ADAPTIVE MECHANISMS OF *Phaseolus vulgaris* AND *Zea mays* SEEDS GROWN IN AGROCENOSES PRONE TO POLLUTION WITH HEAVY METALS

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Abstract. The key goal of our research is the identification and analysis of accumulations of metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}) during their imbibition in seeds of *Phaseolus vulgaris* and *Zea mays*. We take as samples seeds collected from one metalliferrous and respectively from one non-metalliferrous area in Maramures County (North-West of Romania). We will refer following to an area as being "metalliferrous" because of its known high degree of pollution with metallic components, and to the other area as "non-metalliferous" because of being generally known as very much less exposed to pollution in general. We will subsequently name as being "polluted" the seeds originated in the metalliferous area, and respectively as "unpolluted" the seeds originated in the other area. We have thoroughly investigated in quantitative terms the existence of accumulations of each of the metal ions mentioned above during imbibition at three different levels of concentration. The seeds grown in the non-metalliferous area generally display a higher degree of absorption for each of the metal ions than the seeds grown in the metalliferous area. On the other hand, we have concluded that the concentration of heavy metals does not impact significantly the absorption capacity of both the polluted and unpolluted *Z. mays* seeds. The *P. vulgaris* seeds behaved differently, namely the content of metal ions absorbed went proportionally up, as the concentration of the initial solution went higher.

Keywords: *Phaseolus vulgaris*, *Zea mays,* heavy metal accumulation, imbibition, adaptative mechanisms

INTRODUCTION

Anthropogenic factors (industrial activity, mining, sewage disposal, traffic, etc.) are mainly responsible for the growing concentration of heavy metals in soil. Heavy metals inhibit directly (by altering the catalytic function of enzymes, by damaging cellular membranes, by inhibiting the growth of roots) and indirectly (by dampening photosynthesis and mineral nutrient uptake, by structural changes) all physiological processes in plants [10, 14]. Another indirect effect of the contamination of soil with heavy metals consists in the decline of the microbiological activity, activity particularly vibrant in the rhizosphere.

Plants usually show the ability to accumulate large amounts of metals without visible changes in their appearance or yield [13]. In many species of plants the level of metal accumulation can exceed even several hundred times the maximum level accepted for human beings, without a perceived negative impact on their growth or yield [24]. Therefore, it seems that plants can endure a level of environmental pollution that might be even several times higher than the level observed nowadays [9, 16, 21].

Metals accumulate and remain in soil along time frames spanning even hundreds of years, but impact in various ways the plants grown on polluted sites [19]. Various species of plants growing in a metalliferous area are known for their capacity to adapt to a polluted environment, by inactivating ions of heavy metals [3, 4, 26]. This occurs by binding the metal ions in excess and/or by changing the chemical composition and physical organization of cellular membranes [17, 22].

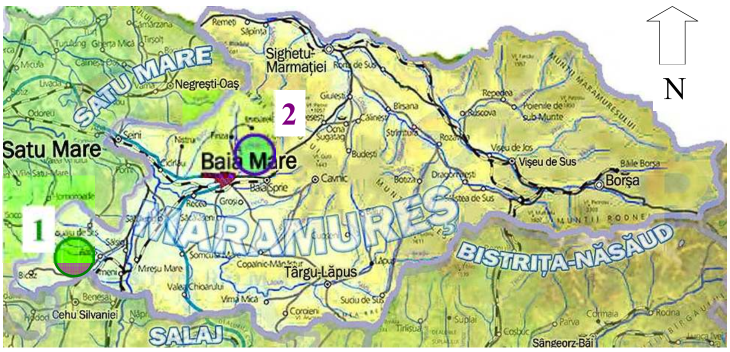
Many research teams [1, 6] have investigated the influence of heavy metals on the metabolism of plants, but data remains scarce about the accumulation of heavy metals in the reproductive organs of plants [5, 20]. Stefanov *et. al.* [22], which studied the accumulation of lead, zinc and cadmium in the seeds of various plants, showed that plants accumulate selectively ions of heavy metals in their seeds. Peanut and corn seeds accumulate mainly lead, pea seeds accumulate mainly cadmium and wheat seeds accumulate mainly zinc. On the other hand, Lane and Martin [11] showed that the coats of *Raphanus sativus* seeds were a strong barrier to lead and helped prevent the contamination of embryos until the coats were torn apart by the germinating embryonic root. There are reports on the inhibitory effect of lead on the germination of seeds of the *Lupinus luteus* [25]*, Oryza sativa* [15] and *Sinapis alba* [7] species.

One of the methods to assess how tolerant the seeds of a plant are to the ions of a metal consists in sowing those seeds in soil containing such ions. This substantiates that heavy metals have a significant effect on germination [2]. The studies mentioned above, unlike those by Lane and Martin [11], point to the significant influence of lead on the germination of seeds. In this situation it seems that lead ions impact the germination process depending on differences in the structure of seeds, more precisely depending on differences in the structure of coats. The role of the coat is to protect the embryo from harmful external factors is well known. But seed coats have a wide range of anatomic forms that do not exist in other organ or tissue of the plant [8]. Wierzbicka *et. al* [24] showed that out of all the families of plants tested, the *Fabaceae* are very sensitive as the concentration of metal ions goes up.

The key goal of our research was to establish how the accumulation of heavy metals occurs during imbibition, the first step of the germination process, in seeds originating from a metalliferous area as compared to seeds from a non-metalliferous area. Our other ongoing concern is to collect evidence about how the adaptive behavior of seeds during imbibition has influenced the growth of the plant, as well as the distribution pattern of the metal ions among the different organs of the plant.

MATERIALS AND METHODS

Seed material. Satu Nou de Sus is a village in the North – East neighborhood of Baia Mare (see upper circle on the map in Figure 1), in the the Maramures County (North-West of Romania). It is known as heavily polluted area due to intensive mining and ore processing activities in the past. Oarta de Jos is a village located about 50 km South - West of Baia Mare (see lower circle on the same map), in an area that is rather clean, just because of being exempted from any sort of mining and industrial activity. Even more, the village is about 10 km far of any significant road traffic, which is by itself a source of pollution. We have collected *P. vulgaris* and *Z. mays* seeds out of the crop harvested in Satu Nou de Sus and respectively in Oarta de Jos, in the fall of 2009.



1. Area free of pollution with ions of heavy metals
2. Area heavily polluted with ions of heavy metals

Source : part of a map edited and posted on the Internet by RestRom SRL

Figure 1. Map of Maramures County. Circles identify the locations from where we have collected the seeds of *P. vulgaris* and *Z. mays* for the research interests of this paper.

Our samples have included ten individual seeds of each type. We have disinfected the seeds with alcohol, washed them three times with distillated water and have introduced them to stay during germination in solutions with different concentrations of ions of heavy metals. The solutions containing the metal ions were prepared using $FeSO_4$, $(CH_3COO)_2Pb$, $CuSO_4$, $ZnSO_4$ salts. We have prepared solutions with the following concentrations: 10, 50 and 250 mg/L for Fe^{2+}; 10, 20 and 25 mg/L for Cu^{2+}; 5, 10 and 15 mg/L for Zn^{2+}; 100, 1000 and 10000 mg/L for Pb^{2+}.

As blanks (witness samples) were used each type of seed (polluted and unpolluted bean, polluted and unpolluted maize) introduced in pots for germination containing distillated water. The imbibition time was 2 days at 22^0C.

The analysis procedure of heavy metals. After imbibition, the seeds were washed with distillated water and dried at 105^0C for 72 hours in a US-made Binder drying oven. A Retsch RM-100 grinding machine was used to prepare the samples of imbibited seeds for the mineralization stage. A Berghof MWS-2 microwave system was used for mineralization. For plant material mineralization, the following parameters were selected: step 1 - 145^0C, 5 minutes, power 75%; step 2 - 190^0C, 10 minutes, power 90%; step 3 - 100^0C, 10 minutes, power 40%. For soil mineralization, the following parameters were selected: step 1 - 180^0C, 25 minutes, power 99%; step 2 - 100^0C, 10 minutes, power 99% were used. The mixture of 10 mL HNO_3 65% (d = 1,4 kg/L, Lach-Ner) and 0,3 g plant powder and 4 g dried soil respectively, were introduced in the microwave system. After mineralization the samples were brought to 100 ml volumetric flask with distilled water. A Perkin Elmer AAS-800 Spectrometer has allowed us to apply a spectrometric method in order to measure the concentration of metals in seeds and soil.

We have relied on a Krúss Optronic binocular microscope in order to investigate the degree of imbibition by optical microscopy. This method required the achievement of a specific color reaction for each metal ion control. Table 1 gives the color assigned to each metal ion and corresponding to each reagent.

Table 1. The specific reagents used to identify the iron, copper, zinc and lead ions.

Heavy metal	Reagent	Color
Fe	$K_3[Fe(CN)_6]$ 5%	Green
Cu	Ditizone 0,12% in $CHCl_3$	Red
Zn	Ditizone 0,12% in $CHCl_3$	Brown
Pb	KI 5%	Yellow

We have maintained the seeds for 10 minutes immersed in reagent, and have dried them up for another 10 minutes in open air. A manual MIC-500 microtome has allowed us to produce mono-cellular microscopic sections. In order to use the microtome efficiently, before sectioning we have immersed the seeds in a paraffin bath, to get them fastened.

RESULTS

Data included in Table 2 and in Figure 2 stand proof that the content of heavy metal in polluted seeds is about 1.2 times higher as compared to the content in unpolluted seeds. With the exception of Zn, this stands for each of the metallic ions considered. The accumulation of copper is 2 times higher in the seeds of maize from the polluted area, as compared to the seeds of maize originating from the pollution - free area.

Also, the content of iron in the seeds of polluted maize is about 1.7 times higher than in the unpolluted seeds. We have established that the ratio between the content of lead in seeds of unpolluted maize and the content of lead in seeds from polluted maize was comparable to the equivalent ratio between seeds of beans.

The surface of bubbles from Figure 2 is proportional to the metal ion content of the sample.

We have extended our analysis on samples of soil collected from each of the two locations, in order to establish the relationship between the content of heavy metals in soil and the content of heavy metals in the seeds originating from each respective sample of soil. We have decided on the concentration of each solution in which the imbibition process took place depending on the content of heavy metals in each sample of soil.

Table 2 includes the average values for the three sets of measurements we have conducted in parallel on each sample.

Table 2. The content heavy metals in the seeds and soil samples for analysis

Samples	Fe (mg/g DW*)	Cu (mg/g DW*)	Zn (mg/g DW*)	Pb (mg/g DW*)
Polluted bean – BP	0.075	0.007	0.009	0.064
Unpolluted bean – BUP	0.064	0.006	0	0.054
Polluted maize – MP	0.015	0.004	0	0.049
Unpolluted maize – MUP	0.009	0.002	0	0.040
Polluted soil (from Satu Nou de Sus)	13.343	0.173	0.836	0.583
Unpolluted soil (from Oarta de Jos)	0	0.014	0.039	0.058

* - DW = dry weight

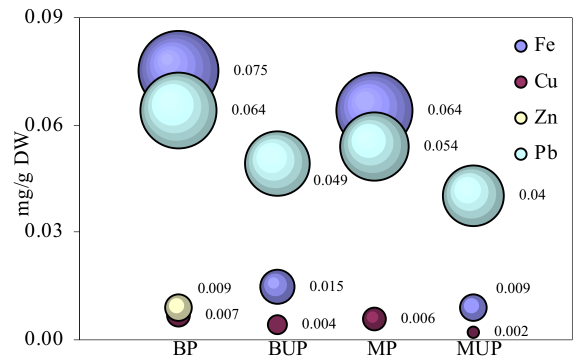


Figure 2. Content of heavy metals, before imbibition, in polluted and unpolluted seeds of bean and maize

In contaminated soil, the content of metallic ions in the crops depends on the degree of pollution as our preliminary data suggests.

Table 3 gives the quantitative dimension of the concentration of heavy metals, during imbibition, for each of the seeds in our samples. The pictures in Figure 3, (a) to (d) give the photographic proof about our samples for each concentration.

We have included the pictures in Figure 3, (e) to (h), in order to reveal in an even more convincing way the close direct relationship between the concentration of each solution and the accumulation of heavy metals. On the other hand, these pictures reveal the dependence of the nature / concentration of metal ions on the land each seed comes from. The accumulation of ions of heavy metals is higher in polluted seeds, as compared to seeds from unpolluted areas, as the Figure 3, (e) – (h) clearly reveal.

Macroscopic and microscopic images of bean seeds during imbibition in the presence of ions of heavy metals conduced to a similar conclusion [23]. The images, especially those microscopic, disclose an imbibition process that starts with the hilum and goes up to the cotyledon (Figure 4). This happens because plants block the metallic ions in excess and/ or change the chemical composition and physical organization of their cell membranes [22]. Moreover, the plants growing on polluted areas develop in time some mechanisms to survive in ecologically – severe environment. The selection of this kind of resistant plants constitutes during the time a particularly ecotype.

In the case of maize, the macroscopic and microscopic images of seeds during imbibition in the presence of ions of heavy metals were not so relevant than the images of bean. Also, the macroscopic and microscopic images of seeds during imbibition in the presence of lead were not relevant because of lead hardly noticeable color (yellow) in presence of KI.

The experiments about the accumulation of heavy metals ions during the imbibition process has conduced us to conclude:

Table 3. The contend of heavy metals in polluted / unpolluted bean and maize seeds after imbibition.

Type of seed	Fe (mg/g DW)			Cu (mg/g DW)			Zn (mg/g DW)			Pb (mg/g DW)		
	10	50	250	10	20	25	5	10	15	100	1000	10000
BP	0.579	0.846	1.486	0.041	0.090	0.827	0.028	0.059	0.094	0.237	7.129	30.059
BUP	0.367	1.097	1.829	0.039	0.048	1.756	0.042	0.052	0.109	0.708	7.435	6.364
MP	0.261	0.321	0.332	0.012	0.015	0.185	0.017	0.027	0.029	0.141	1.832	3.127
MUP	0.162	0.173	0.159	0.003	0.005	0.059	0.021	0.053	0.043	0.258	1.484	8.054

- as regards iron, the seeds analyzed behaved as follows:
  - the unpolluted beans accumulate more ions than the polluted beans in solutions with 50 mg/L and respectively 250 mg/L in concentration;
  - both the polluted and unpolluted seeds of beans accumulate significantly more metal ions at higher concentration;
  - the unpolluted maize accumulates less metal ions than the polluted one;
  - especially in unpolluted sites, the behaviour of the seeds of maize seems to not depend on the concentration of iron;
- as regards copper:
  - in both polluted and unpolluted soil, at 10 mg/L in concentration, the beans accumulate approximately the same amount; however, as the concentration goes up to 25 mg/L, the quantity that unpolluted seeds accumulate amounts to about the double of the quantity that polluted seeds collect; on the other hand, at 20 mg/L in concentration polluted beans accumulate 2 times more ions than the unpolluted beans;
  - the seeds of unpolluted maize accumulate approximately 3 times less than the seeds of polluted maize, if the concentration grows higher (at 20 and respectively at 25 mg/L);
  - as happens for iron ions, the accumulation in the seeds of both polluted and unpolluted maize does not depend on the content of copper at low and medium concentrations (10 and respectively 20 mg/L).

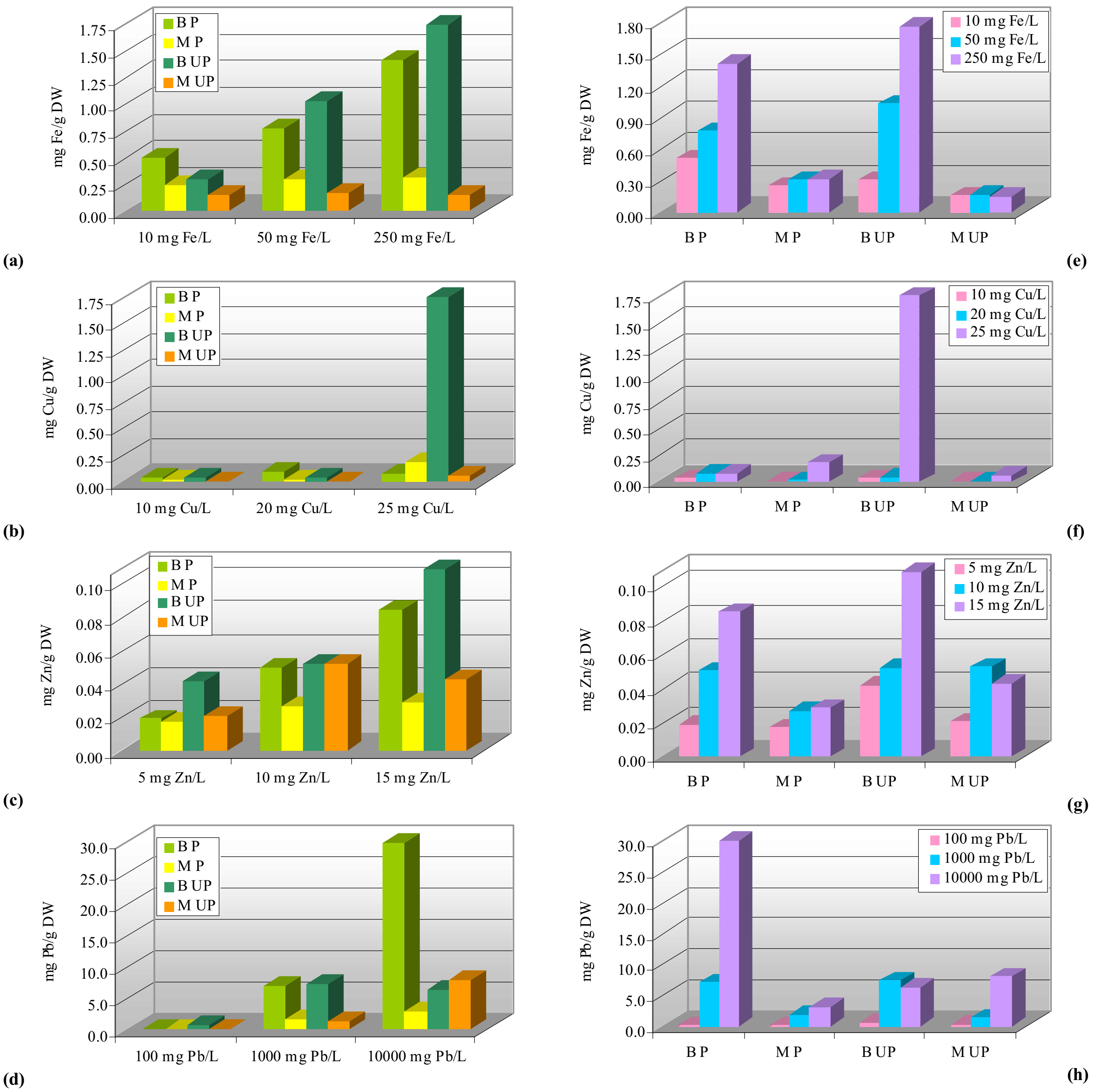


Figure 3. The content of heavy metals in polluted / unpolluted bean and maize seeds after imbibition, where: **B P** – polluted bean; **M P** – polluted maize; **B UP** – unpolluted bean; **M UP** – unpolluted maize.

- as regards zinc, the seeds have behaved in a similar way:
  - the unpolluted seeds of both beans and maize, accumulate zinc ions more than the polluted seeds;
  - all the seeds we have analyzed during imbibition have demonstrated sensitivity to changes in concentration in zinc ions;
- as regards lead, only the polluted beans behave in a different way (showing evidence about their role as bio-accumulators) as compared to both the polluted and unpolluted seeds of beans and maize.

The biosorption of lead (II) ions onto *P. vulgaris* was found by Ozcan et al. (2009), as a spontaneous endothermic process [18]. This finding supports the results of our research that includes the bean in the bio-accumulative plant group, especially for copper and lead ions. Meers et al. (2007) also described this species as one with the high uptake of heavy metal ions, especially copper and lead [12].

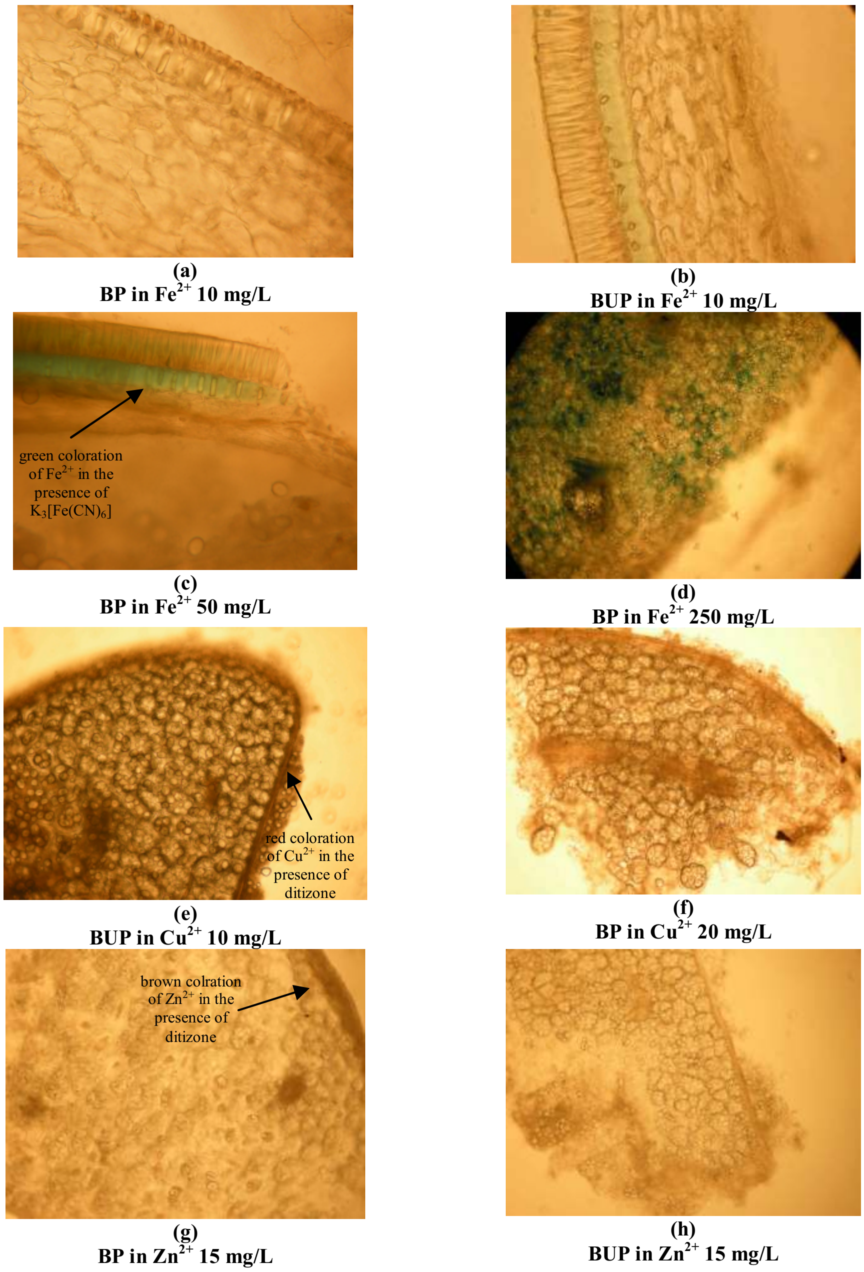


Figure 4. *P. vulgaris* seed – cross sections as captured in optical microscope images at 10 X magnification to detect how the concentration of metal ions impacts the imbibition degree.

The outcomes of our experiments and analyses can be summarized as follows:

a) the beans originating in unpolluted areas display a stronger tendency to absorb iron, copper and zinc ions than those in polluted areas, because plants growing in polluted areas have developed adaptive mechanisms helping them to survive in harsher environments;

b) the seeds of *P. vulgaris* and of *Z. mays* behave in different ways in the presence of different metallic ions accumulated during imbibition; additional differences occur depending on the concentration of those metallic ions;

c) the seeds of beans originating in polluted areas can play the role of bio-accumulators, as demonstrated by their atypical behavior in terms of their capacity to accumulate lead.

Establishing the pattern of distribution of metal ions in the organs of plants growing from such seeds will further contribute to shed light on the behaviors described above. Our expectation is to find the roots as the main host of the metallic ions in the plant. The roots are, we can foresee, part of the adaptive mechanism of the plant. This is to be confirmed in the near future, in our pursuit to bring our research work in this project to an even higher level of complexity.

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REFERENCES

[1] Baker A.J.M., (1987): Metal tolerance. New Phytologist, 106: 93-111.
[2] Baker, A.J.M., Walker, P.L., (1989): Physiological responses of plants to heavy metals and quantification of tolerance and toxicity. A review. Chemical Speciation and Bioavailability 1: 7-17.
[3] Chen, H., Wang, A., (2007): Kinetic and isothermal studies of lead ion adsorption onto palygorskite clay. Journal of Colloid and Interface Science, 307, 309-316.
[4] Engleman C. J., McDiffett W. F., (1996): Accumulation of aluminum and iron by bryophytes in streams affected by acid-mine drainage. Environmental Pollution, 94, 67-74.
[5] Ernst, W.H.O., (1982): Schwermetallpflanzen. pp. 472-506. In: Kinzel, H. (ed.): Pflanzenokologie und Mineralstoffwechsel.
[6] Ernst, W.H.O., Verkleij, J.A.C., Schat, H., (1992): Metal tolerance in plants. Acta Botanica Neerlandica, 41: 229–248.
[7] Fargašová, A., (1994): Effect of Pb, Cd, Hg, As, and Cr on germination and root growth of Sinapis alba seeds. Bulletin of Environmental Contamination and Toxicology, V 52: 452–456.
[8] Grzesiuk, S., Kulka, K., (1981): Seed physiology and biochemistry. PWRiL, Warszawa, 288 p.
[9] Gupta, A.K., Dwivedi, S., Sinha, S., Tripathi, R.D., Rai, U.N., Singh, S.N., (2007): Metal accumulation and growth performance of *Phaseolus vulgaris* grownin fly ash amended soil. Bioresource Technology, 98: 3404-3407.
[10] King, S.O., Match, C.E., Brezonik, P.L., (1992): Changes in trace metal concentrations in lake water and biota during experimental acidification of Little Rock Lake, Wisconsin, USA. Environmental Pollution, 78(1/3): 9-18.
[11] Lane, S.D., Martin, E.S, (1977): A histochemical investigation of lead uptake in *Raphanus sativus*. New Phytologist, 79: 281-286.
[12] Meers, E., Samson, R., Tack, F.M.G., Ruttens, A., Vandegehuchte, M., Vangronsveld, J., Verloo, M.G., (2007): Phytoavailability assessment of heavy metals in soil by single extraction and accumulation by *Phaseolus vulgaris*. Environmental and Experimental Botany, 60: 385-396.
[13] Mohamed, A.E., Rashed, M.N., Mofty, A., (2003): Assessment of essential and toxic elements in some kinds of vegetables. Ecotoxicology and Environmental Safety, 55: 251-260.
[14] Moore, J.W., (1991): Inorganic contaminants of surface water: research and monitoring priorities. Springer-Verlag. ISBN 0-387-97281-1, 334 p.
[15] Mukherji, R., Maitra, P., (1977): Growth and metabolism of germinating rice (*Oryza sativa L.*) seeds as influenced by toxic concentrations of lead. Zeitschrift Pflanzenphysiologie, 81: 26-33.
[16] Murakami, M., Ae, N., (2009): Potential for phyto-extraction of copper, lead and zinc by rice (*Oryza sativa L.*), soybean (*Glycine max* L.) and maize (*Zea mays* L.). Journal of Hazardous Materials, 162: 1185-1192.
[17] Nishizono, H., Kubota, K., Suzuki S., Ishii F., (1989): Accumulation of heavy metals in cell walls of Polygonum cuspidatum roots from metalliferous habitats. Plant and Cell Physiology, 30(4): 595-598.
[18] Ozcan, S.A., Tunali, S., Akar, T., Ozcan, A., (2009): Biosorption of lead (II) ions onto waste biomass of *Phaseolus vulgaris* L.: estimation of the equilibrium, kinetic and thermodynamic parameters. Desalination, 244: 188-198.
[19] Piechalak, A., Tomaszewska, B., Baralkiewicz, D., Malecka, A., (2002): Accumulation and detoxification of lead ions in legumes. Phytochemistry, 60: 153-167.
[20] Searcy, K.B., Mulcahy, D.C., (1985): Pollen-tube competition and selection for metal tolerance in *Silene dioica* (*Caryophyllaceae*) and *Mimulus guttatus* (*Scrophulariaceae*). American Journal of Botany, 72: 1695–1699.
[21] Sinha, S., Saxena, R., (2006): Effect of iron on lipid peroxidation, and enzymatic and nonenzymatic antioxidants and bacoside-A content in medicinal plant *Bacopa monnieri* L.. Chemosphere, 62(8): 1340-1350.
[22] Stefanov, K., Seizova, K., Yanishlieva, N., Marinova, E., Popov, S., (1995): Accumulation of lead, zinc and cadmium in plant seeds growing in metalliferous habitats in Bulgaria. Food Chemistry, 54: 311-313.
[23] Varga, C., Marian, M., Peter, A., Boltea, D., Mihaly-Cozmuta, L., Nour, E., (2009): Strategies of heavy metal uptake by *Phaseolus vulgaris* seeds growing in metalliferous and non-metalliferous areas. Studia Universitatis Babes-Bolyai, Chemia, LIV(3): 223-234.
[24] Wierzbicka M, Obidzinska J, (1998): The effect of lead on seed imbibition and germination in different plant species. Plant Science, 137: 155–171.
[25] Wozny, A., Zatorska, B., Mlodzianowski, F., (1982): Influence of the lead on the development of lupin seedlings and ultrastructural localization of this metal in the roots. Acta Societatis Botanicorum Poloniae, 51: 345-351.
[26] Yetilmezsoy, K., Demirel, S., (2008): Artificial neural network (ANN) approach for modeling of Pb (II) adsorption from aqueous solution by *Antep pistachio* (*Pistacia Vera* L.) shells. Journal of Hazardous Materials, 153(3): 1288-1300.

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