# MIGRATION OF METAL IONS INTO *PHALARIS ARUNDINACEA* WITH THE PURPOSE SOIL DECONTAMINATION

**Cornelia MUNTEAN, Oana GRAD, Adina NEGREA, Mihaela CIOPEC, Narcis DUȚEANU, Mariana CERNICOVA BUCA**

***Abstract:*** *Advances of the science during last decades, made especially since mankind had the wisdom to dedicate its efforts to further improvement of life on earth, had allowed the implementation of some ideas of great importance not only for the present but also for the life perspectives on earth. By definition soil represent the top layer of the earth's crust which is formed from mineral particles, organic matter, water, air and some living organisms. In fact, soil represent an extremely complex, variable and living medium. Due to his extremely slow formation, soil can be considered as a non-renewable resource. Due to the permanent unsustainable use of soil the national and international goals of biodiversity and climate changes are endangers. For all these reasons European Commission adopted a specific strategy on soil (COM (2006) 231) on 22 September 2006 with the aim of soil protection across the EU states, although EU Commission decided in May 2014 to withdraw a proposal for a Framework Soil Directive. In the present paper the migration of metallic ions from soil into the Phalaris arundinaceahas been studied in order to use it for further remediation of polluted soils. Migration of metallic ions into the plant has been proved by using atomic absorption spectrometry and energy dispersive X-Ray spectroscopy (EDX) analysis. Obtained results confirmed that the used plant presents a selective root adsorption of minerals from soil, in order to keep the concentration of metallic ions in admissible limits. When metallic ions concentrations in soil is low adsorption in plant is intense, and for higher concentration has been observed a low adsorption rate.*
***Keywords:*** *pollution, Phalaris arundinacea, bioremediation, heavy metals, soil*

## INTRODUCTION

Human activities and in special the mining activities, metallurgical and chemical one has lead at transformation of fertile soils in contaminated one, unable to sustain life due to higher concentration of heavy metals.

Such contaminated areal's are hostile one for majority of plants, and not all the seed, which germinate there, are leading at mature plants. However, such soils are not completely without vegetation because some species (up to several dozen) are able to survive in such hostile conditions. Some species present a higher genetic variability, which means that some individual's genotypes will adapt to survive in such soils contaminated with heavy metals. Such individuals generally grow much less compared with their potential, but they are able to survive in such hostile conditions (Voina 1981; Petru 1997; Pietraru 1982).

In order to recovery such polluted soils a practical method is represented by the in-situ immobilization of metallic ions. Main advantage of this technology is represented by the affordable price, but has one bigger inconvenient due to the high time framework (Ye 2020:1; Hang 2020: 122065).

Adsorption of different heavy metals from soil is a selective process for biggest majority of cultivated plants. Accumulation of such metals in plants depends mainly on their concentration into the soil, on 69/the interactions between these elements and not at least on their physiological role (Legea protecţiei mediului nr. 137-1995; Duruibe 2007:112).

Radicular adsorption of metallic ions from soil presents a higher importance for plant nutrition and for harvests contamination with heavy metals. Estimation of heavy metals concentration in plants and in soil solution can be done by using mathematical and empirical models. Experimental data proved that during vegetation period plants adsorb heavy metals with different intensity.

Experimental data have been proved that plants absorb heavy metals with different intensities. For example, trees developed on serpentine soils contain up to 3% Ni (II) (determined in dry matter) and trees developed near different mines, highways and non-ferrous metallurgical plants present high accumulation of Pb(II), Cd(II), Zn(II). Among these plants are also different species tolerant to high concentrations of heavy metals, as well species that have adapted to such adverse conditions. Plants capable to accumulate high concentrations of heavy metals, with no visible adverse symptoms, are often referred in literature as metallophytes or hyperaccumulators. *Heavy metals flora* concept refers at plants species that normally grow in natural or anthropogenic soils enriched with heavy metals (Peralta-Videa 2009:1665).

Adsorption of metallic elements, including micronutrients by plants through roots or leaves is influenced by various factors: external and environmental factors (such as temperature, oxygen, soil reaction, concentration of metallic ions in soil solution, water content, light), or internal factors or plant metabolism (metabolism inhibitors, the effect of the root system on the rhizosphere). Generally environmental factors, which have an important influence over plant growth and plant transpiration degree, are strong light and low humidity (drought). All these factors influence the rate of the elements movement from roots, through the woody vessels to the crown of the plant (Eraly 2011: 1489).

Mineral elements from soil have unequal phloem mobility; some of them such as total Mn(II), Mo(VI) and Zn(II) are easily transported from mature organs, being necessary for growth in the plant younger areas. Some other metallic ions such as iron are harder to transport by phloem. Due to that deficiencies in plant nutrition occur at the apex and leaves of young shoots.

Heavy metal ions are transported through plant conducting vessels by the same mechanism as the ions of the other mineral elements. The transport of the metallic ions inside of the plant is accomplished either freely through the wood vessels or in a complex way together with other substances through liberian vessels. Transport of metallic ions inside of plants is influenced by external factors (light, temperature, nutritive elements, humidity, etc.) and as well by internal factors (pH, salt concentration in plant sap, plant photosynthetic capacity, species, etc.) (Peralta-Videa 2009:1665).

A number of methods used for depollution of heavy metals contaminated soils consist of curative techniques used in order to block or neutralize the flow of pollutants generated by different sources. Classification of used techniques has been made on the basis of several specific criteria, of which the following are important: place where the depollution processes are applied in relation with the polluted site, as well as the technical principles applied for soil depollution, such methods can be physical, chemical or biological (Neag 1997:127;Negrea 2013).

Biological depollution methods are based on the action on living microorganisms which are able to convert the organic pollutants mainly into $CO_2$, water and biomass, or which are able to immobilize pollutants by binding them into the soil moisture fraction. Within these depollution methods, three categories present importance: biodegradation, bioleaching and bioaccumulation. Of these three depollution categories, biodegradation has the largest area of applicability due to its net advantages. Such depollution method is based on the existence in soil of microorganism capable to degrade most of organic pollutants and a good part of the inorganic one (Coste 2001:11).

Bioaccumulation involves the biological accumulation of different pollutants in plants or in different microorganisms. These remediation methods are based on various mechanisms such as: assimilation or adsorption of some particles, ions active transport, complexation, ions by adsorption, precipitation, and is mainly suitable for depollution of heavy metals polluted soils. Such products are not destroyed by bioaccumulation, being only concentrated for further destruction by some other methods (Neag 1997:127).

Heavy metals represent one of the most polluting substances and the remediation of soils contaminated with such compounds is not an easy task, especially due to the characteristics they present (Cunningham 1997: 2).

One of the methods often used for remediation of heavy metals contaminated sites is bioaccumulation in higher plants. This remediation method is known as phytoremediation and has been used for at least 15 years (Èechmánková 2011: 120; Zhi-Xin 2007: 961; De Andrade 2009: 1879). For proper phytoremediation is important to use native plants because they have a higher survival rate, good growth and good reproduction rate in comparison with plants newly introduced into the target medium (Joonki 2006: 456).

Depending on plant specificity, phytoremediation process involves four different types of technologies, each of it having its own mechanism of action in the remediation of heavy metals contaminates soils, sediments or water. These mechanisms include:

1) rhizofiltration – a mechanism that involve the use of plants to clean contaminated aquatic environments;

2) phytostabilization – which involves using of plants to stabilize rather than clean polluted soils;

3) phitovolatilization – which involve use of plants for the purpose of extracting certain metals from polluted soils after which they are released into atmosphere by volatilization; and

4) phytoextraction – plants adsorb metals form polluted soil and transfer them either in the stalk, leaves or fruits where they accumulate (Prasad 2003:1; Clemens 2002: 309; Bernal 1994: 251; Wang 2008:299).

Bioaccumulation process is presented in figure 1.

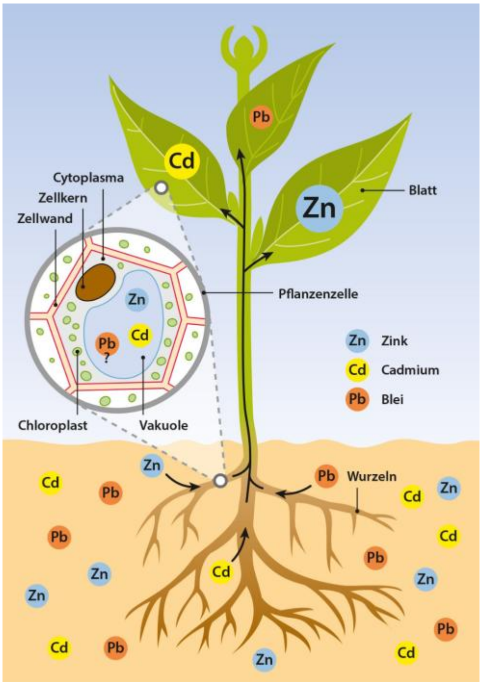



**Figure 1.** Heavy metals bioaccumulation in plants
(https://motherboard.vice.com/de/article/phytomining;Sheoran 2009: 1007)

The mechanism of heavy metals bioaccumulation process in plants is a very complex one and involves several stages schematically presented in figure 2. First stage consists into the solubilization of metals into the soil matrix. Metals which are present in soil can be founded in soils in three different forms: available, potentially available and unavailable. The available form is the form which can be used by microorganisms at any time, those in the potentially available form can be taken from soil only when the fraction of available metals has been eliminated and the metals in unavailable form have an extremely low solubility, being chemically bound to an organic or silicate matrix (Robinson 2003: 117).

The second stage consists into the transport of ionic form of the metals. During this stage, metallic ions can be transformed into less toxic form by some chemical conversion on by complexing with some organic acids (malic, citric) or nicotinamide. Heavy metals with different oxidation states present very different characteristics in term of assimilation, transport and detoxification in plants (Sheoran 2009: 1007).

Once the metals have been translocating into the plant cells, they will be stored in different locations such as apoplast tissue, epidermis, mesophylls, cells wall, etc., where the metallic ions cannot affect cellular processes. Final step in most metallic ions accumulation is represented by their sequestration away from any cellular process that they can disrupt it. Seizure is usually done in the plants vacuoles where the metallic ions were transported over the vacuolar membrane. Metallothionein and phytochelatin play an important role in metallic ions sequestration and also they intensify tolerance and accumulation of metals in plants (Robinson 2003: 117).

More than four hundred plants are known as heavy metals hyperaccumulators including tress, vegetable crops, herbs and some weeds. Hyperaccumulators are defined as those plants that accumulate more than 1000 mg / kg d.s. of Cu(II), Co(II), $Cr_{total}$, Ni(II), P (II), or more than 10000 mg / kg d.s. Mg(II), and Zn(II) (Baker 1989: 81). In general, ideal plant species used for bioremediation of heavy metal polluted soils should produce a large biomass crop and at the same time must tolerate and accumulate pollutants of interest (Ebbs 1997: 776).

*Phalaris arundinacea* is included in the *Gramineae* family, the tribe *Phalarideae Kunth*, genus *Phalaris L*. The popular name of the species *Phalaris arundinacea* in other languages are: reed Canary grass (English), alpiste roseau (French), caniço–malhado (Portuguese), hierba cinta (Spanish), Rohrglanzgras (German) (Figure 3).


**Figure 3**. Different stages of growing the *Phalaris arundinacea* plant

In present paper, the migration method of metallic ions was studied, following the process of bioremediation / bioaccumulation of heavy metals polluted soils from western part of Romania, using *Phalaris arundinacea*.

## MATERIALS AND METHODS

In order to determine the composition of soil sample taken from western part of Romania, approximately 3 grams of soil have been weighed with accuracy of 0.001g and added into a 250 mL reaction vessel. After that this sample was moisten with approximately 1 mL DI water, followed by addition under continuously stirring of 21 mL of HCl, followed by addition of 7 mL of $HNO_3$. Reaction vessel and the refrigerant were kept for 16 h at room temperature in order to get the slow oxidation of organic matter present in taken soil sample. After 16 h the temperature of the reaction mixture has been raised slowly until the reflux conditions were reached and maintained for 2 h, ensuring that the condensation area is less than 1/3 of the height of the refrigerant. After 2 h the entire system is slowly cooled down.

The insoluble residue from reaction vessel is allowed to deposit. The supernatant which is relatively free of sediments is filtered, and the clear liquid is collected in a 100 mL graduated flask. After that all the initial extract founded into the reaction vessel is filtered, then the insoluble residue from filter paper is washed with minimum quantity of $HNO_3$, that last filtrate is collected together with initial one (ISO 11047/1998).

The heavy metal content was determined by atomic absorption spectrometry by using Varian SpectrAAS 280 FS atomic absorption spectrophotometer. After determination of heavy metal content, soil samples (400 g of dry soil, shredded and passed through 2 mm sieve) were placed in vegetation vessels, in which were added seeds from 2 different species of *Phalaris Arundinacea*: *Phalaris Arundinacea Premier* – which is a non-genetically modified single-seeded plant variety and *Phalaris Arundinacea Pollycross* – which is a genetically modified plant obtained by mixing seed from 5 different species. All plants of *Phalaris Arundinacea* used along present study come from the Faculty of Horticulture, King Mihai I University of Agricultural Sciences and Veterinary Medicine of the Banat, Timisoara. Samples thus obtained were periodically watered with tap water. After a period on 3 months of vegetation all plants were separated and dried at room temperature. In order to track the migration of metals in plant initial and final samples (root, stem, and leaf) energy dispersive X-Ray spectroscopy (EDX) analyzes were performed by using a Quanta FEG 250 scanning electron microscope.

## RESULTS AND DISCUSSION

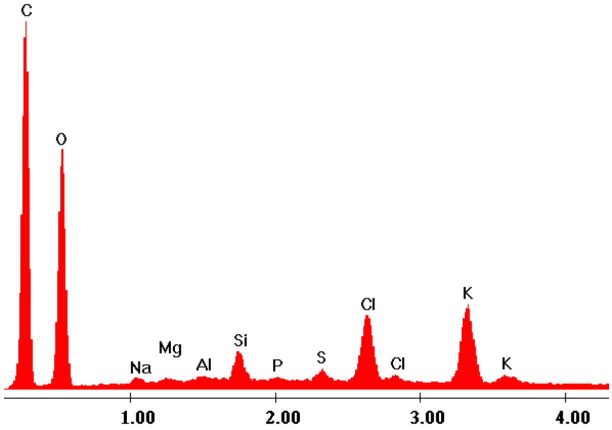
The content of heavy metals in soil samples taken from western part of Romania is presented in table 1.

**Table 1.** Heavy metals initial content of soil samples

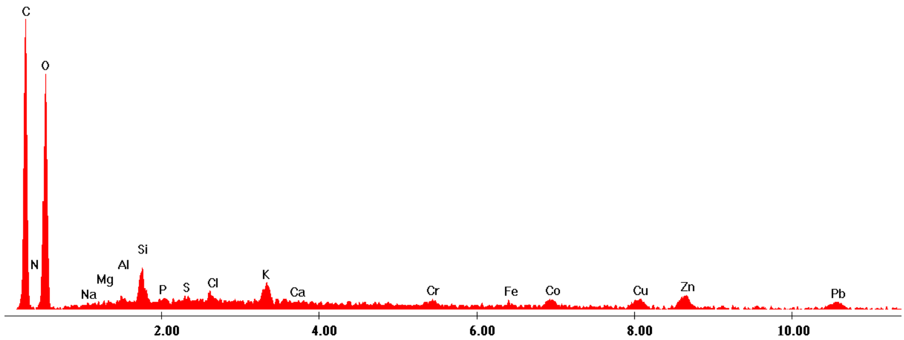
| Metals ions | Limited admission content [mg/kg d.s.] | Heavy metals content from soil sample, [mg/kg d.s] |
|---|---|---|
| $Cu^{2+}$ | 20 | 482 |
| $Co^{2+}$ | 15 | 672 |
| $Cr_{total}$ | 30 | 136 |
| $Pb^{2+}$ | 20 | 61 |
| $Zn^{2+}$ | 100 | $55\cdot10^3$ |
| $Fe^{n+}$ | - | $4,5\cdot10^3$ |
| $Ca^{2+}$ | - | $17\cdot10^3$ |
| $K^+$ | - | $2.8\cdot10^3$ |

From obtained experimental data can be observed that the soil samples taken from western part of Romania have a higher content of heavy metals, in particular Zn(II), which raises the need to apply some remediation methods. Also can be observed that there are exceedances of maximum values allowed by legislation for Cu(II), Co(II), $Cr_{total}$, Pb (II), and Zn(II) ions.

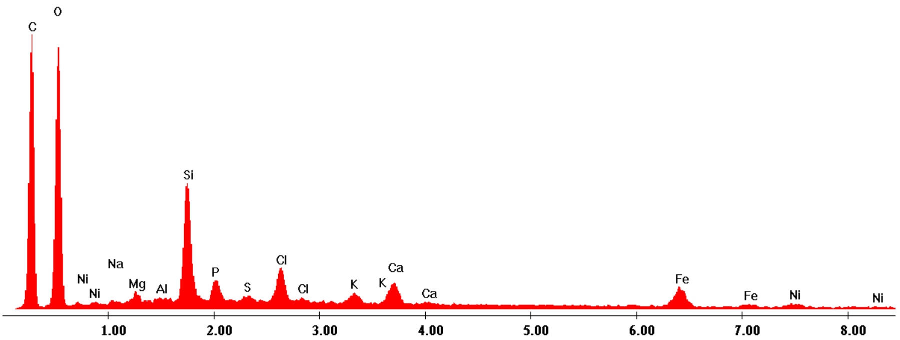
Next migration method of metallic ions into the plant has been followed, in order to be able to improve the heavy metals contaminated soil depollution process. Metallic ions migration from polluted soils into used *Phalaris Arundinacea* plants has been tracked by recording the EDX spectra for control and for plants harvested from contaminated soils. All used plants were separated into the root, stem and leaf. EDX spectra recorded for each part of harvested plants are depicted in figures 4 and 5.



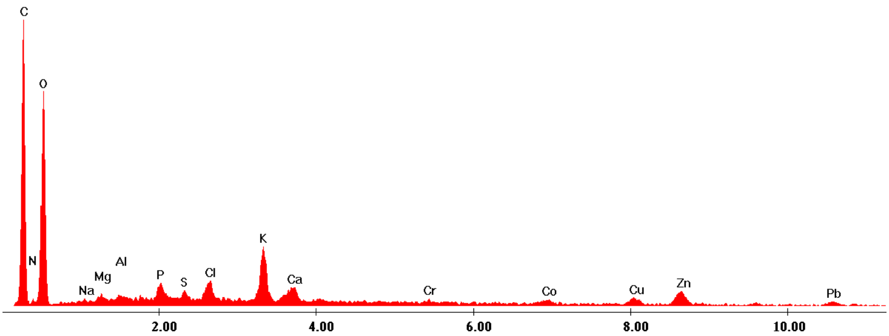

a. root of the control sample



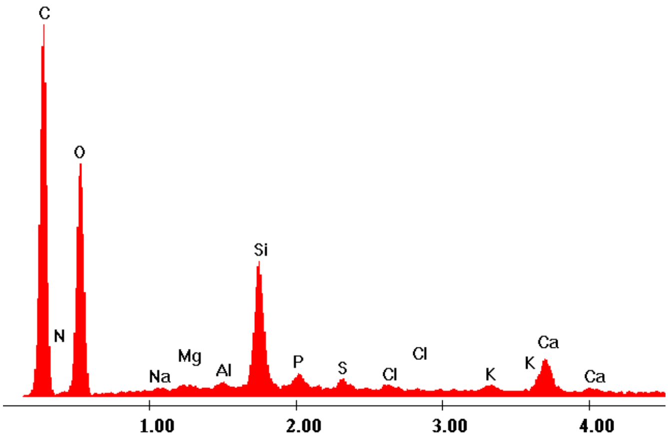

b. root of plant growth on polluted soil



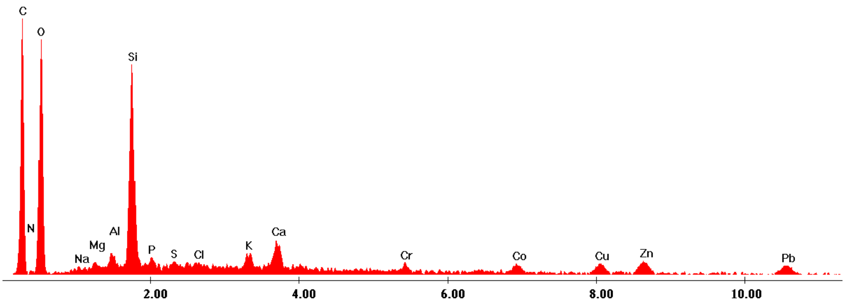

c. stem of the control sample




d. stem of plant growth on polluted soil

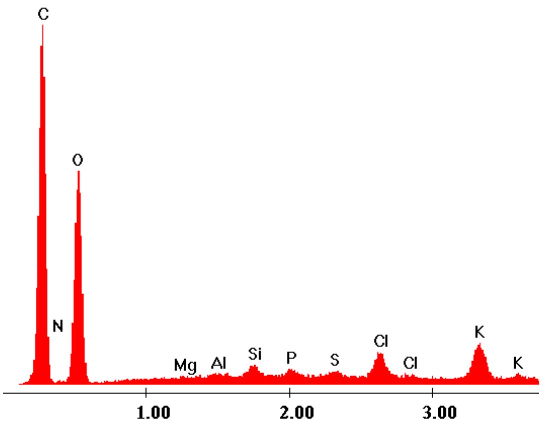



e. leaf of the control sample

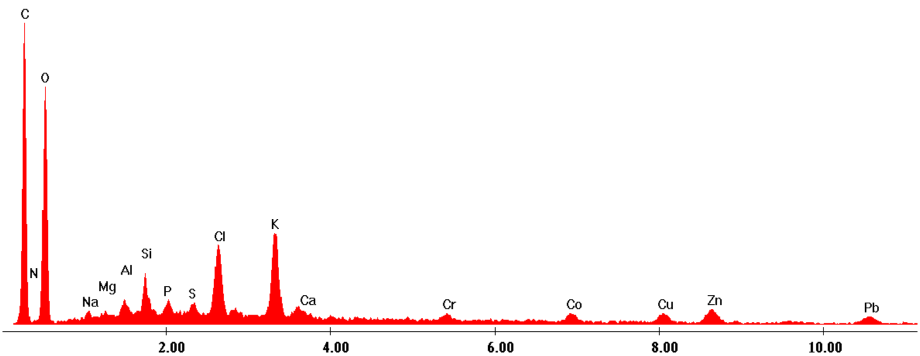



f. leaf of plant growth on polluted soil

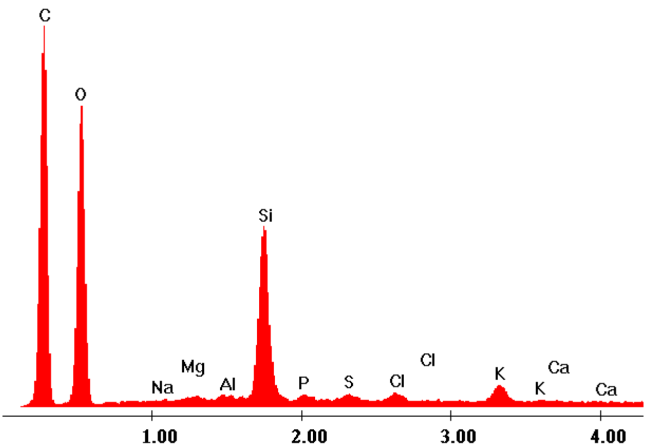
**Figure 4.** EDX spectra for*Phalaris Arundinacea Premier* unchanged plant (control and polluted sample)



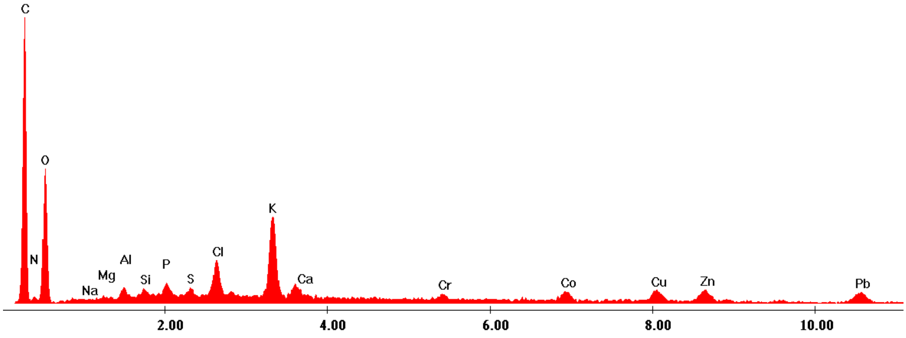

a. root of the control sample



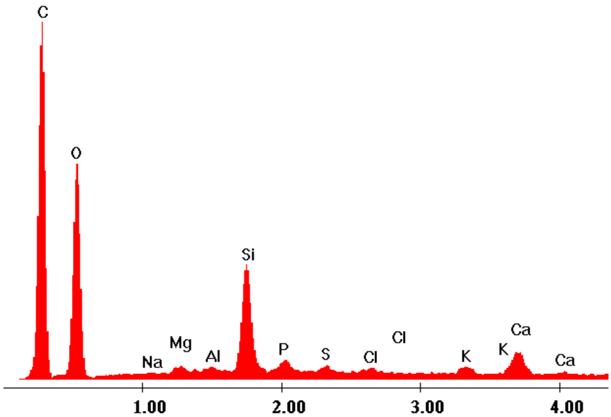

b. root of plant growth on polluted soil



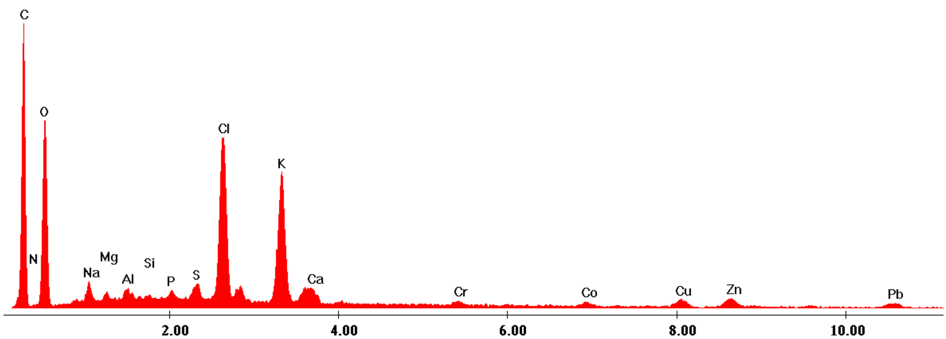

c. stem of the control sample




d. stem of plant growth on polluted soil




e. leaf of the control sample




f. leaf of plant growth on polluted soil

**Figure 5.** EDX spectra for*Phalaris Arundinacea Pollycross* modified by mixing 5 seeds (control and polluted sample)

Analyzing EDX spectra depicted in figures 4 and 5, can observe qualitatively the migration of metallic ions from control and polluted soils into the 2 different plants species used during present study. From EDX spectra recorded for the vegetation growth on control soil, presence of heavy metal ions has not been observed. In this case EDX spectra reveals only the presence of Na (I), K (I), Ca (II), Mg (II), Al (III), Si (IV), P (V) metallic ions, which are specific for used control soil. From the EDX spectra recorded for the vegetation growth on polluted soils, presence of heavy metals ions such as $Cr_{total}$, Cu (II), Zn (II), Co (II) and Pb (II) has been observed. This fact presents a confirmation that the used plants present a good accumulation capacity for all these metallic ions. Has been also observed that the highest quantity of metallic ions was founded in plants roots, followed by stem and leafs. Because the used vegetation time was not along one, can affirm that the migration process can continue in case of an increased vegetation time. At the same time, the migration process of different metallic ions can be selective, depending on the used plant, but in ours, case can observe that all studied metallic ions migrate to plant leaves.

Genetic modification of *Phalaris Arundinacea* plant does not affect the migration process of studied metallic ions. Presence of C and O into the recorded EDX spectra is due to the presence of the organic part of the plant structure.

## CONCLUSIONS

In the present paper the migration process of metallic ions into the *Phalaris Arundinacea*has been studied, in order to use it for further decontamination of soils polluted with heavy metal ions. Migration of metallic ions into plants has been highlighted by using energy dispersive X-Ray spectroscopy, EDX. From obtained experimental data can conclude that the most plant species perform a selective root adsorption of mineral elements from soil, in order to maintain a limited concentration of metals inside the cell. At lower concentration of metallic ions into the soil solution, more intense adsorption occurs, and at higher concentration, the adsorption speed is reduced.

This preliminary analysis can be useful to track the plants behavior in the heavy metals contaminated soil bioremediation process. At same time, it has been found that the used plants, which present good bioaccumulation properties for heavy metals can be also used for stabilizing and inertizing polluted soils or waste dumps as these plants tend to reach heights above 2 m.

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## NOTES ON THE AUTHOR

**prof. dr. habil. Eng. ADINA NEGREA**

**Affiliation:** University Politehnica Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, 2 Piata Victoriei, 300006, Timisoara, Romania

**E-mail address**: adina.negrea@upt.ro

Dr. Adina Negrea is professor at Faculty of Industrial Chemistry and Environmental Engineering, Politehnica University of Timişoara. Her research interest includes environmental engineering and environmental protection fields. Also, she was member in 3 international projects and 18 national projects. She is author or co-author at more than 240 scientific papers, from which 87 articles are indexed in Clarivate Analytics

Journal (h-index 12), 1 book chapter in international publishing houseand 11 books published in national publishing house. As well, she is PhD supervisor.

**Cornelia MUNTEAN[1], Oana GRAD[1,2], Adina NEGREA[1,*], Mihaela CIOPEC[1], Narcis DUŢEANU[1], Mariana CERNICOVA BUCA[3]**

[1]University Politehnica Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, 2 Piata Victoriei, 300006, Timisoara, Romania

[2]Research Institute for Renewable Energy of the Politehnica University Timişoara, 138 Musicescu Street, Timisoara, Romania

[3] University Politehnica Timişoara, Department of Communication and Foreigner Languages, 2 Piata Victoriei, 300006, Timisoara, Romania