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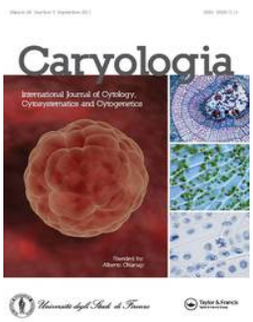

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Cytogenetic effects of ^{99}technetium on meristematic cells of root tips of *Vicia faba* L. andstatistical comparison

Ali Özdemir[a], Bahattın Bozdağ[b], Hakan Sepet[c], Yasemın Parlak[d], Okan Kocabaş[b], F. Serap Ereeş[e] and Canan Özdemir[b]*

[a]*Celal Bayar University, Science and Art Faculty, Mathematics Department, Manisa, Turkey;* [b]*Celal Bayar University, Science and Art Faculty, Biology Department, Manisa, Turkey;* [c]*Ahi Evran University, Engineering-Architecture Faculty, Environmental Engineering Department, Kırşehir, Turkey;* [d]*Celal Bayar University, Department of Nuclear Medicine, School of Medicine, Manisa, Turkey;* [e]*Celal Bayar University, Science and Art Faculty, Physics Department, Manisa, Turkey*

In this study, cytogenetic effects of ^{99}technetium (^{99}Tc) on meristematic cells of root tips belonging to *Vicia faba* L. have been investigated. Seeds of the plant were prepared and kept in ^{99}Tc standard for different time periods: 1/12, 1/4, 1/2, 1, 2, 3, 4, 5, 6 and 12 h. Seeds treated with ^{99}Tc were sprouted and the root tips obtained were prepared for microscopic examination. Some abnormalities e.g. chromosome breaking, chromosome dispersion, bridge chromosomes, chromosome adherence and ring chromosomes were observed. Abnormalities seen for each treatment depended on the time period. The variety and number of abnormalities usually increased with increased treatment time. The results obtained were evaluated statistically.

Keywords: abnormalities; chromosome; ^{99}technetium; ^{99}Tc; *Vicia*

Introduction

^{99}Technetium (^{99}Tc) is a fission product of uranium-235 and plutonium-239 and has been widely distributed in the environment as a result of fall-out from nuclear weapons testing and discharges from nuclear facilities (Bishop et al. 2011). Technetium-99 released into soils has the potential to contaminate food chains, although its radiological effects are normally only considered to be significant at very high activities (Gerber et al. 1989). Plant uptake from aerobic soils is, in fact, so rapid that there is significant potential for quantitative removal of ^{99}Tc from soil by plants (Bennett and Willey 2003). In anaerobic soils ^{99}Tc occurs mostly as insoluble forms (Ishii et al. 2004). Soil oxygen status is, therefore, vital to predicting ^{99}Tc behaviour in soils but other soil factors, in particular NO_3^- concentration, also influence soil-to-plant transfer of ^{99}Tc (Krijger et al. 2000). Various chemical substances used in the fields of medicine, biology and agriculture can affect negatively growth of plants beside their positive effects. İnceer and Beyazoğlu (2000) have investigated cytogenetic effects of copper chloride on root cells of *Vicia hirsuta* (L.) S.F. Gray and they detected that this compound affects cell division negatively and also leads to chromosomal abnormalities (İnceer et al. 2003). Leonard et al. (1983) reported that compounds with mercury affect spindle threads during cell division in *Vicia faba* and *Allium cepa*. Pulhanil et al. (2005) investigated the intake and distribution of some radioactive elements by plants. Some investigations into the effect of heavy metal pollution in plants demostrated that these elements can move to plants from soils (Çelik et al. 2004). In this study, we aimed to examine effects of ^{99}Tc treatments in different time periods on root tip cells of *Vicia faba*.

Material and methods

Seeds of *Vicia faba* were used in the study. ^{99m}Tc standard at 1 mCi was prepared with 500 ml distilled water. Seeds were kept in ^{99m}Tc standard 1/12, 1/4, 1/2, 1, 2, 3, 4, 5, 6, 12 h. Then, seeds were washed by distilled water and germinated in Petri dishes at 20–25°C. After fixation of root tips, they were put in 70% ethyl alcohol. Stock root tips were stained by the Feulgen method (Darlington and La Cour 1976) and were made ready for microscopic examination. Homologous areas were chosen on these preparations for cytogenetic examination; the cells in these areas were counted and the numbers of mitotic cells were also detected. Chromosomal abnormalities were detected in the cells counted. The preparations were photographed with a motorized Leica DM 3000 microscope (Leica Microsystems, Wetzlar, Germany). Pearson correlation coefficients were also calculated. Chromosome abnormalities were coded as C1, C2, C3, C4, C5, C6 and C7 (Table 2). Statistical analyses were performed using MINITAB software package.

Results

^{99m}Tc standard treatment on the seeds at different time periods increased mitotic cell division. Mitotic cell division was observed the highest level at 1/2 h treatment.

*Corresponding author. Email: cozdemir13@gmail.com

Table 1. Chromosome abnormalities in the root tip cells of *V. faba.*

		Investigated abnormality (%)						
Dose of treatment (mCi)	Treatment time (h)	Fish bones C1	Chromosome dispersion C2	Chromosome adherence C3	Chromosome breaking C4	Bridge chromosome C5	Chromosome shrinking C6	Ring chromosome C7
1	1/12	0.59	3.55	1.18	1.18	0.59	0.02	0.0
1	1/4	0.81	2.03	1.22	0.81	0.41	0.04	0.00
1	1/2	1.27	1.91	2.55	0.64	0.64	0.64	0.00
1	1.00	1.82	3.28	1.09	0.73	1.09	1.09	0.36
1	2.00	2.92	1.67	0.42	0.42	1.25	1.67	0.42
1	3.00	0.75	1.50	1.88	0.38	0.94	0.00	0.19
1	4.00	0.00	1.38	2.41	0.00	0.69	0.69	0.00
1	5.00	1.81	1.39	0.14	0.28	0.28	0.14	0.14
1	6.00	1.17	3.29	1.17	0.23	1.17	1.17	0.01
1	12.0	0.98	1.72	0.49	0.49	1.72	0.98	0.00

Abbreviations: C1, C2, C3, C4, C5, C6, C7: codes of chromosome abnormalities.

Table 2. The mitotic index of root tip cells of *Vicia faba.*

Time (h)	Mitotic index ± SD	Time (h)	Mitotic index ± SD
1/12	26.03 ± 19.64	3	20.99 ± 16.26
¼	22.00 ± 8.930	4	16.04 ± 12.25
½	30.26 ± 24.10	5	13.75 ± 9.130
1	25.17 ± 19.82	6	20.30 ± 15.57
2	20.92 ± 10.86	12	16.41 ± 12.84
	Control group		10.68 ± 1.650

Abbreviation: SD, standard deviation.

At 1, 2, 3, 4 and 5 h of treatment, mitotic cell division was decreased. Mitotic division increased again at the sixth hour of treatment and it was decreased again at the 12th hour of treatment. Mitotic cell division was observed at high levels at all treatment times according to the control group (Table 2, Figure 1). ^{99m}Tc caused some chromosome abnormality on *V. faba* seeds such as fish bones chromosome adherence, chromosome dispersions, chromosomal adherence, bridge chromosome, chromosome breaking, chromosome shrinking, ring chromosome at different stages of mitotic division were detected. The most frequently observed abnormality was chromosome dispersion. Chromosome dispersion, chromosome adherence and bridge chromosome were observed at all treatment times. Most chromosome dispersions were observed at 1/12, 1 and 6 h treatment times. Fish bones chromosome adherence and chromosome breaking were seen at all treatment times except 4 h. The fish bones chromosome adherence abnormality was determined at the highest level at 2 h. Chromosome adherence was observed at the highest level at 1/2 and 4 h. Bridge chromosome was determined at a high level at 1, 2, 6 and 12 h. Chromosome breaking was observed at a high level at 1/12 h and this abnormality decreased until 4 h. Ring chromosome was observed at 1, 2, 3 and 5 h. Chromosome shrinking was seen at all treatment times except 1/12, 1/4 and 3 h (Table 1, Figures 2–4). According to the statistical results, there is a considerable positive relation between the treatment time and the chromosome abnormality (%). In addition, it was found that there were statistically important differences between C2-C3 (r = 0,937, P = 0,029), C2-C5 (r = 0,987, P = 0,006), C3-C4 (r = 0,871, p = 0,049) and C2-C7 (r = 0,823, P = 0,003) (Table 3).

Discussion

In this study, cytogenetic effects of ^{99}technetium (^{99}Tc) on meristematic cells of root tips belonging to *Vicia faba* L. have been investigated. We determined that ^{99}technetium (^{99}Tc) caused abnormalities such as fish bones chromosome adherence, chromosome dispersions, chromosomal adherence, bridge chromosomes, chromosome breaking, chromosome shrinking and ring chromosome. Copper chloride has similarly caused some chromosomal abnormalities in root tip cells of *Vicia hirsuta* (L.) Gray. The most frequently observed abnormalities were chromosome adherence and bridge chromosomes (İnceer and Beyazoğlu 2000). In other studies, it was determined that increase of the lead (PbCl2) concentrations cause a decrease of mitotic division and give rise to several mitotic abnormalities such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges on root tip cells of *Lens culinaris* Medik (Kıran and Şahin 2005). That was determined that the germination rates of *Vicia faba* seeds which exposed to uranium tailings were not inhibited. The growth of seedlings was considerably stimulated by uranium tailings at low concentrations rather than at high concentrations. The results revealed that nuclease (RNAse) activity was stimulated at low uranium tailing concentrations and inhibited at high concentrations. So, the uranium tailings have complex effects on nuclease activity (Yı et al. 2007). In another study the cytological

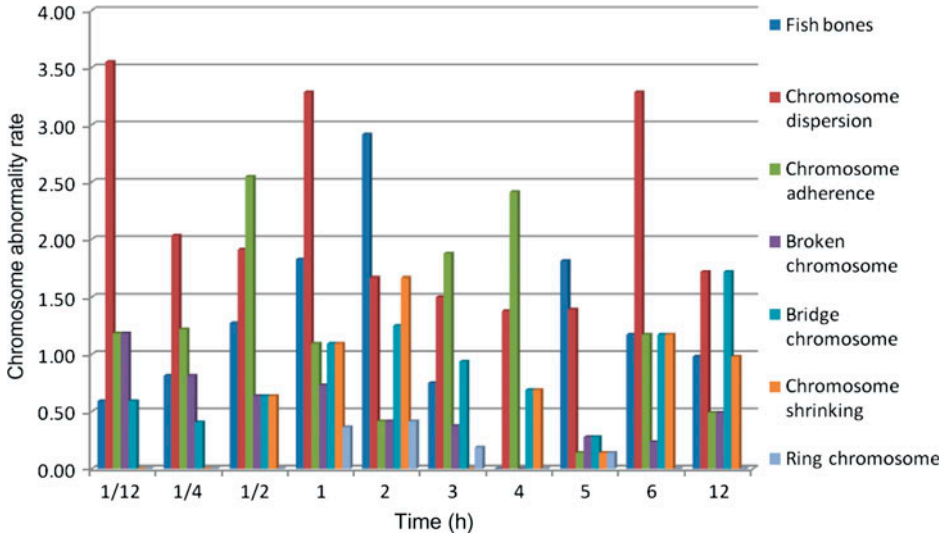

Figure 1. Chromosome abnormalities in the root tip cells of *V. faba.*

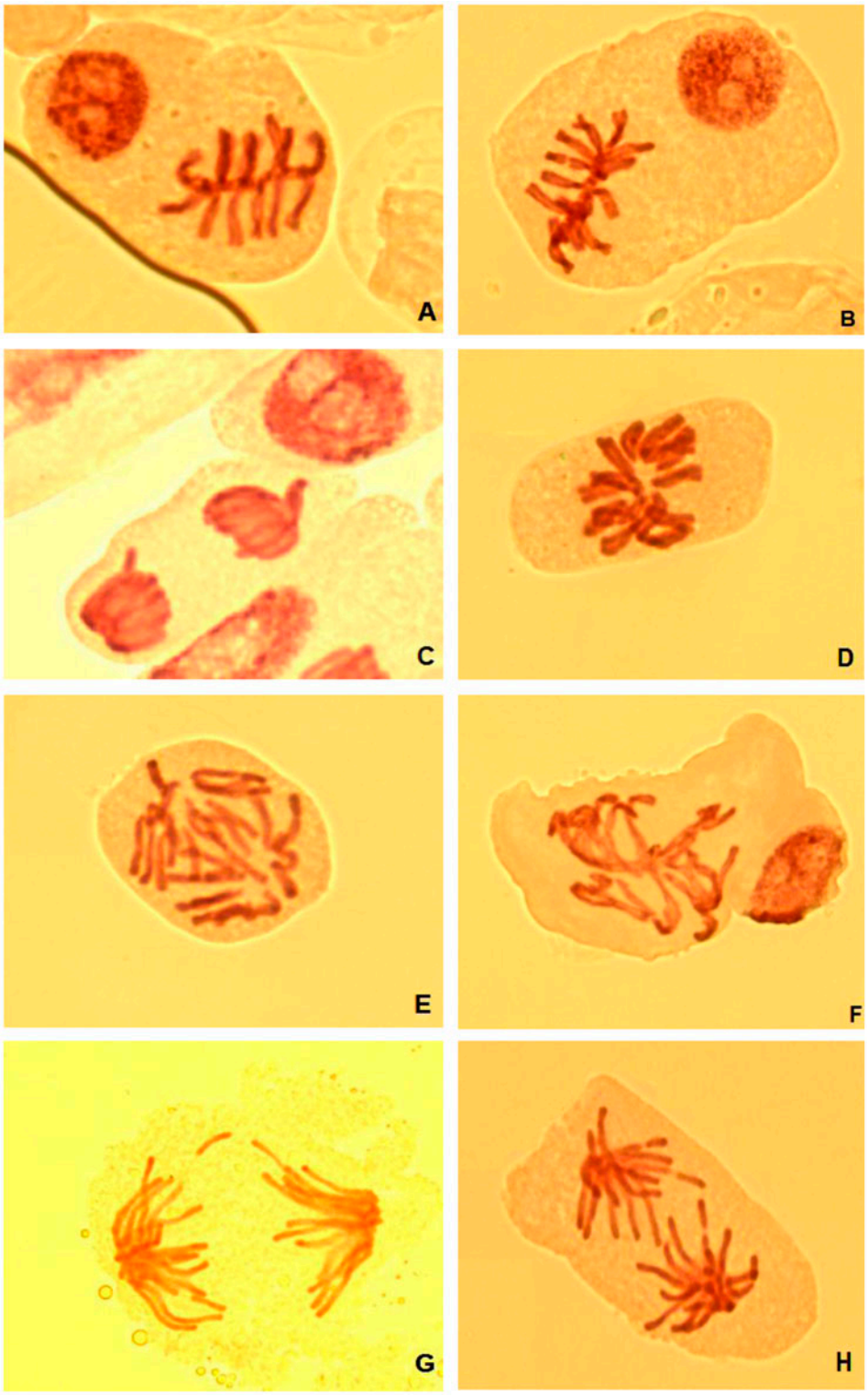

Figure 2. Chromosome abnormality (A, B) fish Bones; (C) chromosome shrinking; (D, E, F) chromosome dispersion; (G, H) chromosome breaking.

effects of the insecticide Phosdrin (mevinphos) and the herbicide Bladex on root tips of *Tradescantia* and *Vicia faba* were observed and compared with those of the chemical mutagen ethyl methane sulphonate (EMS). Plants of *Vicia faba* were sprayed prior to floral initiation and pollen mother cells were examined for chromosomal abnormalities. Phosdrin and Bladex produced the same kinds of chromosome abnormalities as EMS, namely, fragments, bridges, multipolar anaphases and lagging chromosomes (Ahmet and Grant 1972). It has been

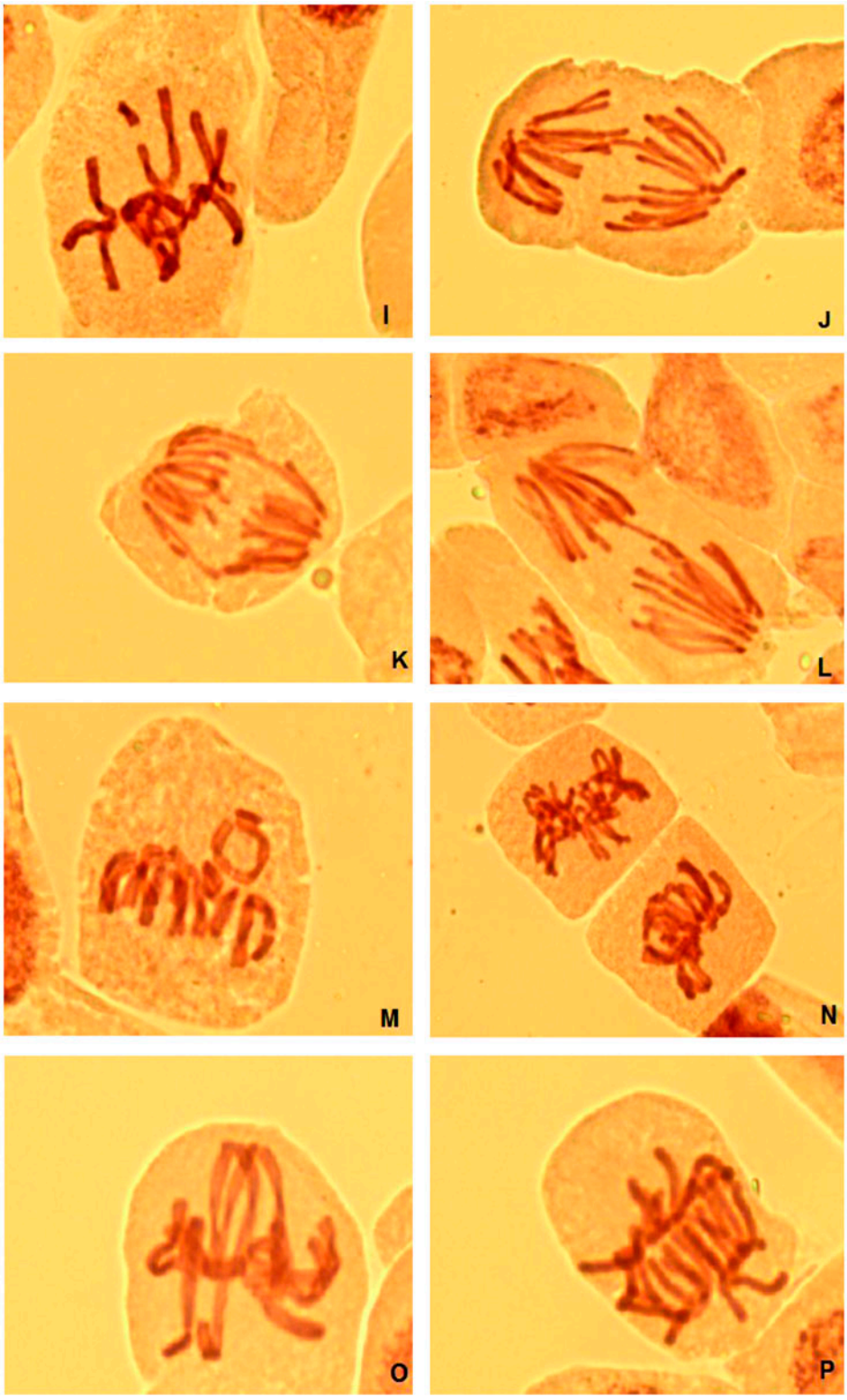

Figure 3. Chromosome abnormality; (I) chromosome breaking; (J, K, L) bridge chromosome; (M) ring chromosome; (N, O, P) chromosome adherence.

shown that the frequency of mitotic cell division is affected by uranium, depending on the treatment time and that uranium led to chromosomal abnormalities in *Vicia faba* cells (Özdemir et al. 2008). In Ukraine, the *Allium cepa* test was used to estimate the impact on plant chromosomes of nuclear pollution in the inhabited zones (Kovalchuk et al. 1998). Strong, significant correlations observed between ^{137}Cs activity of soil samples

Table 3. Pearson correlation based on the chromosome abnormality and ^{99}Tc standard for different time periods.

CA	C1	C2	C3	C4	C5	C6
C2	0.107					
	0.769					
C3	0.288	0.029*				
	0.419	0.937				
C4	0.381	0.598	0.049*			
	0.277	0.068	0.871			
C5	0.479	0.006**	0.577	0.086		
	0.162	0.987	0.081	0.814		
C6	0.603	0.234	0.091	0.248	0.171	
	0.065	0.515	0.803	0.490	0.637	
C7	0.219	0.823	0.109	0.477	0.111	0.118
	0.544	0.003**	0.765	0.163	0.761	0.745

Abbreviation: CA, chromosome abnormality.
**Significant at the level of 0.05.
***Significant at the level of 0.01.

and the percentage of chromosomal abnormalities, r=0.97 ($p < 0.05$) and with the mitotic index, r=−0.93 ($p < 0.05$), in the roots of *A. cepa* (Kovalchuk et al. 1998). Parween et al. (2011) studied the effect of cadmium chloride in the pure germ line of broad bean (*Vicia faba* L.) in relation to chromosomal abnormalities and the rate of cell division. Seeds grown in the nutrient medium for 48 h containing different concentrations of cadmium chloride showed different genotoxic effects such as polyploidy, multipolarity, chromosomal bridge with fragments, lagging chromosome and micronuclei. The relative division rate (RDR) decreased with increasing cadmium concentration (Parween et al. 2011). Radioactive elements can pass to plants through soil and then can reach people (Kasianenko et al. 2005). Studies of the role of uranium in soil and plants are inadequate (Kasianenko et al. 2002). It has been reported that copper, zinc, lead (Arambasic 1995) and chrome (Liu et al. 1992) cause a clastogenic effect in *Allium cepa* root tips. Similarly, Leonard et al. (1983) reported that mercury compounds affect spindle fibres at cell division in *Vicia faba* and *Allium cepa*. It has also been shown that mercury compounds can block DNA replication (De Flora et al. 1994) and chrome compounds can cause broken chromatid (Klasterska et al. 1976). Mercury can inhibit protein synthesis and cause mitotic lagging (Nandi 1985). According to the statistical results presented here, there is a considerable positive relation between the treatment time and the chromosome abnormality (%). In addition, it was found that there were statistically important differences between C2-C3, C3-C4, C2-C5 and C2-C7 at levels of 0.01 P and 0.05 P (Table 3). In this study, we tried to determine the cytogenetic effect of ^{99}technetium (^{99}Tc), arising from radioactivity, on the root tip cells of *Vicia faba* L.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Ahmet M, Grant WF. 1972. Cytological effects of the pesticides Phosdrin and Bladex on *Tradescantia* and *Vicia faba*. Can J Genet Cytol. 14(1):157–165.

Arambasic MB, Bjelicm S, Subakov GS. 1995. Acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on *Allium cepa* L., *Lepidium sativum* L., *Daphnia magna* St. Compar Inves Prac App Wat Res. 29(2):497–503.

Bennett R, Willey N. 2003. Soil availability, plant uptake mechanisms and soil to plant transfer of ^{99}Tc – A review. J Environ Radioact. 65(2):215–231.

Bishop ME, Hailiang D, Ravi K, Kukkadap U, Chongxuan L, Richard E, Edelman N. 2011. Bioreduction of Fe-bearing clay minerals and their reactivity toward pertechnetate (Tc-99). Geochim Cosmochim Acta. 75(18):5229–5246.

Çelik A, Kartal A, Akdoğan A, Kaska Y. 2004. Determining the heavy metal pollution in Denizli (Turkey) by using *Robinio pseudo-acacia* L. Environ Int. 31(1):105–112.

Darlington CD, La Cour F. 1976. The handling of chromosomes. London: Allen and Unwin.

De Flora S, Bennicelli C, Bagnasco M. 1994. Genotoxicity of mercury compounds. A review. Mutat Res. 317(1):57–79.

Gerber GB, Van HM, Garten CT. 1989. Technetium absorption and turnover in monogastric and polygastric animals. Health Phys. 57(2):315–319.

İnceer H, Ayaz S, Beyazoğlu O, Enturk E. 2003. Bakır klorür'ün *Helianthus annuus* (L.) kök ucu hücreleri üzerine sitogenetik etkileri. Turk J Biol. 27(2003):43–46.

İnceer H, Beyazoğlu O. 2000. Bakır klorür'ün *Vicia hirsuta* (L.) S.F. Gray kök ucu hücreleri üzerine sitogenetik etkileri. Turk J Biol. 24(2000):553–559.

Ishii N, Tagami K, Uchida S. 2004. Physicochemical forms of technetium in surface water covering paddy and upland fields. Chemospher. 57(8):953–959.

Kasianenko AA, Kulieva GA. 2002. Ecological consequences of depleted uranium use in ammunition. Bulletin of Russian Peoples' Friendship University, Series Ecology and Life Safety-Moscow, Publishing House of RPFU. 6:90–99.

Kasianenko AA, Kulieva GA, Ratnikov AN, Jiigareva TL, Kalchenko VA. 2005. The migration of U-238 in the system "soil-plant" and its effect on plant growth. International Congress Series. 1276:223–224.

Kıran Y, Şahin A. 2005. 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(1):17–25.

Klasterska I, Natarajan AT, Ramel C. 1976. An interpretation of the origin of subchromatid aberrations and chromosome

stickiness as a category of chromatid aberrations. Hereditas. 83(2):153–162.

Kovalchuk O, Kovalchuk I, Arkhıpov A, Telyuk P, Hohn B, Kovalchuk L. 1998. The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. Mutat Res. 415(1–2):47–57.

Krijger GC, Kolloffel C, Wolterbeek HT. 2000. Effect of nitrate on uptake of pertechnate by tomato plants. J Environ Radioact. 29(3):866–870.

Leonard A, Jacqued P, Lauverys R. 1983. Mutagenicity and teratogenicity of mercury compounds. Mutat Res. 114(1):1–8.

Liu D, Jilang W, Liu M. 1992. Effect of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*. Heredita. 117(1):23–29.

Nandi S. 1985. Studies on the cytogenetic effect of some mercuric fungicides. Cytologica. 50:921–926.

Özdemir, C, Ereeş FS, Cam S. 2008. Cytogenetic effects of uranium on root tip cells of *Vicia faba* L. Bot Lithuan. 14 (3):155–158.

Parween T, Jan S, Mahmooduzzafar Sharma MP, Mujib A, Fatma T. 2011. Genotoxic impact of cadmium on root meristem of *Vicia faba* L. Russ Agric Sci. 37(2):115–119.

Pulhanil VA, Dafauti S, Hedge AG, Sharma RM, Mishra UC. 2005. Uptake and distribution of natural radioactivity in wheat plants from soil. J Environ Radioact. 79(3): 331–346.

Yi S, Wang RL, Feng T, Xiang Y-C, Zhou, H. 2007. Effects of uranium tailings lixivium on early growth of *Vicia Faba*. J Hunan Univ Sci Technol. 4:113–116.