INFLUENCE OF HEAVY METALS PHYTOTOXICITY ON SEED GERMINATION AND PLANTS GROWTH

Mihaela ROȘCA[1], Elena-Diana COMĂNIȚĂ UNGUREANU[1], Raluca-Maria HLIHOR[1,2], Mariana DIACONU[1], Petronela COZMA[1*], Maria GAVRILESCU[1,3*]

Abstract. *Environmental pollution with heavy metals has become a critical concern because of their potential to create negative ecological effects. Such toxic elements are considered pollutants of the soil because of their spread, the appearance and their acute and chronic toxic effect on the cultivated plants. Excessive release of heavy metals into the environment has become a primary issue worldwide, as they cannot be transformed into non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations. In this context, experimental program has been structured to address the problem of heavy metals phytotoxicity and plants tolerance against this aggressive factor. This paper presents the results of the phytotoxicity studies of Cd(II) on three plants: Brassica rapa (rape), Sinapis alba (white mustard) and Amaranthus retroflexus (redroot pigweed) in terms of seeds germination and plants grow. It was observed that plants exhibit some tolerance to heavy metals toxicity, which depends on metal concentration and plant characteristics.*

Keywords: cadmium, seed germination, phytoremediation, phytotoxicity, plant, root, stem

https://doi.org/10.56082/annalsarsciphyschem.2020.2.7

1. Introduction

The rapid growth of industrial sector during the last centuries has led to the release in the environment of a large number of polluting chemical compounds (hydrocarbons, polycyclic aromatic hydrocarbons, halogenated hydrocarbons, pesticides, solvents, metals) which during their manufacture and use are very

* Corresponding authors: petronela_cozma@tuiasi.ro, mgav@tuiasi.ro
[1]"Gheorghe Asachi" Technical University of Iasi, "Cristofor Simionescu" Faculty of Chemical Engineering and Environmental Protection, Department of Environmental Engineering and Management,73 Prof. D. Mangeron Blvd, 700050 Iasi, Romania
[2]"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, Faculty of Horticulture, Department of Horticultural Technologies, 3 Mihail Sadoveanu Alley, 700490 Iasi, Romania
[3]Academy of Romanian Scientists, 3 Ilfov Street, 050044 Bucharest, Romania

dangerous for the health of terrestrial ecosystems along with the intensification of the degradation of different environmental components (water, air, soil) [1]. Some statistics show that nearly 30% of the soil surface is degraded or contaminated with heavy metals, persistent organic pollutants (pesticides, hydrocarbons, polychlorinated biphenyls, drugs etc.), with similar situations for ground and surface waters [2-6].

One of the consequences determined by the current stage of industrialization, road traffic and energy production is represented by the increase of the degree of exposure to the toxic action of the heavy metals [7, 8]. Excessive release of heavy metals into the environment has become a primary issue worldwide, as they cannot be transformed into non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations. Moreover, some heavy metals such as arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc. are not only toxic, but also exhibit carcinogenic and mutagenic effects [9]. Unlike many organic pollutants, metals can be removed, recovered and recycled, moved to a safer location or transformed and changed into valence and species, but they cannot be degraded by any method since they are persistent in the environment [10-13]. The presence of heavy metals in the environment (air, water, soil, ecosystems) is closely related to the deterioration of its quality and, implicitly of the quality of life, thus justifying concerns in the direction of diminishing the impact of this category of pollutants.

2. Environmental pollution with heavy metals

As essential elements, some heavy metals (e.g. copper, selenium, zinc) are vital in maintaining the metabolism of the human body. However, in high concentrations they can be toxic. The negative effect of heavy metals can result, for example, through contaminated drinking water (lead pipes), high levels of heavy metals in the air around emitting sources, or assimilation through the food chain [13, 14]. Contamination of agricultural soil with heavy metals has become a critical concern for the environment because of their potential to create negative ecological effects. Such toxic elements are considered pollutants of the soil because of their spread, the appearance and their acute and chronic toxic effect on the cultivated plants. There are some heavy metal ions which are frequently used in different sectors, but which also are particularly toxic such as lead, cadmium, nickel (Pb(II), Cd(II), Ni(II)).

Lead is the one most common of the heavy metals group. In nature there are several stable isotopes, ^{208}Pb being the most abundant. The average molecular weight of lead is 207.2 g/mol. Lead is a soft metal that is resistant to corrosion and has a low melting point (327°C). Lead being a highly toxic compound can damage the nervous system, kidneys and reproductive system, especially in case of children.

In adults, chronic lead toxicity may occur at blood lead levels of 50-80 μg dL^{-1}, including fatigue, insomnia, irritability, headache, joint pain, and gastrointestinal symptoms. Also, from an environmental point of view, lead can contaminate the environment due to its large-scale use in many important industrial applications, such as the manufacture of batteries, pigments, fuels, photographic or explosive materials, metal coatings, automobiles, aeronautics and steel [15, 16].

Cadmium is a soft metal, silver-white being similar in appearance to zinc and to a certain extent being used in a manner similar to it. Cadmium was discovered in 1817 by the German chemist Friedrich Strohmeyer. Cadmium is present in the environment, exposure to low levels occurring as a result of natural processes, but also due to human activities [17, 18]. Cadmium has proven to be a hazardous pollutant, being one of the most toxic metals to living organisms. Its toxicity generates a variety of syndromes and adverse health effects such as: high blood pressure, renal dysfunction, lung disease, liver injury, teratogenic effects [18]. Cadmium is not considered to be an essential nutrient for metabolism. Several studies in human subjects indicate that 4-7% of a single dose of ingested cadmium is absorbed from the intestine. In animal studies it was found that the uptake of cadmium nitrate or cadmium chloride ranged from 0.5% to 3%.

Nickel (Ni) is the 22nd most widespread element on earth's crust. Ni has several oxidation states, ranging from 1 to 4, but Ni(II) is the most widespread. Numerous compounds containing Ni, such as Ni [Ni (CH_3CO_2)] acetate, Ni carbonate ($NiCO_3$), Ni [$Ni(OH)_2$] hydroxide and Ni (NiO) oxide are widely used in industry for the manufacture of a variety of products such as: fuel production, galvanizing, pigments, ceramics, batteries, jewelry manufacturing, valves, magnets, heat exchangers, medical prostheses, coins, appliances. It can be present in the environment from both natural and anthropic sources. The natural concentration of Ni in soil and surface water is less than 100 mg/kg and 0.005 mg/L respectively. However, anthropogenic activities lead to the release of Ni into the soil from various sources such as burning fossil fuels to obtain electricity, mining, emissions from vehicles, disposal of municipal and industrial waste, steel and cement industry. In the soil, the presence of nickel reduces the microbial biomass, inhibits the nitrification process, mineralizes the carbon, modifies the activity of acid and alkaline phosphatases and arylsulfatase in the soil. For plants, nickel is an essential microelement but at high concentrations it can cause morphological, physiological, biochemical alterations. According to the International Agency for Research on Cancer (IARC), nickel and nickel compounds are carcinogenic to animals and humans [19]. Excessive nickel concentrations cause undesirable effects on children's intellectual ability.

According to data presented by Panagos et al. [20] municipal and industrial waste contribute the most to soil contamination (38%), followed by the industrial/commercial sector (34%). Heavy metals together with hydrocarbons are

the main contaminants of soils (approximately 60% of pollutants detected in soil). The sources of contamination of agricultural areas with heavy metal ions are varied, these pollutants coming from both natural and anthropogenic sources. Also, their presence in the soil poses a significant risk to both human health and the environment [20, 21]. The main sources of pollution of agricultural areas as well as the potential effects on the environment and human health caused by heavy metal ions are presented in fig. 1.

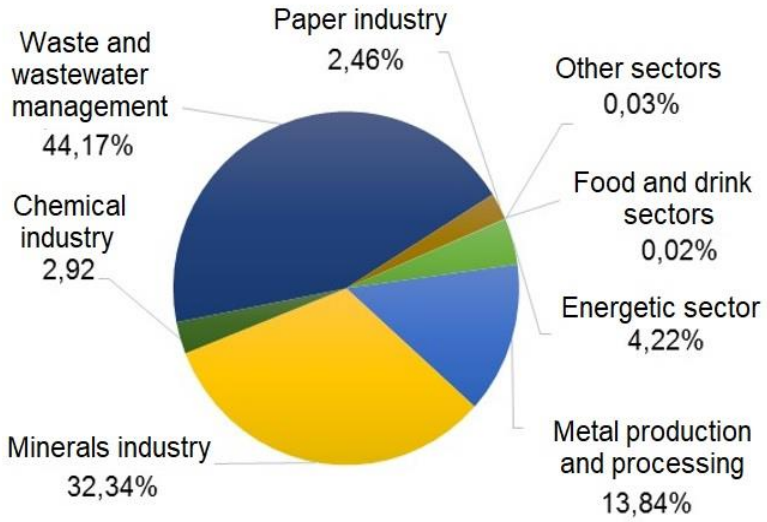

Fig. 1. Lead-based water pollution from various sources in 2015 reported for EU-28 [22]

3. Remediation of soil polluted with heavy metals

The remediation of sites polluted with heavy metals has therefore became a priority for society due to the increase of quality of life standards and awareness of environmental problems. Currently, there are a variety of remediation methods for both solid and liquid media, which are classified into three main classes: *physical*, *chemical and biological methods*. Biological methods have gained a specific attention from researchers in the field, being regarded as feasible alternatives to physico-chemical methods, as they involve a number of ecologically and cost-effective natural processes, without generating toxic waste, and can ensure for example, rectifying and restoring the natural state of the soil [9, 17, 23, 24]

First of all, biological methods (microbial remediation and phytoremediation) use a number of microorganisms and plants to degrade or transform various hazardous contaminants within an environmental matrix into less toxic forms. These methods are embedded in a multidisciplinary approach that includes different

strategies, under controlled working conditions: biostimulation (native microbial population is stimulated by nutrient and / or air and substrate additions), bio-augmentation (artificial introduction of viable population to supplement microbial populations native), biosorption (use of dead microbial biomass), bioaccumulation (use of living cells) and phytoremediation (use of plants) [16].

Investigating the interaction between the natural mechanisms of the biosorption, biotransformation and bioaccumulation processes of persistent pollutants by plants and microorganisms may provide the scientific basis for applying *in situ bioremediation* as a sustainable / feasible / ecological alternative for land and water restoration [17, 25, 26].

4. Phytoremediation applied for heavy metals removal from the environment

Phytoremediation is the process by which plants, which have natural abilities to absorb, accumulate pollutants such as heavy metals, from the environmental component (soil, water), are used for depollution of contaminated sites and their restoration in terms of production capacity. Phytoremediation is not a new concept, but is considered an ecologically and economically feasible alternative in which plants capable of tolerating high levels of heavy metals in the soil can grow in this polluted environment and achieve its depollution, accumulating metals that later can be recovered by chemical, thermal or biological processing of biomass and reused in industry. Plants are exposed to the metals toxicity, generically called phytotoxicity, which can be evaluated to allow the choice of plants with good tolerance to metal toxicity, which can be used for phytoremediation.

Therefore, although natural and/or controlled phytoremediation process can be used effectively to reduce environmental contamination, as well as to prevent and control pollution, there are some difficulties addressed to this approach. The effectiveness of plants in the phytoremediation process is still limited by some shortcomings caused by the toxicity of the target contaminants and by the limited ability of living organisms involved in bioremediation to cope with the contaminated environment [27-30]. Excessive accumulation of contaminants in soil or water can have negative effects on plants, namely phytotoxic effects, which are manifested by growth inhibition, disturbances of the photosynthesis process, decreased biomass, nutrient absorption deficiency etc. [29-31]. The response of plants to heavy metals toxicity depends on the concentration and availability of heavy metals and is a complex process that is controlled by several factors, such as the type of metal, the nature of the environment and the plants and microbial species [9].

5. Principles of phytotoxicity assessment

The basic principles for assessing phytotoxicity are the same, whether the test compound is a heavy metal, herbicide, fungicide, insecticide or any other toxic compound. The difference does not consist in the evaluation method, but only in the experimental program and the working methodology. There are a number of common phytotoxicity criteria such as frequency (number of plants at a given stage or showing a visual symptom) or criteria based on measurements (height, length, diameter, weight of plants or organs in a sample). Other criteria for the assessment of phytotoxicity refer to visual estimates such as: deformation, color change. In this case, the effect is often marked by reference to a standard. The toxic effects of metals can also be assessed by visually comparing a treated batch of plants with an untreated batch, in the sense of exposure to the action of heavy metals.

Among the most commonly applied criteria for assessing symptoms of phytotoxicity are the following: root weight; root length; development of the root system; germination rate; stem length; color changes; the appearance of necrosis; deformation of some organs (stem, leaves). The inhibitory effect of contaminants is determined by comparing the groups tested with the control (control) groups. The measured responses include additive effects of all chemical, physical and biological components of the samples that may affect plant organisms.

Stock solutions are prepared for each compound with a concentration of 1000 mg/L in distilled water or organic solvent as appropriate. By dilution, working solutions with variable concentrations will be made (which will be established later). Solutions of heavy metals and persistent organic compounds will be used in phytotoxicity tests, taking 3 mL for each metal ion or persistent organic compound, which will soak the Whatman filter paper rounds arranged in Petri dishes (90 x 15 mm). In this way it is possible to simulate the interaction between the liquid phase (solution) of the soil in which various concentrations of heavy metals or contaminating organic compounds are found depending on the existing environmental factors.

As test plant species we will use the seeds of *Brassica rapa, Sinapis alba* and *Amaranthus retroflexu*s, by performing seed germination tests [32] for 3-5 or 7 days of exposure depending on the plant species subjected to the experiment. The plants selected for the experiments grow rapidly in laboratory conditions and respond promptly to stressors.

6. Methods for assessing phytotoxicity

6.1. Methods

In general, seed plants are used in tests with terrestrial plants. Tests have been developed for many plant species, most of which are cultivated plants, but plant

species from the spontaneous flora can also be used, if this is necessary and useful for the test. The seeds of the test plants should be selected qualitatively so that no further variability in test results occurs. Thus, the seeds are purchased from commercial producers, and the individual seeds that show signs of poor quality (discolored, deformed, damaged seeds, etc.) were removed and will not be used in experiments.

The effects observed in the phytotoxicity tests with terrestrial plants can be grouped in two categories, namely:

- Quantitative effects in which the results are obtained by measurements or counters. The following effects may be included in this category: the number of germinated seeds; the number of plants sprouted; duration of germination or emergence; survival rate; stem height; root length; number of leaves; the dry biomass produced by the above-ground parts of the plants and the dry biomass of the roots;

- Semi-quantitative effects in which the results are obtained by observations. This category includes observations on abnormal changes in growth, color, appearance of plants compared to plants in control samples. The semi-quantitative effect is assessed (as a percentage of the control) and the results are then processed by statistical methods.

The results of the toxicity tests are recorded in the form of Tables in which the observed effect is noted at certain time intervals for each experimental variant and for each replicate. The basic action in processing the results of toxicity tests, consists in drawing the dose - effect or concentration - effect diagrams. For this purpose, the results recorded in the Tables are used for all experimental variants on the dose or concentration and for the control variant, considered as a zero value of the dose or concentration. Depending on the test protocol, the results recorded at time t are used, which the duration of the test is considered relevant for that test. Also, depending on the number of replicates used in the test, the mean values of the effect were used to draw the dose-effect or concentration-effect curve. In addition, they were transformed into percentage values, which represents a first statistical processing of the results, the average values having different intervals between the maximum and minimum values recorded.

When organizing a phytotoxicity test, the possibilities of statistical processing of the results must be taken into account from the very beginning.

6.2. Germination tests

This test is to determine the inhibitory effect of contaminated liquid or solid samples on the germination and growth potential of fast-growing terrestrial plants. Measurements to determine development are represented by root length, stem length, mass of wet stem material and dry mass [32]. The inhibitory effect of contaminants is determined by comparing the groups tested with the control (control) groups. The measured responses include additive effects of all chemical,

physical and biological components of the samples that may affect plant organisms. Depending on the nature of the study, the test can be performed with a single concentration per sample (single concentration test) or with a series of dilutions (concentration-response test) allowing the determination of different measurement parameters, such as IC25 and IC50 for the germination and EC25 and EC50 for increase in length, wet mass and dry mass. Other measurement parameters can also be used to determine the inhibition or onset of the effect area (threshold effect). After 5-7 days, the number of germinated seeds from each variant is recorded, the averages are calculated and the germination power is determined (% germinated seeds from the total seeds used) and then the relative germination or germination index is calculated, using the relation (1):

$$IG = \frac{S_{germ}}{M_{germ}} \times 100 \quad (1)$$

where: IG is the germination index (relative G);
S_{germ} is the number of seeds germinated in the test sample;
M_{germ} is the number of seeds germinated in the control sample.

6.3. Root and stem length

The length of the roots is measured and the average length of each variant is calculated. Then the relative increase in root length (E_r) is calculated by comparison with the control variant, using the relation (2):

$$E_r = \frac{L_{average}}{L_{control}} \times 100 \quad (2)$$

The same procedure is followed for the length of the stems.

The toxicity indices of the aggregate values are then determined using formula (3):

$$IT_{germ} = \frac{IG \times E}{100} \quad (3)$$

where: E is the index of relative growth of roots (stems) in length (%);
IT_{germ} is the germination toxicity index;
IG is the seed germination index for the test variant.

6.4. Materials

In order to achieve phytotoxicity studies, the working parameters presented in Table 1 were used. The tests were performed using three heavy metals: cadmium,

nickel and lead. In this paper there are presented the results of tests for phytotoxicity of Cd(II) on *Brassica rapa* (rape), *Sinapis alb*a (white mustard) and *Amaranthus retroflexus* (redroot pigweed).

Table 1. Specifications for the toxicity test of heavy metals ions (Cd(II), Ni(II), Pb(II)) to white mustard, rape and redroot pigweed seeds using seed germination and elongation tests (for roots and stems)

Parameters	***Conditions***
Pollutants	Solutions of heavy metals at different concentrations of 25, 50, 100, 150, 200, 250, 300 mg/L were prepared for the Cd(II), Ni(II), Pb(II) ions starting from a stock solution concentration of 1000 mg/L
Plants species	Seeds of *Brassica rapa* (rape), *Sinapis alba* (white mustard) and *Amaranthus retroflexus* (redroot pigweed)
Seeds pretreatment	Sterilized with 96% alcohol for 20 seconds and then with 20% NaClO solution for 20 min, then soaked with deionized water for 10 min and washed with deionized water for seven times, and finally after washing, the seeds were dried for 48 hours in oven at 35°C.
Temperature	25±2ºC
Test vessel	Petri dishes and Whatman filter paper were previously sterilized
Test solution	3 mL
Seeds number	10 seeds in each Petri dishes
Replicates	3
Control and diluent	Deionized water as control (3 mL); Deionized water was renewed at every 24h for plants hydration
Test duration	Five days of seed germination

7. Results of phytotoxicity tests in laboratory

Phytotoxicity of Cd(II) ions and the effects on the germination and elongation degrees were determinated for the tested plants *Brassica rapa* (rape), *Sinapis alba* (white mustard) and *Amaranthus retroflexus* (redroot pigweed). Figures 2-4 illustrate the degree of tolerance of the tested plants (mustard, rape, redroot pigweed) in the presence of Cd(II) at different concentrations, by graphical representation of three suggestive indicators: germination index (*IG*); elongation rate for roots and stems (% from control lengths) or tolerance index (*Er,%*); elongation inhibition rate for roots and stems or inhibition index (*EI,%*).

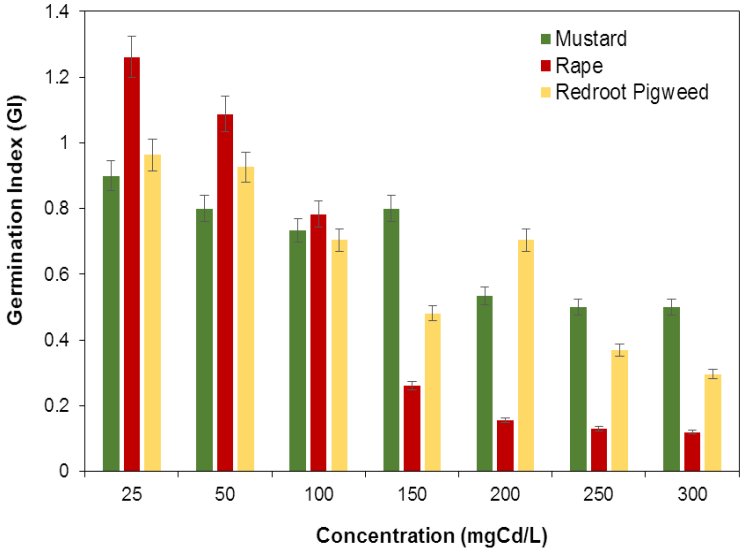

Fig. 2. Cd(II) ions effect on the seeds germination index of tested plant (error bars represent the percentage error)

Analyzing the germination index (*GI*) (fig. 2), a stimulation of rape seed germination degree at low concentrations (25 and 50 mg Cd /L) can be observed compared to both the control sample and the other plant species (mustard and redroot pigweed). On the contrary, at higher metal ion concentrations of 100 and 300 mg Cd(II)/L, the germination index for rape seeds decreases from 0.78 to 0.12. The germination index for the other two plants, white mustard and redroot pigweed, recorded values between 0.9 and 0.5 and 0.96 and 0.296, respectively for concentrations of 25 and 300 mg Cd(II)/L compared to the control sample. The results show that the degree of seed germination is influenced by both the metal concentration and the plant type. Thus, with the increase in Cd(II) concentration, the degree of germination of the tested seeds is significantly reduced. According to our results, we can establish the following hierarchy of toxicity degrees for each plant type in terms of germination index: at concentrations between 25 and 100 mgCd/L: rape < redroot pigweed < white mustard; at higher concentrations, between 150-300 mgCd/L: mustard < redroot pigweed <rape.

The analysis of the elongation rate for roots and stems (or the tolerance index) (*Er*) highlighted the following aspects (fig. 3):

- at low concentrations of 25 mg Cd(II)/L the following order of plant growth can be established: for roots: mustard>rape>redroot pigweed and stems: rape>mustard>redroot pigweed;

- at the concentrations of 50 mg Cd(II)/L the following order of plant growth can be established: for roots: mustard>rape = redroot pigweed and for stems: rape>mustard>redroot pigweed;

- root length significantly decreased for mustard and rape at higher concentrations: for example, at 100 mgCd(II)/L and 300 mgCd(II)/L, *Er* for mustard roots were 8.53% and respectively, 3.07%, and in the case of rape *Er* for the roots at the same concentrations recorded values of 5.68% and 4.46%. The redroot pigweed was apparently more tolerant compared to mustard and rape at higher Cd(II) concentrations regarding the degree of root growth;

- it is certain that at all concentrations tested for Cd(II), **rape** recorded the highest values of stems lengths compared to mustard and redroot pigweed, but at the same time the roots lengths for all the tested plants were much lower compared with the control sample. The highest value of *Er of roots* (38.8%) was recorded for mustard.

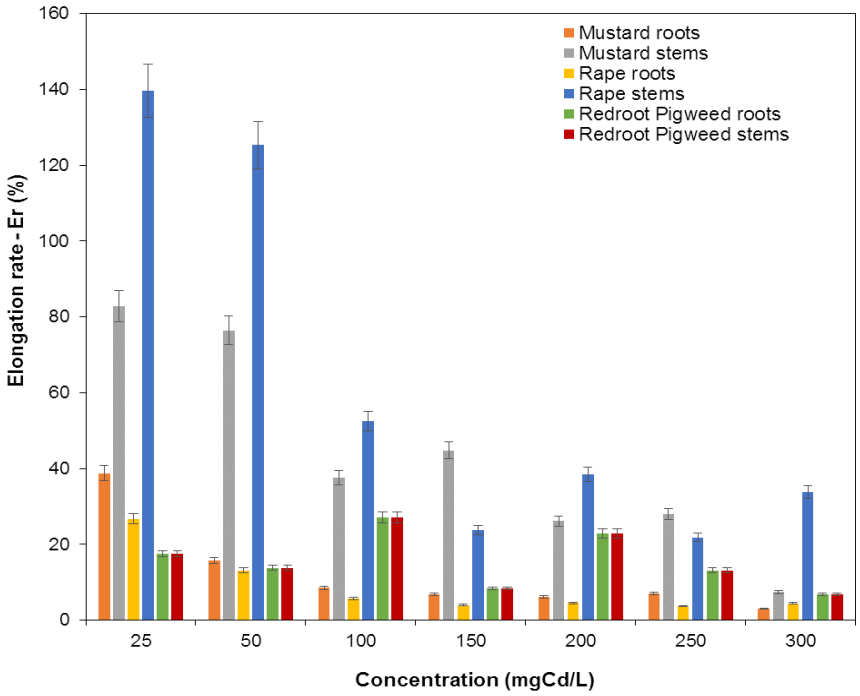

Fig. 3. Cd(II) ions effect on the relative increase in length of the roots and stems of the tested plants (error bars represent the percentage error)

A very well-known and perhaps more suggestive factor in relation to the toxicity of heavy metals on plants is the inhibition index (*EI*). According to the data presented in fig. 4, an increase in the length of rape stems at 25 and 50 mg Cd(II)/L concentrations was observed, in contrast, at higher concentrations greater (300 mg Cd/L), the inhibition rate was approximately 92%. Certainly, at higher

concentrations, starting with 100 mg Cd(II)/L, the degree of root inhibition was greater than 90% for all plants. With some small deviations attributed to experimental errors, we can state that the degree of inhibition of stem lengths was lower for mustard, followed by rape and redroot pigweed.

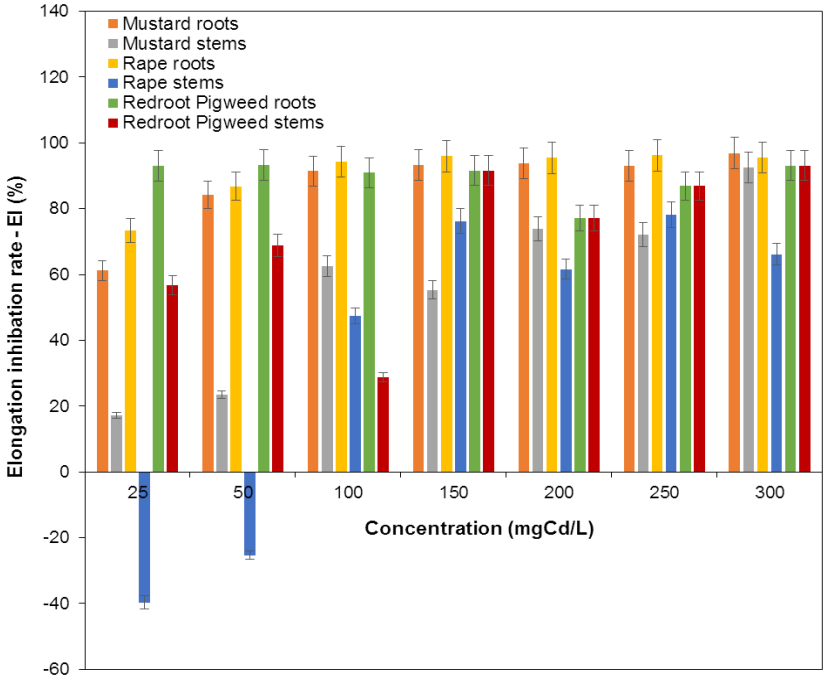

Fig. 4. The elongation inhibition rate of tested plants in the presence of Cd(II) ions

Conclusions

Phytoremediation is a feasible technology for removing heavy metals from the environment that involves the use of plants with a certain degree of tolerance to metal toxicity which can be tested using a series of techniques in the laboratory to demonstrate the extent to which seeds can germinate and plants can grow with biomass formation.

Some heavy metal ions which are frequently used in different sectors, are particularly toxic such as lead, cadmium, nickel (Pb(II), Cd(II), Ni(II)). Excessive accumulation of these contaminants in soil or water can have negative effects on plants, manifested as phytotoxic effects, which are revealed by growth inhibition, disturbances of the photosynthesis process, decreased biomass, nutrient absorption deficiency etc. Based on these considerations, the phytotoxicity of Cd(II) ions and the effects on the germination and elongation degrees were determined for the tested

plants as *Brassica rapa* (rape), *Sinapis alba* (white mustard) and *Amaranthus retroflexus* (redroot pigweed).

The results demonstrated that laboratory phytotoxicity tests which address the degree of seeds germination and plants grow are support for decision making in selecting tolerating plants for soil clean up and elimination of various categories of heavy metals. The resulting biomass can be further valorized by chemical, thermic or biological to recover the metals that can be a critical ones for industry.

Acknowledgements
This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-III-P4- ID-PCE-2016-0683, Contract no. 65/2017, and a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-5239, Contract no. 269PED/2020, within PNCDI III.

R E F E R E N C E S

[1] Bisht S., Pandey P., Bhargava B., Sharma S., Kumar V., Sharma K.D., (2015), Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology, *Brazilian Journal of Microbiology*, **46**, 7-21.

[2] Cachada A., Ferreira da Silva E., Duarte A.C., Pereira R., (2016), Risk assessment of urban soils contamination: The particular case of polycyclic aromatic hydrocarbons, *Science of the Total Environment*, **551-552**, 271-284.

[3] Căliman F.A., Robu B.M., Smaranda C., Pavel L., Gavrilescu M., (2011), Soil and groundwater cleanup: benefits and limits of emerging technologies, *Clean Technology and Environmental Policy*, **13**, 241-268.

[4] Pavel V.L., Sobariu D.L., Tudorache Fertu I.D., Statescu F., Gavrilescu M., (2013a), Symbiosis in the environment: biomanagement of soils contaminated with heavy metals, *European Journal of Science and Theology*, **9**, 211-224.

[5] Stegmann R., Brunner G., Calmano W., Matz G., (2013), *Treatment of Contaminated Soil: Fundamentals, Analysis, Applications*, Springer.

[6] Thomas S.V., (2008), *Water Pollution Issues and Developments*, Nova Publishers.

[7] Chapman E.E.V., Dave G., Murimboh J.D., (2012), Bioavailability as a factor in risk assessment of metal-contaminated soil, *Water, Air, & Soil Pollution*, **223**, 2907-2922.

[8] Hlihor R.M., Apostol L.C., Gavrilescu M., (2017), *Environmental Bioremediation by Biosorption and Bioaccumulation: Principles and Applications*, În: *Enhancing Cleanup of Environmental Pollutants*, Anjum N.A., Singh S., Tuteja G.N. (Eds.), Springer International Publishing AG, pp. 289-315, Elveția.

[9] Dixit R., Wasiullah, Malaviya D., Pandiyan K., Singh U.B., Sahu A., Shukla R., Singh B.P., Rai J.P., Sharma P.K., Lade H., Paul D., (2015), Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes, *Sustainability*, **7**, 2189-2212.

[10] Gavrilescu M., (2010), *Biosorption in Environmental Remediation*, In: *Bioremediation Technology – Theory & Application*, Fulekar M.H. (Ed.), Springer, 35-99.

[11] Hlihor R.M., Diaconu M., Fertu D., Chelaru C., Sandu I., Tavares T., Gavrilescu M., (2013), Bioremediation of Cr(VI) polluted wastewaters by sorption on heat inactivated *Saccharomyces cerevisiae* biomass, *International Journal of Environmental Research*, **7**, 581-594.

[12] Roșca M., Hlihor R.M, Gavrilescu M., (2019), *Bioremediation of Persistent Toxic Substances: From Conventional to New Approaches in Using Microorganisms and Plants,* In: *Microbial Technology for the Welfare of Society,* Kumar A.P. (Ed.*),* Springer Singapore.

[13] Gavrilescu M., Demnerová K., Aamand J., Agathos S., Fava F., (2015), Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, *New Biotechnology*, **32**, 147-156.

[14] Hlihor R.M., Diaconu M., Leon F., Curteanu S., Tavares T., Gavrilescu M., (2015), Experimental analysis and mathematical prediction of Cd(II) removal by biosorption using support vector machines and genetic algorithms, *New Biotechnology*, **32**, 358-368.

[15] Hlihor R.M., (2011), *Procese de sorbție aplicate pentru îndepărtarea metalelor grele din medii contaminate*, Teză de doctorat, Universitatea Tehnică *Gheorghe Asachi* din Iași, România.

[16] Cozma P., Hlihor R.-M., Apostol L.C., (2016), *Biosystems and Bioreactors*, In: *Biosorption and Bioaccumulation. Principles and Applications in Environmental Bioremediation*, Politehnium Publishing House.

[17] Gavrilescu M., (2004), Removal of heavy metals from the environment by biosorption, *Engineering in Life Sciences*, **4**, 219-232.

[18] Hlihor R.M., Simion I.M., (2016), *Poluarea apei și solului: Îndrumar de laborator*, Editura EcoZone, Iași.

[19] IARC, (2016), *Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid*, Vol. 113, IARC Monographs, On line la: http://monographs.iarc.fr/ENG/Monographs/vol113/index.php.

[20] Panagos P., Van Liedekerke M., Yigini Y., Montanarella L., (2013), Contaminated Sites in Europe: Review of the Current Situation Based on Data Collected through a European Network, *Journal of Environmental and Public Health*, **2013**, 1-13.

[21] Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J., (2012), Heavy Metals Toxicity and the Environment, *EXS*, **101**, doi: 10.1007/978-3-7643-8340-4_6.

[22] E-PRTR - European Pollutant Release and Transfer Register, (2017), Pollutant Releases – Lead and compounds, Online la: http://prtr.ec.europa.eu/#/pollutantreleases.

[23] Gianfreda L., Rao A.M., (2004), Potential of extracellular enzymes in remediation of polluted soils, *Enzyme Microbial Technology*, **35**, 339-354.

[24] Hlihor R.M., Bulgariu L., Sobariu D.L., Diaconu M., Tavares T., Gavrilescu M., (2014), Recent advances in biosorption of heavy metals: support tools for biosorption equilibrium, kinetics and mechanism, *Revue Roumaine de Chimie*, **59**, 527-538.

[25] De Vrieze J., (2015), The littlest farmhands, *Science*, **349**, 680-683.

[26] Taiwo A.M., Gbadebo A.M., Oyedepo J.A., (2016), Bioremediation of industrially contaminated soil using compost and plant technology, *Journal of Hazardous Materials*, **304**, 166-172.

[27] Bellion M., Courbot M., Jacob C., Blaudez D., Chalot M., (2006), Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi, *FEMS Microbiology Letter*, **254**, 173-181.

[28] Ma Y., Prasard M.N.V., Rajkumar M., Freitas H., (2011), Plant growth promoting rhizobacteria and endophytes accelaerate phytoremediation, *Biotechnology Advances*, **29**, 248-258.

[29] Manara A., (2012), *Plant Responses to Heavy Metal Toxicity*, In: *Plants and Heavy Metals*, Furini A. (Ed.), Springer, 27-53.

[30] Pavel V.L., Sobariu D.L., Diaconu M., Statescu F., Gavrilescu M., (2013b), Effects of heavy metals on *L. sativum* germination and growth, *Environmental Engineering and Management Journal*, **12**, 727-733.

[31] Boutin C., Aya K.L., Carpenter D., Thomas P.J., Rowland O., (2012), Phytotoxicity testing for herbicide regulation: Shortcomings in relation to biodiversity and ecosystem services in agrarian systems, *Science of the Total Environment*, **415**, 79-92.

[32] Diaconu M., (2016), *Metode şi teste ecotoxicologice*, Ed. Performantica, ISBN 978-606-685-417-7.