LEAD-INDUCED GENOTOXICITY IN WHEAT

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Keywords. Lead acetate, lead nitrate, chromosome aberrations, genotoxicity
Abstract. The changes induced in cytogenetic parameters from root meristems of *Triticum aestivum* cv. *Maruca* seedlings have been studied after treatment with lead acetate and lead nitrate solutions, at four concentrations (10, 25, 50, 100 µM) containing 2.07, 5.18, 10.36, respectively 20.72 µg ml^{-1} Pb^{2+}. Lead induced mitosis disturbances in root meristematic cells of wheat seedlings, expressed mainly in decrease of mitotic index and changes in preponderance of division phases. This heavy metal has genotoxic effects, expressed in the occurrence of many chromosomal aberrations in all Pb^{2+} treated variants. Pb^{2+} nitrate shows a more pronounced genotoxic potential than lead acetate trihydrate.

INTRODUCTION

Lead is naturally found in small amounts in the earth crust and is largely used in the production of containers of foods, stills, batteries, paints, and leathers. Human activities like burning of fossil fuels, mining, and manufacturing are lead sources. Its use as tetraethyl and tetra methyl additives in gasoline to increase octane rating has transformed lead into one of the metals of high toxic risk. In 1965 – 1990 lead consumption increased in the world to 5.6 x 10^6 tones, its concentration in biosphere being 1,000 – 100,000 times higher than the natural level. Since the half-life in biological systems is one of the longest among metals (150 – 5000 years), the consequences of lead pollution can be devastating. Increases in lead content of soils are registered near industrial areas. Lead-contaminated soils induce diminutions in crop productivity.

The primary effect of lead toxicity in plants is a rapid inhibition of root growth, probably due to inhibition of cell division in root tips. Important alterations have been reported in the structure, biochemistry and physiology of plant cells in lead excess. In *Helianthus annuus* L., Pb^{2+} showed the highest phytotoxicity comparatively with Al, Cd, Cu, Ni, Pb and Zn (Chakravarty and Srivastava, 1992). This metal denatures the proteins (Rathore et al., 2007) and alters photosynthesis (Akinci *et al.*, 2010). It causes changes in lipid composition of thylakoid membranes and modifies membrane permeability (Stefanov *et al*., 1995). Root elongation, plant growth, seed germination, transpiration, photosynthesis, mineral nutrition, plant water status and enzymatic activities can be also negatively influenced by lead treatment (Pinero *et al.*, 2002; Kaznina *et al*., 2005; Akinci *et al*., 2010; Jiang and Liu). Increasing concentration of Pb reduced DNA, RNA and protein synthesis in embryo axis and endosperms of germinating rice seedlings (Maitra and Mukherji, 1977).

Some sources (Carruyo *et al*., 2000) consider lead as probable carcinogen for humans, but relatively few data are available on lead genotoxicity in plants and positive as well as negative results have been registered on genotoxic potential of lead in these biological systems, so the mechanisms of lead-induced genotoxicity still need more experimental research. Lead binds strongly to a large number of molecules including DNA and RNA; it disrupts DNA synthesis and alters the transcriptional process and mitotic activity. Genome alterations consist in depolymerizations, generation of abnormal nitrogenous bases, DNA strand breaking, DNA – DNA cross-links, DNA – protein cross-links. DNA damage may result in the production of abnormal bases such as thymine glycol and 8-hydroxyguanine or to strand breakage through a series of reactions initiated by the abstraction of a 4'-hydrogen atom from a ribose residue (Babior, 1997). Indirectly, like other heavy metals, lead can inhibit DNA repair enzymes or DNA replication; consequently they can act as co-clastogens or co-mutagens. This heavy metal binds to SH groups of cell tubulins, modifying the typical arrangement of metaphase chromosomes (Liu *et al*., 2009).

Wheat is a plant of a worldwide economic importance, a main link in trophic chain and a pathway of pollutant ingestion for animals and humans and, like other plant systems, it can be used as plant test in the quantification of effect induced by various xenobiotic factors on its genetic material. The main objective of the present investigation is to evaluate the genotoxic potential of Pb^{2+} provided as lead acetate and lead nitrate, in *Triticum aestivum* L. cv. *Maruca*, by analyzing the frequency and types of mitotic chromosome disturbances in wheat root tips.

MATERIALS AND METHODS

Biological material is represented by wheat seeds (*Triticum aestivum* L. cv. *Maruca*), Agricultural Research Station, Podu Iloaie, Romania). The seeds were 4 h treated with:

1. *Lead acetate trihydrate* $Pb(C_2H_3O_2)_2.3H_2O$, mol. weight=379.33 g/mol
2. *Lead nitrate* $Pb(NO_3)_2$, mol. weight=331.20 g/mol

Four concentrations (10 µM, 25 µM, 50 µM, 100 µM) were prepared for each lead compound and were used for seed treatment. The lead concentration (µg ml^{-1}) in each solution is presented in Table 1. In control, distilled water was used.

Table 1. Lead concentration in tested solutions.

variant	molar concentration of salt solution	lead concentration ($\mu g\ ml^{-1}$)
Control – distilled water		
Lead acetate trihydrate	10 µM	2.07
	25 µM	5.18
	50 µM	10.36
	100 µM	20.72
Lead nitrate	10 µM	2.07
	25 µM	5.18
	50 µM	10.36
	100 µM	20.72

For *cytogenetic analysis*, wheat roots (10 – 15 mm in length) were firstly fixed for 24 h in ethyl alcohol:glacial acetic acid (3:1, v/v), at room temperature, then washed and stored in 70% ethyl alcohol. The plant material was 10 min hydrolyzed in 50% HCl, and then stained in modified charbol fuchsin solution. To prepare the slides – five for each variant - the meristematic regions were carefully squashed into a drop of 45% acetic acid. 10 microscopic fields were microscopically analyzed on every slide. A Nikon Eclipse 600 light microscope was used for this analysis. Photos were taken with a Nikon Cool Pix 950 digital camera, 1600x1200 dpi resolution.

Mitotic indices (MIs), frequencies of mitotic phases (prophase, metaphase, anaphase and telophase indices), ana-telophase chromosome aberrations and metaphase abnormalities were calculated and were used as endpoints for determination of lead-induced genotoxic effects.

These indicators were calculated according to the following formulas:

Mitotic Index = TDC x100/TC

PI% = prophase cells x 100/TDC

MeI% = metaphase cells x 100/TDC

AI% = anaphase cells x 100/TDC

TC% = telophase cells x 100/TDC, where

TC = total (dividing and non-dividing) cells, and TDC = total dividing cells.

The percentages of ana-telophase chromosome aberrations ($A\text{-}T_{CA}\%$) and of metaphase abnormalities ($M_{abn}\%$) were also calculated in relation to the number of cells in mitosis:

$A\text{-}T_{CA}\% = A\text{-}T_{CA} \times 100/TDC$

$M_{abn}\% = M_{abn} \times 100/TDC$

RESULTS AND DISCUSSIONS

Behaviour of mitotic index. Mitotic index is a reliable predictor of cell proliferation in tissue indicating the frequency of dividing cells. Based on the gravity of effects induced on mitosis, Patra *et al*. (2004) classified the heavy metals in three groups. Pb^{2+} is included in the category of relatively low active metals, together Mg, V, As, Mo, Ba.

In this experiment, we found that Pb^{2+} induces mitosis disturbances in root meristems of wheat seedlings (Fig. 1). The lowest tested concentrations of lead acetate and lead nitrate (10 µM) have slight stimulatory effect on cell division (8.58±0.42 %, respectively 8.83±0.34 %), comparatively to untreated control (8.22±0.62, in terms of %). All the other concentrations – except 50 µM lead acetate – influenced in a negative manner the mitotic index. Other studies also confirmed mitodepressive and mitotoxic effects of lead in different plant species (Samardakiewicz and Wozny, 2002; Glinska *et al*., 2007; Rathore *et al.*, 2007). No direct correlation between lead concentration and MI was established in our experiments. Carruyo *et al*. (2008) found that the correlation between lead content and mitotic index was not significant ($p>0.05$) and that the exposure time is a more important factor in induction of mitosis disturbances than lead concentration.

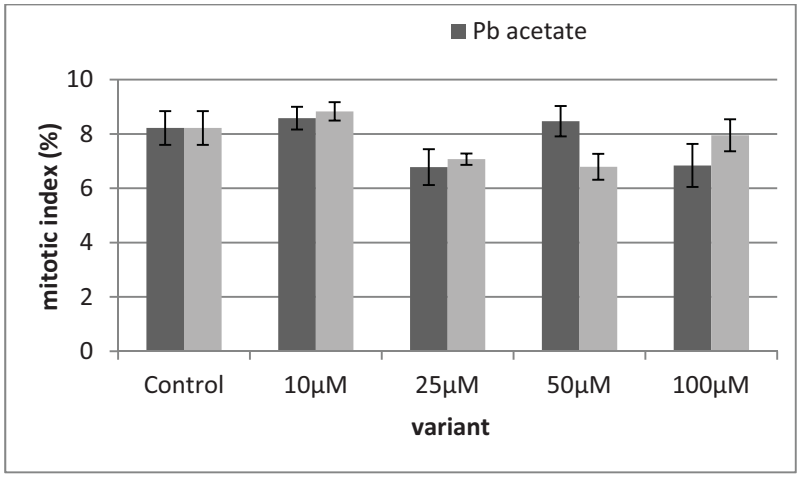

Fig. 1 Graphic representation of evolution of mitotic index (x±SE) in wheat root meristems, after lead treatment (x±SE).

The precise action of lead on cell division is still incompletely known. It is possible that higher MI values in Pb^{2+} exposure to result from extending mitosis rather than from promoting cell entrance into mitosis, while its depression can result from the inhibition of DNA synthesis or of some proteins essential for mitotic cycle (Wozny and Jerczynska, 1991).

Another Pb^{2+}-induced change consists in percentage modification of division phases (Wierzbicka, 1999; Glinska *et al.*, 2007). In this study, in wheat root meristems a decrease of prophase index was induced in all lead-treated variants, whereas metaphase index had values generally close to control (Fig. 2). The decline of prophase index could be a proof that lead prevents mitosis beginning, by stopping interphase cells to enter into prophase. Concerning metaphase index, only in 25 µM lead acetate and 25 µM lead nitrate treated variants this parameter shows slight increase comparatively to control. Ana-telophase indices are higher than control in all variants, but significant increases (over 35%) exhibited those variants exposed to 100 µM lead acetate, 25 µM and 50 µM lead nitrate. Higher values of metaphase and ana-telophases indices could be the effect of lead action on division spindle resulting in stop of division in these stages.

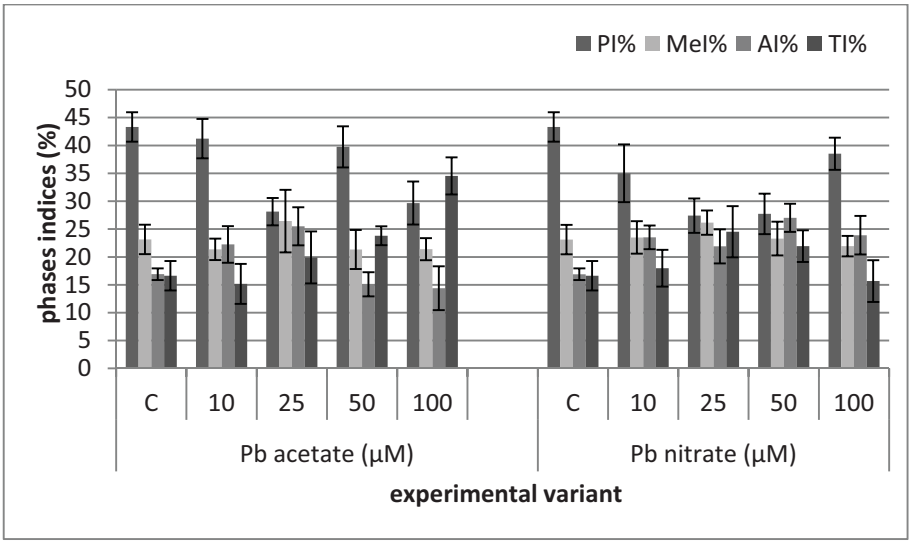

Fig. 2. Graphic representation of frequency of division phases in wheat root meristems, depending on lead compound and lead concentration (x±SE).

Metaphase disturbances. Lead is, at certain doses, an effective turbagen due to the affinity for thiol groups, inducing various types of spindle disturbances (Patra *et al*., 2004). In our study, total aberrant metaphases registered smaller values than control in variants treated with lead acetate (Fig. 3).

In the case of lead nitrate, the minimum tested concentration has values close to control, but the other three surpass control group, slightly at 50 and 100 µM lead nitrate and significantly for 25 µM lead nitrate, where the percentage of anomalous metaphases is 1.80 times higher than control**.** The abnormal metaphases with abnormal configurations are mainly represented by *metaphases with one or more expelled chromosomes* from equatorial plate and *C-metaphases*. Delayed centromere division can induce colchicine-like chromosome configurations which can result in the formation of cells with elevated degrees of ploidy. C-metaphases, result of spindle inactivation, are followed by chromosome scattering in cell. The metaphases showing expelled chromosomes from equatorial plate are numerically predominant, in all lead treated variants.

*Ana-telophase chromosome aberrations***.** Relatively few data exist on lead influence on plant genetic material. Lead is genotoxic itself or it enhances the effect of other DNA-damaging agents. Generally, at high Pb^{2+} doses the antioxidative capacity of plant systems is overcame and the generated ROS can combine with DNA, determining unreliable inter-cross connections and duplications in DNA which result in chromosome aberrations. Pb^{2+}-induced clastogenic effects can cause cell death due to the blocking of genome repair enzymes; the cell cannot recover from the produced damage and thus the number of aberrations increases as a new cycle begins.

Although lead is considered to be rather a co-mutagen or a weak genotoxic agent, our results reflect a considerable genotoxic potential of this metal (Fig. 4), fact confirmed too for other species (Wierzbicka, 1999; Mansour and Kamel, 2005; Glinska *et al.*, 2007). The clastogenic effect was materialized in induction of an important number of ana-telophase chromosome aberrations in wheat root meristems for both compounds and at all tested concentrations. This increase may be explained by preponderant accumulation of lead in plant roots - about 90% of

Pb^{2+} is accumulated in roots, only a small fraction of lead being translocated upward to the shoots and other plant parts (Patra *et al*., 2004; Gichner *et al*., 2008; Jiang and Liu, 2010).

In this study, Pb^{2+} concentration is the same in the two compounds, at correspondent variants (Table 1), but the effect amplitude is different. In lead acetate, the frequency increase is moderate (1.1 – 1.7 times higher than control), whereas in lead nitrate variants the genotoxic effect is higher - the frequency of chromosome aberrations observed in mitotic ana-telophases exceeds 1.7 – 2.0 times the control value. For lead acetate, the 25 µM-treated variant exhibits the highest percentage of ana-telophase aberrations (21.11 %), while for lead nitrate the 50 µM-treated variant shows the most numerous chromosome aberrations (24.86 %). 100 µM lead nitrate variant has 3.06 times more complex aberrations so confirming the strong genotoxic effect of this heavy metal; 10 µM lead acetate variant also shows a percentage of complex aberrations 2.0 times higher than control.

A variety of chromosome abnormalities was registered in wheat root tips (Fig. 5), fact indicating the damage amplitude at chromosome level: laggards, bridges, multipolar A-T, A-T with expelled chromosomes as well as an important number of complex aberrations (A-T with bridges and laggards, A-T with bridges and expelled chromosomes, multipolar A-T with chromosome bridges and expelled chromosomes, A-T with expelled and lagging chromosomes etc). In all variants, the highest preponderance belongs to chromosome bridges followed by laggards. This is a proof that lead alters the normal function of mitotic spindle, so that the chromosome movement to the cell poles is disturbed. Laggards are a potential source of aneuploidy because they lost the ability to attach by spindle fibres; they do not participate to the normal division and cause genetic disequilibriums between daughter cells. The complex chromosome aberrations have more severe repercussions at genetic level and on subsequent plant growth and development.

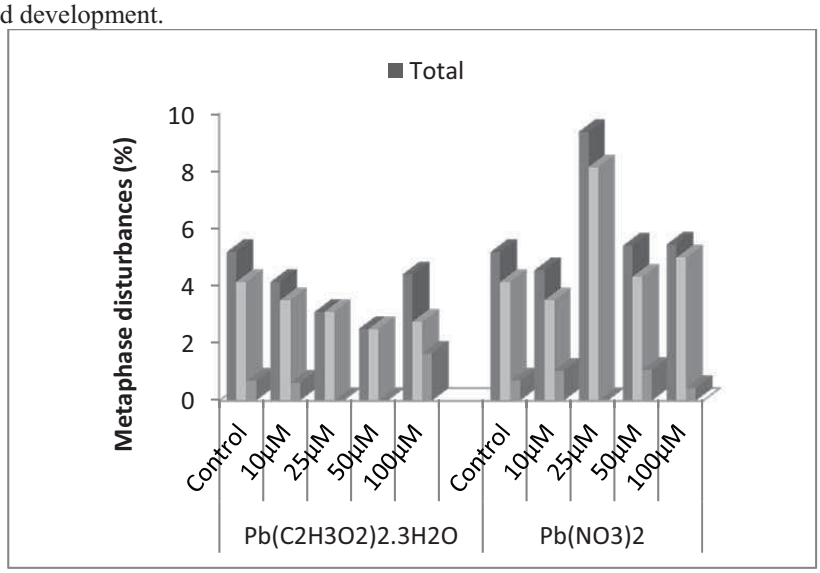

Fig. 3 Percentual preponderance of main types of metaphase disturbances in the total of abnormal metaphases, after lead treatment.

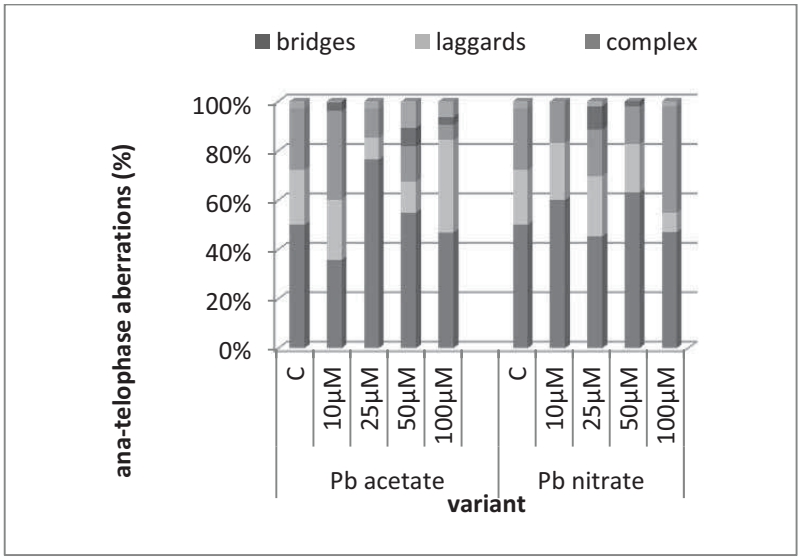

Fig. 4 Percentual preponderance of main types of ana-telophase chromosome aberrations in the total of aberrant ana-telophases (considered 100 %), after lead treatment.

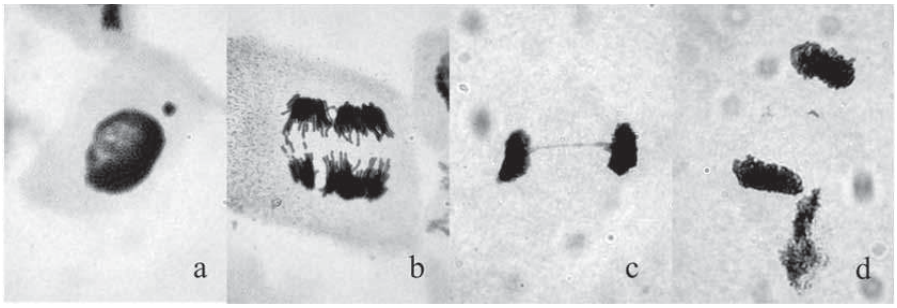

Fig. 5 Types of mitotic disturbances induced by lead in wheat root tip meristems. a. interphase with micronucleus (lead nitrate, 25 µM); b. tetrapolar anaphase with laggards (lead nitrate, 25 µM); c. telophase with bridge (lead nitrate, 10 µM); d. telophase with laggard (lead acetate, 10 µM).

Out of main categories of chromosome aberrations, micronuclei (1.43% in 50 µM Pb^{2+} acetate; 1.62% in 50 µM Pb^{2+} nitrate) (Fig. 5a), fragments, and polar deviations (in the variants treated with 50 and 100 µM Pb^{2+} acetate) represented other mitotic irregularities encountered in our study, but not at significant values. Insufficiently condensed chromatin material was observed in 50 µM Pb^{2+} acetate, 50 µM and 100 µM Pb^{2+} nitrate. Mainly in variants treated with lead nitrate lysis zones of chromatin material and dissolution of chromosomes have been evidenced.

Based on obtained results, we can conclude that lead nitrate was more clastogenic than lead acetate but the aberration frequency was not concentration dependent. If some literature data establish a gradual increment of chromosome aberrations with lead concentration increase (Kumar and Tripathi, 2008, in grass pea), in other approaches a Pb^{2+} dose-dependent relation was described only at small concentrations of metal, whereas at higher Pb^{2+} concentrations a

significant decrease of DNA damage was observed such as in lupin after Pb^{2+} nitrate treatment (Rucinska *et al.,* 2004) or in Pb^{2+} acetate treated tobacco plants (Gichner *et al*., 2008).

Amplitude of lead-induced genotoxic effects depends on metal oxidizing state, exposure duration, the plant parts used for exposure, metal concentration, pH of solution, lead compound type, plant species, features of chromosome set (Patra *et al*., 2004; Azmat and Haider, 2007). The pathways of lead genotoxicity may involve the interaction of Pb^{2+} with DNA, either directly or indirectly *via* oxidative stress, but the mechanism of this interaction is not fully understood (Cenkci *et al*., 2010).

According to some opinions, the chemical form of lead affects only transport of the heavy metal from medium into the plants and all forms had similar effects on mitosis (Patra et al., 2004). In plant systems *in vivo*, water solubility of the salt is of primary importance. The degree of dissociation and the availability of cations influence the aberration number. The lead compounds showing lower water solubility have greater toxic and mutagenic effects than those moderately soluble perhaps because the more soluble compounds dissolve completely in the solution and are supplied as ions, rather than molecules as in the cases of those low soluble (Radecki *et al.*, 1989). Although the water solubility of lead nitrate and lead acetate trihydrate, at 20ºC, is not strongly different [52 g/100 ml, for $Pb(NO_3)_2$; approximately 45 g/100 ml, for $Pb(C_2H_3O_2)_2.3H_2O$] (Hilber *et al*., 2001), and contrary to the opinions considering lead nitrate as a weak mutagen, the effects of lead nitrate concerning the rate of induced chromosome aberrations are more pronounced in our study than those induced by lead acetate. The high genotoxic effect of lead nitrate was also evidenced in other species by molecular studies using multiple biomarker systems such as random amplified polymorphic DNA (RAPD) profiles. These markers indicate that genomic template stability was significantly affected at all Pb^{2+} concentrations (Cenkci *et al*., 2010), in *Brassica rapa* L. Lead acetate is very toxic but the published results are inconsistent concerning its mutagen, clastogen and carcinogen effects.

In this study, heterogeneous responses have been obtained concerning the behaviour of cytogenetic parameters. Although the problem of Pb^{2+}-induced mutagen effects remains in discussion, many authors confirmed the genotoxic potential of this heavy metal, expressed in occurrence of chromosome aberrations and other mitotic disturbances, and their persistence during next generations. However, few works reported production of viable plants carrying lead-induced chromosome abnormalities (Kumar and Tripathi, 2008).

CONCLUSIONS

Pb^{2+} treatment caused mitosis disturbances in root meristematic cells of wheat seedlings, expressed mainly in decrease of mitotic indices and changes in preponderance of division phases. The occurrence of many chromosomal aberrations in all Pb^{2+} treated variants clearly indicates that this heavy metal has genotoxic effects in root meristems of wheat seedlings.

Lead nitrate shows a more pronounced genotoxic potential than lead acetate trihydrate in the studied wheat cultivar.

It was not established a direct relationship between Pb^{2+} concentration and aberration frequency.

REFERENCES

Akinci, I. E., Akinci, S., Yilmaz, K., (2010): *Response of tomato (Solanum lycopersicum L.) to lead toxicity: Growth, element uptake, chlorophyll and water content*. Afr. J. Agric. Res., 5(6), 416-423.

Azmat, R., Haider, S., 2007. *Pb stress phytochemistry of seedlings of Phaseolus vulgaris and Lens culinaris.* Asian J. Plant Sci., 6(2), 332-337.

Babior, B. M, (1997): *Superoxide: a two-edged sword*. Braz. J. Med. Biol. Res., 30(2): 141-145.

Carruyo, I., Fernández, Y., Marcano, L., Montiel, X., Torrealba, Z., (2008): Correlation of toxicity with lead content in root tip cells (*Allium cepa* L.). Biol. Trace Elem. Res., 125(3), 276-285.

Cenkci, S., Ciğerci. I. H., Yildiz, M., Őzay, C., Bozdağ, A., Terzi, H., (2010): *Lead contamination reduces chlorophyll biosynthesis and genomic template stability in Brassica rapa L*. Environ. Exp. Bot., 67(3), 467–473.

Chakravarty, B., Srivastava, S., (1992): *Toxicity of some heavy metals in vivo and in vitro in Helianthus annuus*. Mutat. Res., 283(4), 287-294.

Gichner, T., Žnidar, I., Száková, J., (2008): *Evaluation of DNA damage and mutagenicity induced by lead in tobacco plants*. Mutat. Res., 652: 186–190.

Glińska, S., Bartczak, M., Oleksiak, S., Wolska, A., Gabara, B., Posmyk, M., Janas, K., (2007). *Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of Allium cepa L. roots treated with heavy metals*. Ecotox. Environ. Safe, 68(3), 343-350.

Hilber, T., Marr, R., Siebenhofer, M., Zapfel, W., (2001): *Solid/liquid extraction of zinc from EAF-dust.* Separ. Sci. Technol., 36(5-6), 1323 — 1333.

Jiang, W., Liu, D., (2010): *Pb-induced cellular defense system in the root meristematic cells of Allium sativum L.* BMC Plant Biol., 10, 40-47.

Kaznina, N. M., Laidinen, G. F., Titov, A. F., Talanov, A. V., (2005): *Effect of lead on the photosynthetic apparatus of annual grasses*. Biol. Bull., 32(2): 147–150.

Kumar, G., Tripathi, R., (2008): *Lead-induced cytotoxicity and mutagenicity in grass pea*. Turk. J. Biol., 32, 73-78.

Liu, D., Xue, P., Meng, Q., Zou, J., Gu, J., Jiang, W., 2009. *Pb/Cu effects on the organization of microtubule cytoskeleton in interphase and mitotic cells of Allium sativum L.* Plant Cell Rep., 28, 695-702.

Maitra, P., Mukherji, S., (1977): *Effect of lead on nucleic acid and protein contents of rice seedlings and its interaction with IAA and GA_3 in different plant systems*. Indian J. Exp. Biol., 17, 29-31.

Mansour, M. M., Kamel, E. A. R., (2005): *Interactive effect of heavy metals and gibberellic acid on mitotic activity and some metabolic changes of Vicia faba L. plants*. Cytologia, 70(3): 275-282.

Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., (2004): *Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance*, Environ. Exp. Bot., 2(3), 199-223.

Pinero, H. J. L., Maiti, R. K., Star, M. J. V., Diaz, G. G., Onzalez, A. N., Avila, M. L. C., Orough-Bakhch, R., (2002): *Effect of Pb and Cd on seedling growth, chlorophyll and protein content of common bean (Phaseolus vulgaris L.), alfalfa (Medicago sativa), avena (Avena sativa) and ryegrass (Lolium multiflorum) selected as hyper accumulator of heavy metal*. Crop Res., 3(3), 473-480.

Radecki, J., Banaszkiewicz, T., Klasa, A., (1989): *The effect of different lead compounds on mitotic activity of maize root tip cells*. Acta Physiol. Plant., 11(2), 125–130.

Rathore, H., Punyasi, R., Joshi, P., Rathore, D., Bhatnagar, D., (2007): *Studies on the reversal of lead induced mitostatic effect in Allium cepa root tip cells with myrobalan (fruit of Terminalia chebula, Retz, Combretaceae)*. Internet J. Altern. Med., 4(1), *http://www.ispub.com/ostia/*.

Rucińska, R., Sobkowiak, R., Gwóźdź, E. A., (2004): *Genotoxicity of lead in lupin root cells as evaluated by the comet assay*. Cell. Mol. Biol. Lett., 9, 519-528.

Samardakiewicz, S., Woźny, A., (2005): *Cell division in Lemna minor roots treated with lead.* Aquat. Bot., 83(4), 289-295.

Stefanov, K., Seizova, K., Popova, I., Petkov, V. L., Kimenov, G., Popov, S., (1995): *Effects of lead ions on the phospholipid composition in leaves of Zea mays and Phaseolus vulgaris*. J. Plant Physiol. 147, 243–246.

Wierzbicka, M., (1999): *The effect of lead on the cell cycle in the root meristem of Allium cepa L*. Protoplasma, 207, 186–194.

Wozny, A., Jerczynska, E. (1991): *The effect of lead on early stages of Phaseolus vulgaris L. growth in in vitro conditions*. Biol. Plantarum, 33(1), 32-39.

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Acknowledgements. This study was realized in the framework of project BIODIV 09-36-301/2009 from National Research Program of Romania - National Authority for Scientific Research.