

THE EFFECTS OF TREATMENT WITH LEAD ACETATE ON MITOSIS AT *LARIX DECIDUA* L. AND *PICEA ABIES* L.

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Key ords: *Larix decidua*, *Picea abies*, lead acetate, chromosomal aberationse.

Abstract: The lead acetate is a mutagenic agent that determines the presence of cells with chromosomal aberrations.

The mitotic index at the two species varies in a different way depending on the concentration of lead acetate and time of exposure.

Between the two tested species the highest sensibility at lead acetate was noticed at the spruce fir (the frquency of the cells with aberrations was between 0,30-4,89% while the larch tree was 0,21-3,55%).

INTRODUCTION

Any unusual chemical substance wich enters the human body through the digestive or breathig system is considered a potential mutagenic agent. Since the experiments with mutagenic substances cannot be achieved at human being, firstly because of etical reasons, it is much more easy and certain to use the exposure of other organisms (plants or animals), in vivo or in vitro, to the action of the substance suspected of being possible mutagene and to analyse the resultats wich, afterwards, will be extrapolated to human being. (t is supposed that the effects of the mutagenic substance for bacteria, Drosophila or the colonies of mammals cells, are similar to human being).

The prevention of dangerous effects by discovery and control of the mutagenic substances is a desideratum that hints at humankind and at healt of the environment on the whole.

The study of the lead acetate's effects on individuals of spruce fir [*Picea excelsa* (Lam.) Link sin. *Picea abies* L.] and of larch tree (*Larix decidua* L.), two freqvent species but less studied from a genetics point of view, may give useful data for the assessment of general effects of this poison, inclusively on human being.

THE AIM OF INVESTIGATIONS

The aim of this investigation is to assess the mutagenic effect of lead acetate (salt of a heavy metal), very toxic and frequently present in environment. This substance induces the environmental pollution, poisoning of the vegetation and of the human.

MATERIALS AND METHODS

As biological material was used seeds from *Picea abies* L. and *Larix deciduas* L., obtained from Forest Department of Suceava (harvest of year 2001).

These seeds were treated with lead acetate in different concentrations (0,1%, 0,2% and 0,3%) for different time periods (12, 24 and 48 hours). The control seeds were kept (for the same time) in distillate water.

The seeds were distributed for germination in Petri plates, covered with filter paper moistured with distillate water, at room temperature.

When the roots of the seeds were reached 5 – 10 mm in length, these were treated with lead acetate solution, and then were immersed in distillate water (for washing) for two hours.

After this treatment, the seeds were immersed in absolute ethylic acid / glacial acetic acid (3:1) (for fixation), at room temperature, and then were immersed in ethylic acid 70%, at refrigerator.

For hydrolysis was used a HCl solution (50%), for 2 minutes, and for staining was used Carr stain.

The microscopic slides were realised in accordance with *squash* method, microscopic reading at 20x, and photographs were accomplish at 100x, in immersion, with camera Nikon Eclipse 600.

RESULT AND DEBATES

1. *Larix decidua*

a) The mitotic index (MI)

The treatment for 24 and 48 hours with lead acetate solutions (0,1; 0,2 and 0,3%) determines the decrease of the mitotic index proportionally with the dose's increase.

It is noticed a considerable decrease of mitotic index at the solution treated for 12 hours, at the concentration of 0,1%, afterwards there is an increase of this parameter's value at the solutions 0,2 and 0,3% without exceeding, however, the recorded value at the basic test (fig. 1).

b) The frequency of phases of the mitotic division

It was noticed that at all the tests (Control; 0,1; 0,2 and 0,3%) the highest level belonged to the cells which were in the first phase and had a slightly downward curve together with the increase of the concentration of lead acetate (fig. 2-4)

The cells that were in the second, the third and the fourth phase had been recorded in a very low percentage comparing to those in the first phase, in all the treatment's combinations. Thus, if the cells in the first phase were in a percentage of 96,99% (at the basic test) the cells in the second phase would represent 3,92-9,58%, those in the third phase 2,78-8,50% and the cells in the fourth phase just 0,91-4,78%.

c) The frequency of aberrations cells

The use of the treatment with lead acetate in concentrations of 0,1; 0,2 and 0,3% determined the presence of cells with aberrations in percentages directly proportional with the increase of the concentration and of the treatment's period.

The highest level was found at the solution 0,2% lead acetate in 48 hours exposure, when the percentage of aberrations reached at the value of 3,55%, while at the basic test this value was just 0,21%. There is an increase of almost 17 times (16,99 times) than at the basic test (fig. 5)

The main types of noticed aberrations were: the bridges, the retardatory chromosomes, the expelled chromosomes, the fragments, the multipolar A-T and cells with micronucleus.

2. *Picea abies*

a) The mitotic index (MI)

As a result of the lead acetate's treatment for time periods of 12, 24 and 48 hours it occurs a stimulation of the mitotic cellular division comparing to the control at solutions of 0,1 and 0,2%.

There is a decrease of value of the mitotic index at the maximum concentration of lead acetate (0,3%) but its value is below that of the basic test only at the solution of 48 hours of treatment (fig. 6).

b) The frequency of phases of the mitotic division

As larch tree's case the first phase's cells have the highest level at all the tested solutions (C; 0,1; 0,2 and 0,3%) in 12, 24 and 48 hours time of exposure. The other phases of mitotic division (the second, the third and the fourth) are less represented at the basic test as well as at the treatment's solutions (fig. 7-9).

c) The frequency of cells with aberrations

The treatment with lead acetate led to the increase of the frequency of aberrations. It is noticed an increase proportionally with the dose (0,1 and 0,2%) at the solution in 12 hours treatment, afterwards there is a decrease of the frequency of the cells with aberrations (0,3%).

The percentage of aberrations does not vary proportionally with the concentration of lead acetate at 24 hours' treatment solution. Thus, its value increases at 0,15 decreases at 0,2% so as to the maximum concentration was recorded a treble of the basic test.

The maximum percentage of the cells with aberrations was noticed at the concentration of 0,2% lead acetate and in 48 hours of treatment both in comparison with the control and with the other treatment periods (12 and 24 hours), (fig.10).

CONCLUSIONS

The lead acetate has toxic effects on the individuals of *Larix decidua* L. and *Picea abies* L. Being a mutagenic agent that determines the presence of cells with chromosomal aberrations, it modifies the frequency of cellular divisions.

The mitotic index at the two species varies in a different way depending on the concentration of lead acetate and time of exposure. Thus, at the three periods of treatment at larch tree the minimum concentration (0,1%) lead acetate determines a decrease of MI unlike the basic test, while at spruce fir it brings about its increase. The MI's lowest value at the two species had been recorded at the maximum concentration of lead acetate and in the longest period of exposure (48 hours).

Between the two tested species the highest sensibility at lead acetate was noticed at the spruce fir (the frequency of the cells with aberrations was between 0,30-4,89% while the larch tree was 0,21-3,55%).

The spectrum of the chromosomal aberrations noticed at the two species is similar, being differences concerning just their proportion.

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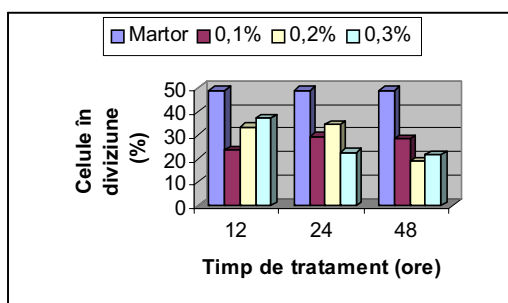
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Fig.1.The mitotic index at *Larix decidua* L. after lead acetate treatment.



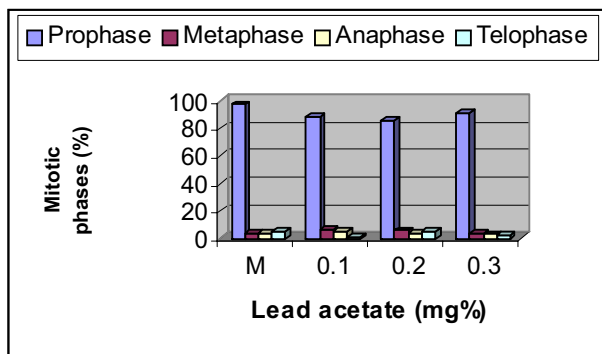


Fig. 2. The frquence of the mitotic phases in *Laix decidua L.*, after lead acetate tretment,for a 12 hours.

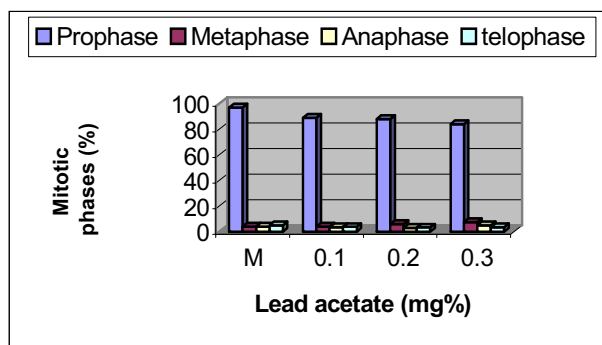


Fig. 3. The frquence of the mitotic phases in *Laix decidua L.*, after lead acetate tretment,for a 24 hours.

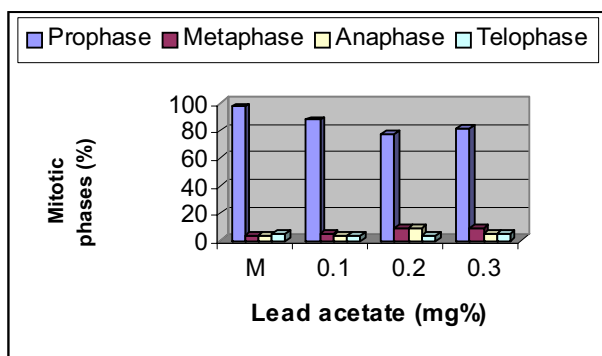


Fig. 4. The frquence of the mitotic phases in *Laix decidua L.*, after lead acetate tretment,for a 48 hours

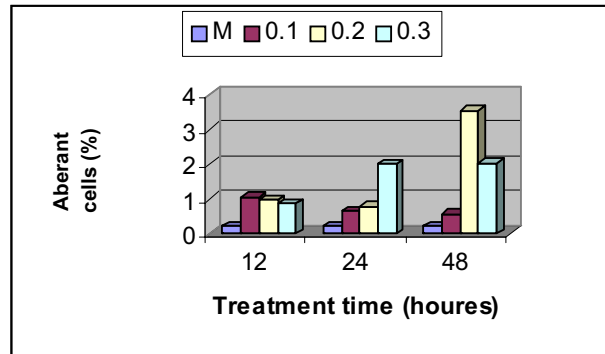


Fig. 5. The frequency of the aberrant cells in *Larix decidua* L. after lead acetate treatment.

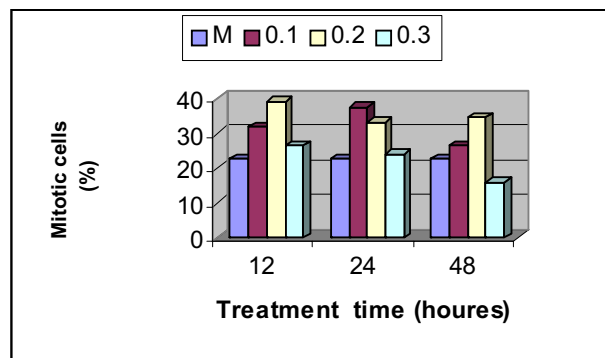


Fig. 6. The mitotic index at *Picea abies* L., after lead acetate treatment.

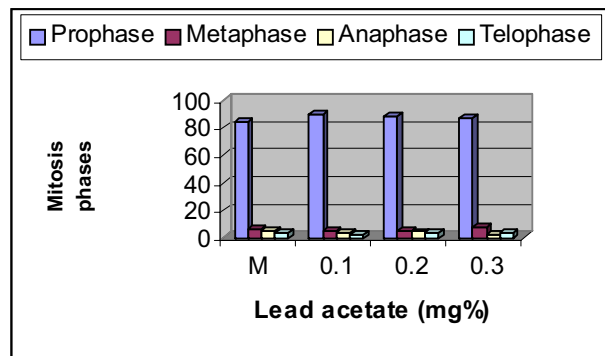


Fig. 7. The frequency of the mitotic phases in *Picea abies* L., after lead acetate treatment, for a 12 hours.

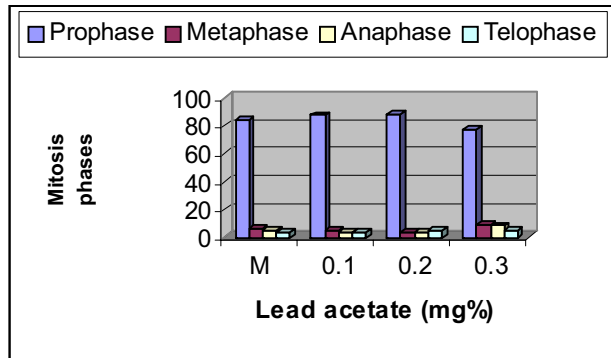


Fig. 8. The frquence of the mitotic phases in *Picea abies* L., after lead acetate tretment,for a 24 hours.

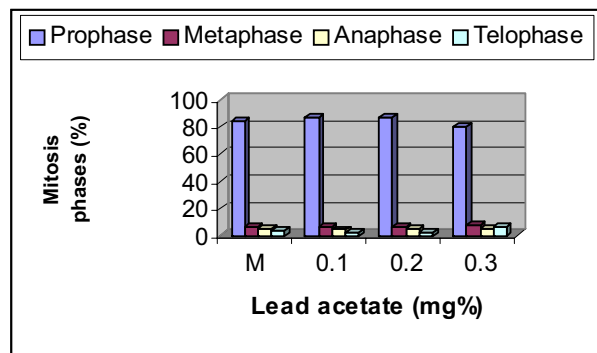


Fig. 9. The frquence of the mitotic phases in *Picea abies* L., after lead acetate tretment,for a 48 hours.

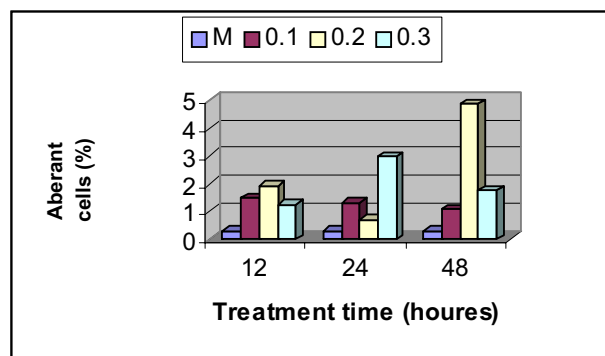


Fig. 10. The frquence of the aberrant cells in *Picea abies* L., after lead acetate treatment.

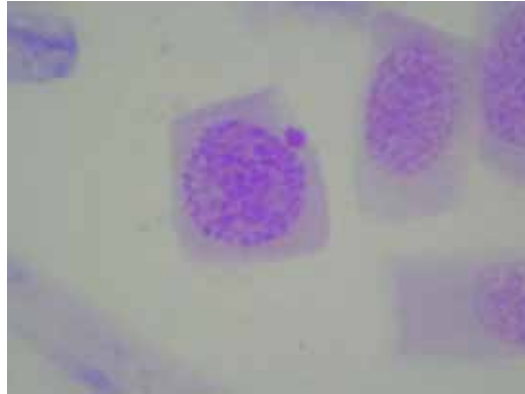


Fig. 11. Prophase with micronucleu (*Picea abies* L.).

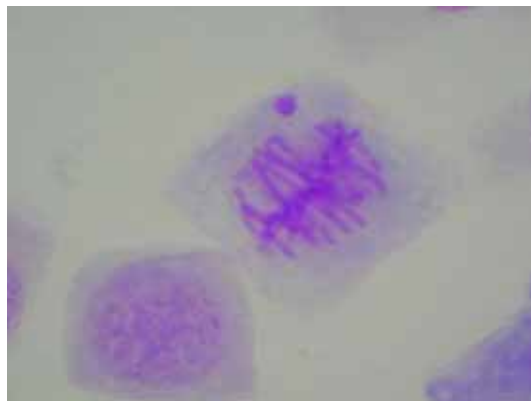


Fig. 12. Metaphase with micronucleu (*Picea abies* L.).

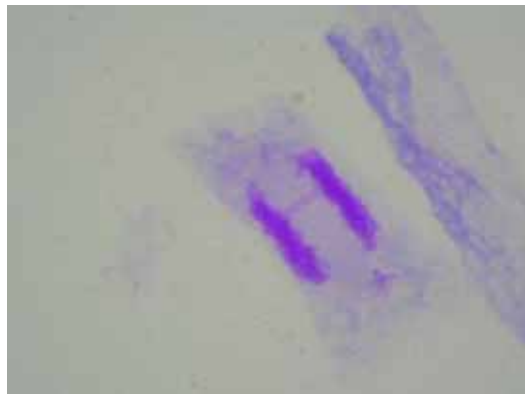


Fig. 13. Telophase with double bridge (*Picea abies* L.).

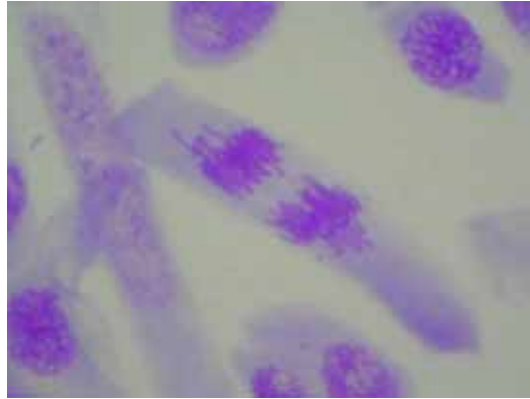


Fig. 14.A-T with expelled chromosomes(*Larix decidua* L)

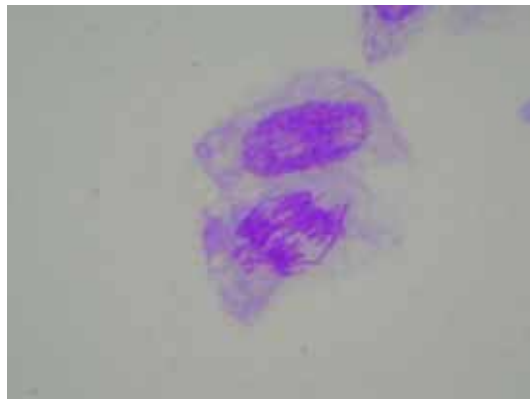


Fig. 15. Anaphase with bridges and expelled chromosomes (*Larix decidua* L.).

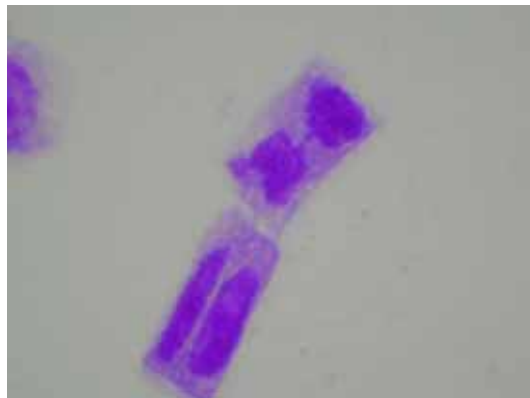


Fig. 16.Telophase with bridge and ring-shaped chromosome (*Larix decidua* L.)