, 203-213 (2002).

Neogy, M., J. Datta, A.K. Roy and S. Mukherji: Studies on phytotoxic effect of Aluminum on growth and some morphological parameters of Vigna radiata L. Wilczek. *J. Environ. Biol.,* **23**, 411-416 (2002).

Obroucheva, N.V., E.I. Bystrova, V.B. Ivanov, O.V. Anupova and I.V. Seregin: Root growth responses to lead in young maize seedling. *Plant Soil,* **200**, 55-61 (1998).

Pandey, S., K. Gupta and A.K. Mukherjee: Impact of cadmium and lead on Catharanthus roseusa - A phytoremediation study. *J. Environ. Biol.,* **28**, 655-662 (2007).

Pietraszewska, T.M.: Effect of Aluminium on plant growth and metabolism. *Acta. Biochim. Pol.,* **48**, 673-686 (2001).

Rana, D.K. and N.C. Aery: Influence of Aluminium on growth and water relations of cowpea. *J. Environ. Biol.,* **26**, 349-353 (2005).

Rodushkin, I., T. Moiseenko and L. Kudravsjeva: Aluminium in the surface waters of the Kola Peninsula, Russia. *Sci. Total Environ.,* **163**, 55-59 (1995).

Rosa, E.V.C., C. Valgas, M.M.S. Sierra, A.X.R. Correa and C.M. Radetski: Biomass growth, micronucleus induction and antioxidant stress enzyme responses in *Vicia faba* exposed to cadmium in solution. *Environ. Toxicol. Chem.,* **22**, 645-649 (2003).

Rosseland, B.O., T.D. Eldhuset and M. Staurnes: Environmental effects of Aluminium. *Environ. Geoch. Hlth.,* **12**, 17-27 (1990).

Sahu, R.K., S. Katiyar, J. Tiwari and G.C. Kisku: Assessment of drain water receiving effluent from tanneries and its impact on soil and plants with particular emphasis on bioaccumulation of heavy metals. *J. Environ. Biol.*, **28**, 685-690 (2007).

Shafiq, M. and M.Z. Igbal: The Toxicity Effects of Heavy Metals on Germination and Seedling Growth of *Cassia siamea* Lamk. *J. New. Seed.,* **7**, 95-105 (2006).

Sharma, A.D., P.K. Gill and P. Singh: DNA Isolation From Dry And Fresh Samples of Polysaccharide-Rich Plants. *Plant. Mol. Biol. Rep.,* **20**, 415-415 (2002).

Sharma, P. and S. Dubey: Lead toxicity in plants. *Braz. J. Plant Physiol.,* **17**, 35-52 (2005).

Staykova, T.A., E.N. Ivanova and I.G. Velcheva: Cytogenetic effect of heavy metal and cyanide in contamined waters from the region of southwest Bulgaria. *J. Cell. Mol. Biol.,* **4**, 41-46 (2005).

Symeonidis, L., M.M. Abou-Audai and T. Yupsanis: Aluminium toxicity effects on *Cucumis melo* and response of diphosphonucleoside kinases. *Biologia Bratislava,* **59**, 133-139 (2004).

Unyayar, S., A. Celik, F.O. Cekic and A. Gozel: Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis,* **21**, 77-81 (2006).

Verma, S. and R.S. Dubey: Lead toxicity induces lipid peroxidation and alters the activites of antioxidant enzymes in growing rice plants. *Plant Sci.,* **164**, 645-655 (2003).

Wei, Q.X.: Mutagenic effects of chromium trioxide on root tip cells of *Vicia faba*. *J. Zhej. Uni. Sci.,* **5**, 1570-1576 (2004).

Wierzbicka, M. and J. Obidzinska: The effects of lead on seed imbibitions and germination in different plant species. *Plant Sci.,* **137**, 155-171 (1998).

Wu, F., G. Zhang and P. Dominy: Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environ. Exp. Bot.,* **50**, 67-78 (2003).

Zengin, F.K. and O. Munzuroglu: Effects of cadmium (Cd) and Mercury (Hg) on the growth of root shoot and leaf of bean (*Phaseolus vulgaris* L.) seedlings. *GU. J. Sci.,* **24**, 64-75 (2003).

Zengin, F.K. and O. Munzuroglu: Effects of Cobalt (Co^{2+}) and Zinc (Zn^{2+}) on the Growth of Root, Shoot and Leaf of Bean (*Phaseolus vulgaris* L. cv. Strike) Seedlings. *Sci. Eng. J. Firat. Uni.,* **17**, 97-106 (2005).