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ORIGINAL RESEARCH PAPER

EFFECTS ON SEEDS GERMINATION AND BIOACCUMULATION OF LEAD IN WHITE MUSTARD IN A LABORATORY STUDY

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Abstract: The paper presents a laboratory study concerning the Pb effects on seeds germination and bioaccumulation in the white mustard plants (*Sinapis alba*). The garden soil rich in nutritive elements was watered with Pb solutions until the concentration of 45 mg·kg^{-1} and 85 mg·kg^{-1}. After 60 days of exposure, the contents of Pb in soil and plant organs were evaluated. The Pb transfer (TC) and translocation (TF) indices were calculated. In addition, the effect of Pb pollution to assimilation of essential metallic elements (Zn, Ca, Mg, Cu, Ni, Fe, Mn) in plants was investigated. Pb (85 mg·kg^{-1}) can cause negative effects on seed germination (40 % inhibition). The Pb was immobilized by plant at root level, both in the control and in the exposed samples (TC and TF < 1). The absorption and translocation processes of the essential metals are influenced by the increasing of Pb concentrations. Their presence decreases the toxicity of Pb to both tested concentrations. Competitive processes were observed between Pb (45 mg·kg^{-1}) and Ca, Mg, Zn, Cu and Mn. Antagonistic processes of Pb were observed in case of Fe, Cu and Ni in the plant. At 85 mg·kg^{-1} Pb inhibition or stagnation of accumulation compared to the control was notice. The photosynthesis process measured through chlorophyll detection reveled not significantly influences of Pb. Phytotoxic effects are reduced. The results reveled that *Sinapis alba* have the capacity to adapt to Pb contamination having a great phytostabilization potential.

Keywords: *nutrients, Pb, Sinapis alba, transfer factor, translocation factor*

INTRODUCTION

Lead (Pb) is one of the most persistent metals, so it has an estimated soil retention time of 150 - 5000 years. Lead is the second most toxic metals after arsenic, which could have harmful effects on living organisms [1]. Activities such as mining, manufacturing of lead acid battery, use of fuels with lead, printing and metallurgic activities has been associated for lead environmental contamination [2].
The soil *p*H, organic matter, soil texture and mineralogy, microbial community, ion exchange capacity and redox potential influence bioavailability of lead to the plant uptake [2].
The mineral component of the soil bound Pb especially on clay minerals, while the organic component of the soil bound Pb on humic acids, in this particular case being strongly restrained. Consequently, the majority Pb concentration from soil is fixed (generally over 80 % of the total Pb in the soil is fixed) and only a small part remains bioavailable to plants. This explains why in lead-polluted soils the toxicity on plants is not proportional to the total lead content [3, 4]. This finding also applies to the mustard tested in soil rich in organic matter, at the end of the lead pollution tests, the added content remains in proportion of 96 % in the soil [3].
On the other hand, Pb inhibits metabolic processes such as nitrogen assimilation, photosynthesis, respiration and water absorption, interfering direct or indirect with the plants, disturbing enzymatic activity, causing oxidative damage [2].
The exposure to high level of Pb affects the mineral nutrition of the plants, inhibits the divalent cations uptake such as manganese, calcium, magnesium, iron. Some studies report the reduction of divalent cations concentration in cauliflower leaves [5], radish, or maize as result to Pb exposure [6].
Karak *et al.* hypothesized that mustard root acts as a buffer, preventing metal contamination in the aerial parts [7]. At the same time, they warn about the risks of consuming edible parts (leaves, grains) as result of the toxic metals accumulation.
Thus, in present laboratory study aims to disclose the effect of Pb on the germination and development processes of white mustard *Sinapis alba*. In addition, the effect of Pb pollution to assimilation of nutrients in white mustard was investigated.

MATERIALS AND METHODS

Materials

Selected *Sinapis alba L.* seeds (Micro Biotests, Belgium) and a universal substrate enrich in nutritive elements for plants cultivation provided by a local producer (Agro CS, Romania) were used in the experimental studies.
For the determination of metallic elements calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) a 100 $mg \cdot L^{-1}$ ICP multi-element standard solution VIII (Supelco, Merck KGaA, Germany) was used.
The quality control of the experimental data was performed using a 100 $mg \cdot L^{-1}$ Ca, Cu, Fe, Mg, Mn, Ni, Pb, Zn Multi-Element Certified Reference Standard, (LGC, Germany).
HNO_3 60 % ultrapure and H_2O_2 30 % for trace analysis were applied in pretreatment process of the plant tissues. The soil samples were pretreated with HNO_3 65 % and HCl 37 % (Sigma-Aldrich, Merck KGaA, Germany) for analysis quality.

Experimental design

Three individual laboratory experiments were performed in laboratory conditions (ambient temperature 23 ± 2 °C, natural light 12 h per day and night, relative humidity of about 25 %). A number of 33 selected mustard seeds were place in 3 kg soil for each control and polluted soil in different flowerpots. Subsequently, *Sinapis alba* seeds were incubated for 60 days in presence of 45 $mg \cdot kg^{-1}$ (Pb I), 85 $mg \cdot kg^{-1}$ (Pb II), or 4.75 $mg \cdot kg^{-1}$ Pb (control), respectively. The selected Pb concentrations were above the normal value (20 $mg \cdot kg^{-1}$), as well as alert threshold for sensitive soils (50 $mg \cdot kg^{-1}$) according to Romanian Legislation [8].
The soil contamination was achieved initial by watering the seeds with Pb solution, and later the seedlings until reaching the interest concentrations, and then the plants were watered with aerated tap water until the end of the experiments (two months).
After 60 days of plant development at Pb exposure, the plant tissues (root, stem and leaves) were separated, rinsed with distilled water and dried approximately 24 h at 50 °C in an oven (Memmert oven UF 110, Germany). Around 0.5 g of vegetal material (root, stem, leaves) was separately digested with 9 mL HNO_3 60 % and 1 mL H_2O_2 30% in order to detect the metallic elements (Ca, Cu, Fe, Mg, Mn, Ni, Pb, Zn).
At the end of experiment, the soil samples were air-dried and 1 g of soil was digested with 10 mL of aqua regia mixture ($HCl : HNO_3 = 3 : 1$) in a microwave oven (Ethos Up Milestone Microwave System, Italy) in order to mineralize organic matter.
Three aliquot samples from both plant tissue and soil, respectively were pretreated and analyzed. The reported results represent the average values.
The Pb content in soil and plant material was performed using ICP-EOS technique (ICP-EOS AVIO 500 Perkin Elmer Spectrometer, USA). In addition, the essential metals (Zn, Ca, Mg, Cu, Ni, Fe, Mn), representing the micronutrients necessary for the germination and development of the plants [9], were determined to estimate the attenuation impact of the stress generated by the toxic metal.
Also, the content of chlorophyll pigments in mustard plants was detected following the protocol described in Dinu *et al.* (2021) in order to evaluate the phytotoxic effects of Pb. [4].

Statistical methodology

The experimental data were expressed as average ± the standard deviation values (SD). Data statistical analysis was carried out using the paired *t* test to assess the differences between the metal contents in control and polluted soils and plants. This statistical test was chosen because all the seeds belonged to the same population. The value of p was considered insignificant for $p > 0.05$ (ns), significant for $p < 0.05$ (*), and very significant for $p < 0.01$ (**). The Pearson correlation (r) was used for the correlation between experiments.

Evaluation of phytoextraction indexes

To assess the phytoextraction potential of the white mustard, two different factors were calculated [10].

The transfer coefficient (TC) defines ability of plants to extract metals from soil and accumulate in the roots (Equation 1). For a specific element, the plant accumulates in the roots if TC value is higher than 1. TC value around 1 indicated that plant was not influenced by the metal uptake.

$$\textit{Transfer coefficient (TC)} = \frac{\text{Me concentration in root}}{\text{Me concentration in soil}} \quad (1)$$

The translocation factor (TF) indicates transfer of metal from roots to aerial parts of the plant (stem, leave, flower, and fruit) (Equation 2). The value higher than 1 indicate that the plant translocate element from roots to a specific tissue.

$$\textit{Translocation factor (TF)} = \frac{\text{Me concentration in aerian tissue}}{\text{Me concentration in root}} \quad (2)$$

RESULTS AND DISCUSSION

Characterization of soil, seeds and tap water

An initial characterization in terms of metallic elements and other nutrients were performed for the enriched substrate, *Sinapis alba* seeds and the tap water used for watering.
The mustard seeds indicated the following concentrations: 4.100 $mg \cdot kg^{-1}$ Ca; 5.73 $mg \cdot kg^{-1}$ Cu; 70 $mg \cdot kg^{-1}$ Fe; 3.150 $mg \cdot kg^{-1}$ Mg; 21.5 $mg \cdot kg^{-1}$ Mn; 1.50 $mg \cdot kg^{-1}$ Ni; < 0.5 $mg \cdot kg^{-1}$ Pb; 46 $mg \cdot kg^{-1}$ Zn. Pb content in seeds was situated below the quantification limit of the applied analytical method (0.5 $mg \cdot kg^{-1}$).
The characteristics of enriched substrate used in the test were *p*H = 6.35; $C_{organic}$ = 12 %; N_{total} =13.400 $mg \cdot kg^{-1}$ dry weight (d.w.); P_{total} = 2.815 $mg \cdot kg^{-1}$ d.w.; 4.75 $mg \cdot kg^{-1}$ d.w. Pb; 108,229 $mg \cdot kg^{-1}$ d.w. Ca; 12.5 $mg \cdot kg^{-1}$ d.w. Cu; 10.713 $mg \cdot kg^{-1}$ d.w. Fe; 3.155 $mg \cdot kg^{-1}$ d.w. Mg; 357 $mg \cdot kg^{-1}$ d.w. Mn; 13.7 $mg \cdot kg^{-1}$ d.w. Ni; 24.3 $mg \cdot kg^{-1}$ d.w. Zn. Same substrate and seeds were used in other study regarding Cd influence on white mustard [11]. As we mentioned in previous paper, the C/N ratio of 17.5 indicated moderate availability of nitrogen for plant uptake [11].
The quality of water used for plants watering during the test period corresponded to free chlorine potable water, and no toxic metals (As, Cd, Cr, Ni, Pb,) were detected, the values were below the quantification limit. Moreover, Ca 23.7 $\mu g \cdot L^{-1}$, Cu 3.4 $\mu g \cdot L^{-1}$, Fe 23.7 $\mu g \cdot L^{-1}$, Mg 5.5 $\mu g \cdot L^{-1}$, Mn 3.9 $\mu g \cdot L^{-1}$and Zn 20.4 $\mu g \cdot L^{-1}$were found in the watering tap water.

Effects of Pb on seeds germination

Pb effects on seed germination were investigated. The germination of seeds occurred at 3 days from planting. While in the control sample, the germination percentage was 84.8 %, in Pb I experiment about 78.8 % of the seeds germinated, and in the case of the Pb II test the percentage was around 60.6 %. Our study confirmed the conclusion of Parmar *et al.* [12], which stated that the excess of Pb concentrations in the soil leads to germination decrease.

Pb accumulation (TC) and translocation (TF) in mustard plants

The Pb extracted by plant from soil was immobilized at root level, both in the control and in the intoxicated samples. The bioaccumulation indices were registered subunit values (TC less than 0.83). In addition, the results indicated that Pb does not accumulate in the aerial parts of the plant (stem and leaves), TF being less than 1, low values indicate that lead has been sequestered in the roots system [2, 13] (Figure 1).
Significant differences in root accumulation were observed (p = 0.015) between the control experiment and the Pb I experiment.

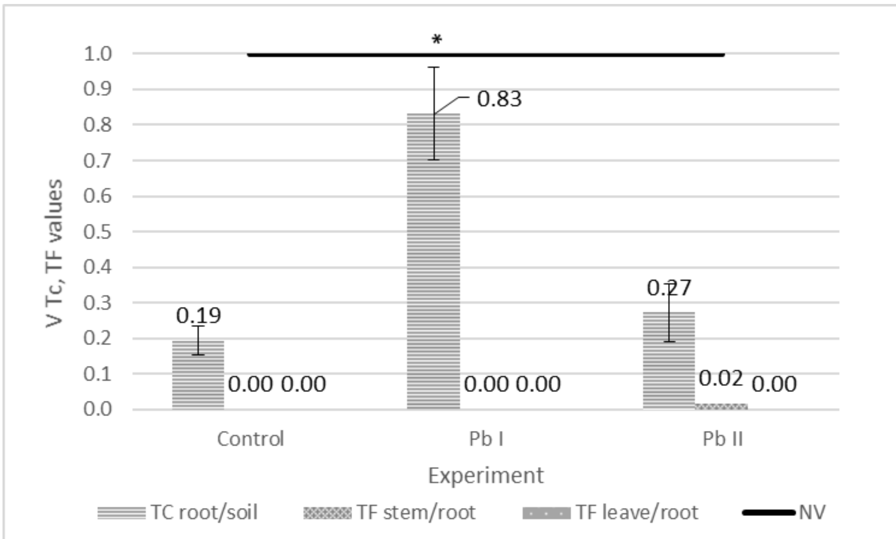


Figure 1. *Pb - TC and TF in control (4.75 mg·kg*$^{-1}$ *d.w. Pb), Pb I (45 mg·kg*$^{-1}$ *d.w.) and Pb II (85 mg·kg*$^{-1}$ *d.w.) tests, mean value ± SD (n = 3), SD =standard deviation,* $p < 0.05$ (*)

To highlight the impact of essential metals from enriched substrate on the Pb bioavailability (accumulation and transfer in plants), the TC and TF indices were also calculated for Ca, Mg, Zn, Cu, Ni, Fe and Mn.

Influence of Pb on the Ca, Mg and Zn accumulation in mustard plants

The total Ca concentrations in entire plant (root, stem and leaves) in polluted tests compare with the control were lower with 8 % in Pb I test, respectively 12.5 % in Pb II test which indicated that pollution of the soil has an insignificant effect on Ca extraction from soil. As presented in Figure 2a, the Ca accumulation in experiments followed the same pattern: $Ca_{leaves} > Ca_{stem} > Ca_{root}$.
According to the TC value (0.31 in control; 0.20 in Pb I; 0.34 in Pb II), Pb does not affect the extraction of Ca from the soil to roots in both control and polluted tests.
Taking into account the TF value, it is observed that Ca translocation potential in the plant (respectively from the root to the stem and leaves) increases in the Pb tests compared to the control. It seems that in the Pb I test ($TF_{stem/root}$ = 2.55, $TF_{leave/root}$ =

5.86), Ca translocation is much more effective than in Pb II test ($TF_{stem/root}$ = 1.87, $TF_{leave/root}$ = 2.18), exceeding also the TC values determined in the control ($TF_{stem/root}$ = 1.47, $TF_{leave/root}$ = 4.48). 45 $mg \cdot kg^{-1}$ Pb in soil has a positively influences on the translocation of Ca in the plant, especially in the leaves. As was reported in literature, Pb absorption is inhibited by Ca, Ca^{2+}- channels being principal route for Pb to penetrate in the root system [13, 14].

Regarding Mg bioaccumulation in aerial parts, while in the Pb I test TF values ($TF_{stem/root}$ =3.12, $TF_{leave/root}$ = 3.57) were higher than in control ($TF_{stem/root}$ =2.27, $TF_{leave/root}$ = 3.29), in Pb II test, TF values ($TF_{stem/root}$ =1.74, $TF_{leave/root}$ = 1.45) were lower than in control. In Pb II experiment, the plants were extracted with 10 % more Mg than the other experiments, around 24 % from whole quantity being retained in the roots (Figure 2b). Mg was the only tested metal that indicates a higher concentration in Pb II test than in Pb I test, respectively in control test.

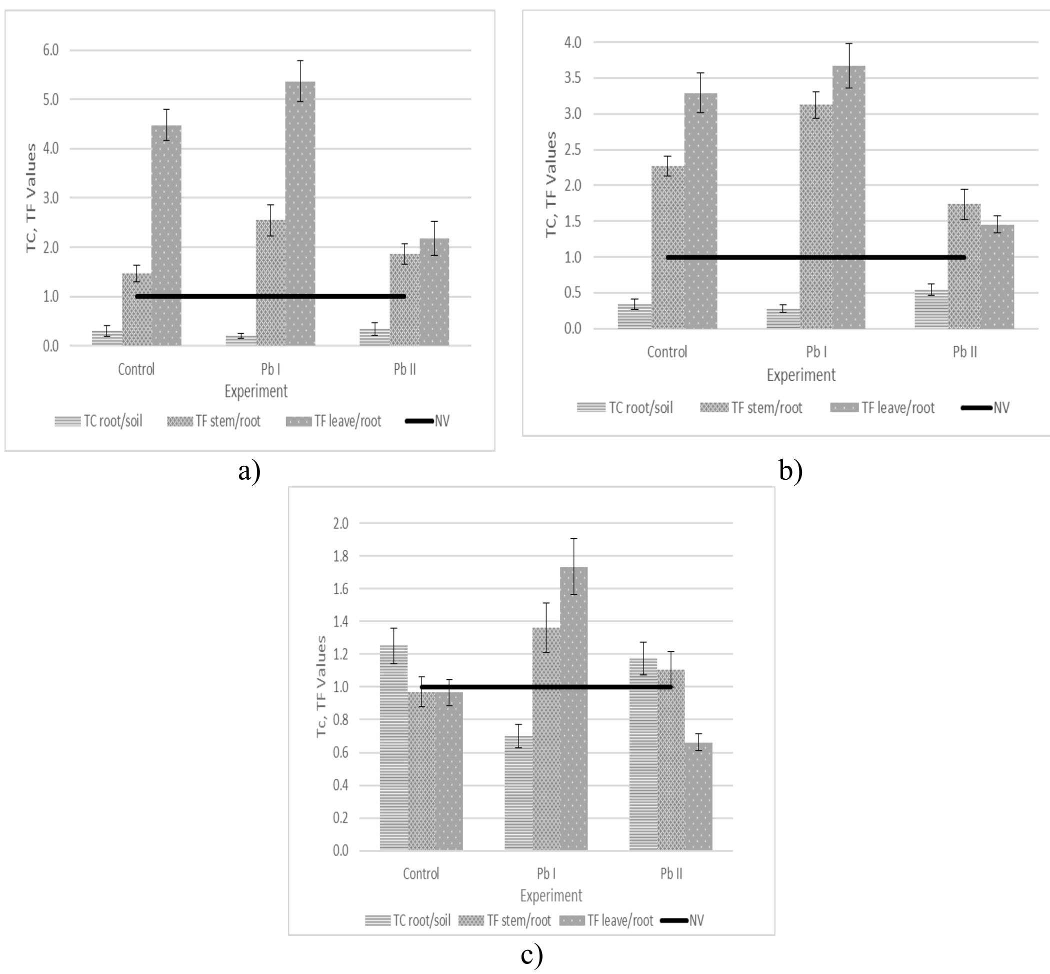
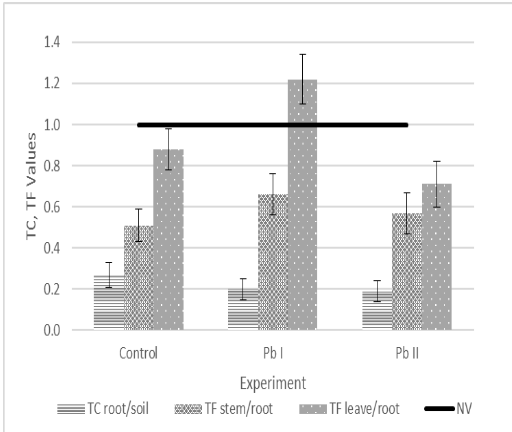


Figure 2. *TC and TF in control a) Ca; b) Mg; c) Zn (4.75 $mg \cdot kg^{-1}$ d.w. Pb), Pb I (40 $mg \cdot kg^{-1}$ d.w.) and Pb II (80 $mg \cdot kg^{-1}$ d.w.) tests, mean value ± SD (n = 3), SD = standard deviation*

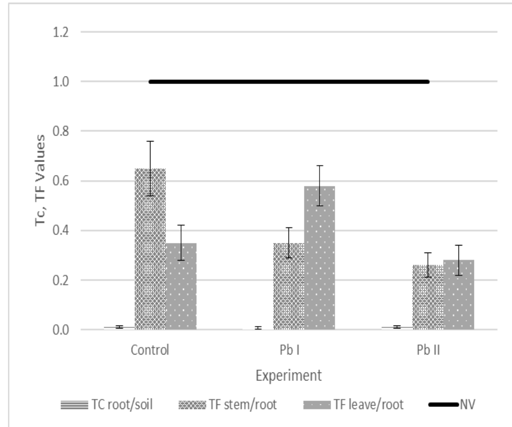
In Pb I test it was observed that Zn was bioaccumulated mainly in the leaves (TF = 1.74), then in the stem (TF = 1.36) and finally in the root (TC = 0.70). In contrast, in the Pb II experiment, Zn was accumulated in the root (TC = 1.17) and in the stem (TF = 1.11) and less in the leaves (TF = 0.66). As is observed in Figure 2c, Zn in control test was accumulated in roots (TC =1.25), the TF values for stem and leaves being less than 1 (TF = 0.97). The total Zn content in the plant ranged between 65 $mg\cdot kg^{-1}$ d.w. to 75 $mg\cdot kg^{-1}$ d.w. thus, the concentration was lower that the phytotoxic concentration (200 $mg\cdot kg^{-1}$) [15].
The presence of Pb in soil negatively influence the extraction of Zn from the soil in Pb I test. In the case of Pb II test compared with control no significant differences were reported (p = 0.4359). The concentration of 45 $mg\cdot kg^{-1}$ Pb induced more efficient translocation of Zn in stems and leaves.

Cu, Fe, Mn, Ni in plant tissues in polluted experiments compared with the control

Regarding Cu, the transfer from the soil to the plant tissues was done in the order leaves > stem > root (Figure 3a). The only tissue in which Cu accumulates was the leaf from the Pb I experiment, but overall there are no significant differences (p > 0.05) between control and polluted tests. The concentration of 45 $mg\cdot kg^{-1}$ Pb in soil influence positively the translocation of Cu in the leaves (TF = 1.22), while a slight decrease in translocation compared to control was found in the leaves of Pb II test ($TF_{control}$ = 0.88, $TF_{Pb\ II}$ = 0.71). As total Cu concentration in entire plant, 9.39 $mg\cdot kg^{-1}$ Cu in control, 8.59 $mg\cdot kg^{-1}$ Cu in Pb I test, respectively 6.83 $mg\cdot kg^{-1}$ Cu in Pb II test was reported.
The concentration of Fe from plant tissue decreased in the polluted tests compared to the control, the highest Fe concentration was found in the root in all tests. Although the statistical tests (*t* test) do not indicate significant differences between the experiments (p = 0.1404; 0.2818), it was observed that 41 % less Fe was accumulated in the plants from Pb I test, respectively 28 % in the plants from Pb II test than in the control test (Figure 3b). Pb at 45 $mg\cdot kg^{-1}$ does not cause effects on Fe accumulation and translocation in mustard plants. Negative influences in the translocation of Fe in stems and leaves were founded in the case of 85 $mg\cdot kg^{-1}$ Pb.
For Mn, in the control test compared to the polluted tests no significant differences (p-values > 0.05) were registered, but a decrease of 7 % (Pb I test) and 17 % (Pb II test) in total concentration was reported (Figure 3c). The highest concentration of Mn was recorded in the leaves both in control (25.3 $mg\cdot kg^{-1}$) and polluted tests (20.4 $mg\cdot kg^{-1}$ in Pb I; 15.5 $mg\cdot kg^{-1}$ in Pb II). Pb decreased Mn translocation in leaves, which it is involved in photosynthesis processes [2].
Ni did not bioaccumulate in any part of the plant, the largest amount of Ni was found in the root in all tests. Therefore, as the Pb concentration increases, the mustard plants extract less Ni from the soil to use for growth and photosynthesis processes (Figure 3d).



a)



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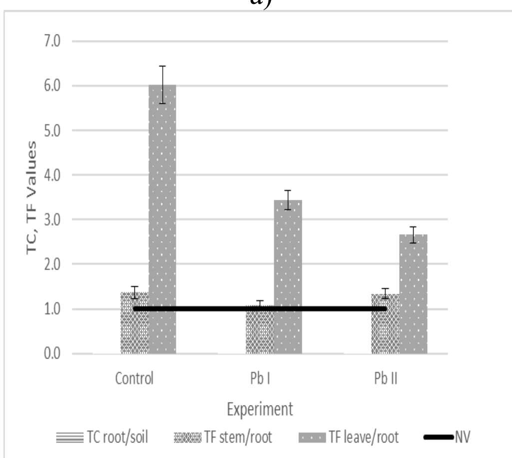
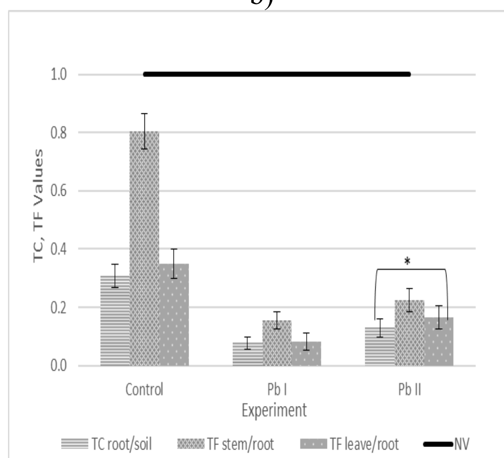


c)



d)

Figure 3. *TC and TF in control a) Cu; b) Fe; c) Mn; d) Ni;*
$p < 0.05$ (*), *(4.75 mg·kg^{-1} d.w. Pb), Pb I (40 mg·kg^{-1} d.w.) and Pb II (80 mg·kg^{-1} d.w.) tests, mean value ± SD (n = 3), SD = standard deviation*

Significant differences were reported between control results in root, stem and leaves compared with plant tissue results in Pb II test ($p = 0.0412$). A total of 9.5 mg·kg^{-1} Ni was detected in plants from control test compared with 6.16 mg·kg^{-1} Ni in Pb I test, respectively 2.63 mg·kg^{-1} Ni in Pb II test. Pb in both tested concentrations affects the accumulation and translocation of Ni into mustard plants, similar conclusion with other reported study [16].

Consequently, competitive processes were observed between Pb and essential metals. Pb was in competition with Ca, Mg, Zn, Cu and Mn, whose accumulation and translocation in the plant became more efficient in the presence of 45 mg·kg^{-1} Pb. The accumulation and translocation of Fe, Cu and Ni in the plant were diminished compared to the control test, which indicates antagonistic processes with Pb, the most affected metal being Ni. Our study indicates that Pb toxicity significantly affects rate of nutrient uptake, translocation and assimilation processes in plants.

Chlorophyll detection

The results for tests (control and polluted) are correlated regardless of which set of two results were analyzed ($r > 0.978$) and no significant differences ($p > 0.05$) were found after performing *t* test. The analysis of the concentration of chlorophyll (chlorophyll *a*, chlorophyll *b*) showed a very small variation (± 1.88 $mg \cdot mL^{-1}$ in case of chlorophyll *a* and ± 2.93 $mg \cdot mL^{-1}$ in case of chlorophyll *b*) among experiments polluted with Pb compared to the control sample (Figure 4), which allows us to appreciate that the photosynthesis process was not significantly influenced ($p > 0.05$, $p = 0.454$).

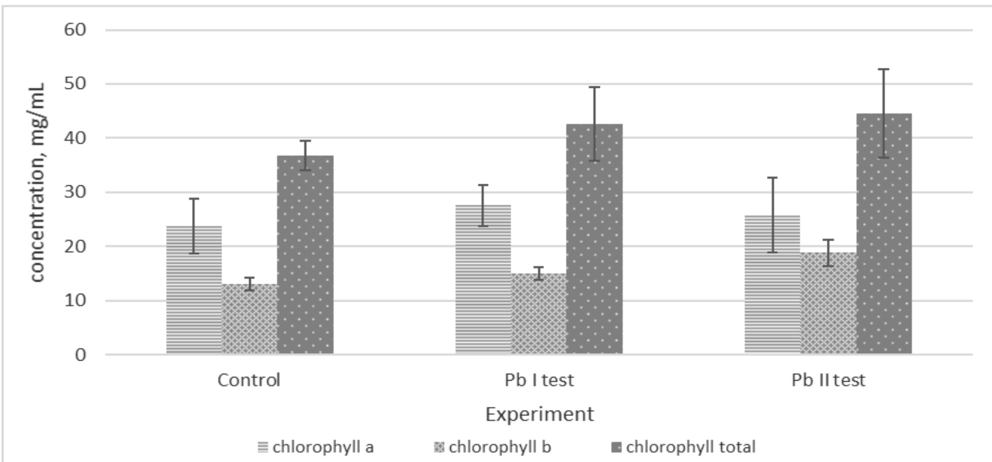


Figure 4. *Chlorophyll a, Chlorophyll b and total concentrations in control (4.75 mg·kg^{-1} d.m. Pb), Pb I (45 mg·kg^{-1} d.m.) and Pb II (85 mg·kg^{-1} d.m.) tests, mean value ± SD (n = 3), SD = standard deviation*

Effects of phytotoxicity

An increase in the biomass of the plants intoxication with Pb compared to the control was observed, due to the growing conditions, respectively the agglomeration of plants per test / flowerpot (Figure 5).

a) b) c)

Figure 5. *Chlorosis effects on control (4.75 mg·kg^{-1} d.m. Pb) and contaminated tests Pb I (45 mg·kg^{-1} d.m.) and Pb II (85 mg·kg^{-1} d.m.)*

As result of the lower germination percentage (60.6 %) in Pb II test, the plants that have been developed were more vigorous, the biomass was more abundant and the chlorosis was reduced compared to the control and Pb I test.
In control and Pb I plants, chlorosis was found correlated with the determined total chlorophyll concentration, which increases slightly in Pb II plants, and with high values of translocation factors for Mn, Mg, Zn, Fe, metals involved in the photosynthesis processes [17].

CONCLUSIONS

The white mustard presents adaptation mechanisms to toxic metals by immobilization at the root level and as result of developing antagonistic and competitive mechanisms for nutrients used in growth and development processes.
The absorption and translocation processes of the essential metals are influenced by the increasing Pb concentrations. While at 45 $mg \cdot kg^{-1}$ Pb stimulation effects for the translocation of the essential metals were observed, inhibition or stagnation processes compared to the control was noticed at 85 $mg \cdot kg^{-1}$ Pb.
Pb can cause negative effects on seed germination, but developed plants grow more vigorously compared to the control. The hypothesis we state is correlated with the fact that Pb contributes positively to the efficiency of absorption and translocation of essential metals (Ca, Mg, Zn, Cu, Mn) in plants.
The presence of essential metallic elements decreases the toxicity of lead to both tested concentrations.

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