SEEDS GERMINATION AND ROOTS LENGTH IN CADMIUM POLLUTED SOILS

GERMINAREA SEMINȚELOR ŞI ALUNGIREA RĂDĂCINILOR ÎN CONDIȚIILE SOLURILOR POLUATE CU CADMIU

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Abstract*: The most frequently soils pollution consist the heavy metal ions contamination, having extremely serious effects both on ecosystems and human health. In this context, a method for decontamination of soils contained heavy metal ions is phytoremediation, which consist in the use of plant species in order to extraction, stabilization and/or neutralization of soil pollutants. For biomonitoring and establishing the parameters of this method, first, the phytotoxicity tests are needed. This causes the maximum dose of heavy metal ions which do not cause a negative effect on plants, even from germination process. Phytotoxicity tests measure the decrease or absence of germination and root length, in just a few days of exposure seeds to contaminated soil, in comparison with unpolluted soil. This study aimed to determine the Cd^{2+} toxicity in seeds germination and root length of bioaccumulating plants such as white mustard (Sinapis alba), rape (Brassica juncea) and triticale (Triticosecale rimpau), at different Cd^{2+} concentrations.*
Key words*: polluted soil, cadmium, germination seed, phytotoxicity, root length*

Rezumat*: Cea mai frecventă formă de poluare a solurilor o constituie contaminarea cu ioni de metale grele, având efecte deosebit de grave atât asupra ecosistemelor cât şi a sănătății umane. În acest context, o metodă de depoluare a solurilor contaminate cu ioni ai metalelor grele este fitoremedierea, care constă in folosirea unor anumite specii de plante cu scopul extracției, stabilizării şi/sau neutralizării substanțelor poluante aflate în soluri. Pentru biomonitorizarea şi stabilirea parametrilor acestei metode, mai intai, sunt necesare testele de fitotoxicitate. Acestea determină doza maximă de ioni de metale grele care nu cauzează un efect negativ asupra plantelor, chiar de la nivelul procesului de germinare. Testele de fitotoxicitate măsoară scăderea sau absența germinării şi creşterea rădăcinilor, in doar câteva zile de expunere a semințelor la soluri contaminate, în comparație cu un sol nepoluat. Acest studiu a avut ca scop determinarea toxicității Cd^{2+} asupra germinării semințelor şi alungirii rădăcinilor unor plante bioacumulatoare cum ar fi muştarul alb (Sinapis alba), rapița (Brassica juncea) şi triticale (Triticosecale rimpau), la concentratii diferite de Cd^{2+}.*
Cuvinte cheie*: sol poluat, cadmiu, germinatia semintelor, fitotoxicitate, alungirea rădăcinilor*

INTRODUCTION

Soils polluted by heavy metals ion is a worldwide problem. That soils is becoming one of the most significant environmental hazard with negative impact

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on biodiversity, surface and groundwater, agricultural sites and finally human health. This is the result of increasing population and industrial technology, finally increased waste quantity on earth`s terrestrial, atmospheric and aquatic systems. While some organic compounds in waste can be directly degraded (Salt et al., 1995), the heavy metal ions are nonbiodegradable. They accumulate in the environment and are mutagenic, carcinogenic and teratogenic (Hazrat et al. 2013). The inorganic pollutants, including heavy metal ions, can be degraded in the soil, for the first, these must either be stabilized (Pohontu, 2013). One of the most serious contamination of the ground is produced by heavy metal, which have a particularly badly both on terrestrial ecosystem vegetation physiology, and on humans and animals coming into directly or indirectly contact with contaminated sites. As a result of this situation, science and technology in environmental world has developed a number of methods and techniques preventing and / or combating pollution with heavy metal ions.

Cadmium is one of the common heavy metals affecting the soil quality. The sources of cadmium in the soil are especially from industrial operation, including steel, alloy, electroplating, motor vehicles, pigments, aircraft paint, chemicals and textiles, from combustion and from phosphate fertilizers (Mani and Freitas, 2008).

The biogeochemical transformations in soil including more processes such as: translocation, transformation, chelation, immobilisation, solubilisation, precipitation, volatilization and complexation of heavy metals (Alkorta and Garbisu, 2001). Several methods and technologies have been used and improved for rehabilitation, decontamination and removed pollutants from soils. These methods consist in physical removal of soil through excavation, uncovering, or extraction by chemical means. These technologies are unfortunately more expensive and have a negative impact on soil physical, chemical, biological properties. The classical soil treatments are most expensive. Better option and an alternative cost effective for decontaminated soils polluted by heavy metal ions is phytoremediation through hyperaccumulating plants (Hazrat et al. 2013). To control this process easier, are necessary phytotoxicity tests, one of the standard methods to determine the maximum dose of heavy metal ions, that not causing a negative effects on plants, even in the germination and seedling, consists in using phytotoxicity tests. These tests are the easiest method of biomonitoring of environmental and may be important in environmental engineering, to establish methods of phytoremediation, in agriculture for the decontamination of polluted soils. The phytotoxicity tests measure the decrease or absence of germination and root length. (Bialowiec and Randerson, 2010) Phytoremediation using the plants to remediation polluted soils is relatively inexpensive, efficient and potentially clean techniques. The major processes of phytoremediation including: phytodegradation, phytostabilisation and bioaccumulation in the rizosphere through root-microbes interaction. (Wild et al. 2005; Xiaoe et al. 2005)

White mustard (*Sinapsis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*) is a species of vascular plant with considerable capacity to grow in a heavily polluted soils as well its capacity for metal ions hypeaccumulation. (Blaylock and Huang, 2000)

The present work aims to investigate the effects of Cd^{2+} in different soil concentrations for germination process of white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*) seeds.

MATERIAL AND METHOD

The experiments was performed at laboratory scale, after a few days of exposure of the seeds to the contamined soil, compared with non-polluted soil representing the control of the experiments.

Phytotoxicity kit consists of plastic plates, transparent, with dimensions of 21,00 cm x 15,50 cm x 0,80 cm and two compartments according to figure number 1. In the bottom compartment of the plate sits soil making sure the entire surface is covered, using a spatula for smoothing.

Fig. 1 - Kit for phytotoxicity determination

The soil used in these experiments was a ground reference OECD (*Organisation for Economic Co-operation and Development*) which is used frequently for standard phytotoxicity tests, provide by MicroBioTests Inc, Belgium. The reference soil composition is presented in table number 1. The initial analysis of OECD reference soil didn`t contain heavy metal ions, which facilitated the accuracy results.

Table 1

Soil composition

Soil compounds	Quantity (%)
Dry quartz sand	85
Kaolin	10
Peat moss *Sphagnum*	5
Calcium carbonate ($CaCO_3$)	To obtain a pH of 6,5 - 7

A second type of substrate used in germination tests was sand. Sand initial analyzes do not contain traces of heavy metal ions, leading to higher accuracy of results.

The palnt seeds used in this study was white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*). Selected seeds have been used, with high germination rate capacity, provided from the Bank of Vegetable Genetic Resource Suceava.

Soil was polluted in a controlled mode by adding a cadmium Cd^{2+} solution. For preparing the pollutant solution was used $3CdSO_4 \cdot 8H_2O$, finally obtained *50 mg/kg* concentration. The soil used in the experiments was placed in the bottom of the plate and saturated with cadmium solutions of various dilutions: 1/1(100%); 1/2 (50%); 1/4 (25%); 1/8 (12.5%) and 1/16 (6.25%). After that, soil was covered with a filter paper.

Distilled water was used for dilution an control the process, representing 0% cadmium concentration plate. On the filter paper was placed 10 seeds of plants at a distance of 1 cm from each other. The seeds of the plants used were white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*). After closing the transparent cover plates, it was placed vertically in a thermostat and incubated at a temperature of 25 ± 1 °C for 3 days. At the end of incubation, the plates were photographed, for measurement of seedlings with ImageTool 3.0 soft.

Each group of experiment was carried out in three times for results reproductibility, and calculated root elongation.

RESULTS AND DISCUSSIONS

After three days of incubation of analyzed plant seeds it could be seen that the pollutants affected seed germination and root elongation. Germination rate of seeds for each plant, at different Cd^{2+} concentrations, in OECD soil and sandy substrate can be observed in figures 2 – 7. As it can be seen, seed germination rate decreases with increasing concentration of the pollutant and depend on the type of soil used.

In the case of rape and white mustard seeds, the germination rate was Higher reaching 70.66% and 65.00% for sandy substrate, than in the case of using OECD soil which was 62.83% and 55.50%, according to figures 2 - 5.

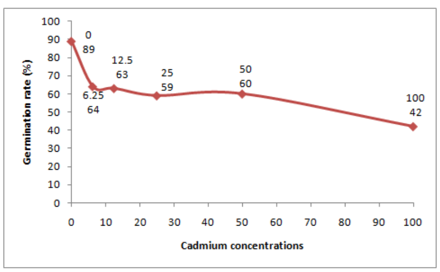

Fig. 2 - Variation of germination rate for white mustard seeds on OECD soil with different Cd^{2+} concentrations

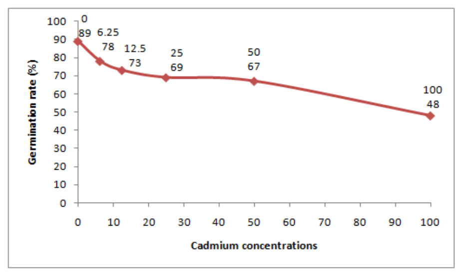

Fig. 3 - Variation of germination rate for white mustard seeds on sand with different Cd^{2+} concentrations

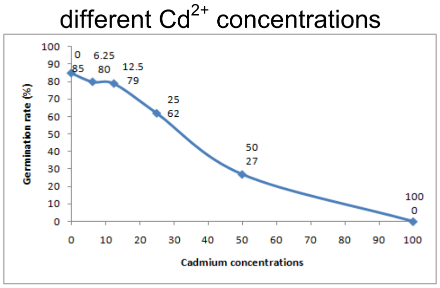

Fig. 4 - Variation of germination rate for rape seeds on OECD soil with different Cd^{2+} concentrations

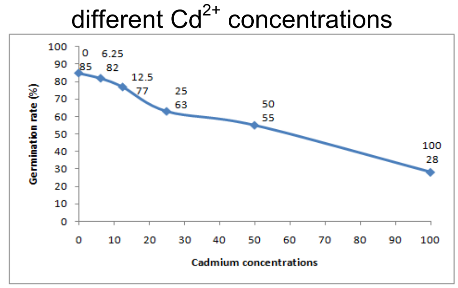

Fig. 5 - Variation of germination rate for rape seeds on sand with different Cd^{2+} concentrations

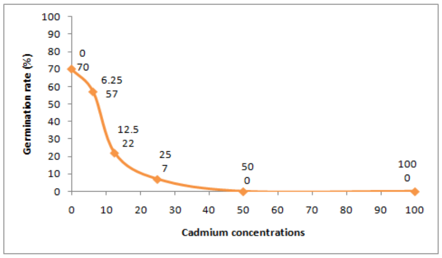

Fig. 6 - Variation of germination rate for triticale seeds on OECD soil with different Cd^{2+} concentrations

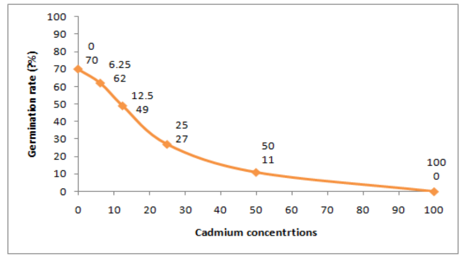

Fig. 7 - Variation of germination rate for triticale seeds on sand with different Cd^{2+} concentrations

Analyzing the figures 6 and 7, we may notice that triticale seeds germination rate was lower when we used OECD soil, it was 26,00% while when we used sand, germination rate reaching we used 36,50 %. Triticale seeds have not germinated in a sand at most concentration of cadmium [1/1 (100%)], while on the OECD soil seeds have not germinated starting at a concentration of cadmium [1/2 (50%)].

Inhibition of plant roots by pollutant, was calculated with the equation number 1 (Bialowiec et al. 2010):

$$I = \frac{C - T}{C} \cdot 100(\%) \ (1), \text{ where:}$$

C - mean of root length of the control, blank (0%);
T - average root length for each pollutant concentration applied.

According to equation 1 was calculated inhibition rate for each situation (type of plant – type of soil), the values are presented in table 2.

Table 3

Inhibition rate

Plant type	Substrate	Inhibition (%)
Sinapis alba	OECD soil	41,64
	sand	28,23
Brassica juncea	OECD soil	35,28
	sand	24,71
Triticosecale rimpau	OECD soil	75,42
	sand	57,42

Inhibition values can determine the lowest effective concentration of cadmium ions in the soil which not causing a toxic effects in a seeds germination process. Thus, can find the optimum solution would be suitable plant for phytoremediation process.

CONCLUSIONS

1. The germination rate of plants seeds studied and their root elongation depend on soil type and decreased with increasing concentration of the pollutant. All seeds had a higher rate of germination on sandy soil compared with the

substrate consists of reference soil OECD, because the capacity of sand drainage, actually pollutant has no contact with seeds surface.

2. When using as germinative substrate OECD reference soil, germination rate was lower than sand, in fact due to both retention in the upper layers of the cadmium pollutant solution and because its complexation with organic substances in the soil.

3. White mustard seeds had a germination rate for OECD soil polluted by cadmium 55,50%, while the use of sand, germination rate is slightly increased, reaching 65,00%, but germinated at every concentration of cadmium in the soil.

4. Rape seeds do not germinate in the OECD soil have the most concentration of cadmium [1/1(100%)]. The average length of the roots began to decline significantly from the pollutant concentrations of 25% corresponding to 12.5 mg / kg Cd, both the OECD soil and the sand.

5. Triticale seeds were most affected by the cadmium toxicity, the germination rate of the sand was of 36,50% and on OECD soil 26,00%. The seeds have not germinated in a sand at most concentration of cadmium [1/1(100%)], while on the OECD soil have not germinated starting at a concentration of cadmium [1/2 (50%)].

6. Considering the inhibition rate, the least affected by the toxicity of cadmium in soil was rape and white mustard seeds.

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