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Protective role of *Ginkgo biloba* on petroleum wastewater-induced toxicity in *Vicia faba* L. (Fabaceae) root tip cells

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Abstract: *The Melet is one of Turkey's economically important rivers. Most of the petroleum plants are located at fairly nearby of the river. This situation is considered as main source of heavy metal pollution in the river. The present study was designed to evaluate the protective role of Ginkgo biloba (GB) on cytotoxicity induced by petroleum wastewater in Vicia faba root tip cells. For this aim, we used the germination percentage, root length, weight gain and micronucleus (MN) frequency as indicators of cytotoxicity. Additionally to the cytological analysis, lipid peroxidation analyses were also performed in V. faba roots. Heavy metal concentrations in wastewater were measured by atomic absorption spectrophotometer (AAS). The V. faba seeds were divided into six groups. They were treated with petroleum wastewater and 10, 20 and 30 µM doses of GB. As a result, the mean concentrations of heavy metals in wastewater were observed in the order: Pb>Al>Ni>Cr>Fe>Cu>Zn>Cd. The highest germination percentage was observed in the seeds of the control and positive control groups (in proportion as 98 and 96%, respectively). Wastewater treatment caused a significant decrease in the germination percentage of Group III (in proportion as 44%). The highest root length and weight gain were observed in the seeds of the control and positive control groups at the end of the experimental period. The least root length and weight gain were observed in the seeds of Group III treated with wastewater alone. In the control group, the final weights of all the seeds increased about 4.08 g according to initial weight. The root lengths of the control seeds were measured as 6.80 cm at the end of the experimental period. The final weights of the seeds exposed to wastewater alone increased about 0.90 g according to initial weight. Besides, there was a significantly increase in the MDA levels of the roots exposed to wastewater. Heavy metals in wastewater significantly affected the MDA production indicating lipid peroxidation. But, GB-treatment caused amelioration in indices of the germination percentage, root length, weight gain, MN frequency and lipid peroxidation when compared with group III. Each dose of GB provided protection against wastewater toxicity, and its strongest protective effect observed at dose of 30 µM. In vivo results showed that GB is a potential protector against toxicity induced by petroleum wastewater, and its protective role is dose-dependent.*

Key words: *Cytotoxicity, Ginkgo biloba, Heavy metal contamination, Lipid peroxidation, Melet river, Vicia faba*
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Introduction

The river and lake systems are the most important surface water resources in Turkey. The most of the waters is commonly used for irrigation purposes of agriculture, landscape, public parks or for drinking. Nowadays, industrial, agricultural and domestic wastes are continuously discharged into the water systems. These waste materials also contain heavy metal compounds (Altundogan *et al.*, 1998; Shrivastava *et al.*, 2003; Ozdilek *et al.*, 2007).

Heavy metals have long been recognized as major pollutants for both of aquatic and terrestrial habitats. They may affect organisms directly by accumulating in their body or indirectly by transferring to the food chain (Memon *et al.*, 2001; Shah and Altindag, 2005; Obasohan *et al.*, 2006; Akinola and Ekiyoyo, 2006). Most of these metals are highly toxic and no know biological function. Hence, they tend to accumulate in soil, sediment and different tissues of plant and animals (Memon *et al.*, 2001; Sharma and Dubey, 2005). They can cause inhibition of photosynthesis in water plants, effect on phytoplankton growth in water, cause to chromosomal aberrations in terrestrial plants and induce carcinogenesis in human (Kiran and Sahin, 2005; Obasohan *et al.*, 2006). Despite regulatory measures carried out in many countries, heavy metals continue to increase in the environment (Sharma and Dubey, 2005).

The use of certain materials may help to decrease the toxicity created by chemical agent such as heavy metals (Aslanturk and Celik, 2005). Recently, bio-polymers of various biological materials such as *Phyllanthus* fruit extract, *Ocimum sanctum* L. leaf extract, grape seed, royal jelly and lycopene have been used for this aim (Pillai and Damodharan, 2007; Madhavi *et al.*, 2007; Babu and Uma Maheswari, 2006). *Ginkgo biloba* is considered the oldest living tree in the world, dating back at least 200 million years. Some ginkgo trees have been known to live well over an average of 1000 or more years. *GB* leaf extract is the most widely sold phytomedicine in Europe, where it is used to treat the symptoms of early–stage Alzheimer's disease, vascular dementia, peripheral claudication and tinnitus of vascular origin (Sierpina *et al.*, 2003).

Although there are many published clinical studies on *GB* in literature, unfortunately, the protective mechanisms of *GB* on

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heavy metal toxicity in plants are still poorly understood. The aim of the present study was to evaluate the protective role of *GB* on toxicity induced by petroleum wastewater in *V. faba* root tip cells.

Materials and Methods

Wastewater sampling procedure: The wastewater samples were collected once in February 2008. The first point discharged into the Melet River of petroleum wastewater for sampling was chosen. The wastewater samples were taken down to a depth of at least 10 cm. The water samples were stored in sterile 1000 ml amber-colored plastic bottles, transported to the laboratory on the same day and stored in the dark at 4°C until the experimental procedure. The wastewater samples were filtered through 0.45 µm Millipore filter paper. The measurement conditions were optimized for each metal (Yilmazer and Yaman, 1999). The concentrations of Pb, Al, Ni, Cr, Fe, Cu, Zn and Cd were measured by *AAS*. 20 ml of sample solution was used for the determination of heavy metals in wastewater.

Preparation of root tips and exposure to wastewater: Healthy and proximate equal-sized *V. faba* seeds were selected. The seeds were sterilized with 2.5% sodium hypochlorite solution for 10 min and washed for 24 hr in ultra-distilled water. The seeds in each treatment group were placed on filter paper in Petri dishes. 50 seeds of *V. faba* were planted in each Petri dish. The *V. faba* seeds were divided into six groups. Group I (control) was treated with tap water, Group II (positive control) was treated with 30 µM dose of *GB*, Group III was treated with petroleum wastewater alone, Group IV was treated with 10 µM dose of *GB* and wastewater, Group V was treated with 20 µM dose of *GB* and wastewater, Group VI was treated with 30 µM dose of *GB* and wastewater, for 7 consecutive days. At the end of 7th day, the root lengths of the germinated seeds were measured with a milimetric ruler. The root length was determined by radicula formation bases of *V. faba* seeds non-exposed and exposed to wastewater. Besides, the weight gain of seeds was measured using a sensitive electronic balance. For the cytological analysis, when the roots attained a length of approximately 1–2 cm, they were washed in distilled water and temporary squash preparations were made.

MN analysis: 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 ethanol (96%) and stored in ethanol (70%) in the fridge at +4°C until making the microscope slides. The root tips were hydrolyzed in 1N HCl at 60°C for 17 min, treated with 45% CH_3COOH solution for 30 min and stained for 24 hr in Acetocarmine. After staining, the root meristems were separated and squashed in 45% CH_3COOH solution (Wei, 2004; Staykova *et al*., 2005). For the MN analysis, 1000 cells were scored for each slide to calculate the frequency of MN. Micronucleated cells were evaluated under a binocular light microscope (Japan, Olympus BX51) at X500 magnification. For the scoring of MN the following criteria were adopted from Fenech *et al.* (2003): (i) the diameter of 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.

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.

Statistical analysis: For the statistical analysis, data were analyzed using the SPSS for Windows software, Version 10.0 (SPSS Inc., Chicago, USA). Statistically significant differences between groups were compared using analysis of variance (ANOVA) and Duncan test. The data are displayed as means ± standard deviation (SD), and p-values less than 0.05 are considered significant.

Results and Discussion

The Melet river is the longest river of Ordu province in Turkey. The river is exposed to petroleum wastewaters discharged from the gasoline industry. Periodical analysis of water, plankton, fish and sediment samples showed that the river is contaminated by various heavy metal ions (Atlas and Büyükgüngör, 2007).

Table 1 shows the metal concentrations in petroleum wastewater in the order: Pb>Al>Ni>Cr>Fe>Cu>Zn>Cd. The average concentrations measured in the wastewater samples were about 66.45±3.87 for Pb, 37.21±3.35 for Al, 27.17±3.35 for Ni, 22.98±2.25 for Cr, 18.09±1.91 for Fe, 14.50±1.28 for

Table - 1: Heavy metal concentrations (mg l^{-1}) in petroleum wastewater collected from the sampling site

Heavy metal	Minimum	Maximum	Average
Pb	60.23	70.43	66.45±3.87
Al	30.56	40.43	37.21±3.35
Ni	20.86	30.42	27.17±3.35
Cr	20.44	25.34	22.98±2.25
Fe	15.47	20.44	18.09±1.91
Cu	11.56	15.96	14.50±1.28
Zn	6.14	9.14	7.25±0.91
Cd	0.78	4.12	2.41±1.13

All values the mean ± SD (20 ml of sample solution was used for the determination of heavy metals in wastewater)

Table - 2: The effect of petroleum wastewater on germination (%) of *V. faba* seeds at the end of 7^{th} day

Groups	Number of seeds	Number of germinated seeds	Number of not germinated seeds	Germination percentage	Percent germination increase
I	50	49	1	98	54
II	50	48	2	96	52
III	50	22	28	44	–
IV	50	28	22	56	12
V	50	34	16	68	24
VI	50	39	11	78	34

Group I: control, Group II: positive control, Group III: wastewater, Group IV: 10 µM *GB* + wastewater, Group V: 20 µM *GB* + wastewater, Group VI: 30 µM *GB* + wastewater

Table - 3: The effect of petroleum wastewater on root length (cm)

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
Root length	6.80 ± 0.63^{a}	6.83 ± 0.61^{a}	2.22 ± 0.18^{e}	3.14 ± 0.16^{d}	3.90 ± 0.11^{c}	4.84 ± 0.14^{b}

All values the mean ± SD (n: 50). Group I: control, Group II: positive control, Group III: wastewater, Group IV: 10 µM *GB* + wastewater, Group V: 20 µM *GB* + wastewater, Group VI: 30 µM *GB* + wastewater. Statistical significance between means was performed using One-Way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test ($p<0.05$). Means with the same letter within the same parameter are not significantly different

Table - 4: Mean weight gain (g) of *V. faba* seeds at the end of 7^{th} day

Groups	Initial weight of seeds	Final weight of seeds	Difference
I	1.82±0.14	5.90 ± 0.13^{a}	4.08
II	1.83±0.15	5.92 ± 0.16^{a}	4.09
III	1.84±0.13	2.74 ± 0.26^{e}	0.90
IV	1.83±0.12	3.14 ± 0.16^{d}	1.31
V	1.84±0.11	3.88 ± 0.15^{c}	2.04
VI	1.85±0.13	4.50 ± 0.20^{b}	2.65

All values the mean ± SD (n: 50). Group I: control, Group II: positive control, Group III: wastewater, Group IV: 10 µM *GB* + wastewater, Group V: 20 µM *GB* + wastewater, Group VI: 30 µM *GB* + wastewater. Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test ($p<0.05$). Means with the same letter within the same parameter are not significantly different

Cu, 7.25±0.91 for Zn and 2.41±1.13 for Cd, mg l^{-1}. The average value of Pb was higher than values of the other heavy metals.

The high concentrations of these heavy metals can inhibit the seed germination, root length and seed gain. As shown in Table 2, the germination percentages of the control and positive control groups were fairly similar. But, the germination percentages of the seeds treated with wastewater were rather different than control groups. The highest germination percentage was observed in the seeds of the control and positive control groups (in proportion as 98 and 96%, respectively). Heavy metal-polluted wastewater caused a significant decrease in the germination percentage of *V. faba*. But, supplementation of three different doses of *GB* extract prevented decrease in the germination percentage compared to Group III. 10, 20 and 30 µM doses of *GB* caused 12, 24 and 34% increase in the seed germination according to Group III, respectively. Even so, these increases were still lower than the control values. In *GB*-supplemented group, the largest effect of supplementation particularly at 30 µM dose of *GB* was seen. The results showed that the germination percentage can be considered as most sensitive indicator of heavy metal toxicity. This information is parallel with the other toxicity data available so far. The research results clearly indicate that heavy metal ions can lead to decrease of the germination percentage in seeds of different plant species (Munzuroglu and Geckil, 2002; Kiran and Sahin, 2005; Wierzbicka and Obidzinska, 1998; Aybeke and Olgun, 2004; Neelima and Reddy, 2003).

The visual non-specific symptoms of heavy metal toxicity on plant are inhibition of the root growth and seed weight (Burton *et al.*, 1984). In the present study, we observed changes in the weight gain and root growth in the seeds exposed to wastewater. The results related to root length is given in Table 3 and the weight gain of seeds (*V. faba*) is given in Table 4. The data showed that wastewater treatment significantly reduced the root length and weight gain of seeds. The highest root length and weight gain were observed in the seeds of the control and positive controls at the end of the experimental period. The least root length and weight gain were also observed in the seeds of Group III treated with wastewater alone. In the control group, the final weights of all the seeds increased about 4.08 g according to initial weight. The root lengths of the control seeds were measured as 6.80±0.63 cm at the end of the experimental period. The final weights of the seeds exposed to wastewater alone increased about 0.90 g according to initial weight. But, *GB* supplementation caused again increase in the root length and weight gain according to Group III, and differences were statistically significant ($p<0.05$). Besides, there was a positive correlation between *GB* doses and the increase in the root length and weight gain. Besides, these increases were statistically significant ($p<0.05$). The inhibitory effect of heavy metals on the root growth

Table - 5: The effect of petroleum wastewater on micronucleus (MN) frequency at the end of 7th day

Groups	Number of scored cell	Minimum	Maximum	Average ± SD
I	1000	0	0	00.00±0.00[e]
II	1000	0	0	00.00±0.00[e]
III	1000	55	66	60.87±3.52[a]
IV	1000	43	53	49.33±2.88[b]
V	1000	33	40	37.37±2.53[c]
VI	1000	25	32	28.87±2.37[d]

All values the mean ± SD. Group I: control, Group II: positive control, Group III: wastewater, Group IV: 10 µM *GB* + wastewater, Group V: 20 µM *GB* + wastewater, Group VI: 30 µM *GB* + wastewater. Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test (p<0.05). Means with the same letter within the same parameter are not significantly different

Table - 6: The effect of petrol wastewater on lipid peroxidation MDA content

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
MDA	14.65±2.30[e]	14.45±2.09[e]	33.40±4.01[a]	27.10±2.65[b]	24.10±3.28[c]	19.40±3.33[d]

All values the mean ± SD (5 g root tip sample from each treatment group were used for MDA content). Group I: control, Group II: positive control, Group III: wastewater, Group IV: 10 µM *GB* + wastewater, Group V: 20 µM *GB* + wastewater, Group VI: 30 µM *GB* + wastewater. Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test (p<0.05). Means with the same letter within the same parameter are not significantly different

was widely reported by bio-monitoring studies. These studies showed that toxic concentrations of Pb, Hg, Zn, Cd and Cu may lead to inhibition of vegetative organ growth rates in plants (Dimitrova and Ivanova, 2003). Shafiq and Igbal (2006) determined decrease of the root length at all concentrations (25-100 ppm) of Pb and Cd in *Cassia siamea*. Malekzadeh *et al.* (2007) investigated the inhibitory effect of Cd^{+2} on root growth in maize plant. As a result, they indicated inhibition of the root growth in maize seedling treated with Cd^{+2}. Besides, Godbold and Kettner (1991) observed a significant decrease in primary, secondary and tertiary root growth of *Picea abies* seedlings treated with different Pb solutions. Although the ultimate mechanism of metal toxicity on the weight gain is completely unknown, it seems plausible that these metals act as a blocking agent by interaction with the cell components. Sharma and Dubey (2005) reported that heavy metal ions may block the entry of cations and anions, cause a decline in transpiration rate and water content resulting into significant alterations in nutrient status and nutrient contents of tissues, thereby reducing seed weight.

The MN formation in *V. faba* root tip meristem cells was not observed in both the control and positive control. But, a significant increase in the MN formation was observed in all the seeds exposed to petroleum wastewater (Fig. 1) and the frequency is indicated in Table 5. The maximum frequency of MN was observed in the seeds treated with wastewater alone and minimum frequency of MN was observed in the seeds treated with different doses of *GB*. The MN frequency showed a decrease with rising in *GB* dose. There was a strong dose-effect relationship between the MN frequency and *GB* doses. It also appeared that 30 µM dose of *GB* had greater effect than those observed in 10 and 20 µM doses on MN frequency. There was statistically significant difference between MN frequencies of the control and treatment groups (p<0.05). There was also statistically significant difference among 10–30 µM doses of *GB* (p<0.05). These findings suggested that heavy metals in petroleum wastewater had cytotoxic activity induced MN formation in the root tips of *V. faba*. These observations are also in agreement with cytotoxicity data reported by other authors so far. In most of the studies, results indicated that the test substances as heavy metals ions can produce chromosomal or spindle damage and mitotic apparatus damage leading to formation of MN (Inceer *et al.*, 2003). Especially, the inhibition of spindle formation has been shown to lead to severe abnormalities such as stickiness, unequal distribution, multipolar anaphase, chromosal bridges and laggards. Heavy metal ions interact with biomolecules and bind them via reactive groups such as hydroxyl and sulphydryl. As a result, they cause breaks and conformation changes in 3D structure of biomolecules (protein, nucleic acid) or alteration in metabolic pathways (Kark, 1979). In our opinion about this matter, heavy metals may enter into cell nucleus and may bind to purine and pyrimidine bases or proteins such as 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. This knowledge is also in agreement with results reported by Staykova *et al.* (2005). They reported high 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 the MN frequency and chromosome aberrations with increased concentration of CrO_3 in *V. faba* (Wei, 2004). In another study, Rosa *et al.* (2003) observed a significant increase in the MN frequency at 20 µM dose of Cd^{++}.

It is known that heavy metal stress causes molecular damage to plant cells either directly or indirectly through the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl and superoxide radicals (Zhang *et al.*, 2007). Harmful

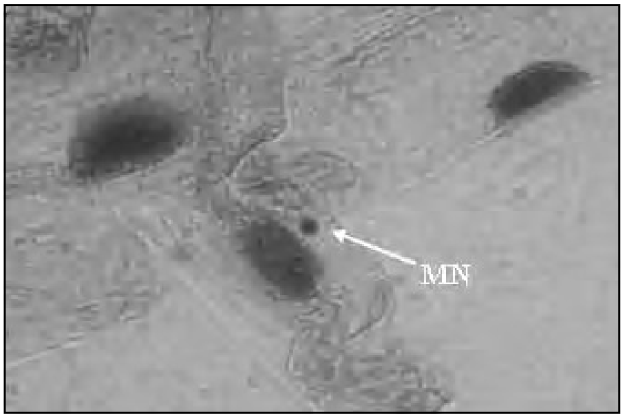

Fig. 1: The appearance (arrow) of MN in *V. faba* acetocarmine staining, X 500

ROS can damage biological molecules such as lipids which are altered by peroxidation. Measurement of MDA levels is routinely used as an indicator of lipid peroxidation under stress conditions (Meng *et al*., 2007). Lipid peroxidation as MDA data are given in Table 6. The results showed that there was a significantly increase in the MDA levels of the roots exposed to wastewater. Heavy metal ions in wastewater significantly affected the MDA production indicating lipid peroxidation. There was a statistically significant difference between control and treatment groups (Table 6, $p<0.05$) for MDA content. *GB* treatment decreased the toxic effects of heavy metal ions manifested by lower lipid peroxidation, lesser production of hydrogen peroxide and reduction in the generation of superoxide radicals. In decreased MDA content was observed in roots at all three dose of *GB*. In *GB*-treated roots, the level of MDA reduced about 19, 28 and 42% according to Group III at 30 μM dose of *GB*, respectively. These observations are also in agreement with results reported by Hong *et al.* (2000), Choudhury and Panda (2004), Unyayar *et al.* (2006), Meng *et al.* (2007) and Pandey *et al.* (2007) on the generation of lipid peroxidation products under different heavy metal stresses. These researchers reported a significantly increase in the MDA content in the roots treated with different doses of heavy metals.

Consequently, *GB* had a protective effect on the cytotoxicity and lipid peroxidation induced by petroleum wastewater in seeds of *V. faba*, and its protective effect was dose-dependent. The protective role of *GB* on wastewater-induced toxicity may be explainable with the antioxidant properties of *GB*. Antioxidants are significant molecules that act as free radical scavengers and they trap the free radicals and give up own electrons. Thus, antioxidants with stated functions, are protect molecules as protein, lipid, enzyme, chromosome and DNA against free radical damage (Feri, 1994; Halliwell *et al.*, 1995) Although it is not a general rule, antioxidants and *GB* appear to share similar mechanisms of protection against the toxicity. Researchers have shown that *GB* contains antioxidant properties (Mix and Crews, 2002). *GB* is also known to be efficient in helping to treat or prevent diseases associated with free radicals. Pharmacologically, there are two groups of substances which are significant compounds found in *GB*. These are the flavonoids as myricetin and quercitin which give ginkgo its antioxidant action and the terpenes which help to inhibit the formation of blood clots (Oberpichler *et al.*, 1990). These compounds have the capability of decreasing cell damage which results from the presence of free radicals. Besides, these compounds scavenge and destroy free radicals and reactive forms of oxygen, such as $O_2^{\cdot-}$, $^{\cdot}OH$ and lipid peroxide radicals (Oberpicher *et al.*, 1990; Sierpina *et al.*, 2003). No study so far has been undertaken to examine the protective role of *GB* in plant tissues. But, most human laboratory tests and animal studies were performed on antiradical or antioxidant properties of *GB*. Sener *et al.* (2006) investigated the possible beneficial effect of *GB* against the analgesic acetaminophen (AAP) toxicity in mice. Ergun *et al.* (2005) investigated the anti-apoptotic effect of *GB* in gossypol-treated human lymphocytes. Harputluoglu and Demirel (2006) investigated the protective effects of *GB* on thioacetamide (TAA) induced fulminant hepatic failure in rats.

The results of the present study indicated that there was a serious pollution problem caused by petroleum wastewater in the Melet river. This pollution caused significant toxic effects in the root tip cells of *V. faba*. Nevertheless, our observations indicated that *GB* enhanced the antioxidant status and decreased the incidence of heavy metals-induced toxicity. In conclusion, *GB* supplementation may decrease toxic damages induced by petroleum wastewater. Therefore, its protective role may be used as "a toxicity-limiting agent" in the future.

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References

Atlas, L. and H. Büyükgüngör: Heavy metal pollution in the Black Sea shore and offshore of Turkey. *Environ Geol.,* **52**, 469-476 (2007).

Akinola, M.O and T.A. Ekiyoyo: Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odo-nla area of Ikorodu, Lagos state, Nigeria. *J. Environ. Biol.,* **27**, 597-599 (2006).

Altundogan, H.S., M. Erdem, R. Orhan, A. Ozer and F. Tumen: Heavy metal pollution potential of zinc leach residues discarded in Çinkur plant. *Turkish J. Eng. Environ. Sci.,* **22**, 167-177 (1998).

Aslanturk, O.S. and T.A. Celik: Preventive effect of lycopene on chromosome aberrations in *Allium cepa*. *Pakistan. J. Biol. Sci.,* **8**, 482-486 (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).

Babu, K and K.C. Uma Maheswari: *In vivo* studies on the effect of *Ocimum sanctum* L. leaf extract in modifying the genotoxicity induced by chromium and mercury in *Allium* root meristems. *J. Environ. Biol.,* **27**, 93-95 (2006).

Burton, K.W., E. Morgan and A. Roig: The influence of heavy metals on the growth of sitka-spruce in South Wales forests. *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).

Ergun, U., E. Yurtcu and M.A. Ergun: Protective effect of *Ginkgo biloba* against gossypol-induced apoptosis in human lymphocytes. *Cell. Biol. Int.,* **29**, 717-720, 2005.

Fenech, M., W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi and E. Zeiger: Human micronucleus 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).

Feri, B.: Natural Antioxidants in Human Health and Disease. Academic Press, San Diego (1994).

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

Halliwell, B., M.A. Murcia, S. Chirico and O.L. Aruoma: Free radicals and antioxidants in food and *in vivo*: What they do and how they work. *Crit. Rev. Food Sci. Nutr.,* **35**, 7-20 (1995).

Harputluoglu, M.M.M. and U. Demirel: Protective effects of *Gingko biloba* on thioacetamide-induced fulminant hepatic failure in rats. *Human. Exp. Toxicol.,* **25**, 705-713 (2006).

Hong, Z., K. Lakkineni, Z. Zhang and D.P.S. Verma: Removal of feedback inhibition of Δ^1-Pyrroline-5-Carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *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).

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

Kiran, Y. and A. Sahin: The effects of the lead on the seed germination, root growth and root tip cell mitotic divisions of *lens culinaris* MEDIK. *GU. J. Sci.,* **18**, 17-25 (2005).

Madhavi, D., K.R. Devil, K.K. Rao and P.P. Reddy: Modulating effect of Phyllanthus fruit extract against lead genotoxicity in germ cells of mice. *J. Environ. Biol.,* **28**, 115-117 (2007).

Malekzadeh, P., J. Khara, S. Farshian, A.K. Jamal-Abad and S. Rahmatzadeh: Cadmium toxicity in maize seedlings: Changes in antioxidant enzyme activities and root growth. *Pakistan. J. Biol. Sci.,* **10**, 127-131 (2007).

Memon, A.R, D. Aktopraligul, A. Ozdemur and A. Vertii: Heavy metal accumulation and detoxification mechanisms in plants. *Turk. J. Bot.,* **25**, 111-121 (2001).

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).

Mix, J.A. and W.D. Crews: A double-blind, placebo-controlled, randomized trial of *Ginkgo biloba* extract EGb 761 in a sample of cognitively intact older adults: Neuropsychological findings. *Hum. Psychopharmacol.,* **17**, 267-277 (2002).

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

Neelima, P. and K.J. Reddy: Differential effect of cadmium and mercury on growth and metabolism of *Solanum melongena* L. seedlings. *J. Environ. Biol.,* **24**, 453-460 (2003).

Obasohan, E.E., J.A.O. Oronsaye and E.E. Obano: Heavy metal concentrations in *Malapterurus electricus* and *Chrysichthys nigrodigitatus* from Ogba river in Benin City, Nigeria. *Afr. J. Biotech.,* **5**, 974-982 (2006).

Oberpichler, H., D. Sauer, C. Rossberg, H.D. Mennel and J. Krieglstein: PAF antagonist ginkgolide B reduces postischemic neuronal damage in rat brain hippocampus. *J. Cereb. Blood. Flow. Metab.,* **10**, 133-135 (1990).

Ozdilek, H.G., P.P. Mathisen and D. Pellegrino: Distribution of heavy metals in vegetation surrounding the Blackstone river, USA: considerations regarding sediment contamination and long term metals transport in freshwater riverine ecosystems. *J. Environ. Biol.,* **28**, 493-502 (2007).

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

Pillai, T.G. and D.P. Damodharan: Prevention of radiation-induced chromosome damage in mouse bone marrow by aqueous leaf extract of *Chicorium intybus*. *J. Cell. Mol. Biol.,* **6**, 59-64 (2007).

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).

Sener, G., G.Z. Omurtag, O. Sehirli, A. Tozan, M. Yuksel, F. Ercan and N. Gedik: Protective effects of *Ginkgo biloba* against acetaminophen-induced toxicity in mice. *Mol. Cell. Biochem.,* **283**, 39-45 (2006).

Sierpina, V.S., B. Wollschlaeger and M. Blumenthal: Ginkgo Biloba. *Am. Fam. Phys.,* **68**, 923-926 (2003).

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).

Shah, S.L. and A. Altindag: Effects of heavy metal accumulation on the 96 hr LC_{50} values in *Tench Tinca tinca* L. *Turk. J. Vet. Anim. Sci.,* **29**, 139-144 (2005).

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

Shrivastava, P., A. Saxena and A. Swarup: Heavy metal pollution in a sewage-fed lake of Bhopal, (M. P.) *India. Lakes Reservoirs Res. Manage.,* **8**, 1-4 (2003).

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).

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).

Wei, Q.X.: Mutagenic effects of chromium trioxide on root tip cells of *Vicia faba*. *J. Zhejiang. 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).

Yilmazer, D. and S. Yaman: Heavy metal pollution and chemical profile of Ceyhan river (Adana-Turkey). *Turk. J. Eng. Environ. Sci.,* **23**, 59-61 (1999).

Zhang, F.Q., Y.S. Wang, Z.P. Lou and J.D. Dong: Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere,* **67**, 44-50 (2007).