Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: I. Sarac, E. Bonciu, M. Butnariu, I. Petrescu, E. Madosa (2019) Evaluation of the cytotoxic and genotoxic potential of some heavy metals by use of *Allium* test. *Caryologia* 72(2): 37-43. doi: 10.13128/cayologia-256

Published: December 5, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Evaluation of the cytotoxic and genotoxic potential of some heavy metals by use of *Allium* test

Ioan Sarac[1], Elena Bonciu[2,*], Monica Butnariu[1], Irina Petrescu[1], Emilian Madosa[1]

[1] *Banat's University of Agricultural Science and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania*
[2] *Department of Agricultural and Forestry Technology, University of Craiova, Craiova, Romania*
*Corresponding author: elena.agro@gmail.com

Abstract. The present study aimed to evaluate the cytogenetic effects induced by heavy metals nickel (Ni) and lead (Pb) to crop plants, using the *Allium sativum* (garlic) as a test plant. For this purpose, were used solutions of nickel nitrate - $Ni(NO_3)_2$ - and lead nitrate - $Pb(NO_3)_2$ - at concentrations of 50, 150 and 450 ppm for 72 hours, along with an untreated control variant immersed in plain water. The biological material was immersed from the beginning in the tested solutions. The results obtained showed a strong inhibitory effect of these heavy metals on the process of rhizogenesis, as well as a significant mitodepresive effect in the meristematic cells, both phenomena being correlated with increasing concentration of the tested solutions. At the same time, several types of chromosomal aberrations (c-mitosis, vagrants, star-anaphase, star-telophase, fragments, clumping, stickiness, bridges) have been recorded in all treatment variants. The presence of these chromosomal aberrations in all treatment variants indicates the aneugenic effects of nickel nitrate and lead nitrate in the meristematic cells of *A. sativum*. The results suggest the ecotoxicity potential of nickel and lead on plants even at low concentrations and confirm the suitability of *A. sativum* as a test plant for assessing the cytotoxicity and genotoxicity of heavy metals to plants.

Keywords. Genotoxicity, chromosomal aberrations, lead, mitotic index, nickel.

INTRODUCTION

The concept of heavy metals has refers to any metallic chemical element that has a relatively high density and is toxic or poisonous in low concentrations. As natural water pollutants, heavy metals are among the most toxic pollutants due to their prolonged persistence in solutions and the difficulty of being converted into insoluble compounds in surface waters (Bilal et al. 2014). The contamination of soil by heavy metals is also a major environmental problem (Lassoued et al. 2014) and have a toxic action on the aquatic organisms, meanwhile inhibiting the self-purification processes (Nemcsók

et al. 2010). Heavy metals are dangerous because they tend to bioaccumulate (Coroian et al. 2017; Hariri et al. 2018; Marinova et al. 2018). Bioaccumulation means the increase in time in biological organisms of the concentration of the substance in an amount compared to the concentration of this substance in the environment.

The presence of heavy metals in soil, is one important factor that can cause altered physiological and metabolic processes to plants or disturbing the metabolism of essential elements (Dong et al. 2006; Mohanpuria et al. 2007; Wójcik and Tukiendorf 2014; Petrescu et al. 2015; Sarac et al. 2015; Georgieva et al. 2018; Nikolova and Georgieva 2018).

Symptoms of heavy metal toxicity are the result of harmful effects of metals on physiological processes including: inhibiting respiration and photosynthesis, altering the plant-water relationship that causes stress, decreased plasma membrane permeability in the root cells, adverse effects on the metabolic activities of enzymes (Arduini 1994).

Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydril groups (Yadav 2010). High lead exposure is harmful, particularly for children; its effects include damage to the nervous system, liver and kidney damage and developmental delays. Also, the lead exposure is associated with an increased risk of several cancers, in particular, meningioma, brain cancer, and kidney cancer (Liao et al. 2016).

Nickel (Ni) is considered to be an essential micronutrient for plants (Eskew et al. 1983) but at excess concentrations this metal becomes toxic for majority of plant species and triggers oxidative damage (Zornova et al. 1999; Nakazawa et al. 2004; Gajewska et al. 2006; Sachan and Lal 2017). On the other hand, some authors reported cytotoxic effects even at low doses (20 to 100μM) of nickel ions as well as antioxidative enzyme changes in *Allium cepa* roots (Gantayat et al. 2017). Concentrations of Ni could increase by human activities such as application of phosphate fertilizers and pesticides (Gimeno-García et al. 1996) or industrial and agricultural wastewater discharges, domestic sewage discharge and atmospheric deposition (Yan et al. 2018).

The vegetal meristematic tissues that are used for testing the effects of chemicals on chromosomes should be easy to obtain and less expensive. From this point of view, the species *A. sativum* and *A. cepa* are well suited to cytogenetic studies because the meristematic roots appear lightly, have relatively large chromosomes in small numbers and can be easily observed by optical microscope (Doroftei et al. 2010; Bonciu 2012; Bonciu et al. 2018).

MATERIALS AND METHODS

Plant material

The biological material consisted of garlic bulbs, clean and without traces of pests or diseases that had been spread in several bulbils. They were cleaned from the dried leaves and formed by removing any roots after which they were transferred to small glass bottles containing the heavy metal solutions: nickel nitrate - $Ni(NO_3)_2$ and lead nitrate - $Pb(NO_3)_2$ in concentrations of 50, 150 and 450 ppm for each of them.

Three treatment variants with 4 repetitions were performed for each of the heavy metals experienced, along with an untreated control immersed in plain water. In each variant, four garlic bulbils were immersed directly into the treatment solutions for 72 hours, time required for the meristematic roots to be emitted.

Microscopic preparations

After sampling, the meristematic roots were fixed with a mixture of absolute ethyl alcohol and glacial acetic acid in a volume ratio of 3: 1 for 16 hours in the refrigerator, followed by acid hydrolysis with 1 N HCl for 5 minutes and HCl 50% consisting of equal parts of HCl and distilled water for 16 min at room temperature. Roots' staining was performed by the Feulgen technique with Schiff's reagent; the staining time was 90 minutes, followed by the intensification of the coloration in plain water for 20 minutes.

Statistical analyses

After 72 hours, the meristematic roots were counted and measured at each variant. The cytogenetic effects of heavy metals were assessed by calculating the mitotic index (MI) and analysing the chromosomal aberrations observed in the various stages of mitosis. The microscopic preparations have been studied using a microscope with digital camera Kruss (Kruss manufacturer Hamburg, Germany). Five preparations for each variant and 500 cells were analysed for calculating the mitotic index and the chromosome aberration frequency.

Statistical analysis was done using MS Excel 2007. The obtained data were analysed statistically with one-way analysis of variance (ANOVA). The differences between treatment means were compared using the LSD-test at a probability level of 0.05% subsequent to the ANOVA analysis.

The mitotic index and chromosomal aberrations were calculated using the following formulas:

Mitotic index (MI%) = total number of cells in division / total number of analysed cells x 100;

Chromosomal aberrations (CA%) = total number of aberrant cells / total number of cells in division × 100.

RESULTS

The treatment of *A. sativum* bulbils with nickel and lead depending on the concentration negatively influenced the process of issuing meristematic roots.

The treated roots was smaller in size and they had a smaller number than the control. Thus, their number decreased as the concentration of the heavy metal solutions tested in all variants increased: from 46 registered roots to the control variant, to 14-28 roots to the variant treated with $Ni(NO_3)_2$ respectively 5-18 roots to the variant treated with $Pb(NO_3)_2$ (Figure 1).

The results showed that both heavy metals treatments caused a decrease in MI at all the treatment groups (Table 1). Thus, the value of the MI decreased with the increase concentration of heavy metal solutions. The intensity of mitotic activity was decreasing in order of treatment with lead nitrate to nickel nitrate treatment. However, the strongest mitodepresive effect was seen in the treatment of $Pb(NO_3)_2$ at the concentration of 450 ppm, when MI was 5.32%, ie with 46.4% lower mitotic activity compared to the control variant.

Heavy metals tested induced a high number of CA when compared with control. The increase of CA was dependent on the increasing treatment concentrations (Table 1). The types of CA identified in meristematic cells of *A. sativum* were the following: C-Mitosis (Figure 2A); fragments and vagrants (Figure 2B); star-anaphase; star-telophase (Figure 2C); clumping; stickiness (Figure 2D); bridges (Figure 2E).

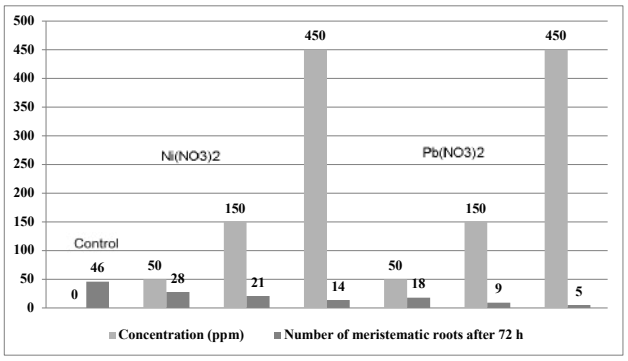

Fig. 1. The inhibitory effect of different concentrations of $Ni(NO_3)_2$ and $Pb(NO_3)_2$ on the rhizogenesis to *A. sativum.*

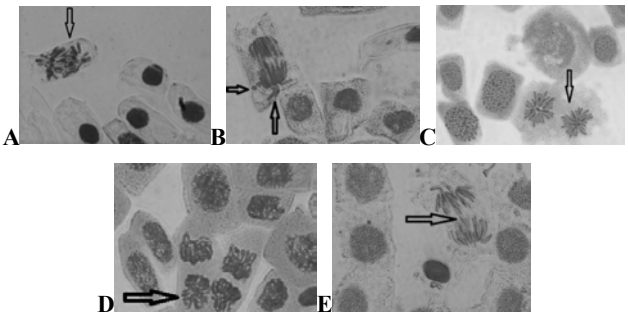

Fig. 2. Some chromosomal aberrations identified in meristematic cells of *A. sativum* exposed to $Ni(NO_3)_2$ and $Pb(NO_3)_2$: C-Mitosis (A), fragments and vagrants (B), star-telophase (C), stickiness in telophase (D), anaphase bridge (E).

As can be seen from the data of Table 1, the most common types of CA were stickiness, C-Mitosis and bridges, and the least frequent were vagrants chromosomes. Compared with the control variant, the total CA rate recorded insignificant values for the variant treated with 50 ppm $Ni(NO_3)_2$, significant for the variant treated with 150 ppm $Ni(NO_3)_2$ and distinctly significantly positive for the variant treated with 450 ppm $Ni(NO_3)_2$. On the other hand, in case of the $Pb(NO_3)_2$ treated variants compared to the control variant, the total CA recorded significantly positive values for the variant treated with 50 ppm $Pb(NO_3)_2$ and strongly positive for the variants treated with 150 and 450 ppm $Pb(NO_3)_2$ respectively.

DISCUSSION

We chose to study the cytotoxic effects of Ni and Pb heavy metals on *A. sativum* because, according to many authors (Saxena et al. 2004; Gul et al. 2006; Unyayar et al. 2006; Liu et al. 2009), the evaluation of CA in *A. sativum* meristematic roots is a reliable biotest example that can be applied to detect a wide range of genetic damage.

At a macroscopic level, heavy metals induced inhibition of the growth of meristematic garlic roots. Generally, heavy metals are known to decreasing the plant growth and ground cover (McGrath et al. 2001). Some effects of lead on growth, physiology, metabolism and yield attributes of plants are the following: inhibition in seed germination, fresh and dry biomass, leaf area, chlorophyll and growth to *Helianthus annuus* (Mahmood et al. 2013); decline in growth, chlorophyll, carotenoids and prolinecontent to *Brassica juncea* (John et al. 2009); decrease in plant growth, root hair to *Vigna unguiculata* (Kopittke et al. 2007), etc.

In our experiment, reducing the number of meristematic root has been accentuated with increasing con-

Table 1. Mitotic index, type and percentage of mitotic aberrations induced by some heavy metals on the meristematic roots to *A. sativum.*

Treatment / Exposure time (hours)	Conc. (ppm)	MI ± SD (%)	CA (%)								Total aberrations (%)
			C-M	V	S-A	S-T	F	CL	S	B	
Ct / 72	0	11.45±0.5	2.35	0	0	0	0	0	0	0	2.35
$Ni(NO_3)_2$ / 72	50	10.66±0.4	1.61	0.65	0.42	0	0	0	3.62	2.31	8.61
	150	8.13±0.3	1.90	0.83	1.81	0.83	0	1.23	5.11	3.22	14.93*
	450	6.84±0.8	3.15	1.40	2.73	1.43	2.11	3.41	5.42	3.91	23.56**
$Pb(NO_3)_2$ / 72	50	8.73±0.4	4.65	1.62	3.90	1.70	2.80	3.96	6.28	4.82	29.73**
	150	6.51±0.8	6.23	2.11	4.31	2.82	3.70	4.20	6.50	5.31	35.18***
	450	5.32±0.5	7.51	2.64	4.85	4.20	4.21	6.52	7.40	6.10	43.43***

Ct = Control; Conc. = Concentration; MI = Mitotic index; SD = Standard deviation;
CA = Chromosomal aberrations; C-M = C-Mitosis; V = Vagrants; S-A = Star-Anaphase; S-T = Star-Telophase; F = Fragments; CL = Clumping; S = Stickiness; B = Bridges;
The differences between treatment means were compared using the LSD-test at a probability level of 0.05%: *significant at P<0.05, **significant at P<0.01, ***significant at P<0.001 as compared to the control variant.

centrations of test solutions (especially to treatments with lead nitrate). Proportionately with increasing concentrations, intensity of the mitotic division has been decreased to all variants, as an active protection reaction of the plants exposed to heavy metals action. These findings are in agreement with Doroftei et al. (2010) who have tested the cytogenetic effects of lead nitrate to *A. cepa*. The lead and nickel inhibited cell division to other plants too, like *Zea mays* (Kozhevnikova et al. 2007).

The root growth is an integrative process depending on whole-organism signalling and individual growth trajectories of cells (Beemster et al. 2003). The intensity of the mitotic division is directly related to the growth of plant roots; conceptually, mitotic division in the apical root meristem provides cells during longitudinal growth (Sanz et al. 2012). The number of dividing cells in the root apical meristem generates a cell flux with importance in modulating root growth (Baskin, 2013). Studies of cell length profiles have shown that the proliferative fraction of dividing cells in the apical root meristem proliferation domain is indistinguishable from one, even in response to moderate levels of stress (Ivanov and Dubrovsky 2013).

The results of this study highlight the increasing of CA frequencies to *A. sativum* dependent on different concentrations of nickel nitrate and lead nitrate. Stickiness, C-mitosis and bridges were most often identified in all treatment variants but the highest frequency was recorded to variants treated with lead nitrate. According to some authors, sticky chromosomes might have resulted from increased chromosome contraction and condensation or possibly from depolymerisation of DNA and partial dissolution of nucleoproteins (Kuras et al. 2005; Turkoglu 2013b). Asita and Mokhobo (2013) suggested that the induction of *Allium*'s sticky chromosomes under the influence of pesticides indicates abnormal DNA condensation, abnormal chromosomal wrapping, and inactivation of the axes, and all these anomalies cell division can have adverse effects on the environment. C-Mitosis indicates a chemical-inhibited spindle formation similar to the effect of colchicine and induction of these aberrations suggests a turbogenic effect (Shahin and El-Amoodi 1991; Turkoglu 2013b). Regarding the anaphase bridges, these chromosomal aberration cause structural chromosome mutations and may lead to loss of genetic material (George 2000; Pampalona et al. 2016). According to Turkoglu (2013b), bridges could form due to dicentric chromosome presence or due to the breakage and fusion of chromosomes and chromatids.

In our experiment, the stickiness had a frequency of 3.62-5.42% at the nickel nitrate treatment, while for treatment with lead nitrate the frequency of these CA was at the level of 6.28-7.40% at all concentrations (50, 150, 450 ppm). Sticky chromosomes can probably lead to cell death (Singh 2015). These results suggest the strong genotoxic effect of lead nitrate even at low concentrations of 50 and 150 ppm. The current findings agreed well with other reports which showed cytotoxicity and genotoxicity of lead in plant cells (Choudhury and Panda 2004; Arya et al. 2013).

Some nickel compounds have been established as human carcinogens (Coogan et al. 1989), but at the same time, some plant seeds, such as soybeans, can act as protectors when introduced into diet of treated mice, by reducing the percentage of CA (Fahmy et al. 2014). Certain hormones can act as agents for inducing resistance

of plants to heavy metal toxicity. Thus, some authors have reported that *Brassica juncea* plants sprayed with 28-homobrassinolide hormone, showed improved resistance against the some heavy metal toxicity (Hayat et al. 2007). Also, the organic acids (citrate and malate) have been reported to have a role in the plant protection against heavy metal stress (Haydon et al. 2007).

CA called C-mitosis had a frequency of 1.61-3.15% at the nickel nitrate treatment, while for treatment with lead nitrate the frequency of C-mitosis was at the level of 4.65-7.51%, these results demonstrating the high genotoxic potential of lead to plants. C-mitosis is the result of damaged mitotic apparatus due to genotoxic substances in the cells and is stimulated by many chemicals (Fiskesjö 1993; Firbas and Amon 2014).

Cytogenetic tests on *A. sativum* reveal a decrease in the mitotic index following heavy metal treatments. Mitosis analysis indicates the occurrence of a large number of CA identified at various stages of mitosis, the cell division process being significantly affected. The most of the CA identified indicates aneugenic effects, because of in the mitotic spindle disorders (Sharma and Panneerselvan 1990; Fernandes et al. 2007).

The results obtained in this study reflect cytotoxic and genotoxic potential of Ni and Pb to higher plant A. *sativum*. Changes induced by the heavy metals analysed in the genetic material can be much deeper but unnoticeable with our investigative means.

REFERENCES

Arduini I, Goldbold DL, Onnis A. 1994. Cadmium and cooper change root growth and morphology at *Pinus pinea* and *Pinus pinaster* seedling. Physiol Plant. 92(4): 675-680.

Arya SK, Avisek Basu A, Mukherjee A. 2013. Lead induced genotoxicity and cytotoxicity in root cells of *Allium cepa* and *Vicia faba*. *Nucleus*. 56(3): 183-189.

Asita AO, Mokhobo MM. 2013. Clastogenic and Cytotoxic Effects of Four Pesticides Used to Control Insect Pests of Stored Products on Root Meristems of *Allium cepa*. Environ Nat Resources Res. 3(2):133-145.

Baskin TI. 2013. Patterns of root growth acclimation: constant processes, changing boundaries. Developmental Biology 2(1): 65–73.

Beemster GTS, Fiorani F, Inzé D. 2003. Cell cycle: the key to plant growth control? Trends in Plant Science 8(4): 154–158.

Bilal E, Guenolé–Bilal I, Bounahkla M, Iatan LE, Machado de Mello F, Doumas M, Tahri M, Gallice F, Grail-lot D, Piegay H, Lassoued N. 2014. The freshwater mussels are a good bioindicator for monitoring the water pollution by heavy metal (the Saint–Victor–sur–Loire Lake, France). Banat¢s Journal of Biotechnology. 5(10): 5–11.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A. 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia. 71(3): 191-209.

Bonciu E. 2012. Cytological effects induced by Agil herbicide to onion. J Horticult For Biotechnol. 16(1):68-72.

Choudhury S, Panda SK. 2004. Induction of oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* (Schwaegr) broth under lead and arsenic phytotoxicity. Curr Sci 87(3): 342-348.

Coogan TP, Latta DM, Snow ET, Costa M. 1989. Toxicity and carcinogenicity of nickel compounds. Crit Rev Toxicol. 19(4): 341-384.

Coroian A, Miresan V, Cocan D, Raducu C, Longodor AL, Pop A, Feher G, Andronie L, Marchis Z. 2017. Physical-chemical parameters and the level of heavy metals in cow milk in the Baia Mare area. Banat¢s Journal of Biotechnology. 8(16): 69–74.

Dong J, Wu FB, Zhang GP, 2006. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). Chemosphere. 64(10): 1659-1666.

Doroftei E, Antofie MM, Sava D, Arcus M. 2010. Cytogenetic effects induced by Manganese and Lead microelements on germination at *Allium cepa*. Botanica Serbica. 34(2): 115-121.

Eskew DL, Welch RM., Cary EE. 1983. Nickel: an essential micronutrient for legumes and possibly all higher plants. Science. 222(4624): 621-623.

Fahmy MA, Hassan NHA, Melek FR, Hassan ZM, Al-Ashaal HA. 2014. Studies on the Genotoxic Effect of Nickel Chloride in Mice and the Possible Protective Role of Soybean Seeds Extracts. Global J Pharmacol. 8(4): 625-634.

Fernandes TCC, Mazzeo DEC, Marin-Morales MA. 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pesticide Biochemistry and Physiology. 88(3): 252-259.

Firbas P, Amon T. 2014. Chromosome damage studies in the onion plant *Allium cepa* L. Caryologia. 67(1): 25-35.

Fiskesjö G. 1993. The *Allium* test – A potential standard protocol of assessment of environmental tox-

icity. In: Gorsuch JW, Dwyer FJ, Ingorsoll CG, La Point TW, editors. Environ Toxicol and Risk Assess, Vol. 2. Philadelphia: American Society for Testing and Materials. p. 331–345.

Fisjekö G. 1982. Evaluation of short term tests of toxicity and mutagenity with special reference to mercury and selenium. Hereditas. 95: 155-162.

Gajewska E, Sklodowska M, Slaba M, Mazur J. 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biologia Plantarum. 50(4): 653-659.

Gantayat S, Mania S, Pradhan C, Das AB. 2017. Ionic Stress Induced Cytotoxic Effect of Cadmium and Nickel Ions on Roots of *Allium cepa* L. Cytologia 83(2):143-148.

George NM. 2000. Evaluation on Mutagenic Effects of the Three Heavy Metals on *Vicia faba* Plants. Cytologia 65(1): 75-82.

Georgieva N, Nikolova I, Naydenova Y. 2018. Possibility for weed control by using of an organic product with herbicidal effect. Banat¢s Journal of Biotechnology 9(17): 40-49.

Gimeno-García E, Andreu V, Boluda R. 1996. Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environ Pollut. 92(1): 19-25.

Gul T, Kaymak F, Fulya D, Muranli G (2006). Genotoxic Effects of Avenoxan on *Allium cepa* L. and *Allium sativum* L. *Caryologia.* 59(3): 241-247.

Hariri Moghadam F, Khalghani J, Moharramipour S, Gharali B, Mostashari Mohasses M. 2018. Investigation of the induced antibiosis resistance by zinc element in different cultivars of sugar beet to long snout weevil, *Lixus incanescens* (Col: *Curculionidae*). Banat¢s Journal of Biotechnology. 9(17): 5–12.

Hayat S, Ali B, Hasan SA, Ahmad A. 2007. Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot. 60(1): 33–41.

Haydon MJ, Cobbett CS. 2007. Transporters of ligands for essential metal ions in plants. New Phytol. 174(3): 499–506.

Ivanov VB, Dubrovsky JG. 2013. Longitudinal zonation pattern in plant roots: conflicts and solutions. Trends in Plant Science 18(5): 237–243.

John R, Ahmad P, Gadgil K, Sharma S. 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int J Plant Product. 3(3): 65-76.

Kopittke PM, Asher CJ, Kopittke RA, Menzies NW. 2007. Toxic effects of Pb2+ on growth of cowpea (Vigna unguiculata). Environ Pollut. 150(2): 280-287.

Kozhevnikova AD, Seregin IV, Bystrova EI, Ivanov VB. 2007. Effects of heavy metals and strontium on division of root cap cells and meristem structural organization. Russ J Plant Physiol. 54(2): 257–266.

Kuraś M, Nowakowska J, Sliwinska E, Pilarski R. 2005. Changes in chromosome structure, mitotic activity and nuclear DNA content from cells of *Pallium* Test induced by bark water extract of *Uncaria tomentosa* (Willd.) DC. J of Ethnopharmacol. 107(2): 211-221.

Lassoued N, Khelil MN, Rejeb S, Bilal E, Chaouachi M, Rejeb MN, Guenolé–Bilal I. 2014. Transfert heavy metal sewage sludge as fertilizer from soil to ray grass. Banat¢s Journal of Biotechnology. 5(9): 86–95.

Liao LM, Friesen MC, Xiang YB, Cai H, Koh DH, Ji BT, Yang G, Li HL, Locke SJ, Rothman N, Zheng W, Gao YT, Shu XO, Purdue MP. 2016. Occupational Lead Exposure and Associations with Selected Cancers: The Shanghai Men's and Women's Health Study Cohorts. Environ Health Perspect. 124(1): 97-103.

Liu D, Jiang W, Meng Q, Zou J, Gu J, Zeng M (2009). Cytogenetical and ultrastructural effects of copper on root meristem cells of *Allium sativum* L. *Biocell.* 33(1): 25-32.

Mahmood S, Ishtiaq S, Malik MI, Ahmed A. 2013. Differential growth and photosynthetic responses and pattern of metal accumulation in sunflower (*Heliathus annuus* L.) cultivars at elevated levels of lead and mercury. Pak J Bot. 45(SI): 367-374.

Marinova DH, Ivanova II, Zhekova ED. 2018. Evaluation of Romanian alfalfa varieties under the agro–environmental conditions in northern Bulgaria. Banat¢s Journal of Biotechnology 9(18): 56-64.

McGrath SP, Zhao FJ, Lombi E. 2001. Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant and Soil. 232(1-2): 207-214.

Mohanpuria P, Rana NK, Yadav SK 2007. Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. Environ Toxicol. 22(4): 368-374.

Nakazawa R, Kameda Y, Ito T, Ogita Y, Michihata R, Takenaga H. 2004. Selection and characterization of nickel-tolerant tobacco cells. Biol Plant. 48(4): 497-502.

Nemcsók J, Editare H, Zsanett J, Băneş A, Horablaga M, Bostan C. 2010. The pollution of the soils, the underground and surface waters, from Hungarian–Romanian Cross–Border area. Banat¢s Journal of Biotechnology. 1(2): 70-78.

Nikolova I, Georgieva N. 2018. Effect of biological products on the population of aphids and chemical components in alfalfa. Banat¢s Journal of Biotechnology 9(18): 38-45.

Pampalona J, Roscioli E, Silkworth WT, Bowden B, Genescà A, Tusell L, Cimini D. 2016. Chromosome Bridges Maintain Kinetochore-Microtubule Attachment throughout Mitosis and Rarely Break during Anaphase. PLOSOne. 11(1): e0147420.

Petrescu I., Sarac I, Chereches B, Madosa E. 2015. Effect of lead on the growth of *Coriandrum sativum* L. J Horticult For Biotechnol. 19(4): 93-95.

Sachan P, Lal N. 2017. An Overview of Nickel (Ni2+) Essentiality, Toxicity and Tolerance Strategies in Plants. Asian J Biol. 2(4): 1-15.

Sanz L, Murray JAH, Dewitte W. 2012. To Divide and to Rule; Regulating Cell Division in Roots during Post-embryonic Growth. In: Lüttge U, Beyschlag W, Büdel B, Francis D (Eds.), Progress in Botany: 73: 57-80. Springer-Verlag Berlin Heidelberg.

Sarac I, Petrescu I, Madosa E, Antonie IF. 2015, *Copper effects at seed germination of Secale cereale* L. J Horticult For Biotechnol. 19(4): 84-88.

Saxena PN, Chauhan LKS, Chandra S, Gupta SK. 2004. Genotoxic effects of diuron contaminated soil on the root meristem cells of *Allium sativum*: a possible mechanism of chromosome damage. *Toxicology Mechanisms and Methods.* 14(5): 281-286.

Shahin SA, El-Amoodi KHH. 1991. Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides Nimrod and Rubigan-4 in root tips of *Vicia faba* L. Mutation Research 261(3): 169–176.

Sharma CBSR, Panneerselvan N. 1990. Genetic toxicology of pesticides in higher plant systems. Critical Reviews in Plant Sciences. 9(5): 409-442.

Singh P. 2015. Toxic effect of chromium on genotoxicity and cytotoxicity by use of Allium cepa L. IJREAS. 5(10): 1-10.

Unyayar S, Celik A, Cekic FO, Gozel A. 2006. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis. 21(1): 77–81.

Türkoglu S. 2013b. Evaluation of genotoxic effects of five flavour enhancers (glutamates) on the root meristem cells of *Allium cepa*. Food and Chemical Toxicol. 49(4): 763 – 769.

Wójcik M, Tukiendorf A. 2014. Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. Plant Growth Regulation. 44(1): 71-80.

Yadav SK. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany. 76(2): 167-179.

Yan X, Liu M, Zhong J, Guo J, Wu W. 2018. How Human Activities Affect Heavy Metal Contamination of Soil and Sediment in a Long-Term Reclaimed Area of the Liaohe River Delta, North China. Sustainability: 10(2): 338.

Zornoza P, Robles S, Martin N, 1999. Alleviation of nickel toxicity by ammonium supply to sunflower plants. Plant and Soil. 208(2): 221-226.