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Original Research

Influence of lead acetate on seed germination and growth of young alfalfa plants

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Abstract: This study presents the results of testing the effect of different concentrations of lead-acetate on seed germination and young Seedlings of alfalfa. The experiment was organized so that in the first variant, only lead-acetate of different concentrations (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 2 x 10^{-2} M) was used, and in the second, next to the lead acetate EDTA concentration of 0.012 % was added. The results of this study in which natural conditions are imitated showed that the percentage of alfalfa seed germination decreases with increasing lead-acetate concentrations. In a medium of high concentration of lead acetate 10^{-2} and 2 x 10^{-2}, a small number of seeds (39% and 32 (32%) germinated. In the same treatments with EDTA, the toxic effects of lead acetate were reduced, and seed germination was better (59% and 43% seeds germinated). It has been noticed that lead has toxic effects on the growth of alfalfa roots and stems. In the variant in which EDTA was used, the negative influence of lead on and growth of alfalfa seedlings was significantly mitigated.

Keywords: alfalfa; lead acetate; seeds; seed germination

1. Introduction

In recent decades, the problem of heavy metals in ecosystems has become increasingly important. This is the development of scientific and public awareness and the technical possibilities of precise measurement of their concentrations [1]. It often applies indirect methods such as the presence of metal in some living organisms such as plants [2]. Plants are important components of an ecosystem and one of the main entry routes of heavy metals into the food chain [3]. The toxic effects of heavy metals are based on their irreversible binding to metabolically active groups in amino acids, polypeptides, and proteins [4]. They use different morphological, physiological, biochemical mechanisms to cope with the heavy metals that enter their cells [5].

Lead is a toxic metal found in biological systems and abiotic components of the environment and is available to plants from soil and aerosol sources [6]. The inhibitory effect of lead is manifested in the earliest stage of morphogenesis, seed germination, and the growth and development of plants during the vegetation period [7]. Lead negatively affects the synthesis of chlorophyll, chloroplast ultrastructure [8], auxin metabolism and transport [9], photosynthesis, disrupts mineral nutrition and plant water status [10]. Lead disturbs mineral nutrition and water balance [11], processes transpiration, respiration, membrane structure and properties, and gene expression [12]. Excess concentrations of lead significantly affected enzyme inhibition [13]. Some plant species can absorb and accumulate large amounts of heavy metals without visible symptoms [14]. Only when the concentration of these metals exceeds a certain critical limit, the life processes of plants and organic matter production decrease [15].

Alfalfa is a perennial legume important for animal husbandry, growth in significant areas in conditions of anthropogenic pollution. Its root system is strong and developed, permeating the soil and making it loose [16] so that the plant species using the phytoremediation of toxic metals [17, 18]. Alfalfa can reduce the oxidation state of toxic metals such as Cr and Au [19]. This research examines different concentrations of lead acetate influencing the alfalfa seed germination and the growth of young plants, under controlled conditions.

2. Experimental

Alfalfa (*Medicago sativa* L.) seeds were used to test seeds under the influence of lead-acetate. In each of the three repetitions, 100 healthy seeds were sown (300 per treatment). The seeds were first immersed in distilled water for two hours and then sown on filter paper in Petri dishes. After that, one batch of seeds was treated with 20 ml of lead-acetate of different concentrations (10^{-5}, 10^{-4}, 10^{-3}, $10^{-2,}$ and 2 x 10^{-2} M). The second batch of seeds was treated with some concentrations of lead-acetate solutions, but a 10 ml ethylenediaminetetraacetic acid - EDTA concentration of 0.012 % was added. At the same time, a series of seeds were prepared for germination, which was treated only with

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water (control) and was placed in a thermostat for germination at a temperature of 22ºC and relative humidity of 95%. After 72 hours of germination, the germinated seeds were counted. Changes in seedlings were observed as a function of time, and the length of the root and shoot ’were measured on the 10th day of germination.

The results were processed using the STATISTICS 10.0 program. The significance of the difference between the investigated probes was determined upon the variance analysis, i.e., the LSD test.

3. Results

The results obtained in this study indicate that high concentrations of lead acetate had inhibitory activity on the germination of seeds alfalfa (**Figure 1**).

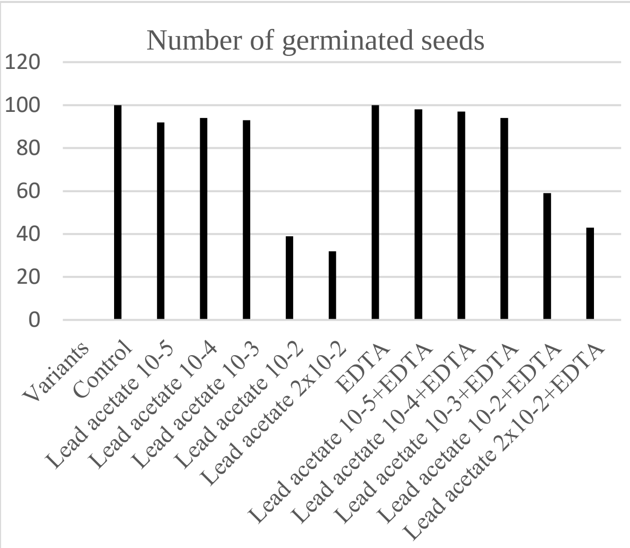
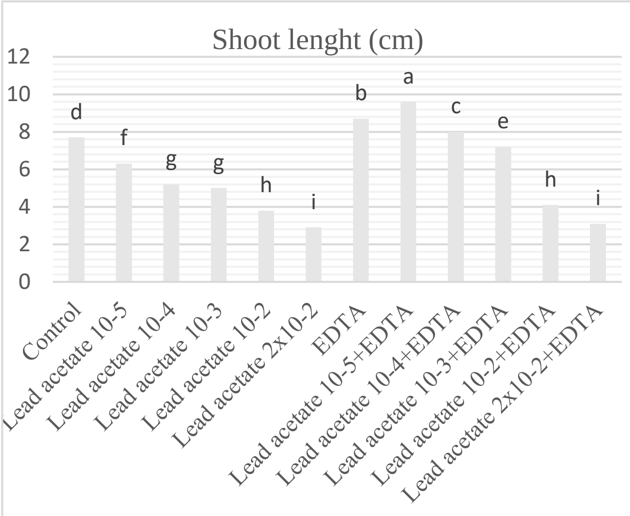


Figure 1. Germination of alfalfa in the presence of different concentrations of lead acetate and EDTA in lab conditions

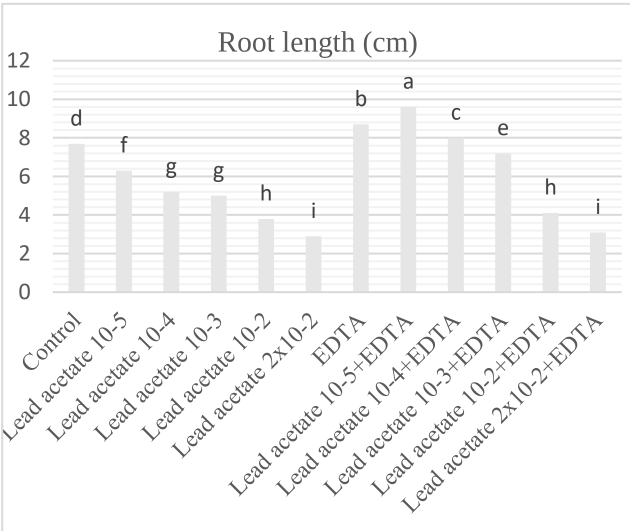
Low concentrations of lead acetate (10^{-3}, 10^{-4},10^{-5}) did not significantly reduce alfalfa germination, only a few seeds less than the control. However, the concentration of lead-acetate 10^{-2} M caused a significantly higher decrease in germination; only 39 seeds germinated. In the presence of a twice as high concentration of lead-acetate 2 x 10^{-2}, only 32 seeds germinated. In the treatments with EDTA, the toxic effects of lead decreased. The number of germinated seeds increased so that in the presence of 10^{-2} M lead-acetate, 59 seeds germinated, 20 more than the variant without EDTA, and at a concentration of 2 x 10^{-2} with EDTA 43 seeds germinated, *i.e.*, 11 seeds more than that in the treatment with lead acetate alone (**Figure 1**).

The results in the present study showed that for the examined parameters, a statistically significant difference in the negative sense was observed in relation to the control and all the probes with lead acetate (**Figure 2**). The maximum shoot length (9.6 cm) in the treatment with lead acetate concentrations of 10^{-5} and EDTA was recorded. In addition, two more treatments with lead acetate concentrations of 10^{-4} and EDTA (8.0 cm) and EDTA without lead acetate (8.7 cm) shoot length were higher than control (water; 7.7cm). According to the Fishers test, there was no statistically significant difference between treatments lead acetate concentrations of 10^{-3} and 10^{-4} on stem height (5.0 and 5.2 cm). The lowest shoot length was recorded in the variant with the concentration of lead-acetate 2x10^{-2} M of 2.9 cm. Also, there was no statistically significant difference among these and treatment with lead acetate same concentration and EDTA.



*The same letters in superscript indicate high homology, *i.e.*, the absence of statistically significant differences ($p<0.05$)

Figure 2. Shoot length of young seedlings of alfalfa in the presence of different concentrations of lead acetate and EDTA (0.012%)



*The same letters in superscript indicate high homology, *i.e.*, the absence of statistically significant differences ($p<0.05$)

Figure 3. Root length of young seedlings of alfalfa in the presence of different concentration of lead acetate and EDTA (0.012%)

The statistically significant difference in a positive sense was observed in probes with concentrations of lead acetate 10^{-5} (13.7 cm) and 10^{-4} with EDTA (12.0 cm) and control with only EDTA (12.6 cm) in relation to the control (11.1 cm; **Figure 3**). The negative effects of lead acetate on root

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length manifested in all other treatments. The minimum root length (3.9 cm) was recorded in probes with lead acetate concentration 2 x 10^{-2}. In comparison, the same concentration of lead acetate in the presence of EDTA had a less harmful effect on the root alfalfa length, which was 7.4 cm.

4. Discussion

In the present study, low concentrations of lead acetate did not significantly reduce alfalfa germination, while high concentrations (10^{-2} and 2 x 10^{-2}) caused a decrease in germination. According to Sethy and Ghosh, lead strongly affects the seed morphology and physiology, inhibits germination, root elongation, seedling development, and plant growth [20].

Also, in our research, it was observed that the presence of EDTA reduced the toxic effect of this lead compound. Titov *et. al.* states that elevated concentrations of $Pb(NO)_3$ from 10^{-6} to 10^{-2} and an increase in treatment length of 1-7 days cause a slowdown in the growth of winter wheat, and barley [21]. Kabir *et al.* reported the negative effect of cadmium and lead on the germination of seed of *Thespesia populnea* L and seedling growth [22]. Alfalfa shoot biomass has demonstrated the ability to bind an appreciable amount of heavy metals from aqueous solutions [23].

All used concentrations of lead acetate in the present study had a negative effect on shoot length and root length of alfalfa plants. The presence of toxic substances in plants changes the function of cellular organelles and has a negative impact on plant growth [24]. Sêdzik *et al.* found that toxic effects lead to the seed germination, root, and growth processes [25]. Peralta-Videa *et al.* reported the effects of heavy metals on alfalfa depending on the plant's growth stage [26]. Sêkara *et al.* found significant correlations between the levels of cadmium and lead in the roots and shoots of alfalfa (r = 0.968 and r = 0.610) [17]. According to Yang *et al.*, roots represent a barrier for metals for most plant species [8]. Roots work as a primary passageway for all fluids and nutrients spread to the plant tissues, so the concentration of heavy metals in roots is usually higher than that of stems and leaves [2, 27]. In our study, thanks to the influence of EDTA, the effect of lead acetate on shoot length and root length alfalfa was reduced. According to Begonia *et al.*, synthetic chelating agent EDTA forms a complex with the lead that enhances its mobility through the plant and translocation of lead from roots to leaves [28].

5. Conclusion

The present study results showed that lead acetate was a toxic effect and caused a significant reduction in stem height and root length in young seedlings of alfalfa. Also, high concentrations of this compound caused a decrease in germination seeds of this plant species. However, low concentrations of lead acetate in the presence of EDTA acted as a stimulating on root and stem alfalfa. EDTA reduces lead toxicity and disables its inhibitory influence on the enzymes responsible for germination. Considering that the concentration of heavy metals like lead in the environment has been increased due to automobiles, agrochemicals, and other anthropogenic activities, the information from the present studies would be helpful in understanding the response of alfalfa plants to the presence of lead in polluted soil.

Declarations

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Author Contribution: SA wrote the manuscript with support from SB and VZ; FB and MP carried out the experiments. JM and OP were involved in planning and supervising the work. All authors discussed the results and contributed to the final manuscript.

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Conflict of Interest: The authors declare no conflict of interest.

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