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The effects of the lead ($PbCl_2$) on mitotic cell division of Anatolian Black Pine (*Pinus nigra ssp. pallasiana*)

Ersin YÜCEL[1], Ayşe HATİPOĞLU[*1], Emel SÖZEN[1], Şükrü Teoman GÜNER[2]

[1] Anadolu University, Faculty of Science, Depart. of Biology, 26450, Eskişehir, Turkey

[2] Forest Land and Ecology Research Institute, Eskişehir, Turkey

Abstract

In this study, the effects of lead ($PbCl_2$) one of the significant environmental pollutant, on mitotic divisions of Anatolian Black pine was investigated. Different concentrations (300µM, 500µM and 700µM Pb^{+2}) of lead were applied. Due to the increase of the lead concentrations, cell division was decreased, several mitotic anomalies such as c-mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges were increased.

Key words: Toxic, Lead, Mitotic cell division, Pinus, Pine

1. Introduction

Woody species can be very sensitive to moderate concentrations of heavy metals. These elements may reduce biomass accumulation in tree seedlings. Inhibit root growth, decrease the availability of essential elements and modify root morphology and architecture, compromising root capacity to explore soils . The excess or deficiency of essential metals may also inhibit protein and enzyme function, and thus impair photosynthetic electron transport at the reaction centers. Heavy metals may indirectly affect seedling performance by reducing plant ability to access and transport soil resources, particularly water (Fuentes et al., 2007).

Among non-nutrient heavy metals, Cd and Pb are the most widespread. Most of Pb and Cd contamination results from four human economic activities: burning liquid and solid fuels, smelting and foundry works, sewage high in Pd and Cd, and soil-applied chemicals, including fertilizers. Pb enter food chains mostly from plants, which often accumulate heavy metals to concentrations exceeding their levels in the soil by many times. Plant capacities to accumulate heavy metals and tolerate their abundance are species-specific traits. Based on these plant capacities, plant-derived technologies were worked out to decontaminate the environment of the heavy metals. In addition, the plants are used as indicators sensitive of soil pollution with heavy metals (Seregin at al., 2001).

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: bitkilerim@gmail.com

Uptake of this metal can cause destructive changes in plants, in particular, inhibition of root growth, which is considered to be one of the earliest morphological effects of metal toxicity (Samardakiewicz et al.,2005).

Pb produces mitotic disarrays, such as C-mitoses, resulting in a higher metaphase percentage. The result resembles the weak effect of colchicine. Pb causes disarray in the pattern of the mitotic cycle and produces chromosome aberrations, such as the development of micronuclei, chromosome bridges, chromosome stickiness, etc.. Other metal ions, such as Al^{3+}, Cr^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, and Hg^{2+} produce similar effects (Seregin et al., 2001).

The air and soil contaminations cause direct damage of the vegetative and generative organs of plants as well as influence physiological processes. Gaseous air pollutants, by acidification of soil, induce changes in its chemical and mechanical properties and release harmful metal ions, which in turn damage the soil microflora and mycorrhizal fungi. These adverse changes affect the vitality and fertility of trees. This is manifested in the case of *Pinus sylvestris* in decreased biomass increments, linked to a significant economical loss, decrease in seed production, and their diminished germination. When stress caused by the pollution exceeds a certain level, then individual trees and whole tree stands start to decline (Prus-Gowacki et al.,2006).

2. Materials and methods

The Black Pine (*P. nigra* ssp. *pallasiana*) seeds used in this study were collected from Anadolu University campus. The experiments were carried out in plant growth chambers (MLR-350 Model Sanyo, Japan). For the duration of the experiments a constant temperature (+25°C) and photo-period of 8 hours light, 16 hours darkness were maintained.

300µM, 500µM and 700µM concentration of lead chloride ($PbCl_2$) were prepared with distilled water. Planted petri dishes were filled with 9 ml of lead solution containing different lead concentrations. Control groups were filled with only distilled water. They were covered and kept into plant growth cabinet for 5 day.

The root tips of germinated seeds were cut and were fixed in acetic acid-alcohol (1:3) for 24h and were transferred in 70 % alcohol and stored in the fridge. For mitotic preparation, root tips were removed from alcohol and washed with tap water and hydrolised with 5 N HCl, at 60 °C for 15 min. Then it was dyed with Feulgen reactive for 1h. After that the root tips were kept in tap water for 15 min. Finally the last parts of root tips which dyed very densely were cut and their crushing preparates in 45 % acetic acid were made. Specimens were observed with a light microscope and photographs taken.

The results are expressed as means ± standard error. The data were compared with ANOVA test using a significant level of $p<0.01$.

3. Results

Mitotic index was decreased with inrease of lead concentration. Abnormalities in root tips exposed to lead and control group were observed. Mitotic division number was decreased in treatment groups according to control group in Table 1 and 2 ($p<0.05$). When compared with control group , mitotic index was significantly decreased by depending on concentration of lead in mitotic index results of treatment groups ($p<0.05$) (Table 3 and 4). In different lead concentration, a relationship was found second grade in groups (Figure 1). In different lead concentration, a relationship was found fisrt grade in groups (Figure 2).

Table 1. ANOVA results of mitotic division

	Sum of Squares	df	Mean square	F	Sig
Between Groups	37978,267	3	12659,422	19,233	,000
Within Groups	36860,133	56	658,217		
Total	74838,400	59			

Table 2. Mitotic division of treatment groups according to control group

Dunnett t testi (2-sided)[a]	2	1	-33,5*	9,37	,002
	3	1	-52,00*	9,37	,000
	4	1	-67,53*	9,37	,000

Table 3. ANOVA results of mitotic index

	Sum of Squares	df	Mean square	F	Sig
Between Groups	6029,950	3	2009,983	26,986	,000
Within Groups	4170,991	56	74,482		
Total	10200,940	59			

Table 4. Istatistical results of mitotic index in treatment groups compared with control group

Dunnett t testi (2-sided)[a]	2	1	-7,88473*	3,15134	,041
	3	1	-18,16540*	3,15134	,000
	4	1	-26,42407*	3,15134	,000

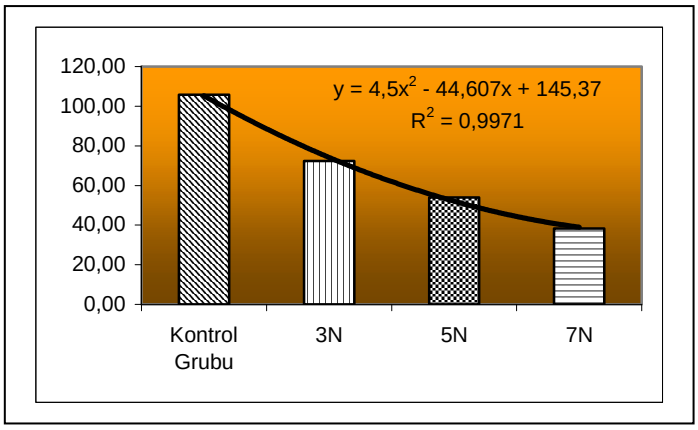

Figure 1. Results of regression analyse of normal dividing cells in each group

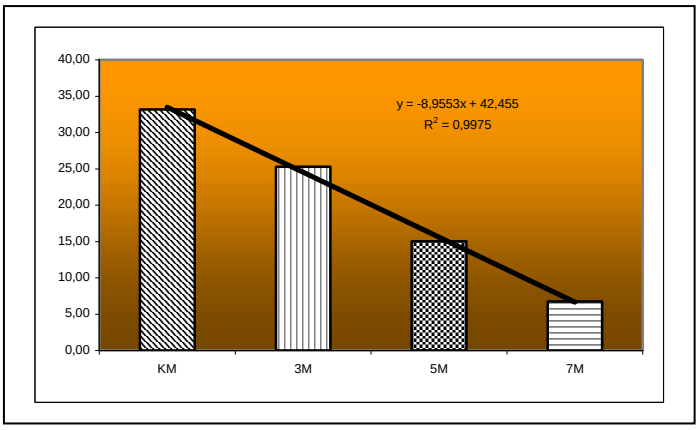

Figure 2. Mitotic index of groups

4. Discussion

In this study, it was investigated cytogenetic effects of lead chloride on root tip cells of *Pinus nigra ssp. pallasiana* seed.

It was found that cell division was inhibited by lead chloride. It caused chromosomal changes and normal cell division was affected. Mitotic index decreased by increasing of lead concentration.

In parallel with our findings, cytological analyses demonstrated numerous aberrations of chromosomes in meristematic root tissue of seedlings developed from seeds collected from trees in the polluted area. The aberrations included chromosome bridges and stickiness, laggards, retarded and forward chromosomes, and their fragments (Prus-Gowacki et al.,2006). There are many study about lead which affects plants. In these studies, lead causes decreases of seedgermination and root elongation, inhibits biosynthesis of chlorophlly and photosynthesis and affects cell structure and chromosome (Munzuroglu et al., 2000 and Kıran et al.,2005).

There are reports on the inhibitory effect of lead concentrations on the germination of seeds of *Phaseolis vulgaris*, *Pisum sativum* and *Brassica napus* var. zerowy. Inhibiting effects of lead for seed germination were observed pollen germination and also pollen tube elongation. For example; this effect was observed on pollens of *Quercus cerris*, *Picea abies*, *Pinus nigra* and *Malus sylvestris* (Kıran et al.,2005).

In conclusion, the result of this study showed that lead concentrations significantly increase mitotic cell division so that decrease mitotic index and also cause various mitotic abnormalities. Our findings about lead are parallel with other investigations in many plant species. These data show that lead damages development of plants and so that leads loss of crop.

Observed chromosomal abnormalities were shown in Figure 3.

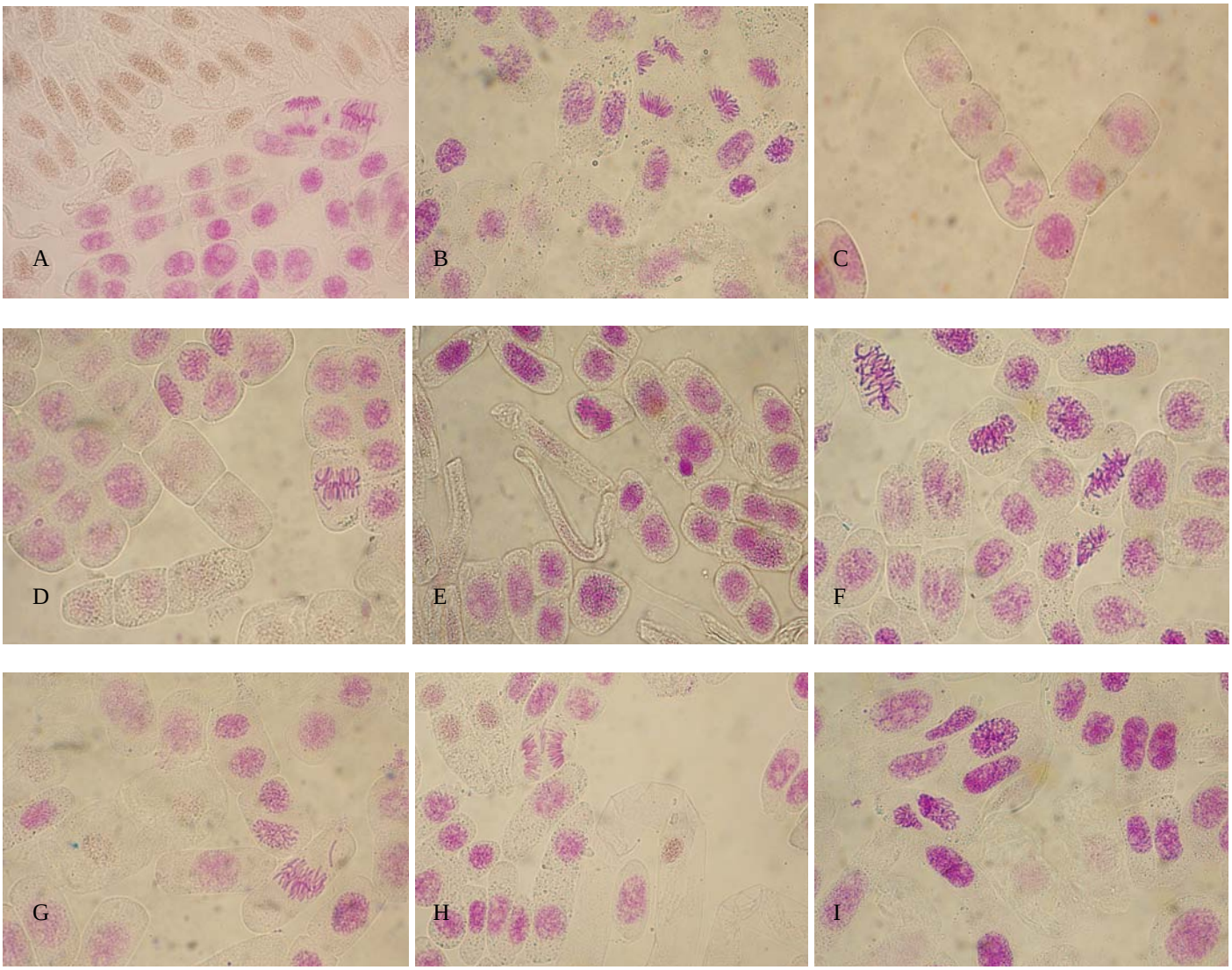

Figure 3. Mitotic cell abnormalities by lead . A-C) chromosome bridges B) multiple polar anaphase D-G-H) breaking chromosomes E) micronuclei F) c-mitosis I) fragmented nucleus

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