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# The investigation of cytotoxic effects of refinery wastewater on root tip cells of *Vicia faba* L.

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***Abstract:*** *The present study was carried out to evaluate the cytotoxic effects of refinery wastewater collected from different stations of the Kizilirmak river on Vicia faba L. 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, DNA analyses were performed in root tips meristems of Vicia faba seeds treated with refinery wastewater. Heavy metal concentrations in the water samples were determined using atomic absorption spectrophotometer (AAS). The concentrations of heavy metals in the water were in the order of Pb>Zn>Fe>Cu>Ni>Cd>Hg. The highest germination percantage was observed in the control group (in proportion as 96%). Heavy metals in the water samples collected from Station I, II and III caused a decrease in the germination percentage as 48, 18 and 30%, respectively. The highest root length and weight gain was observed in the control group at the end of the experimental period. The least root length and weight gain was observed in seeds treated with wastewater collected from Station I. In the control group, the weights of all the seeds increased about 4.08 g when compared with initial weight. The root lengths of the control seeds were determined as 6.38 cm at the end of the experimental period. The weights of the seeds exposed to wastewaters obtained from Station I, II and III increased about 1.08, 3.03 and 2.01 g according to initial weight, respectively. Microscopic examination of V. faba root tip meristem cells showed that any example of the MN formation was not seen in the control group. The highest frequency of MN was observed in group treated with wastewater collected from Station I and least frequency of MN was observed in group treated with wastewater collected from Station II. It was also observed that the yields of DNA in the seeds exposed to wastewater were lower than recorded in the controls. Hence, DNA yields exposed to wastewater were run ahead on agarose gel according to the control group. The results clearly indicate that refinery wastewater had important cytotoxic effects on V. faba root tip cells. It was also observed that V. faba seeds are very sensitive and useful biomarkers for monitoring these effects in waters contaminated with heavy metals.*

***Key words:*** *Cytotoxicity, Heavy metal contamination, Kizilirmak river, Micronucleus assay, Refinery wastewater, Vicia faba L.*
*PDF of full length paper is available online*

## Introduction

Most of the water systems (*e.g.* river and lake) are commonly used for purposes, such as irrigation of agriculture, landscape, public parks and as drinking water. Industrial, agricultural and domestic wastes are discharged directly into the water systems. These wastes are particularly rich in heavy metals (Shrivastava *et al*., 2003; Altundogan *et al*., 1998; Nath *et al*., 2009).

Heavy metals have long been recognised as one of the major sources of pollution in the aquatic and terrestrial environment (Arun-Kumar and Achyuthan, 2007). Heavy metals are natural components of the Earth's crust and cannot be degraded or destroyed. Heavy metals may affect organisms directly by accumulating in their bodies or indirectly by transferring to the food chain (Shah and Altindag, 2005; Obasohan *et al*., 2006; Memon *et al*., 2001; Agoramoorthy *et al*., 2009). They tend to accumulate in soils, sediments and certain tissues of plants and animals (Memen *et al*., 2001; Sharma and Dubey, 2005; Nas, 1978). Despite regulatory measures carried out in many countries, these substances continue to rise in environment (Sharma and Dubey, 2005).

Wastewaters contain heavy metals as Pb, Zn, Hg, Cu, and Ni which are produced by many manufacturing processes and find their way into the environment (Ozdilek *et al*., 2007). These metals can be harmful to human and aquatic life even at very low concentrations. They inhibit photosynthesis in water plants, prevent phytoplankton growth in water, cause to chromosomal and tissue damage in terrestrial plants and induce carcinogenesis in human (Kiran and Sahin, 2005; Singh and Singh, 2006).

There are many methods available for determining the effects of water contamination. Recently, there has been an increase in use of biological materials to research the effects of water contamination (Metcalfe-Smith, 1994; Ahmad *et al*., 2003). The aim of the present study was to evaluate the cytotoxic effects of the heavy metals released by welding refinery in the water and their effect on *V. faba* seeds. The water samples were collected from different regions of the Kizilirmak river (Stations I,II and III).

## Materials and Methods

**Sample collection:** The wastewater samples for heavy metal analyses were collected once in October 2007 and three sampling sites were defined in different locations of the Kizilirmak river for sampling (Fig. 1). The sampling sites were coded as Station I

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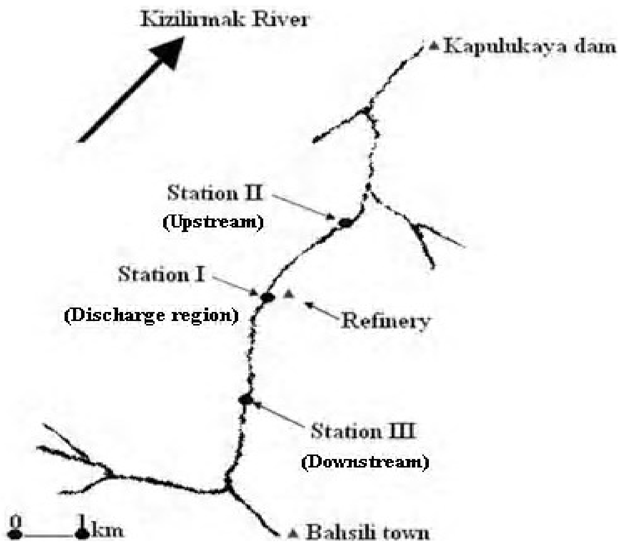


**Fig. 1:** Sampling stations in the Kizilirmak river

(region discharged of refinery wastewater), Station II (upstream or above 1000 m of discharge region) and Station III (downstream or below 1000 m of discharge region). Three bottles of wastewater from each station were collected for heavy metal analysis. The wastewater samples were taken using a gouge from the depth 10 cm. The samples were stored in polyethylene plastic bottles, transported to the laboratory on the same day and stored in the dark at 4°C until making the experimental procedure. The wastewater samples were filtered through a 0.45 µm Millipore filter paper, and the concentrations of Pb, Ni, Fe, Cu, Zn, Cd and Hg were measured by AAS using the procedures of Yilmazer and Yaman (1999) and Agtas *et al*. (2007).

**Root tip preparations:** Healthy and proximate equal-sized *V. faba* (beans) seeds were selected. The seeds were sterilized with 2.5% sodium hypochlorite solution for 10 min and washed for 24 hr in ultradistilled water. The seeds in each treatment Groups (I, II and III) were placed on filter paper in Petri dishes. 50 seeds were planted in each Petri dish and treated with wastewaters collected from Station I, II and III, for 7 consecutive days at 23°C in incubator. Petri dishes were controlled and treated with 2 ml of wastewater once daily (a 24 hr period) during 7 days. The control group seeds were treated in the similiar manner with tap water.

**Determination of root length, weight gain and germination percentage:** The root lengths of germinated seeds were measured by a milimetric ruler. The root length was determined by radicula formation bases of *V. faba* seeds non-exposed and exposed to wastewater. The weight gain was determined by measuring the differences between the seed weights before and after wastewater-treatment, using a sensitive balance. The germination percentage of seeds exposed to wastewater was calculated as the following equation.

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

For the cytological analysis, when the roots attained a length of approximately 1–2 cm, they were treated with distilled water, and temporary squash preparations were made.

**MN assay:** The root tips were fixed for 6 hr in Clarke's fixator (3:1 ratio of glaciale acetic acid and distilled water respectively), washed for 15 min in 96% ethanol and stored in 70% ethanol at 4°C until making the microscopic slides. The roots were hydrolyzed in 1N HCl at temperature of 60°C for 17 min, treated with 45% $CH_3COOH$ for 30 min and stained for 24 hr in Acetocarmine. After staining, the root meristems were separated and squashed in 45% $CH_3COOH$ solution (Staykova *et al*., 2005; Wei, 2004). For MN analysis, 1000 cells were scored in each slide to calculate the MN frequency. Micronucleated cells were evaluated under a binocular light microscope (Japan, Olympus BX51) at X 500 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.

**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, 5 M NaCl, 1M β-merkaptoetanol, distilled $H_2O$ and incubated at 65°C for 30 min. After centrifugation at 6800 rpm at room temperature for 15 min., the supernatant carefully transferred into a fresh polypropylene tube and 5 M potassium acetate was added. The solution was incubated in ice bath then centrifugated again 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:** The statistical *analysis* was carried out *using SPSS for Windows version* 10.0 statistical *software* (*SPSS Inc, Chicago*, USA). Statistically significant differences between the groups were compared using one-way analysis of variance (ANOVA) and Duncan's test. The data are displayed as means ± SD and p values less than 0.05 are considered "statistically significant".

## Results and Discussion

**Heavy metal content:** Heavy metal ions in the wastewater samples taken from different stations in the Kizilirmak River were analyzed by using AAS, and the mean concentrations of heavy metals were given in Table 1. The results showed that the highest concentrations of Pb, Zn, Fe, Cu, Ni, Cd and Hg were measured in Station I and the lowest metal pollution was measured at Station II. Besides, the concentrations of heavy metals in wastewaters were in the order of Pb>Zn>Fe>Cu>Ni>Cd>Hg.

The results obtained from the present study showed that, concentrations of heavy metals such as Pb, Zn, Fe, Cu and Ni in

**Table - 1:** Heavy metal concentrations (mg $l^{-1}$) in the water samples collected from the Kizilirmak river

| Station/Element | Average ± SD |
|---|---|
| **Station I** | |
| Pb (56.43-60.05) | 58.42±1.28 |
| Zn (23.98-26.43) | 24.98±0.74 |
| Fe (18.86-19.48) | 19.12±0.22 |
| Cu (15.53-16.68) | 16.14±0.41 |
| Ni (10.76-11.58) | 11.13±0.28 |
| Cd (7.98- 8.92) | 8.50±0.32 |
| Hg (4.23-5.13) | 4.65±0.28 |
| **Station II** | |
| Pb (15.61-17.67) | 16.49±0.80 |
| Zn (7.97- 9.24) | 8.70±0.38 |
| Fe (5.96-6.65) | 6.30±0.25 |
| Cu (3.91-4.55) | 4.20±0.20 |
| Ni (1.89-3.06) | 2.37±0.43 |
| Cd (0.76-1.96) | 1.20±0.43 |
| Hg (0.05-0.53) | 0.18±0.18 |
| **Station III** | |
| Pb (30.56-35.42) | 33.13±1.62 |
| Zn (15.78-17.76) | 16.43±0.59 |
| Fe (11.86-12.56) | 12.18±0.27 |
| Cu (9.43-10.32) | 9.88±0.27 |
| Ni (5.68-6.64) | 6.27±0.34 |
| Cd (3.95-5.76) | 4.68±0.76 |
| Hg (1.65-2.31) | 2.03±0.19 |

Values in paranthesis are the minimum and maximum, mean ± SD

**Table - 2:** The effects of wastewaters on germination percentage of *V. faba* seeds

| Groups | Number of seeds | Number of germinated seeds | Number of not germinated seeds | Germination percentage (%) |
|---|---|---|---|---|
| Control* | 50 | 48 | 2 | 96 |
| Treatment I | 50 | 26 | 24 | 52 |
| Treatment II | 50 | 41 | 9 | 82 |
| Treatment III | 50 | 35 | 15 | 70 |

* = The control group seeds were treated with tap water; Treatment I group seeds were treated with wastewater collected from Station I; Treatment II group seeds were treated with wastewater collected from Station II, Treatment III group seeds were treated with wastewater collected from Station III

the Kizilirmak River exceeded the reference values determined by TSE–266 (Turkish Standard, 1997) guidelines. The mean metal concentrations in wastewater samples of Station I were about 58.42 for Pb, 24.98 for Zn, 19.12 for Fe, 16.14 for Cu, 11.13 for Ni, 8.5 for Cd and 4.65 for Hg, mg $l^{-1}$. From these results it was observed that heavy metal concentrations measured in Stations I were higher when compared with the results obtained from Station II and III. The levels of Pb, Zn, Fe, Cu, Ni, Cd and Hg fairly decreased with increasing distance from Station I (refinery region).

**Effects on germination:** As shown in Table 2, the germination percentages of the seeds treated with wastewater were rather different from the control group. The highest germination percantage was observed in the control group (in proportion as 96%). The germination percentage significant decreased in the wastewater-treatment groups. Heavy metals in the water samples collected from Station I, II and III caused a decrease in the germination percentage as 48, 18 and 30%, respectively.

**Effects on root length and weight gain:** At the end of the experimental period, the root lengths of the germinated seeds were measured by a milimetric ruler. The root lengths were determined by radicula formation bases of *V. faba* seeds in the control and treatment groups. Besides, the weight gain of seeds was measured with a sensitive balance. The results related with the weight gain and root lenght were given in Table 3. These data showed that wastewater treatments significantly prevented the root growth and weight gain of seeds. A correlation was determined among heavy metal concentrations with the root length and weight gain. The highest root length and weight gain was observed in the control group at the end of the experimental period. The least root length and weight gain was observed in the seeds treated with wastewater collected from Station I. In the control group, the weights of all the seeds increased about 4.08 g when compared with initial weight. The root lengths of the control seeds were determined as 6.38±0.48 cm at the end of the experimental period. The weights of the seeds exposed to wastewaters collected from Station I, II and III increased about 1.08, 3.03 and 2.01 g according to initial weight, respectively. Namely, the control group seeds showed an increase in the weight gain about 332%, while the seeds treated with wastewater collected from Station I, II and III showed an increase of 161, 271 and 214% according to initial at the end of the experimental period, respectively.

**Effects on MN frequency:** Microscopic examination of *V. faba* root tip meristem cells showed that any example of the MN formation was not seen in the control group. But, the MN formation was observed in all the seeds exposed to wastewater taken from different points of the river (Fig. 2). In all tested groups, the MN frequency was increased with increasing the heavy metal levels. The MN frequency was indicated in Table 4. The highest frequency of MN was observed in group treated with the water samples of station I and least frequency of MN was observed in group treated with the water samples of Station II. There was a statistically significant difference between the MN frequency of the control and treatment groups ($p<0.05$). To determine the effect of the MN formation on DNA concentration, DNA isolation protocol was applied to root tips of all the seeds. In Fig. 3, it was observed that the yield of DNA in the seeds treated with wastewater were lower than recorded in the controls.

Data obtained in this study were compared and evaluated statistically (Table 5). It was observed that the differences defined in the germination percentage, root lenght, weight gain and MN frequency when compared with the control group were statistically significant ($p<0.05$).

The Kizilirmak is an economically important river of Turkey. One of the biggest petrol plants of Turkey is located fairly nearby. Therefore, it is the main source of heavy metal pollution in the river.

**Table - 3:** Mean weight gain and root length of *V.faba* seeds at the end of 7th day

| Groups | Number of seeds | Initial weight of seeds (g) | Final weight of seeds (g) | Difference (g) | Root length (cm) |
|---|---|---|---|---|---|
| Control* | 50 | 1.76 ±0.14 | 5.84 ±0.12 | 4.08 | 6.38±0.48 |
| Treatment I | 50 | 1.76 ±0.13 | 2.84 ±0.19 | 1.08 | 2.30±0.16 |
| Treatment II | 50 | 1.77 ±0.12 | 4.80 ±0.14 | 3.03 | 5.08±0.32 |
| Treatment III | 50 | 1.76 ±0.14 | 3.77 ±0.14 | 2.01 | 3.64±0.31 |

* = The control group seeds were treated with tap water; Treatment I group seeds were treated with wastewater collected from Station I; Treatment II group seeds were treated with wastewater collected from Station II, Treatment III group seeds were treated with wastewater collected from Station III

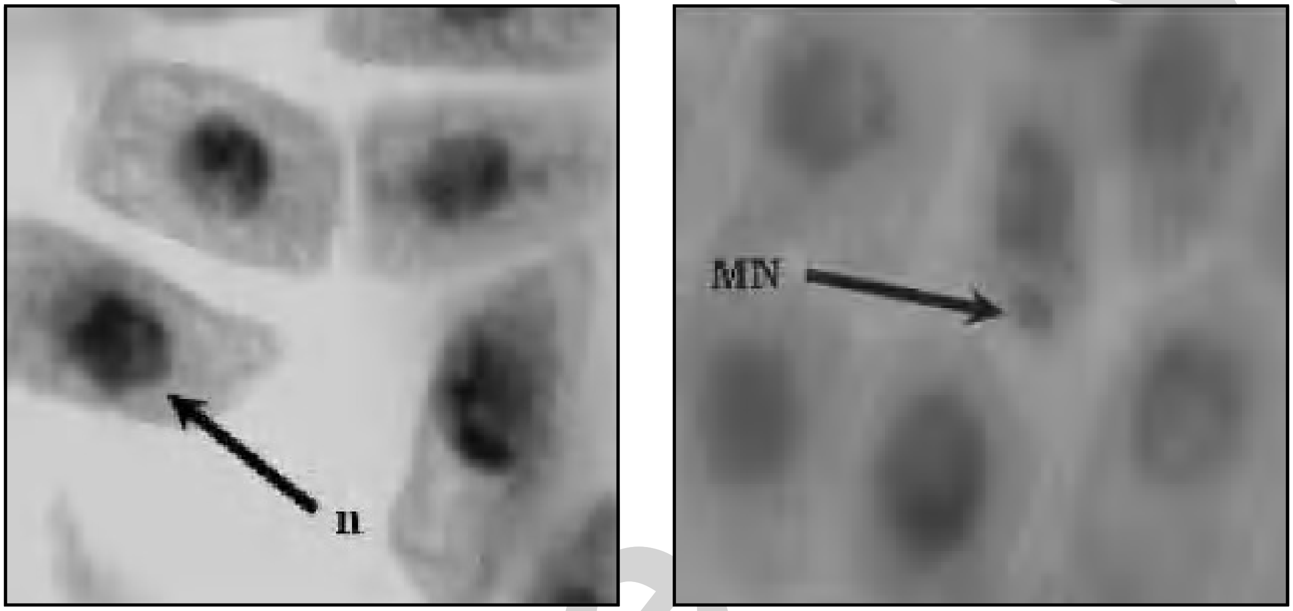


**Fig. 2:** The appearance of nucleus (a) and micronucleus (b) in *V. faba* seeds treated with wastewater sample (magnification, X 500)

**Table - 4:** The effects of wastewaters on *MN* frequency in root tips

| Groups | Number of scored cell | Minimum | Maximum | Average ±SD |
|---|---|---|---|---|
| Control* | 1000 | 0 | 0 | 00.00±0.00 |
| Treatment I | 1000 | 35 | 46 | 41.83±3.27 |
| Treatment II | 1000 | 10 | 15 | 12.33±1.63 |
| Treatment III | 1000 | 25 | 30 | 26.97±1.83 |

* = The Control group seeds were treated with tap water; Treatment I group seeds were treated with wastewater collected from Station I; Treatment II group seeds were treated with wastewater collected from Station II, Treatment III group seeds were treated with wastewater collected from Station III

The Kizilirmak water has been used as irrigation water in agriculture, so high amounts of heavy metals have been accumulated in the soils of this region. Eventually, the high levels of these metals may increase health risks for human and animals.

In the present study, we found a positive correlation between heavy metal concentrations and the germination percentage of *V. faba* seeds. With the increase of heavy metal levels the germination rates continuously decreased. The lowest germination rate was observed in the seeds treated with wastewater collected from Station I. The results showed that the germination percentage can be considered as a sensitive indicator for heavy metal toxicity. This information is parallel with other genotoxicity data available so far. In most study, results indicated that, the test substances as heavy metals can be decreased the germination percentage in different plant seeds. For example, Kiran and Sahin (2005) observed a dose-dependent decrease in the germination percentage of *Lens culinaris* seeds after exposure to different doses of Pb. Munzuroglu and Geckil (2002) reported reduce with increasing concentrations of Hg, Co, Cu, Pb, Cd and Zn of the germination percentage in *Triticum aestivum* and *Cucumis sativus* plants. Moreover, many similar studies were designed to investigate the effects of heavy metals on the germination percentage of *Phaseolus vulgaris*, *Pisum sativum and 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 the root growth and seed weight (Burton *et al*., 1984; Pandey *et al*., 2008). In this study, we investigated the changes in weight gain and root growth of *V. faba* seeds treated with wastewater. The increase in heavy metal concentrations inhibited the root growth. In the seeds treated with wastewater collected from Station II, the root growth was decreased about 1.26 times lower than in the controls. The decrease in the root growth was pronounced with the increase in heavy metal concentrations. The rooth growth was decreased about 64, 20 and 43% in the seeds treated with wastewater from Station I, Station II and Station III, respectively.

**Table - 5:** Statistically comparision of datas root length, weight gain and *MN* frequency determined in treatment group seeds at the end of $7^{th}$ day

| Parameters | Control* | Treatment I | Treatment II | Treatment III |
|---|---|---|---|---|
| Root length (cm) | $6.38 \pm 0.48^{a}$ | $2.30 \pm 0.16^{d}$ | $5.08 \pm 0.32^{b}$ | $3.64 \pm 0.31^{c}$ |
| Weight gain (g) | $5.84 \pm 0.12^{a}$ | $2.84 \pm 0.19^{d}$ | $4.80 \pm 0.14^{b}$ | $3.77 \pm 0.14^{c}$ |
| MN frequency | $00.00 \pm 0.00^{d}$ | $41.83 \pm 3.27^{a}$ | $12.33 \pm 1.63^{c}$ | $26.97 \pm 1.83^{b}$ |

* = The control group seeds were treated with tap water; Treatment I group seeds were treated with wastewater collected from Station I; Treatment II group seeds were treated with wastewater collected from Station II, Treatment III group seeds were treated with wastewater collected from Station III. Values presented as mean±SD. Means denoted with different superscripts are within the same column are statistically significant ($p<0.05$)

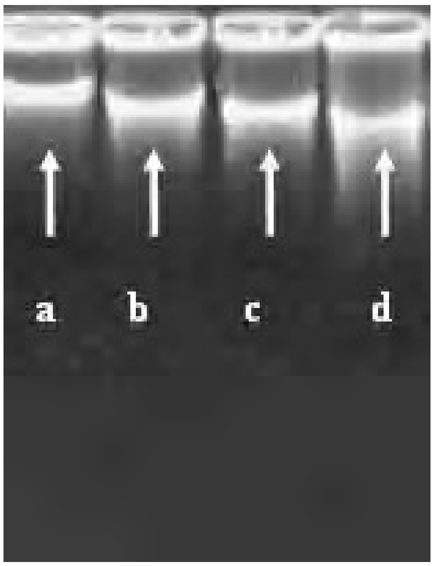


**Fig. 3:** Isolated DNA from *V. faba* resolved on 0.8% agarose gel. a: control group, b: treatment group exposed to Station II samples, c: treatment group exposed to Station III samples, d: treatment group exposed to Station I samples

The effects of heavy metals on root growth were widely reported by biomonitoring studies. The previous studies were reported that high concentrations of Pb, Hg, Zn, Cd and Cu may lead to inhibition of vegetative organ growth in some plant specieses (Dimitrova and Ivanova, 2003). For example, Shafig and Iqbal (2006) determined decrease according to the control group at all concentrations (25-100 ppm) of Pb and Cd of the root length in *Cassia siamea*. Zengin and Munzuroglu (2003) 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. 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. In a similar study, Obroucheva *et al.* (1998) demonstrated inhibition of primary root growth by heavy metals.

The findings obtained from this experiments showed that heavy metals affected the weight gain depending on their concentrations in wastewater. The results indicated that heavy metals depressed and significantly rate decreased the weight gain of the seeds. Moreover, the seeds treated with wastewater collected from Station I showed a lower weight gain than the seeds treated with wastewater collected from Station II and III. In previous studies related to the weight gain, the effects of heavy metals on seed weight were not reported with deserve. 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. For example, Sharma and Dubey (2005) reported that heavy metal ions may block the entry of cations and anions into plant tissues. They also determined that heavy metals may cause a decline in transpiration rate and water content of plant tissues. These conditions may cause significant alterations in nutrient status and nutrient contents of tissues. As a result, may be the reason for reduce the weight gain of plant and seeds.

In our present study, the frequency of MN was also recorded. The results showed that there was a dose-related increase in the MN frequency of the seeds with wastewater includes heavy metals. On the other hand, the MN frequency increased with increase in the heavy metal concentrations. These findings suggested that heavy metals in refinery 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 studies, the results indicated that, the test substances as heavy metals 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. Besides, 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 MN rate and chromosome aberrations with increased concentration of $CrO_3$ in *V. faba* root tip cells (Wei, 2004). In our study, it was concluded that the *V. faba* MN assay may be used as an endpoint biomarker acceptable in biomonitoring environmental pollutives such as heavy metal ions.

Moreover, this study is designed to investigate a possible correlation between the MN formation and the DNA concentration. Therefore, we extracted DNA samples from nuclei of root tip meristems of *V. faba* seeds non-exposed and exposed to refinery wastewaters, and used agarose gel DNA electrophoresis technique to measure DNA band length. As a result, it was found that DNA was rather sensitive to heavy metals in wastewater. We suggest that the length of DNA bands in the seeds treated with wastewaters were longer than recorded in the controls. Besides, the band lengths of DNA were shorterin meristematic tissues of root tips exposed to wastewaters collected from Station II and III when compared with Station I. This diversity may be associated with loss of genetic material. Because, MN formation is a condition which termination with loss of genetic material in nucleus and originated from chromosome fragments or a whole chromosome. This observation suggests that the MN formation is direct related with the length of DNA bands, and this data has not detailed reported by other researchers so far. This information may serve as reliable indicators or may provide new insights into the molecular mechanisms of heavy metal toxicity.

The results of this study indicated that there was a serious pollution problem welding rafinery in the Kizilirmak river. Especially, heavy metal pollution in Station I and III was fairly serious, and Station II was moderately polluted. Hence, this pollution caused serious cytotoxic effects in the root tip cells of *V. faba*. Furthermore, this situation may create a potential healthy risk for human and animals utilizing the water from the Kizilirmak River.

## References


Ahmad, A., A. Inam, I. Ahmad, S. Hayat, Z. Azam and M. Samiullah: Response of sugarcane to treated wastewater of oil refinery. *J. Environ. Biol.,* **24**, 141-146 (2003).

Agoramoorthy, G., F.A. Chen, V. Venkatesalu and P.C. Shea: Bioconcentration of heavy metals in selected medicinal plants of India. *J. Environ. Biol.,* **29**, 175-178 (2009).

Agtas, S., H. Gey and S. Gul: Concentration of heavy metals in water and chub, *Leuciscus cephalus* (Linn.) from the river Yildiz, Turkey. *J. Environ. Biol.,* **28**, 845-849 (2007).

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

Arun Kumar, K. and H. Achyuthan: Heavy metal accumulation in certain marine animals along the east coast of Chennai, Tamil Nadu, India. *J. Environ. Biol.,* **28**, 637-643 (2007).

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).

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

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).

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

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. Elsevier North-Holland Biochemical Press, New York (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).

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).

Metcalfe-Smith, J.L.: Biological water quality assessment of rivers: Use of macroinvertebrate communities *In*: The rivers Handbook (*Eds*.: P. Callow and G.E. Petts). Blackwell Science Publications. pp. 144-150 (1994).

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).

NAS: An Assessment of Mercury in the Environment. National Academy of Sciences Press, Washington DC (1978).

Nath, K., D. Singh, S. Shyam and Y.K. Sharma: Phytotoxic effects of chromium and tannery effluent on growth and metabolism of *Phaseolus mungo* Roxb. *J. Environ. Biol.,* **29**, 227-234 (2009).

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. Biotechnol.,* **5**, 974-982 (2006).

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

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.N., B.D. Nautiyal and C.P. Sharma: Pollution level in distillery effluent and its phytotoxic effect on seed germination and early growth of maize and rice. *J. Environ. Biol.,* **29**, 267-270 (2008).

Shafiq, M and M.Z. Iqbal: 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, 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).

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

Singh, V.K. and J. Singh: Toxicity of industrial wastewater to the aquatic plant *Lemna minor* L. *J. Environ. Biol.,* **27**, 385-390 (2006).

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).

TSE (Turkish Standard Institute): Turkish water intended for human consumption TS-266, Ankara, Turkey. (Available: http://www.tse.org.tr/Turkish/Abone/Standard.htm) (1997).

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).

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).