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EFFECTS OF SOIL POLLUTION WITH HEAVY METALS ON PLANT SEEDS OF *BRASSICA NAPUS*, *PISUM SATIVUM* AND *SECALE CEREALE*

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Abstract

In the paper we studied the effects of soil pollution with cadmium, chromium and zinc on the germination and roots elongation of rape (Brassica napus), peas (Pisum sativum) and rye (Secale cereale). The soil used in the experiments was an OECD reference soil. The soil was contaminated with solutions of $CdCl{\cdot}H_2O$, $K_2Cr_2O_7$ and $ZnSO_4{\cdot}7H_2O$ in concentrations from 200 mg/kg to 1200 mg/kg. At the maximum pollutant concentration in the respective soil, 1200 mg/kg, the lowest germination rate was obtained for pea seeds 34% (in the case of chrome soil pollution), for rye seeds 50% and 60%, respectively for seeds of rapeseed (in case of soil pollution with zinc). As far as elongation of the roots is concerned, zinc was the metal that most affected their growth. Thus, the root length decreased in the case of Pisum sativum from 44.08 mm (0 mg/kg) to 0.86 mm (at the concentration of 1200 mg/kg). Chromium was the only metal whose effect on rye seeds was the reverse, namely, to stimulate germination and to lengthen the roots with increasing soil concentration.

Key words: *effect, germination, root elongation.*

INTRODUCTION

Heavy metal pollution is a global problem, degrading the environment and being a serious threat to human health. The underlying causes are anthropic activities such as mining, irrigation with wastewater, applying sludge to wastewater and applying chemical fertilizers, as well as rapid industrialization (Zhang et al., 2019; Onyenmechi et al., 2020; Wuana and Okieimen, 2011; Antonkiewicz et al., 2018).

Thus, heavy metals can cause serious ecotoxicological problems because they are non-degradable and persistent in the environment (Rana, 2008; Cortés-Eslava et al., 2018).

In different plant species heavy metals can alter the membrane permeability, reduce enzyme activities, disturb mineral nutrition, damage the photosynthetic apparatus, and generate oxidative stress, therefore affecting the morphology, growth and photosynthetic processes of plants. Excess of heavy metals in cells induces molecular damage to plants mainly through the synthesis of reactive oxygen species such as superoxide radical, hydroxyl radical and hydrogen peroxide (Verma et al., 2003; Steliga et al., 2020).

Heavy metal contamination not only affects the functioning of the ecosystem, but also represents potential threats to human health, because these metals could be absorbed by humans through the food chain. Therefore, remediation of heavy metal-contaminated soil is important for improving the health of the ecosystem and humans (JunKang et al., 2020).

Physical and chemical methods of soil depollution involve high costs and can cause the damage of soil structure (Smolinska, 2015; Liu, 2019).

Phytoremediation is a cost-effective and environmentally friendly strategy for decontamination of soil contaminated with heavy metals, which involves the use of plants that have the ability to absorb metals from the soil and transfer them to its upper parts (Farrag et al., 2012; Salati et al., 2010; Turgut et al., 2004). Anyway, the efficiency of phytoremediation may be limited by low plant biomass, low rate of root translocation, and low bioavailability of metals in soils (Khan et al., 2000; Zaier et al., 2010). One method of determining the metal concentration in the soil from which the plants are affected, both at germination and root lengthening is the use of Phytotoxkit toxicity tests.

These tests presents the following advantages: set-up is simple and rapid; little bench space and incubation space are required; transparent test plates allows direct observation of the germinated seeds without any manipulation; images of the test plates with the germinated seeds are easily stored and accessed using computers; rapid, automated root-length measurements are feasible using image-analysis techniques; multiple tests are carried out concurrently (Wadhia and Clive Thompson, 2007; Janssen et al., 2000).

The objective of this study is to determine the toxicity of cadmium, chromium and zinc on the germination and elongation of the roots of rapeseed (*Brassica napus*), peas (*Pisum sativum*) and rye (*Secale cereale*).

MATERIALS AND METHODS

The soil used in the performed Phytotoxkit toxicity tests was purchased by order from MicroBioTests Inc in Belgium. This is a reference soil (Figure 1) used especially in this type of tests and has the following composition, according to the distributor: air-dried quartz sand (85%), kaolin clay (10%), sphagnum peat (5%), calcium carbonate (to obtain an initial pH of 6.5-7.0).

Figure 1. The soil used in experiments

25 g of soil was introduced into the bottom of the Phytotoxkit plate. It was contaminated with solutions of $CdCl \cdot H_2O$, $K_2Cr_2O_7$ and $ZnSO_4 \cdot 7H_2O$ in concentrations from 200 mg/kg to 1200 mg/kg. Distilled water was used to saturate the soil of the control sample. The polluted soil was then covered with a filter paper on which 10 plant seeds were placed in the case of rape and rye and 6 seeds in the case of peas, at a distance of 1 cm one from another. The plant seeds were purchased from the Plant Genetic Resources Bank Suceava, Romania. Pictures of seeds used in experiments can be seen in Figures 2, 3 and 4.

Figure 2. Seeds of *Pisum sativum*

Figure 3. Seeds of *Secale cereale*

Figure 4. Seeds of *Brassica napus*

After closing the plates with the transparent lid, they were placed vertically in a support and incubated in the dark at a temperature of 25 ± 1ºC for 3 days. Each experimental variant was performed in 3 repetitions. At the end of the incubation period, photos of the germinal plants were taken for each Phytotoxkit plate.

The percentage of seed germination inhibition (Rg) and root elongation (RI) for each plant was calculated with Eq. 1 (Oleszczuk, 2008):

$$Rg/RI = \frac{A-B}{A} \cdot 100 \quad (1)$$

where:

- A is the seed germination or the average length of the roots in the control sample;
- B seed germination or average root length under the influence of the applied pollutant.

Root elongation was measured using the Image Tool 3.0 software.

RESULTS AND DISCUSSIONS

After the 3 days of incubation, we can first visualize the germinated seeds and the elongation of the studied plants roots. The figures below present the effect of the pollutant for each plant studied separately (Figures 5, 6, 7).

Figure 5. Seed germination and root lengthening of *Pisum sativum* under the influence of soil pollution with Cd from 0 mg/kg to 1200 mg/kg

Figure 6. Seed germination and root lengthening of *Brassica napus* roots under the influence of soil pollution with Zn from 0 mg/kg to 1200 mg/kg

It can be seen that the most affected by pollution soil with the studied metals in terms of germination, was pea.
The germination rate of the above mentioned three types of seeds, under the influence of the applied pollutant, was calculated and the obtained results are presented in Table 1.

Figure 7. Seed germination and elongation of *Secale cereale* roots under the influence of soil pollution with Cr from the concentration of 0 mg/kg to 1200 mg/kg

At the maximum pollutant concentration in the respective soil, 1200 mg/kg pea seeds had a germination rate of 68% in case of soil pollution with Cd, 34% in case of soil pollution with Cr and 35% in case of soil pollution with Zn.
The germination rate of rapeseed was 80% in case of soil pollution with Cr and 60% in case of soil pollution with Zn.
Germination was not affected in the case of soil contamination with Cd, the germination rate being 100%. In the case of rye seeds, the germination rate of the soil with Zn was 50% and the soil pollution with Cd of 80%.
The only metal that did not affect the germination of the rye seeds was chromium, its effect being inverse, to stimulate germination as the pollutant concentration in the soil increased.
As for the root's elongation, their length has depended on the metal concentration in the soil. The higher the concentration, the smaller the length of the roots was, except for the rye in the case of pollution of the soil with chromium, which stimulates the growth of the roots.
For each studied plant, it was determined the average of the roots length and the obtained results can be seen in the following figures (Figures 8-10).

Table 1. Germination rate of the studied plant seeds

Plant seeds	Pollutant	Pollutant concentration (mg/kg)						
		0	200	400	600	800	1000	1200
		Germination rate (%)						
Pea	Cd	100	100	100	100	100	100	68
	Cr	100	100	100	100	100	100	34
	Zn	100	100	86	86	86	86	35
Rape	Cd	100	100	100	100	100	100	100
	Cr	100	100	100	100	100	100	80
	Zn	100	100	100	100	90	80	60
Rye	Cd	100	100	90	90	90	90	80
	Cr	60	60	80	90	100	100	100
	Zn	100	100	100	100	100	90	50

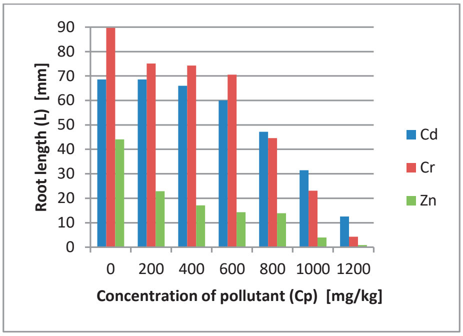

Figure 8. Elongation of pea roots

Zinc was the metal that most affected the elongation of the studied plants roots.
Thus, the root length decreased in the case of *Pisum sativum* from 44.08 mm (0 mg/kg) to 0.86 mm (conc. 1200 mg/kg), from 56.2 mm (0 mg/kg) to 9.72 mm (mg/kg) in the case of *Secale cereals* and from 69 mm (0 mg/kg) to 4.2 mm (1200 mg/kg) in the case of *Brassica napus*.

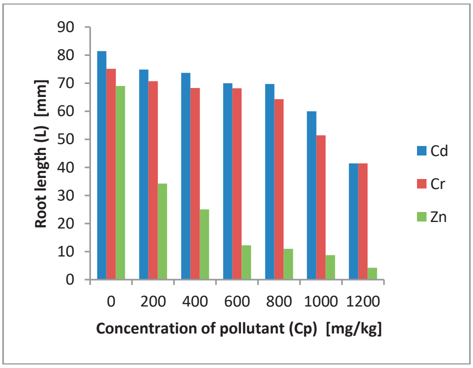

Figure 9. Elongation of rape roots

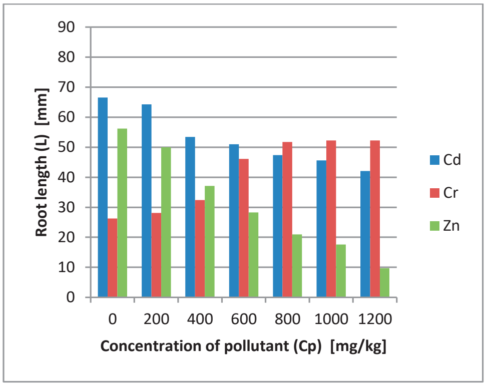

Figure 10. Elongation of rye roots

The correlation between the pollutant concentration and the inhibition of plant seeds under its influence can be seen in the figures below (Figures 11-13).

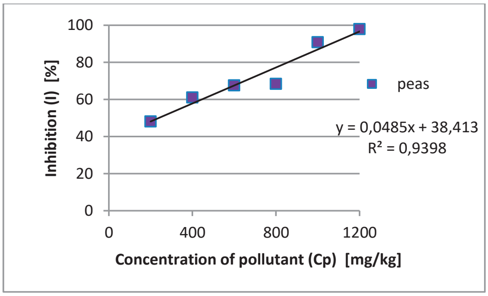

Figure 11. Correlation between the pollutant concentration (Cp) and inhibition (I) of pea seeds under the influence of soil pollution with Zn

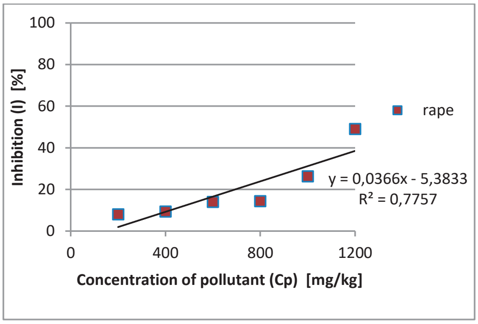

Figure 12. Correlation between the pollutant concentration (Cp) and inhibition (I) of rape seeds under the influence of soil pollution with Cd

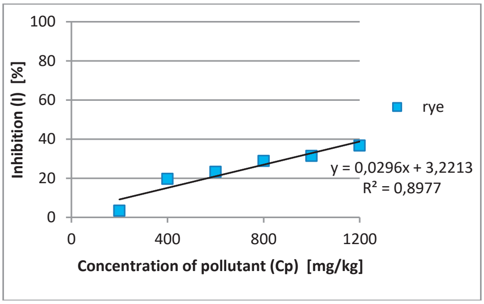

Figure 13. Correlation between the pollutant concentration (Cp) and inhibition (I) of rye seeds under the influence of soil pollution with Cd

CONCLUSIONS

The carried out and presented research in this paper led to the following conclusions:

➢ Seeds germination and root elongation decreased with increasing soil pollutant concentration except rye (in the case of chrome soil pollution).

➢ Chromium has positively contributed to both germination and elongation of rye roots.

➢ Zinc, on the other hand, affected the most studied plant seeds.

➢ At the maximum concentration of zinc in the soil, the root length decreased by 93.05% in the case of *Pisum sativum*, by 93.91% in the case of *Brassica napus* and by 82.70% in the case of *Secale cereale* compared to the control sample.

➢ Of the studied plants, the least affected plant by soil pollution with metals was *Secale cereale* both at germination level and at the root elongation stage.

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