Rehabilitation of degraded soils containing Lead (Pb^{2+}) ions based on phytoremediation with *Fagopyrum esculentum Moench* in presence of Ethylene-diamine-tetracetic acid (EDTA)

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Abstract: - In this paper the lead removal from soil by bioremediation was investigated. At a laboratory scale, this process was experimented using buckwheat (*Fagopyrum esculentum Moench*) plants and polluted soil with lead ions. Buckwheat is a vascular plant that shows affinity for lead and has a high biomass productivity. The EDTA is a chelating agent and has a greater affinity for lead and facilitates higher metal availability and uptake by plant tissue. The main objective of this study is to show the removal performances of lead ions of the plants involved in the soil remediation and to establish the optimal experimental conditions. The main investigations carried out in order to establish the lead ions removal efficiency were: seed germination, quantity of biomass and quantity of lead ions accumulated in all plant tissues mattered. The lead ions were determined using Atomic Absorption Spectrometry. The results of the study show that *Fagopyrum esculentum Moench* is a plant with a high capacity for lead ion accumulation and the efficiency of phytoremediation process is enhanced in the presence of EDTA.

Key-Words: phytoremediation, lead, soil, pollutant, heavy metal ion, bioaccumulation, ethylenediaminetetracetic acid

1 Introduction

Lately, environmental pollution by heavy metals has become a very complex problem, because most heavy metals are found in different environments with high toxicity and health impact of all forms of life. Most of the heavy metal ions lie in the soil.

The pollutants in the soil are subject to bio-geo-chemical transformation process. These processes affect the structure of the pollutant through a chemical process that occurs in a geological environment and can be achieved by a biological organism.

Phytoremediation - use of plants to remove toxic metals from soil is emerging as a cost-effective alternative to conventional methods. Phytoremediation, commonly known as “green clean technology” is a new promising technology used for toxic contaminants removal from the environment such as heavy metals, adopting suitable plants. This concept is increasingly being adopted as it is a cost effective and environmentally friendly alternative to traditional methods of treatment.[1]

The conventional procedures for remedying soils polluted with heavy metals consists in : scraping of the surface layer, storage in another isolated zone, chemical treatment by soil washing, resulting in large quantities of infertile soil, but also large quantities of water loaded with heavy metals ions. The increase of solubility of metals, so that to leave the arable layer, can also it can cause contamination with heavy metals from the groundwater. Other conventional procedures applied to polluted soils include covering with concrete asphalt and geotextiles, then with one layer of uncontaminated fertile soil. [2-8]

Phytoremediation is a simple, environmentally friendly and less expensive solution for removing heavy metal ions and restoring degraded soils.[9]

Soils phytoremediation involves using plants to remove processes, transfer, stabilize, and destroy contaminants in soil.

Hyperaccumulator plants extract or absorb the pollutants. Phytoextraction is a mechanism using plants accumulator from contaminated soil with heavy metal ions and other pollutants.

Translocation of heavy metal ions takes place first in plant tissues and finally in plant shoots.

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Process efficiency is determined by plants type and it is chemically enhanced by the chelators added.
The plants recommended for bioaccumulation must have some characteristics such as: seeds germination capacity, the ability to grow rapidly, high amounts of biomass, the ability to accumulate in different tissues high quantities of pollutants, inclusively heavy metals ions. [10, 11].
Buckwheat (*Fagopyrum esculentum Moench)* is an annual herb, up to 1 m tall, branched, glabrous. Leaves are petiolate, blades are ovate-triangular to triangular, 2-8 cm long, with acuminate tips, bases are cordate or approximately hastate; upper leaves are smaller, sessile. Inflorescences are terminal and auxiliary, branch in dense corymbose or paniculate cyme. Flowers are white or pink, 6 mm in diameter; pedicel is 2-3 mm long, articulate; perianths are 3 mm long; 8 nectaries are yellow, alternating with stamens; being heterostyly, capitate stigma. Achene is triquetrous, acute angle, longer than 5 mm, more than twice the length of the persistent perianths, brown or black-brown, lucid. [12] That plant with high bioaccumulation capacity is classified as hyperaccumulator and hypertolerant for lead ions. hypertolerants and hyperaccumulatos.[13] Buckwheat is a promising plant to be used for lead extraction for polluted soils. In fact it has the ability both to tolerate and accumulate Pb^{2+}, from the growth substrate, adapted to a humid climate.
In this experiment, Lead has been selected as pollutant, because it is a common industrial metal residual which spread in all environments: air, water, soil, and food. But particularly this metal dispersed in soil.
In nature, it is easily found in oxidized forms (salts and oxides) that have been used for metal extractions almost since the beginning of industrialization. Lead (Pb^{2+}) often exists as a divalent cation, in soil, which is prone to the cation exchange; the sorption/solubility phenomena occur. So, the activity of free Pb^{2+} in soils varies, being conditioned by the solubility of the controlling lead phase [14].
The chelators are added to the contaminated soil to enhance heavy metal uptake by plants. [15–17].
Ethylene-diamine-tetraacetic acid (EDTA) is a well known synthetic species broadly used for improved efficiency heavy metal ions translocation. Among different chelators, EDTA is used for environment contaminated by Cd, Ni, Pb, Cu, Zn [18]. The addition of EDTA to soil induces accumulation of Pb within plant green parts known as shoots [19]. In soil EDTA forms Pb–EDTA, a stable complex, which is highly water-soluble and becomes therefore available to plants [20–22].

2 Problem Formulation

After suffering geochemical transformations, the heavy metals from soil, because of their toxicity, can damage the health of all the forms of life. This phenomenon has created resistant plants, which over time have become hyperaccumulators for heavy metal ions. Buckwheat plants are excellent candidates for soils phytoremediation because they accumulate high amounts of trace metal ions in their aboveground biomass when growing in metal enriched habitats. [23-24]

2.1 Growing conditions and soil treatment

At laboratory scale, this study was conducted in two successive processes.
For the first process were used especially glasslike plastic pots and reference soil for germination process. The ground with composition as in table 1, was placed at the bottom and saturated lead acetate trihydrate solution $(C_2H_3O_2)_2$-Pb-$_3H_2O$ in a three different concentrations (2000, 5000, 7000 ppm). In order to compare the results, one of the samples was saturated only distilled water, representing 0 % Pb^{2+} concentration. After covering the soil with filter paper, 10 buckwheat seeds were placed there and then closed with glasslike cover. Selected seeds with 99% germination capacity, provided by Bank of Vegetable Genetic Resources, have been used. The germination process was carried out for 3 days at thermostat at a temperature of 25 °C.

Table 1. The characteristics of the soil used in the germination process.

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

For the second process, growing plants and lead bioaccumulation, were used plastic pots each containing 1 kg of soil with the characteristics stated in table 2 and 3.
These soils provided by Micro Bio Tests Laboratories Inc, Belgium do not contain other metal ions that might interfere with lead ions added, which facilitated the accuracy of results.

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Table 2. The general characteristics of the soil used in the process of phytoremediation

Soil	Characteristics of soil
Sampling depth, cm	**0-15**
Soil type/subtype	**Cernisol/ typical chernozem**
Texture	**Clay – loam**
Structure	**Granular – medium**
Actual use	**Cultivation different grass**
Anthropogenic modification	**Weak**
Pollution	**Unpolluted**
ρ, g/cm^3	***1.480***
pH(Soil:H_2O = 1:10)	***7.160***
EC (Soil:H2O=1:5)	***0.159***
Moisture content, %	**4**

Table 3. Chemical and mineralogical composition

Soil sample	Compounds		%
Clay minerals	Total crystalline minerals		***33,2***
Carbonates, [%, w /w]	Crystalline		***1,56***
	Amorphous		***0,3***
	Total carbontes		***1,86***
Iron oxides and oxyhydroxides [%, w/w]	Crystalline		***0,65***
	Amorphous		***1,39***
	Total		***2,04***
Silica (SiO_2), [%, w / w]	Crystalline		***3,5***
	Amorphous		***0,7***
	Total		***4,2***
Organic matters, [%, w / w]	Total humus		***3,6***
	Other organic compounds		***0,46***
	Total		***4,06***
Soluble salts, [mg/100 g sol]	Cationic composition (soluble salts extract)	Na^+	***10,52***
		K^+	***24,12***
		Ca^{2+}	***25,43***
		Mg^{2+}	***12,03***
		Other cations	***5,82***
		Total	*77.92*
	Anionic composition (soluble salts extract)	Cl^-	***5,82***
		NO_3^-	***16,45***
		SO_4^{2-}	***15,67***
		CO_3^{2-}	***18,24***
		HCO_3^-	***3,78***
		PO_4^{3-}	***15,56***
		Other anions	***1,54***
		Total	***77.06***

Soil pollution has been done in a controlled mode by adding the same solution contained lead: lead acetate trihydrate solution $(C_2H_3O_2)_2$-Pb-$_3H_2O$ and after that mixture in soil. Lead was added to the soil in a three different concentrations (2000, 5000, 7000 ppm). In order to emphasize the efficiency of the phytoremediation process, one of the pots was not contaminated with lead solution. Instead, distilled water was used. That plastic pot represented the 0% concentration of lead in soil used as a starting point for comparing all the other results. EDTA was applied in a 5 mmol/kg concentration, after duplication the plastic pot samples for each concentration of Pb^{2+} in soil. Afterwards in each plastic pot were sown 5 buckwheat seedlings, about 5 cm between them. Each group was replicated three times. Tested plants was daily followed to differentiate morphologically between the plants growth on lead most contaminated soil and the plants growth on EDTA containing soil.
The experiments were performed for a 30 days period at an average temperature about of 20 ± 3 °C. The main investigations carried out in order to establish the lead ions removal efficiency were: seed germination, quantity of biomass and finally quantity of lead ions accumulated in all plant tissues.

2.2 Sample preparations and analysis conditions

Harvested plant tissue were carefully rinsed with distilled water to remove soil, dried in the oven for 24 hours at a temperature of 60 °C and grinding for mineralization process. For acid digestion, were used nitric acid (6mL), hydrochloric acid (2mL) and mineralized at a temperature of 105°C. Each sample was heated to boiling for at least 3h. The solution was filtered to remove residue and diluted to 100mL in a volumetric flask.
Elemental analysis was performed by GBS Avanta Ultra Z Atomic Absorption Spectrometer, using graphite furnace electrothermal.

3 Problem Solution

Due to a great capacity to solubilise nutrients from the root zone, buckwheat is able to metabolize the microelements from soil, including heavy metal ions. *Fagopyrum esculentum Moench* is a vascular plant that shows high tissue concentration of lead, when grown in contaminated soil and which has a high biomass productivity.

3.1 Seeds germination

After 3 days of buckwheat seeds incubation, could be seen, by visual observations pollutant effects on seed germination and root elongation.

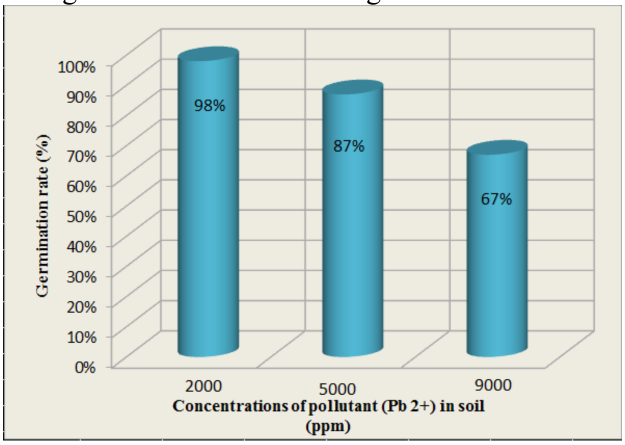

Fig. 1. Seeds germination rate

As it can be seen in figure 1, germination rate of seeds is influenced by the concentrations of pollutant in the soil. Thus, the higher the concentration of Pb $^{2+}$ ions, the lower of germination rate of both seeds.

3.2 Accumulation capacity of plants

After 30 days, the buckwheat plants developed on lead contaminated soil and accumulated in their tissues lead ions, as shown in figure 2.

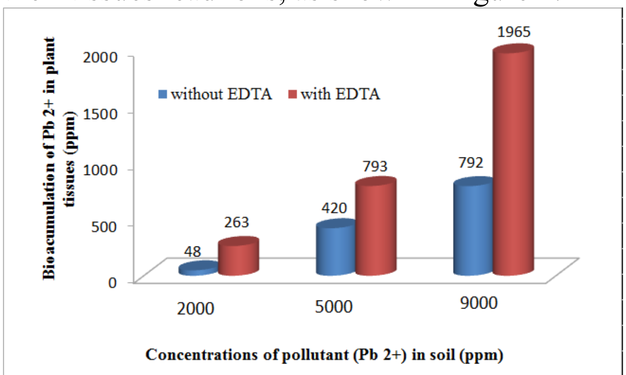

Fig. 2. Plants bioaccumulation capacity

Thus, at lower concentrations of pollutants in the soil and the amount of Pb 2+ in plants is lower, so biaccumulation rate is also lower. But the higher amount of pollutant in soil increases with both buckwheat plants accumulate in tissues Pb 2 + ions. Bioaccumulation process of lead ions is visibly improved in the presences of EDTA in soil, so that at 2000 ppm concentration of Pb 2+ in soil, the bioaccumulation process with EDTA is almost five time more active and at the high concentration, 9000 ppm Pb 2+ in soil, the bioaccumulation process is almost 2,5 times more active.

After 30 days, obtain efficiency in the process of removal of lead ions from soil, expressed in a percentage in table 4.

Table 4. Efficiency of phytoremediation process

Lead soil concentration	Without EDTA	With EDTA
2000 ppm	***2,1%***	***13,15%***
5000 ppm	***8,4%***	***15,86%***
9000 ppm	***8,8%***	***21,83%***

The best yield has been achieved at a concentration of Pb 2 + in soil at 9,000 ppm and in the presece of EDTA.

4 Conclusion

This experiments confirmed that the use of ethylene-dyamine-tetraacetic acid EDTA is a chelator that enhanced Pb^{2+} extraction by *Fagopyron esculentum Moench*. The examined plant proved to be suitable for rehabilitation of degraded and contaminated soil with lead ions.

It has been demonstrated, taking into consideration the time factor, that phytoremediation is the most effective method for soil polluted, even without chelator addition.

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