ECOLOGIA BALKANICA
2018, Vol. 10, Issue 2 December 2018 pp. 73-81

The Effects of Lead and Cadmium on Cell Division and Chromosomal Structure in Allium cepa Test System In Vivo

Ivan Y. Stoyanov, Penka L. Vasileva*, Teodora P. Popova, Betina N. Slavova

University of Plovdiv "Paisii Hilendarski", Faculty of Biology, 24, Tzar Assen Str., Plovdiv, BULGARIA
*Corresponding author: pepa_vasileva@abv.bg

Abstract. The present work focuses on the study of the potential mito-inhibiting and genotoxic effect of lead and cadmium in the root meristem of *Allium cepa* L. *in vivo*. A cytotoxic effect of lead and cadmium was registered, as evidenced by the lower mitotic index values in all test concentrations compared to the control. The analysis of the microscopic preparations for the experimental variants of the two heavy metals showed a significant genotoxic effect. All analyzed samples of lead and cadmium demonstrate an increased frequency of chromosomal aberrations compared to the control, as a positive dependence was established between the frequencies reported and the concentration of the studied metals. Lead and cadmium solutions cause a wide range of chromosomal aberrations, varying with the dose of the metal. The most common observed disorders are: acentric fragments, lagging and "vagrant" chromosomes, micronuclei, chromosome bridges, and asynchronous mitosis, demonstrating the genotoxic potential of the studied heavy metals. For both metals a maximum frequency of aberrations for the concentration limit is established. By comparing the two heavy metals, it has been found that lead has a greater cytostatic potential than cadmium by more effectively inhibiting cell division. The results obtained regarding the frequency of chromosomal aberrations show a higher genotoxic effect of cadmium compared to lead.

Key words: heavy metals, chromosomal aberration, mitotic index, *Allium* test.

Introduction

Heavy metals are a group of chemical elements that are known to be one of the most significant causes of environmental and health risks. They accumulate in the environment as a result of active production activity. Some heavy metals such as lead and cadmium are not specific to living organisms that have no developed mechanisms to eliminate them. These metals can not be metabolized and accumulate in organisms, causing intoxication. In view of the negative biological effect that heavy metals have on living organisms, monitoring programs are in place to determine the effect of their impact using biomarker systems. The existing dependence between the increase in the concentration of heavy metals in the environment and the stimulation of genotoxicity and carcinogenicity has been reported in a number of ecotoxicological studies (KUNGOLOS *et al.*, 2006; ŽEGURA *et al.*, 2009). Chromosomal analysis of plants is a commonly used tool for assessing the damaging properties of heavy metals. According to RANK (2003), the occurrence of aberrations in anaphase and telophase cells in *Allium cepa* represents a rapid screening for the detection of chemical substances in environmental samples. GLINSKA *et al.* (2007)

use the *Allium cepa* test system to analyze the influence of heavy metals on lead and cadmium. They found reduction in mitotic index, changes in phase indices and abnormalities during mitosis, consistent with that reported by LIU *et al.* (1994) and PĂDUREANU (2005). KUMAR & SRIVASTAVA (2015) found clastogenic and mito-inhibiting effect of heavy metals lead and cadmium in plant cells, taking into account specific variations in chromosomal behavior depending on the concentrations of the metals used for seed treatment. ÜNYAYAR *et al.* (2006) identified cadmium as one of the most toxic pollutants of the environment affecting cytogenetically different organisms. The analysis of chromosomal aberrations in cells of *Allium cepa* L. root apical meristem is accepted by the International Program on Plant Bioassays (IPPB) for monitoring or testing environmental pollutants (MA, 1999). The present study aims to investigate the influence of various lead and cadmium concentrations on *Allium cepa* L. root meristem cells at cytological cellular and chromosomal levels.

Materials and Methods

For the purpose of the study, annual seeds of *Allium cepa* and solutions with different concentrations of $Cd(NO_3)_2$ и $Pb(NO_3)_2$ were used. The maximum permissible concentration (MPC) has been analysed for the respective metal, according to the Ordinance on environmental quality standards for priority substances and certain other pollutants dated 2010, as well as 75%, 50% and 25% of the MPC for the metal for determination of cytotoxic and genotoxyc effect and at lower concentration of metals compared to MPC. The onion seeds are placed in petri dishes on a three-layer filter paper under humid chamber conditions. The filter paper is moistened with the test solutions with different concentrations of $Cd(NO_3)_2$ and $Pb(NO_3)_2$, and in the control variant with distilled water. For the preparation of temporary microscopic preparations, the root apical meristem is used. The mutagenic effect of the pesticide is reported using the *Allium* test (according to FISKESJÖ, 1988, with modifications by IVANOVA *et al.* 2005; STAYKOVA *et al.* 2010). Microscopic preparations were observed under a light microscope at 400x magnification and were photographed with a digital camera for microphotographing. At least 2000 cells were analyzed for each experimental variant and for the control.

Results

Cytotoxicity of heavy metals lead and cadmium

To analyze the cytotoxic activity of the tested heavy metals, the mitotic index and the phase indices that are indicative of cell proliferation are used. Tables 1 and 2 give data on mitotic and phase indexes in the growing root tip of *Allium cepa* in control and experimental variants treated with different concentrations of lead and cadmium.

Table 1. Mitotic index and phase indices (in %) in *Allium cepa*, treated with different concentrations of lead.

Samples	Mitotic index, IM	Phase indices			
		IPph	IMph	IAph	ITph
Control	68.45	91.45	3.39	1.67	3.49
Pb 25 1.8 µg.l^{-1} $Pb(NO_3)_2$	40.54	91.19	3.22	2.84	2.75
Pb 50 3.6 µg.l^{-1} $Pb(NO_3)_2$	34.07	88.37	4.31	4.31	3.01
Pb 75 5.4 µg.l^{-1} $Pb(NO_3)_2$	36.49	86.11	5.27	4.16	4.46
Pb 100 7.2 µg.l^{-1} $Pb(NO_3)_2$	35.95	91.66	2.61	2.91	2.82

Table 2. Mitotic index and phase indices (in %) in *Allium cepa*, treated with different concentrations of cadmium.

Samples	Mitotic index, IM	Phase indices			
		IPph	IMph	IAph	ITph
Control	68.45	91.45	3.39	1.67	3.49
Cd 25 0.1 µg.l^{-1} $Cd(NO_3)_2$	53.99	89.35	4.12	2.86	3.7
Cd 50 0.225 µg.l^{-1} $Cd(NO_3)_2$	62.89	92.65	2.64	2.15	2.57
Cd 75 0.3375 µg.l^{-1} $Cd(NO_3)_2$	61.7	93.14	2.51	2.08	2.27
Cd 100 0.45 µg.l^{-1} $Cd(NO_3)_2$	50.91	91.23	3.62	2.15	3.0

Genotoxicity of heavy metals lead and cadmium

In the present study, metal-induced genotoxic effects were assessed by determining the type and frequency of chromosomal aberrations in the root meristem cells of *Allium cepa*. By anaphase analysis and micronucleus test, cells with disorders of the type of "vagrant" and lagging chromosomes, asynchronous mitosis, anaphase and telophase bridges, micronuclei and acentric fragments were reported (Fig. 1 and 2).

Tables 3 and 4 represent the calculated total chromosome aberration frequency and the frequency of each individual structural aberration type (in %) relative to the total number of cells analyzed and the number of dividing cells in the test and control samples of the two test metals.

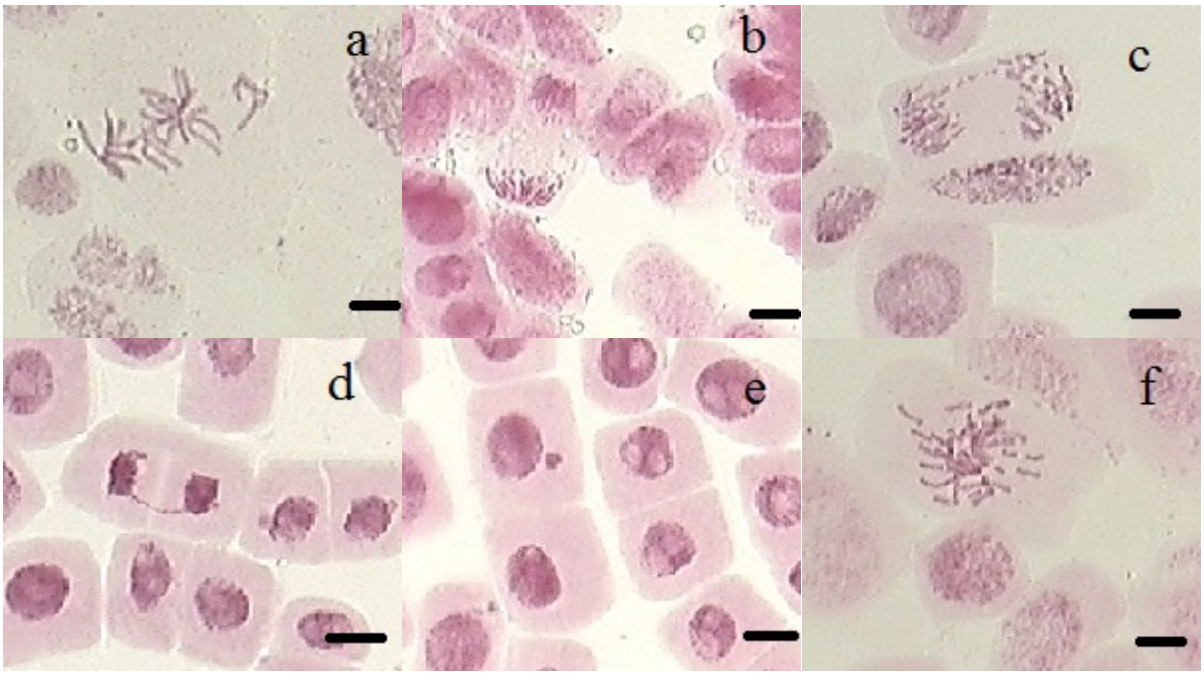

Fig. 1 (a-f). "Vagrant" chromosomes in sample Pb25 (a), asynchronous mitosis in sample Pb75 (b), anaphase (c) and telophase (d) bridges in samples Pb100, micronuclei in sample Pb50 (e), c-mitosis and fragments in sample Pb75 (f), magnification 400x, scale bar 10 µm.

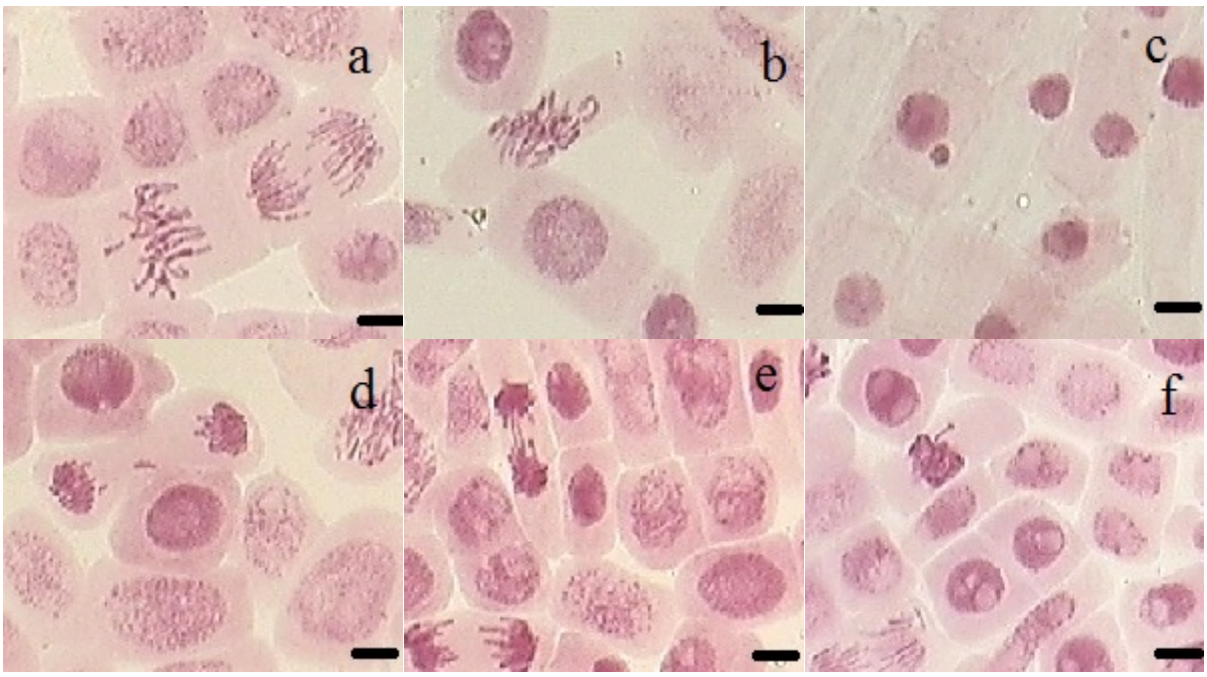

Fig. 2 (a-f). Fragments in sample Cd100 (a), "Vagrant" chromosomes in sample Cd100 (b), micronuclei in sample Cd100 (c), fragment in sample Cd75 (d), anaphase bridges in sample Cd75 (e), "Vagrant" chromosome in sample Cd50 (f), magnification 400x, scale bar 10 µm.

Table 3. Frequency of chromosome aberrations, analized by means of *Allium* test relative to the total number of analyzed cells (on the first row) and the dividing cells (on the second row).

Samples	Cells with micro-nuclei	Asynchro-nous mitosis	Cells with chromo-somal fragments	Cells with chromo-somal bridges	Cells with lagging chromo-somes	Total number of cells with aberra-tions
Control	0.25	0.00	0.00	0.06	0.16	0.48
	0.37	0.00	0.00	0.09	0.23	0.70
Pb 25	0.19	0.08	0.04	0.23	0.12	0.65
1.8 µg.l^{-1}Pb$(NO_3)_2$	0.47	0.19	0.09	0.57	0.28	1.61
Pb 50	0.24	0.00	0.07	0.03	0.38	0.72
3.6 µg.l^{-1}Pb$(NO_3)_2$	0.70	0.00	0.20	0.10	1.10	2.11
Pb 75	0.15	0.07	0.04	0.11	0.30	0.67
5.4 µg.l^{-1}Pb$(NO_3)_2$	0.41	0.20	0.10	0.30	0.81	1.38
Pb 100	0.18	0.11	0.29	0.11	0.07	0.76
7.2 µg.l^{-1}Pb$(NO_3)_2$	0.50	0.30	0.80	0.30	0.20	2.11

Table 4. Frequency of chromosome aberrations, analized by means of *Allium* test relative to the total number of analyzed cells (on the first row) and the dividing cells (on the second row)

Samples	Cells with micro-nuclei	Asynchro-nous mitosis	Cells with chromo-somal fragments	Cells with chromo-somal bridges	Cells with lagging chromo-somes	Total number of cells with aberra-tions
Control	0.25	0.00	0.00	0.06	0.16	0.48
	0.37	0.00	0.00	0.09	0.23	0.70
Cd 25	0.33	0.03	0.06	0.03	0.33	0.77
0.1 µg.l^{-1} $Cd(NO_3)_2$	0.60	0.05	0.11	0.05	0.60	1.43
Cd 50	0.31	0.04	0.26	0.09	0.35	1.09
0.225 µg.l^{-1} $Cd(NO_3)_2$	0.49	0.07	0.42	0.14	0.55	1.73
Cd 75	0.29	0.12	0.20	0.20	0.38	1.20
0.3375 µg.l^{-1}$Cd(NO_3)_2$	0.47	0.19	0.33	0.33	0.61	1.94
Cd 100	0.37	0.03	0.49	0.09	0.32	1.30
0.45 µg.l^{-1}$Cd(NO_3)_2$	0.74	0.06	0.96	0.17	0.62	2.55

Discussion

Cytotoxic effect of lead

The suppression of mitotic activity is one of the indicators of cytotoxicity (SMAKA-KINCL *et al*., 1996). The results we have obtained show that lead has a depressive effect on cell proliferation, which is consistent with that reported by LIU *et al.* (1994) and KUMAR & SRIVASTAVA (2015). All concentrations of lead correlate with lower values of the mitotic index compared to the control, which is evidence of cytostatic effect (Table 1). This effect is probably due either to cell cycle disorders or chromatin dysfunction induced by the interaction between heavy metals and DNA (MESI & KOPLIKU, 2014). The highest lead depressive activity with respect to cell proliferation was recorded in samples Pb50, Pb75 and Pb100. We found the highest prophase index (88.37 - 91.66) in the phase indices in the control and experimental variants, indicating the longest duration of this phase of cell division.

Conclusions

Economic losses from eaten and damaged fish and the lack of compensation for fish producers from the government generate a negative attitude towards fish-eating birds in the study area, motivating fish farmers to exterminate piscivorous birds, opposing to nature conservation legislation.

Cormorants were the species with the highest potential threat for extermination from fish farmers.

Cytotoxic effect of cadmium

Analysis of the mitotic index and phase index results in control and experimental variants shows an inhibitory effect of cadmium on cell division (Table 2). We found the lowest degree of cell proliferation at the maximum admissible cadmium concentration (Cd100), which corresponds to the lowest mitotic index - 50.91. We recorded the largest number of dividing cells in the control variant, where the value of the mitotic index was higher (68.45) than all the experimental variants. We found a gradual decrease in the value of the mitotic index at all tested cadmium concentrations, except for sample Cd 25. This provoked a higher cytostatic effect of all tested concentrations except for the maximum admissible. According to BADR &

IBRAHIM (1987), lower mitotic index values indicate that the test compound inhibits the ability of cells to enter mitosis. Changes in normal cell cycle length may result from stopping DNA synthesis, blockage of processes in postsynthetic interphase period (TÜRKOĞLU, 2007), microtubule formation disorders or ATP levels decrease (JAIN & ANDSORBHOY, 1988). The reduction of the mitotic index in *Allium cepa* depending on the concentration of heavy metals was also found by other authors (CHANDRA *et al.*, 2005; TÜRKOĞLU, 2008; BABU *et al.*, 2008).

Genotoxic effect of lead

The substantial increase in the frequency of dividing cells with aberrations is considered to be one of the reliable indicators of the genotoxic potential of the test compounds (RANK, 2003). Table 3 shows the frequency of each type of chromosome aberration compared to the number of cells analyzed and the number of dividing cells. The data show a high percentage of abnormal mitotic cells in all experimental groups. The total incidence of chromosomal aberrations in the experimental variants exceeded their control frequency significantly, with the highest values reported in the Pb100 samples. The disorders related to the formation and functions of the division spindle are the most common type. We found the presence of lagging and "vagrant" chromosomes (Fig. 1, a) and cells with asynchronous mitosis (Fig. 1, b). We also observed anaphase and telophase bridges (Fig. 1, c and 1, d) and micronucleus cells (Fig. 1, e). Formation of micronuclei is considered an important cytogenetic indicator for a mutagenic effect. They are formed from fragments and lagging chromosomes that are not included in the daughter nuclei during telophase and may cause cell death (MA *et al.*, 1995). In control cells, we found aberrations that result from auto-mutagenic effect and have a lower incidence. The Pb50 solution provoked deviations and aberrations with an incidence exceeding the control and that of the Pb25 and Pb75 experimental variants. At this concentration, we recorded the most cells with lagging chromosomes. Among the aberrations in Pb25-treated cells, the anaphase bridges and the micronucleus prevailed. In some of the Pb25, Pb75 and Pb100-treated cells, we observed asynchrony with regard to the despiralization of chromosomes and the formation of the two daughter nuclei. We also recorded cells of varying degrees of compaction of the genetic material in the segregating chromosomal complexes. The largest number of cells with abnormalities was found in samples with Pb100 concentration, as we recorded micronucleus cells, asynchronous mitosis, chromosomal bridges and fragments (Fig. 1, c, d). The results obtained in this study indicate that lead has a cytostatic effect on *Allium cepa* cells and has a mutagenic effect causing chromosomal aberrations and disorders during mitosis.

Genotoxicity of cadmium

The cadmium induced genotoxic effect is assessed by the frequency of chromosomal disorders in the treated cells. We recorded various deviations, ranging from chromosomal fragmentation to disorganization of the division spindle, and therefore of all subsequent phases of the mitotic cycle. Table 4 shows the relative frequency of chromosomal aberrations found against the number of cells analyzed and the number of dividing cells. As can be seen from the data presented, cadmium exhibits genotoxic effect on *Allium cepa* meristem cells, inducing high frequency disturbances. In all experimental variants, the chromosome structural change rate was higher than in the control. We detected an auto-mutagenic effect in the control variant, with predominance of "vagrant chromosomes" type and micronuclei. We established a direct proportionality between the concentration of cadmium solutions and the frequency of aberrations and deviations during mitosis. The highest percentage of structural chromosomal deviations were reported in experimental variant Cd100 - mainly fragments (Fig. 2, a), "vagrant chromosomes" (Fig. 2, b) and micronuclei

(Fig. 2, c). From the chromosomal aberrations found, micronuclei were predominant in all samples, which were evidence of structural changes occurred leading to the formation of acentric chromosomes and fragments (Fig. 2, d). An increased number of micronuclei in *Allium cepa* cells as a result of the clastogenic effect of heavy metals has also been reported by CHANDRA *et al.* (2005) and TÜRKOĞLU (2008). In all experimental variants we found intact and broken anaphase bridges (Fig. 2, e) as well as cells with "vagrant" chromosomes and fragments (Fig. 2, f). The Cd75 solution provoked the greatest number of aberrations of the type of lagging chromosomes and chromosomal bridges (Fig. 2, e). Chromosomal bridges arise as a result of chromosomal and/or chromatid break and subsequent adhesions (LEME & MARIN-MORALES, 2009). Among structural chromosomal alterations in Cd50-treated cells prevail acentric fragments, micronuclei and "vagrant" chromosomes (Fig. 2, f).

The analysis of the obtained results shows that cadmium has a pronounced cytotoxic and genotoxic effect on the onion root meristem, which is evidenced by the lower mitotic index and the higher frequency of chromosomal aberrations recorded in the experimental variants.

Analyzing the results obtained for the two heavy metals tested, the following findings can be made: the *Allium cepa* test is successfully applied to assess the induced cyto- and genotoxicity of heavy metals lead and cadmium by taking into account changes in cell division rate, the formation of the cell division apparatus and the integrity of the chromosomes, which is consistent with the findings of other authors (FISKEJSÖ, 1988; WIERZBICKA & ANTOSIEWICZ, 1993; LIU *et al.*, 1995; PALACIO *et al.*, 2005; KOPLIKU & MESI, 2013).

Conclusions

Heavy metals lead and cadmium have a cytostatic effect on the *Allium* test system, reducing the intensity of cell division. In all analyzed concentrations, a lower mitotic index was established compared to the control variant.

A genotoxic effect of lead, expressed by the higher frequency of chromosomal aberrations recorded in the experimental variants, was reported. The induced disorders are of the type: chromosomal bridges, fragments, lagging and "vagrant" chromosomes, asynchronous mitosis and micronuclei.

Heavy metal cadmium induces a wide range of cytogenetic abnormalities (chromosomal bridges, micronuclei, lagging and "vagrant" chromosomes and fragments) with a higher incidence in the test samples than the control which is evidence of a genotoxic effect. There is a tendency to increase the incidence of chromosomal aberrations in proportion to the cadmium concentration in the experimental solutions.

The comparative analysis of the effects of the two heavy metals shows a greater cytostatic effect of lead, expressed in a significant decrease in the value of the mitotic index and a more pronounced genotoxic effect of cadmium inducing chromosomal aberrations with a higher frequency.

References

BABU K., M. DEEPA, S. SHANKAR, S. RAI. 2008. Effect of Nano-Silver on Cell Division and Mitotic Chromosomes: A Prefatory Siren. - *The Internet Journal of Nanotechnology*, 2 (2): 25-29.

BADR H., A.G. IBRAHIM. 1987. Effect of herbicide on mitosis chromosome and nuclei acids in *A. cepa* and *V. faba* root meristem. - *Cytologia*, 52: 293-302. [DOI]

CHANDRA S., L.K.S. CHAUHAN, R.C. MURTHY, P.N. SAXENA, P.N. PANDE, S.K. GUPTA. 2005. Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. - *Science Total Environment*, 347:46–52. [DOI]

FISKESJO G. 1985. The *Allium* test as a standard in environmental monitoring. – *Hereditas,* 102: 99-112. [DOI]

FISKESJO G. 1988. The *Allium* test - an alternative in environmental studies: the relative toxicity of metal ions. - *Mutation Research*, 197(2): 243-260. [DOI]

GLINSKA S., M. BARTCZAK, S. OLEKSIAK, A. WOLSKA, A. GABARA, M. POSMYK, K. JANAS. 2007. Effects of anthocyanin-rich extract from red cabbage leaves onmeristematic cells of *Allium cepa* L. roots treated with heavy metals. - *Ecotoxicology and Environmental Safety*, 68: 343–350. [DOI]

IVANOVA E., T. STAIKOVA, I. VELCHEVA. 2005. Cytogenetic testing of heavy metal and cyanide contaminated river waters in a mining region of Southwest Bulgaria. - *Journal of Cell and Molecular Biology*, (4): 99-106.

JAIN A.K., R.K. ANDSORBHOY. 1988. Cytogenetical studies on the effects of some chlorinated pesticides III. Concluding Remarks. – *Cytologia*, 53: 427-436.

KOPLIKU D., A. MESI. 2013. Assessment of cytotoxic and genotoxic potency of Cr (VI)-doped river water of Nën-Shkodra lowland, Albania, on *A. cepa*. - *Journal of Environmental Research and Development*, 7(4): 1322-1332.

KUMAR G., A. SRIVASTAVA. 2015. Clastogenic and mito-inhibitory effect of heavy metals in root meristems of *Vicia faba* G. - *Chromosome Botany*, 10 (1): 23-29. [DOI]

KUNGOLOS A. G., C. A. BREBBIA, C. P. SAMARAS, V. POPOV. 2006. *Environmental Toxicology*. Southampton: WIT Press.

LEME D. M., M.A. MARIN-MORALES. 2009. *Allium* test in environmental monitoring: A review on its application. - *Mutation Research*, 682: 71-81. [DOI]

LIU D., W. JIANG, W. WANG, L. ZHAI. 1995. Evaluation of metal ion toxicity on root tip cells by the *Allium* test. - *Israel Journal of Plant Sciences*, 43(2): 125-133. [DOI]

LIU D.H., W.S. JIANG, W. WANG, F.M. ZHAO, C. LU. 1994. Effects of lead on root growth cell division and nucleolus of *Allium cepa*. - *Environmental Pollution*, 86: 1-4. [DOI]

MA T. H., Z. XU, C. XU, H. MCCONNELL, V. E. RABAGO, A. G. ARREOLA, H. ZHANG. 1995. The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. - *Mutation Research*, 334(2): 185-195. [DOI]

MA T.H. 1999. The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China. - *Mutation Research*, 426(2): 103-106. [DOI]

MESI A. D., D. KOPLIKU. 2014. Toxic Potency Evaluation of Metal-Doped River Water (Cr, Cu and Pb), on *Allium cepa* L. - *Athens Journal of Sciences*, 1(4): 255-267. [DOI]

PĂDUREANU S. 2005. Cytogenetics effects induced by nitrate of lead on mitotic division at Allium cepa L. - *Anale şt. Univ. "Al.I. Cuza" Iaşi, Genetică şi Biologie moleculară*, 6: 201-206.

PALACIO S.M., F.R. ESPINOZA-QUIÑONES, R.M. GALANTE, D.C. ZENATTI, A.A. SEOLATTO, E. K. LORENZ, C.E. ZACARKIM, N. ROSSI, M.A. RIZZUTTO, M.H. TABACNIKS. 2005. Correlation between heavy metal íons (Copper, Zinc, Lead) concentrations and root length of *Allium cepa* L. in polluted river water. - *Brazilian Archives of Biology and Technology*, 48: 191-196. [DOI]

RANK J. 2003. The method of *Allium* anaphase-telophase chromosome aberration assay. - *Ekologija (Vilnius)*, 1:38–42.

SMAKA-KINCL V., P. STEGNAR, M. LOVKA, M.J. TOMAN. 1996. The evaluation of waste, surface and ground water quality using the *Allium* test procedure. - *Mutation Research*, 368: 171–179. [DOI]

STAYKOVA T., E. IVANOVA, G. PANAYOTOVA, I. CVETKOVA, S. DZHOGLOV, B. DZHAMBAZOV. 2010. General toxicity and genotoxicity of Nodularia moravica (Cyanoprokaryota, Nostocales). - *Biotechnology & Biotechnological Equipment*, 24/SE: 397-400. [DOI]

TÜRKOĞLU Ş. 2007. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. - *Mutation Research - Gene-*

tic Toxicology and Environmental Mutagenesis, 626: 4-14. [DOI]

TÜRKOĞLU Ş. 2008. Evaluation of genotoxic effects of sodium propionate, calcium propionate, and potassium propionate on the root meristem cells of *Allium cepa*. - *Food and Chemical Toxicology*, 46: 2035–2041. [DOI]

ÜNYAYAR S., A. ÇELIK, F.O. ÇEKIÇ, A. GÖZEL. 2006. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. – *Mutagenesis*, 21(1): 77-81. [DOI]

WIERZBICKA M., D. ANTOSIEWICZ. 1993. How lead can easily enter the food chain - a study of plant roots. - *Science of the Total Environment Suppl. 1*: 423-429. [DOI]

ŽEGURA B., E. HEATH, A. ČERNOŠA, M. FILIPIČ. 2009. Combination of *in vitro* bioassays for the determination of cyto- and genotoxic potential of wastewater, surface water and drinking water samples. – *Chemosphere*, 75: 1453-1460. [DOI]

Received: 31.10.2018
Accepted: 21.12.2018