Methods using plants to clean the soils that are polluted with heavy metals

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Abstract. The soil contamination with heavy metals is a major worldwide issue as a result of mining processes, car industry, glass and paper industry, non-compliant waste dumps and heavy car traffic. This study will focus on the main polluted areas with heavy metals from Romania as well as the maximum permited limits of heavy metals in soils. We will describe the methods of using plants to clean the soils that are polluted with heavy metals, examples of plants that can be used, as well as the phytotoxicity tests used to determine the influence of heavy metals on plants. Another topic will focus on presenting the areas where soil decontamination using plants was applied on national as well as international scale.
Key Words: maximum limits of heavy metals in soil, alert limit, intervention limit, hyperaccumulator plants, phytoremediation.

Introduction. Soil contamination is a major problem world-wide, as a result of extraction processes, mining products but also due to the urban activities in the past two centuries. In the European Union there are about 3 million contaminated sites, from which 250.000 are being decontaminated through different methods (Gomes 2012). Activities causing soil pollution in Europe are presented in Figure 1.

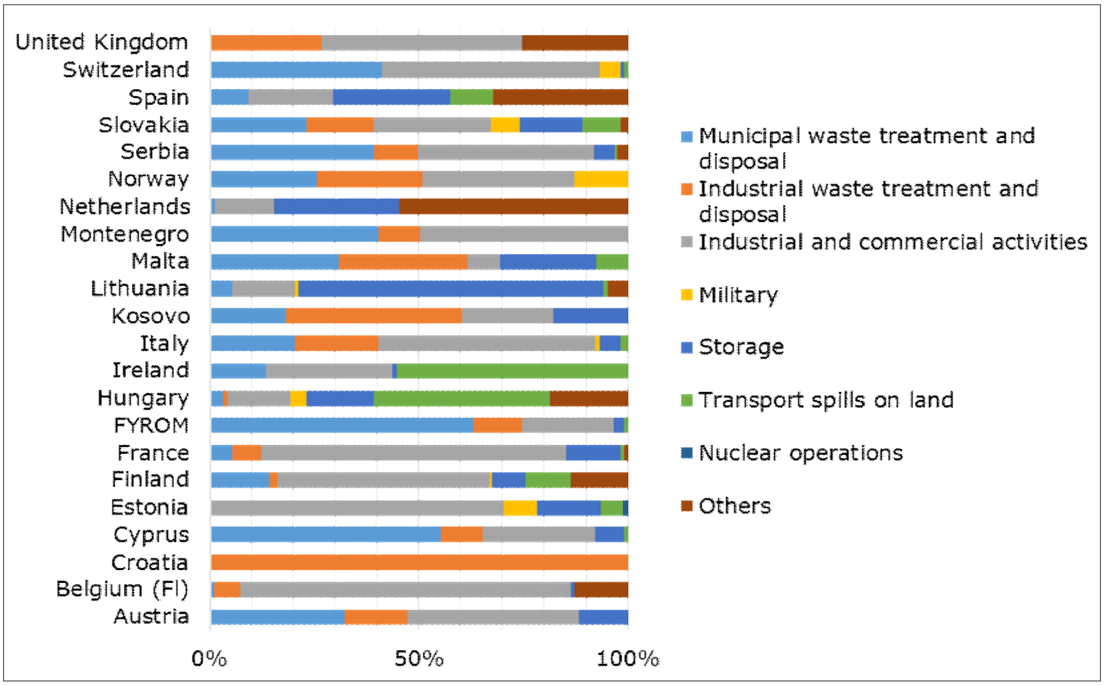

Figure 1. Breakdown of activities causing soil contamination (http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment).

In the EU countries there are differences concerning the maximum admitted concentrations (M.A.C.) of heavy metals; these differences are due to the soil types for agricultural land. After 1997, Romania introduced the terms of normal value, alert threshold and intervention threshold, through the Ministry Order no 756/1997.

Alert threshold represents the concentrations of air, water, soil pollutants or emissions/evacuations, that have the purpose of warning the competent authorities of a potential threat to the environment and prompt an additional monitoring and/or the decreasing of pollutants from emissions/evacuations.

Intervention threshold represents the concentrations of air, water, soil pollutants or emissions/evacuations, to which the authorities will order the risk assessment studies and the decreasing of pollutants from emissions/evacuations.

According to the Ministry Order no 756/1997 the reference values for the most common heavy metals are shown in the Table 1.

Table 1

Reference values for heavy metals in soil (mg kg^{-1} dry substance) (Minister Order No. 756 from 11.03.1997)

Heavy metal	*Normal values*	*Alert treshold/Use types*		*Intervention tresholds/Use types*	
		Sensitive	*Less sensitive*	*Sensitive*	*Less sensitive*
Cadmium	1	3	5	5	10
Copper	20	100	300	300	600
Manganese	900	1500	2000	2500	4000
Mercury	0.1	1	4	2	10
Lead	20	50	250	100	1000
Zinc	100	300	700	600	1500

The soils contaminated with heavy metals pose a big threat to the health of humans and animals around the world because of the possibility of entering the food chain. This is one of the reasons why it is so important to establish the bioaccumulation potential of the plants (Islam et al 2007; Jadia & Fulekar 2008).

Plants that respond to contaminants uptake are classified in: hyperaccumulators, accumulators, indicators and excluders (Baker 1981; Meuser 2013). It is necessary to identify the hyperaccumulators in order to have a high performance in soil treatment. A hyperaccumulator is defined as a plant capable to grow in soils with high amounts of metals, accumulating these metals through their roots in the aerial parts, phytotoxic effects not being visible (Rascio & Navari-Izzo 2011).

In the future, the purpose is to identify plant species that can be used for different types of contaminated areas (Goel et al 2009).

Heavy metals pollution in Romania. Heavy metals can end up in soils through human activities and other ways. The most common situation when heavy metals get in the soil is through atmospheric dust deposition (Tiwari et al 2008). Besides the previous mentioned situation, heavy metals can reach the soil also from wastewater ponds, following accidents (Science Communication Unit 2013).

The most polluted areas with heavy metals in Romania are: Copsa Mică, Baia Mare si Zlatna. In the town of Copsa Mică, pollution with heavy metals occurred due to the two main factories: Întreprinderea Metalurgică de Metalurgie Neferoasă and Uzina Carbosin. Pollution with heavy metals in the Copsa Mică area impacted the soil in both the surface layers as well as the depth layers. This makes the Copsa Mică area heavily polluted on an area of 3400 ha and medium polluted on an area of 7600 ha. The negative impact of heavy metals can be observed on vegetation and also on the soil's physical, chemical and biological properties. The non-ferrous metals that have the biggest impact on soil are the lead and cadmium. The negative impact of these metals depends on several factors like: the distance from the source, what the soil is used for, slope exposure, meteorogical conditions, etc. (Barbu & Sand 2004).

In the Baia Mare area the main pollution sources are: S.C. Phoenix S.A, S.C Romplumb Firiza S.A, mining wastewater ponds, mining tailings and mining wastewaters filled with heavy metals (Coman et al 2010; Big et al 2012). As a result of heavy pollution in the past years, the affected surface is 32,300 ha. Test results throughout the years indicated high concentrations of heavy metals in soils and vegetation in these areas (Oros 2011).

Zlatna area is known as a rich source of non-ferrous metals like: Au, Ag, Cu, Zn, Pb, Cd, As, Sn, Sb that were heavily mined to this day. Because of this exploitation the result was heavy pollution with residual gasses, acid rain with a high content of heavy metals and also the spreading of the mining tailing dust after flotation. According to Barbu & Sand (2004), there were 3500 tons of heavy metals charged dust emitted in the atmosphere.

Cleaning of the sites contaminated with heavy metals using phytoremediation. Decontamination methods nowadays in the western countries are made using big amounts of soil or by using some chemical agents that make the soil use its fertility, both of these options being very expensive (Sharma & Pandey 2014). So there is a need to have cheaper techniques that are more environmentally friendly.

Phytoremediation techniques*.* Phytoremediation is a long term solution for soil treatment and it is described as a process of removing the pollutants by using plants, their associated microorganisms, enzymes and soil amendments (Kvesitadze et al 2006; Dordio & Carvalho 2011; Kadukova & Kavuličova 2011). Phytoremediation is used for metals like: Ni, Zn, Cu, Cr, Cd and Pb (EPA 2000).

Depending on the influence on pollutants and the location in which it takes place, we have the following phytoremediation techniques:

Phytostabilization – is based on the attribute of the plants to stabilize the pollutants, lowering their mobility and bio-availability. The remediation technique consists in using some plant species to immobilize the contaminants through absorbing sand storing them in the roots, or immobilizing the contaminants in the root area. Depending on the area in which the accumulation is, there are different types of phytostabilization: phytostabilization in the rhizosphere, phytostabilization on the root membranes and phytostabilization in the root cells (Barbu & Sand 2004).

Phytoextraction – it happens when plants with large biomass are involved in the process. If the metals are bioavailable they are stored in the aerial parts of the plant. There are two types of metal categories: readily bioavailable (Zn, Cd) and less bioavailable (Pb) (Lasat 2000).

Phytomobilization – represents a combination between phytoextraction and phytomobilization, meaning that the plants are used to extract heavy metals from the soil but they are not harvested. Phytoimmoblization is the use of plants to decrease the mobility and bioavailability of contaminants by altering soil factors that lower contaminant mobility by formation of precipitates and insoluble compounds and by sorption onto the roots. After applying this technique, by using some plant species that tolerate the targeted pollutant a "green carpet" is formed in the areas where the vegetation is absent due to the high concentration of pollutants. This method is already successfully used in the areas of mining tailings, or non-ferrous materials, being much more economical than a classic sealing wrap (Barbu & Sand 2004; Gonzaga 2006).

Evapotranspiration – is used by the plants to process rich nutrient water and is dependent on humidity, temperature, wind and season. As the name implies, there are two processes involved in this: evaparation and transpiration (Rock 2003). According to Barbu & Sand (2004) plants that have high intercept capacity are: alfalfa (*Medicago sativa*) and pine trees (Coniferae).

Rhizodegradation – also known as phytostimulation, is the degradation of contaminants in the rhizosphere by means of microbial activity which is enhanced by the presence of plant roots (https://knowhowtogmo.wordpress.com/2011/01/31/rhizodegradation).

Rhizofiltration – is used for the remediation of waste water by aquatic or land plants. Pb, Cd, Cu, Ni, Zn and Cr can be extracted using rhizofiltration. Some plants examples are: sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), spinach (*Spinacia oleracea*), rye (*Secale cereale*) and Indian mustard (*Brassica juncea*) (Surriya et al 2015).

Phytodegradation – this process involves the feature of the plants to synthesize some organic pollutants and to turn them into more simple and less toxic compounds. In order for an organic pollutant to be absorbed by the plants through the roots, it has to be soluble in the soil (Barbu & Sand 2004).

Phytovolatilization – is based on the contaminates release into the atmosphere after they were absorbed by the plant from the soil (Vijayaraghavan 2012).

Lots of plants naturally contain some quantities of essential heavy metals. Some of there plants are: gooseberry (*Ribes uva-crispa*), cocoa tree (*Theobroma cacao*), green pepper (*Capsicum annuum*), coffee *(Coffea arabica*), potato (*Solanum tuberosum*), sweet chestnut (*Castanea sativa*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), yarrow (*Achillea millefolium*), marjoram (*Majorana hortensis*), carrot (*Daucus carota*), plantago (*Plantago media*), sage (*Salvia officinalis*) etc. (Parvu 1997).

The metals in the soil resulted from the mining tailings may be absorbed by the plants and exhibit symptoms of toxicity. These symptoms of toxicity are distinguishable by: slow growth, yellow leaves, some plants don't blossom, growing the roots more in depth in order to find non-polluted zones (Malschi 2009).

Phytotoxicity tests. Phytotoxicity tests are done to determine the influence of heavy metals on plants. There are several types of tests: tests involving terrestrial plants, testing the effect of seed germination, growing plants in hydroponic liquid and testing the germination and growth rate of plants (ASTM 1994; Oros 2011; Roccottielo et al 2011).

Terrestrial plants tests. These types of tests can be used to establish the phytotoxicity of soil samples from polluted areas and also for the phytotoxicity of the mud, sediments and wastes that contain toxic compounds. These types of tests are useful to determine the resources necessary to decontaminate polluted areas or to establish the possibility to use them for fertilizing waste lands. The terrestrial plants test can also be used to determine the phytotoxicity of some substances that will end up in the soil. The presence of the pollutant will inhibit sensitive plant growth and will encourage the growth of sturdy plants (http://www.oecd-ilibrary.org/docserver/download/9720801e.pdf?expires=1453389171&id=id&accname=guest&checksum=29594E52C6BBE81C0F82AE0523605D67).

In most situations, in the terrestrial plants' tests, seeded plants are used. There were developed tests for lots of species of plants, cultivated plants in majority, but there can also be used plants from common vegetation if that is useful for the given situation. Standard tests with terrestrial plants were developed and can be found in the guides belonging to: ASTM, EPA, OECD and ISO. Standardized tests target mostly the following: seed germination, early plant growth, root length, life cycle, woody plants (Oros 2011).

Seeds from different species of superior plants may be used. The most common ones used are: rye grass (*Lollium perenne*), salad (*Lactuca sativa*), cucumber (*Cucumis sativus*) etc. (Oros 2011).

Seed germination and early plant growth test (Test OECD 208). This method is used to evaluate the potential effects on germination and early growth of the plants. It does not target the chronic effects or reproduction effects (Gong et al 2001; OECD 2006).

The seeds are placed in direct contact with the soil that was treated and the results are evaluated after 14 or 21 days (time measuring is started after 50% of the control lot have germinated). The germinated seeds are recorded, the dry part of the plant (above the ground), in some cases plant height. The results are then compared with the control lot.

Worldwide phytoremediation. National reports of European countries indicate that heavy metals and oils are the most common pollutants found on investigated soils, while oils and chlorinated hydrocarbons are the main pollutants of groundwater (http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites/progress-in-management-of-contaminated-1).

In Table 2 some phytoremediation projects with high efficiency are presented.

Table 2

European phytoremediation field projects (Van Der Lelie et al 2001)

Site name and location	*Institution*	*Plant species*	*Metals contained*
Czechowice refinery from Katowice, Poland	Phytotech Florida University Greening institution for industrial areas	*Brassica juncea*	*Pb, Cd*
Former landfill, Switzerland	General institution of technology, Switzerland	*Salix viminalis*	*Zn, Cd*
Sludge deposit from UK	Glasgow University	*Salix* sp.	*Ni, Cu, Zn, Cd*
Contaminated sites with Zn and Cd, Switzerland	More institutions	Tobacco plants	*Cu, Cd, Zn*
Zinc/cadmium – contaminated playing ground, Overpelt, and zinc smelter zite, Lommel, Belgium	Limburgs University	Grasses for phyto-stabilization	*Zn, Cd, Pb, Cu*
Zinc/cadmium – contaminated soil, Balen, Belgium	Limburgs University	*Brassica napus* for phytoextraction	*Zn, Cd, Pb*

Marchiol et al (2013) carried out a study on an extremely contaminated site with heavy metals from Crotone, Italy. The study was based on heavy metal accumulation in coojong (*Acacia saligna*), carrot (*Daucus carota*), false yellowhead (*Dittrichia viscosa*), river red gum (*Eucalyptus camaldulensis*), sweet pea (*Lathyrus odoratus*), reed (*Phragmites australis*), smilograss (*Piptatherum miliaceum*) and French honeysuckle (*Sulla coronaria*). The results showed that common reed and river red gum accumulated important amounts of heavy metals in the leaves and stalks, metals like Sb and Tl. Based on the study, the maximum limits for Ca, Cu, Hg, Pb, Sb and Tl were overtaken and a high quantity of heavy metals was accumulated in plants roots.

According to Bidar et al (2007), a field experiment was carried out on two different sites: one unpolluted, located at 20 km from the polluted area and one located at 200 m from a Zn contaminated area. *Trifolium repens* (white clover) and *Lolium perenne* (rye-grass) were planted on both sites and after six months, the plants have been collected and transported in the laboratory. The roots were washed with deionized water and divided in two: one group was stocked until the lab experiments at - 80^{o}C and another one was dryed at 105^{o}C. The result is that in both sites large amounts of Pb were accumulated in the analyzed plants.

Another study mentions 50 plant species that were studied for remediation purposes. From all the studied plants, there was not found a hyperaccumulator for Pb. The experiments showed that many species were capable to extract heavy metals. In the industrial area from Islamabad, the extraction capacity of *Hyacinthus orientalis* was studied. The results revealed heavy metals accumulation in buds (Cu, Zn, Cu, Pb, Co and Ni). In the same study, it was confirmed the existence of *Brassica juncea* in Pakistan area, plant known as a hyperaccumulator for Pb, Zn and Cu (Kamran et al 2013).

Ho et al (2008) have demonstrated the suitability of kenaf (*Hibiscus cannabinus*) to be used for building materials, adsorbents, textiles and fibers in new and recycled plastics. Although root biomass was only between 8% to 19% of the total biomass, accumulation of Pb in root was between 38 and 97% of total Pb/plant. Kenaf could be used for phytoremediation of Pb. Phytostabilization proved to be the best technique for the recovery of waste dumps and produces high efficiency when is accompanied by amendaments such as compost and lime.

In Flanders (Belgium, Europe) large-surface areas are diffusely polluted with inorganic pollutants like heavy metals. Van Ginneken et al (2010) used oil-producing plant species, such as rape seed (*Brassica napus*) for phytoextraction purposes, which represents a sustainable use of metal-contaminated land. If oil crops (such as *Brassica* sp.) are used in the phytoremediation of soils contaminated with heavy metals, biodiesel production from the resulting plants could be a viable option to generate bioenergy and also an environmental friendly approach.

Phytoremediation in Romania. In Romania, few studies that involve plants are known. Babeș Bolyai University from Cluj-Napoca used cucumber (*Cucumis sativus*) seeds on soil polluted with Fe. In the same University, Biotechnology laboratory used *Lolium perenne* to depollute soils from SC Minbucovina SA Vatra Dornei and Târnăveni Chemical Plant. The exposure was made for 14 - 28 days using potted plants grown from seeds (6 - 8 weeks old plants) (Malschi 2011).

The experiments from Copșa Mică area involved *Miscanthus sinesis* plant. This is a plant with high energy potential, but does not accumulate large amounts of heavy metals. Studies show that this plant extracts 2.12±0.44 mg kg^{-1} dry matter for Cd and 3.71±0.73 mg kg^{-1} dry matter for Pb while the soil contains 13.47 mg kg^{-1} dry matter Cd and 682.50 mg kg^{-1} dry matter for Pb (Grama et al 2010).

ICPA Bucharest has done a research in phytoremediation domain analyzing the soil in the industrial-battery Neferal, located 13 km from the highway Bucharest – Brăneşti. They established nine sampling points located in different directions from the source and harvested plants, proving resistance to heavy metal pollution. Pb values were between 4.8 and 1178 mg kg^{-1}, but most values being over 10 mg kg^{-1} (maximum limit in plant). The chemical parameters from the soil samples were: pH – 5.70; C – 1.24%; Ni – 0.158%; Cu_t – 379 mg kg^{-1}; Zn_t – 650 mg kg^{-1}; Pb_t – 573 mg kg^{-1} (t representing the total element content) (Biotech 2006).

Conclusions. Phytoremediation can be applied for the conversion of polluted industrial areas in green areas due to low cost and aesthetically pleasing appearance. The main disadvantage is that the process is longer compared to other technologies.

Future research may discover new species of plants that can be used in the process of phytoremediation. In most studies, cereals and leguminous plants were used because of their heavy metal tolerance, easy establishment, low acquisition and support costs and they can cover large areas.

A higher biomass may result if chelating agents are used in phytoremediation.

Most research on decontamination yield has been conducted over several years and it can be concluded that the rate of the first year of experiments on soil pollution with heavy metals is relatively low, but it may increase with continued research over several consecutive crops.

The amount of the metals extracted from the polluted soil surface depends on the density of the plants.

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