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Cytogenetic effects induced by Manganese and Lead microelements on germination at *Allium cepa*

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ABSTRACT: The study gives insight into the effects of manganese and lead microelements treatment on germination of *Allium cepa* L. The cytogenetic effects were studied by the calculation of the mitotic index, by the study of the interphase and chromosomal aberrations on the mitotic cells. We used $MnSO_4$ and $Pb(NO_3)_2$ solutions with different concentrations: 0.0003, 0.003, and 0.03%. The *Allium* bulbs were preliminary imbued in water, and then they were treated for 12, 24 and respectively 72 hours in these solutions. The control group was treated with water. We prepared five cytological slides, for each slide we have studied 10 microscopic fields with good density of cells for the mitotic index and another 10 different microscopic fields for abnormal interphases and chromosomal aberrations.

In the analyzed meristematic cells we observed an almost totally inhibition of cell division and the mitotic index was smaller in comparison with the control variant. The study of the frequency of the cells in different phases of the mitotic division showed that the highest percent was registered by prophases, followed at distance by telophases. The radicles formed in $MnSO_4$ solutions were characterized by the presence of disorganized nuclei, with an unusual structure of the chromatin fiber and metaphases with expulsed chromosomes. In $Pb(NO_3)_2$ solutions there are observed anaphases with small bridge and telophases with small bridge and one micronucleus. We can conclude that the heavy metals Mn and Pb have a significant mutagenic activity *in vivo* upon the radicles of *A. cepa*.

Key words: cytogenetic effects, *Allium cepa* L., manganese, lead, chromosomal aberrations

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INTRODUCTION

Considering the present overextended anthropogenic activities there is a main qualitative difference among the natural environmental changes observed long time ago compared with those observed today. Today neither human nor other superior organisms do not find genetic solutions to fight against the polluting factors of antropic origin such as chemicals resulting from industry emissions and some of them never occurred in nature being considered xenobiotics.

Aside pesticides - the most important „stress indicators" which are especially used in agriculture, other very important indicators are heavy metals. The residual waters resulting from the galvanic industry contain a real "hurricane" of heavy metals such as: mercury, cadmium, zinc, copper, lead and chrome. Generally the water pollution sources for heavy metals are as following: galvanic industry, mining, metallurgy and car industry. Copper water pollution is especially due to viticulture as the copper sulphate is used for pests' control.

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Once the heavy metals are eliminated into the water they quickly reach the external tissues and organs of aquatic fauna. It is already very well known that they have a very low mobility and once they are introduced into ecosystems they are very slowly eliminated. Part of them is up-taken by the plants through the root systems and accumulated into their tissues being then consumed by the herbivores and later by the carnivores. Due to their low mobility, heavy metals are concentrating at every trophic stage as following: their level in plants being higher compared to the soil, for herbivores being higher compared to the plants and for carnivores being higher compared to the herbivores, the higher level being reached at the pics of the trophic chains, for raptors and humans. This is bioaccumulation and for each group of organisms, high levels of heavy metals being able to induce grave diseases which may lead to morbidity and disrupting the ecosystems equilibrium.

Lead is eliminated mostly as a result of burning gasoline, petrol and different dyes, affecting the central nervous system in humans, creating behaviour problems and convulsions, at higher levels being lethal. Lead is spearing no organ or system being the first incriminated in boosting or getting worse a series of diseases through diminishing the body resistance. Lead effects are usually irreversible.

Manganese is a nutritionally essential chemical element but also in certain conditions it can be potentially toxic. Manganese name is originating from Greek language meaning "magic" and this feature is still adequate because the scientists are still working to understand different effects of its deficiency and toxicity effects for living organisms. However, without doubt in high levels manganese is highly toxic causing a series of pathologies based on reactive oxygen species (ROS) generation.

Long term oxidative stress consequences in human where associated to the different diseases pathogenesis and toxicities namely atherosclerosis, diabetes, chronically inflammatory diseases, neurological disturbances and cardiovascular diseases. Manganese induces the oxidative stress in a time and concentration depending manner, according to the cytotoxic parameters measurements, lactate dehydrogenase and lipid peroxidation. Also, manganese may accumulate into the cell causing cytotoxic effects and cell destruction. Following different activity enzyme alteration and the alteration of gene expression the intracellular disruptions caused by manganese include DNA helix broken up, chromosomes destruction and lipid peroxidation (Brooks 1994).

Our research focused in detecting the mutagenic effects induced by heavy metals such as manganese (Mn) and lead (Pb) on higher plants using the cytogenetic analysis in *A. cepa* L. as plant indicator for heavy metals polluting degree in crops.

Heavy metals absorption and distribution processes were carefully studied in crops and heavy metals accumulation from soils are not following particular ways varying towards the metal and being influenced by the species and the plant organ too (Doroftei *et al.* 2008; Russel *et al.* 1984). The toxicity symptoms induced by heavy metals in plants are the results of some negative effects on physiological processes including: respiration and photosynthesis inhibition, water – plant relationship disruption, decreasing plasmalema permeability in root cells, adverse effects on the metabolic enzymes (Arduini 1994; Chardonneres *et al.* 1999; Ouzounidou 1994; Vangronsveld & Clijsters 1994; Vennitt & Parry 1984).

MATERIALS AND METHODS

The chemical effects on chromosomes are often studied on plant material such as root tips as they are easily produced through seed germination, the experiments may be conducted all over the year and are not costly (Bateman 1977; Tomulescu *et al.* 2004).

For studying the heavy metal effect on mitosis, we used solutions of $MnSO_4$ and $Pb(NO_3)_2$ in different concentrations (0.0003%; 0.003% and 0.03%) in which the *A. cepa* bulbs were submersed for 12, 24 and 72 hours. As control it was used tap water.

We preferred to use the *Allium* test as it is cheap, rapid and the very well prepared slides can be easily studied (Fiskejö 1982). Also, as the chromosomes belonging to *A. cepa* are relativelly large and in small number are easily to be observed under the light microscopy hence the root meristems contain a large number of dividing cells.

The meristematic tissue were cut in fragments of 2mm/2mm and were prefixed in tampons of sodium cacodylate 0.1N with glutaraldehyde 2.5%. After the prefixation the specimens were washed in tampon cacodylate (0.1N) and fixed in solution of osmium tetra-oxide 2%. The specimens were washed again in tampon cacodylate (0.1N) to remove the osmium excess and dehydrated in serial baths of alcohol of 30%, 50%, 70%, 90%, 95%, 100% 15-30 min each. The first three baths are executed at 4°C and the rest were carried out at the room temperature. After the dehydration the samples were kept overnight in a mixture of propylene oxide with epoxidic resins of the type Epon 812 and DMP-30 as a hardening agent in order to introduce the warm resin polymerization. Subsequently they were placed in plastic capsules, covered with Epon 812 and then placed for polymerization in sterilizers at 67°C for 48-60 hours.

The semifine sections were obtained with the aid of an Ultracut-R ultramicrotome and then were plucked out of the bath with a thin wire loop and put on a degreased

port-object blade. The coloration is made applying on the blade 2-3 drops of the solution of toluidine blue. Then the blades are set back in the thermostat at 60°C for 10-30min, are washed in flowing water, then in acetone (100%), and rapidly passed through xilol, blotted, assembled and examined under the photonic microscope. The slides were analyzed in light microscopy for the cytogenetic effects of heavy metals by calculating the mitotic index and revealing the chromosomal aberrations for different mitotic stages. A Novex Holland digit camera was used for taking photographs. In this study 5 slides per variant were analyzed and for each slide 10 microscopically fileds were used for mitotic index calculation and for chromosomal aberrations study.

RESULTS AND DISCUSSIONS

Analyzing the control untreated roots, it was revealed the normal feature of the chromosomes and also normal cell division behaviour with a mitotic index of 15.4%. Roots development was lower when the *Allium* bulbs were immersed into the tested solutions, according to macroscopical differences observed compared to the control. Thus, the treated roots were smaller and in a low number compared to the control. For the heavy metals variants in concentration of 0.03% and a period of 72h, the treated bulbs developed 10-15 roots compared to the 30-50 roots of the control.

The mitotic index significantly decreased especially in the case of 0.03% and 72h treatment duration for manganese and lead too, supporting the idea that cell division slowly progressed compared to the control (table 1). These microscopically observations are supported by those macroscopically (roots number and size).

The chromosomal aberrations are relatively diverse, being aleatory distributed and depending on manganese and lead concentration and treatment period. It was observed cells with big nuclei and unorganized features (fig.2F), interphases with micronuclei, cells with lagging chromosomes (figs.1C,1D,1E,2C,2F), expulsed and picnotic chromosomes with uncoiled and sticky aspect (figs.1A,1B,1C,2B) cells with telophase bridges (fig.2E) as a result of errors in chromosomes separation during anaphase (figs.1D,2D).

Table 1. Mitotic division phase's frequency in *Allium cepa* treated with different solutions of $MnSO_4$ or $Pb(NO_3)_2$

Experimental variant			Frequency of prophase cells	Frequency of metaphase cells	Frequency of anaphase cells	Frequency of telophase cells
-	Control		6.2	4.1	3.1	2.0
$MnSO_4$	12 h	0.0003%	1.5	1.0	0.5	0.4
		0.003%	1.1	0.6	0.4	0.8
		0.03%	1.1	0.6	0	0.3
	24h	0.0003%	1.0	0.9	0	0.8
		0.003%	1.1	0.5	0.4	0.3
		0.03%.	1.0	0.3	0.3	0
	72h	0.0003%	1.1	0.5	0.2	0.4
		0.003%	1.0	0.5	0	0.3
		0.03%	0.8	0.3	0	0.2
$Pb(NO_3)_2$:	12h	0.0003%	1.1	0.4	0.3	0.8
		0.003%	1.2	0.6	0	0.3
		0.03%	1.2	0.5	0.1	0.5
	24h	0.0003%	1.0	0.5	0.4	0.1
		0.003%	1.1	0.6	0.3	0.4
		0.03%	1.2	0.9	0	0.4
	72h	0.0003%	1.2	0.4	0.1	0.3
		0.003%	1.2	0.4	0	0.1
		0.03%	0.9	0.3	0	0

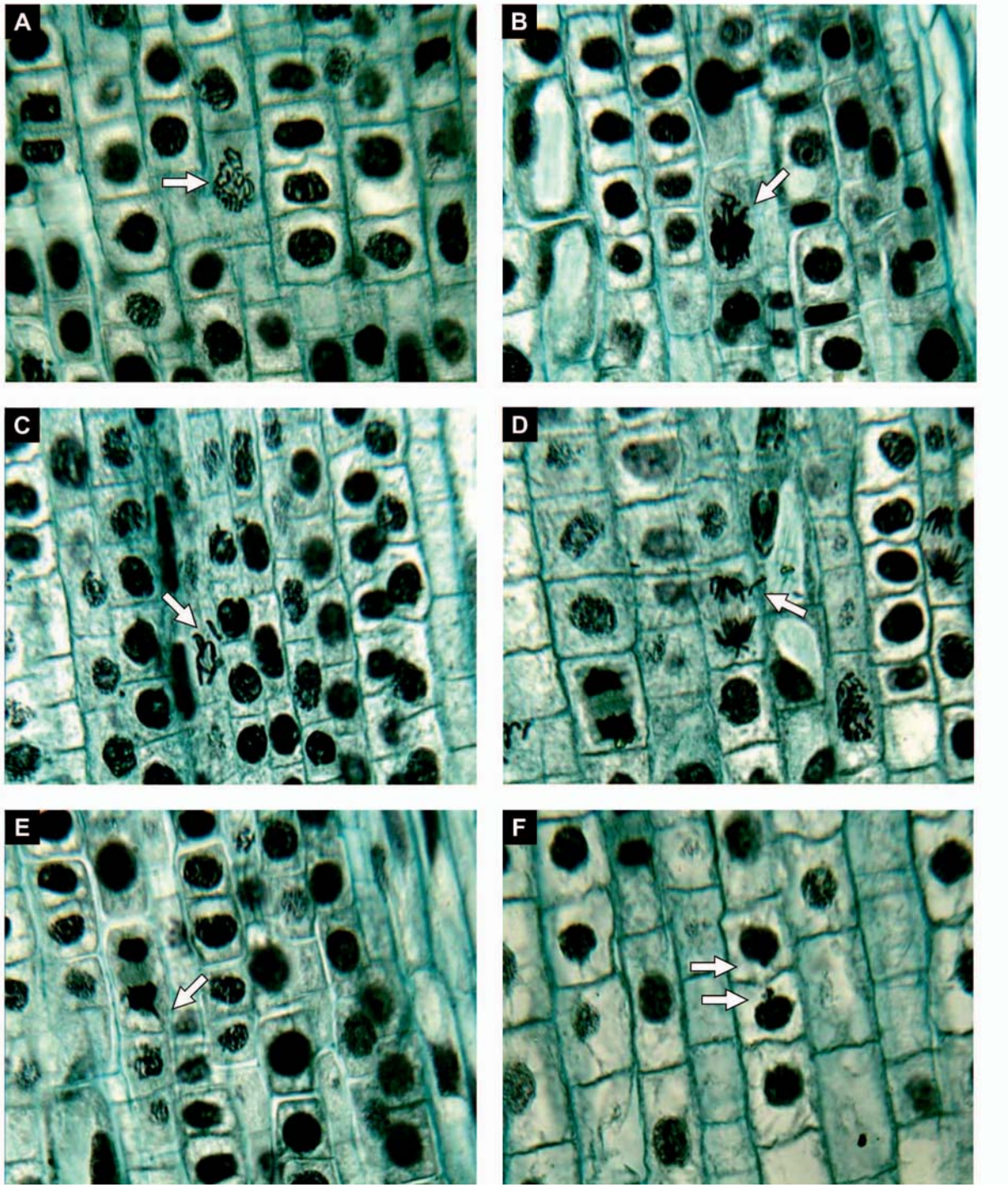

Fig.1 Root meristematic cells in *Allium cepa* treated with $MnSO_4$

A. Treatment with 0.003% for 24h, central prophase with picnotic chromosomes with uncoiled and sticky aspect.
B. Treatment with 0.03% for 24h, central prophase with picnotic chromosomes.
C. Treatment with 0.0003% for 24h, central methaphase with lagging and expulsed chromosomes.
D. Treatment with 0.003% for 24h, central anaphase cell with lagging chromosomes.
E. Treatment with 0.03% for 24h, central telophase with lagging chromosomes.
F. Treatment with 0.0003% for 72h, central cytokinesis with micronuclei.

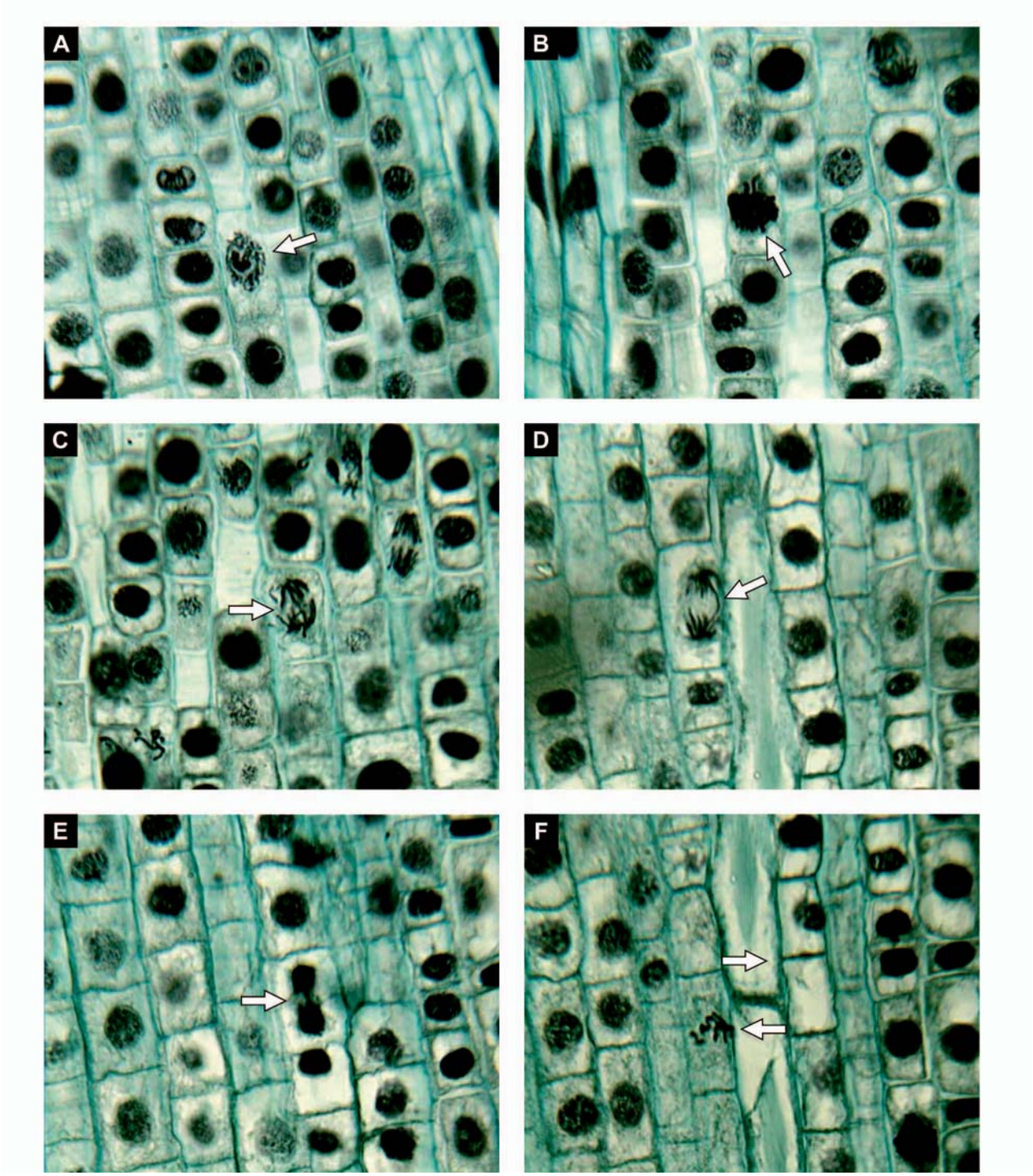

Fig.2 Root meristematic cells in *Allium cepa* treated with $Pb(NO_3)_2$

A. Treatment with 0.03% for 24h, central prophase with obviously hetero-chromatinisations.
B. Treatment with 0.003% for 24h, central promethaphase with picnotic chromosomes.
C. Treatment with 0.003% for 24h, central anaphase cell with lagging chromosomes.
D. Treatment with 0.003% for 72h, central cell with lagging chromosomes and anaphase bridges.
E. Treatment with 0.0003% for 72h, central telophase with a small telophase bridge.
F. Treatment with $Pb(NO_3)_2$ 0.003% for 72h, central metaphase with lagging chromosomes and elongated plasmolytic cells.

The treatment with $MnSO_4$ solution in concentration of 0.0003% and 0.003%, for a 72h treatment, it was observed cells with anaphase bridges due to some errors in chromosomes separation or to the joining of the ending parts belonging to sister chromatide suffering terminal deletions (figs.1D,2D). For a treatment with $MnSO_4$ solution in concentration of 0.03% for 12h treatment, the cells with big nuclei and unorganized and vacuolated features were observed. The un-organizing process is probably due to some disequilibrium occurred as a consequence of genetic material accumulation in a too big quantity. The treatment with $MnSO_4$ solution in concentration of 0.03% for 24h treatment it was observed that the majority of cells were in interphase and prophase (table 1) and after 72h of treatment, cell plasmolysis occurred for non dividing cells. These data support the idea that among heavy metals, manganese in large quantities impedes the normal roots growth for higher plants as a consequence of cell division negative effects induced for the meristematic cells in *A. cepa*.

The treatment with $Pb(NO_3)_2$ solution in concentration of 0.0003% and 0.003%, for 12 or 24h treatments, induced a decrease in mitotic division frequency (table 1). For a treatment with $Pb(NO_3)_2$ of 0.03%, for 72h a significant decrease cells in mitotic division frequency it was registered as a result of summing the effects of high concentration and long period of treatment (table 1).

For all manganese tested variants it were registered prophases and metaphases with picnotic chromosomes (figs.1A,1B,1C), the higher frequency of over 55% being observed for the concentration of 0.03% and 12h period of treatment. For this later variant, nuclei unregulated in shape and size were observed and chromosome appeared either big with a relaxed chromatin or small but presenting a compact chromatin and unregulated shape.

For lead too, for a concentration of 0.03% for 24h there were observed predominantly cells in interphase or prophase (table 1) and after 72h of treatment, the cell plasmolysis occurred in the non-dividing cells (fig.2F).

The studied heavy metals solutions may have according to our results the following negative effects:

- slowing down the cell division rate (figs.1,2);
- frequent cell degradation appearance (figs.1B,1D,1E,1F,2B,2D,2F);
- dehydration effect at cell level frequently inducing cell plasmolysis more drastically at 72h (figs.1F,2D,2F);
- heterochromatinization during prophase (fig.2A);
- changes in the nuclei shape becoming elongated (figs.1B,1D,2B,2F);
- degradation of the nucleic material in the completed destroyed cells (fig.2F).

In all variants, comparing to the control, a decreasing in the mitotic index was observed. We recorded the lack of cells in anaphase for the following variants: 3, 4, 8, 9, 11, 15, 17, and 18 and of cells in telophase for variants 6 and 18. For the control as well as for the treated variants the predominance of prophase and metaphases towards the anaphases and telophases was registered (table 1). The highest percentage of dividing cells is registered for the variant no. 1 (3.4%). The biggest number of cells was registered in prophase in variant 1(table 1). For metaphase the biggest number of cells was registered in variant 1 followed by in variants 4 and 15 (table 1).

CONCLUSIONS

Based on the results of this study we may conclude that:

- the heavy metals solutions used in this experiment have a great mutagenic effect on the root meristematic cells of *A. cepa*;
- after heavy metals solution treatment, the decrease in cell division in rate was recorded;
- heavy metals have a dehydration effect at cellular level;
- in all variants a decrease in the mitotic index compared to the control was observed;
- the mutagenic effects depend on the used heavy metals in the treatment and the treatment duration.

Cytogenetic tests on *A. cepa* reveal a decrease in mitotic index after the treatment with the heavy metals solutions. The mitosis analysis reveals the appearance of a low number of aberrations during interphases and chromosomal aberrations identified for different mitotic stages, the division process being significantly affected. These microscopically observations are also supported by the macroscopically observation: the root size and number being lower. These results revealed that the studied heavy metals present a significant mutagenic activity. The inhibition of mitotic division in the root apex induces the root growth inhibition as an active reaction of the plant, when plants are exposed to the action of heavy metals in soil. Heavy metal effects are more profound but they may become visible using further molecular techniques.

These results are sufficient serious arguments in the elaboration of prophylactic methods for pollution combating of surface land water, underground water as well as for grounding the protection measures for ecosystem maintaining.

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REZIME

Citogenetički efekat na klijanje crnog luka *Allium cepa* uzrokovan manganom i olovom

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Rade pruža uvid u uticaj mangana i olova na klijanje crnog luka *Allium cepa* L. Citogenetički efekat proučavan je na osnovu mitotičkog indeksa, proučavanja interfaze i hromozomskih aberacija.. Koriće je rastvor $MnSO_4$ i $Pb(NO_3)_2$ rayličitih koncentracija (0.0003, 0.003 i 0.03%). Lukovice *Allium* su pre tretmana dryane u vodi, a onda tretirane 12, 24 i 72 sata odgovarajućim rastvorom. Kontrolna grupa ostala je u vodi. Pet citoloških slajdova i 10 vidnih polja korišteno je za obradu podataka, računanje mitotičkog indeksa a drugih deset vidnih polja za posmatranje abnormalne interfaze i hromozomskih aberacija.

Kod analiziranih ćelija uočena je gotovo potpuna inhibicija deobe a mitotički indeks bio je manji nego kod kontrolnih biljaka. Najveći broj ćelija je bio u profazi, pa telofazi. Korenovi formirani u tretmanima sa rastvorima $MnSO_4$ karakterišu se prisustvom dezorganizovanih jedara, neobičnom strukturom hromatinskih niti i metafazom sa ekspulzovnim hromozomima. U tretmanu sa rastvorima $Pb(NO_3)_2$ uočavaju se neobična anafaza i telofaza i mikronukeus. Teški metali, mangan i olovo imaju mutagenu aktivnost *in vivo* na korenove *A. cepa*.

Ključne reči: citogenetički efekat, *Allium cepa*, mangan, olovo, hromozomske aberacije