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Recovery of contaminated soils with leachate after closing a municipal landfill

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Abstract. In this paper, one of cost effective and environmental friendly method for rehabilitation a polluted soil with landfill leachate was investigated. Old municipal landfills waste stored directly on the ground and are not equipped with leachate collection systems. Leachate is a liquid mixture provided by precipitations which penetrated waste and taking over more pollutants including heavy metal ions with high capacity of remaining in the soil. The process was experimented ex situ, at laboratory scale and is based on phytoremediation and bioaccumulation of heavy metals by rape (*Brassica napus*) and white mustard (*Sinapis alba*). The main objective of this experiment is to show the removal performance of heavy metals in the plant species and after that establish the optimum process conditions. Thus, the investigations carried out were established: lowest effective leachate concentrations not causing a toxic effect in seeds germination process, rate of biomass development and the uptake capacity of heavy metal ions in the tissues of the plants.
Key Words: landfill, leachate, phytoremediation, germination, heavy metal ions.

Introduction. Solid waste management in worldwide is achieved by landfilling (Mariam & Nghiem 2010) A municipal waste storage practice varies considerably depending on the degree of economic development of each country. Thus in developed countries we have to do with sanitary landfills, designed by controlled storage and installation of leachate collection and treatment. While in developing countries predominate uncontrolled storage in open land, generators large quantities of leachate with adverse effects on the environment (Pohonţu 2011)

The leachate is a liquid mixture with complex composition generated from rainfall water that crossing through wastes stored in open space. Thus, water crossing through a waste collects the pollutants produced by biological decomposition processes facilitated by microorganisms (Jones et al 1983). Generally speaking, leachate pollutants are characterized by high concentrations of organic compounds, ammonia nitrogen, heavy metal ions, as well as bad odor and dark-brown color (Raghab et al 2013).

Soil contamination by landfill leachate is a very important environmental problem, because it can be affected not only soil quality, but also surface and ground water, air, biodiversity and human health.

The newest European legislation and strategy that can be applied according to European Directives 2006/12/CE, about waste and 99/31/EC about solid waste landfilling which stipulating to close old landfills and building of sanitary landfills, method of controlled disposal of municipal solid waste refuse on land and is designed to collect, transport and treatment system of leachate. However, following decommissioning of old landfills remain the large areas of soil contaminated with leachate that could be given back to agriculture or other activities.

The classical treatments of contaminated soils are more expensive with negative impact on ecosystems, seriously affecting soil parameters. These included physic-mechanical methods consist in uncovering the surface layer, excavation, transport and storage in another isolated zone and the ex situ treatment of pollutants, or physic-chemical removal “in situ” processes by washing, when results large quantities of waste water and infertile soil (Schiopu & Gavrilescu 2010). The chemical procedures are dangerous, because it can be affect groundwater quality (Lombi et al 2001; Caraiman (Cojocaru) et al 2012). The alternative is cost effective and green clean solution, when soil decontamination is carried out by biological organisms (Pohonţu 2013; Białowiec et al 2011).

Soil bioremediation involves all biological processes of the pollutants transformation from soil to plant tissues. This mechanism is based on degradation of main pollutants by rizosphere bacteria and after that absorption by radicular system, accumulation and transformation of pollutants. Degradative bacteria creating stabilization of soil pollutants, toxicity reducing, thus, preventing penetration of pollutants in groundwater (Rajkumar & Freitas 2008; Rajkumar et al 2012) In the absence of plants this process would be slowed and inefficient (Glick 2010).

Rape (*Brassica napus*) and white mustard (*Sinapis alba*) are two species of vascular plants, part of the family Brassicaceae. They are annual plants with well developed root system in soil, resistant to adverse environmental conditions and known as high bioaccumulation capacity especially for a large variety of heavy metal ions. In association with rizosphere degradative bacteria from soil, heavy metal accumulation process becomes much more efficient (Rajkumar et al 2012; Glick 2010).

The present work aims to investigate the efficiency of the remediation process of soils polluted with leachate by plant in presence of rizosphere plant growth promoting bacteria. Thus, the investigations carried out have determined: leachate toxicity, calculating vigor index of seeds germination process, plant biomass development according to degree of soil pollution by leachate and finally heavy metal ions uptake in plant tissues.

Material and Method. Studies took place in March–June 2015 and were carried out “ex situ” at laboratory scale in Applied Ecology Lab of University Stefan cel Mare Suceava.

The experiments consist of two consecutive methods. The first of them supposed phytotoxicity tests checking seeds rape and white mustard germination ability, thus determining vigor index and leachate concentration not causing a toxic effect in physiologic plant processes.

The second process represents a continuation of the first, by which will be soil remediation of pollutants with plants and inoculated rizosphere growing promoting bacteria, isolated from soil.

The leachate was sampled from Botoşani old municipal landfill, which has been decommissioned. The physicochemical parameters of this leachate are presented in Table 1.

Table 1

Leachate physicochemical parameters

Parameters	*Values*
CCO-Cr (mg O_2 L^{-1})	4,992.5
pH	7.4
Ammonium (mg L^{-1})	1816
Total nitrogen (mg L^{-1})	1,956
Nitrate (mg L^{-1})	28
Total Phosphorus (mg L^{-1})	10.9
Chlorides (mg L^{-1})	5,320.39
Sulfates (mg L^{-1})	200.15
Conductivity (mS cm^{-1})/Temperature (25°C)	44.17
Fixed residue (mg L^{-1})	896.42

The initial leachate analyzing by atomic absorption spectroscopy was determined heavy metal ions concentration, as described in Table 2.

Leachate phytotoxicity test kit (Figure 1) is composed of a plastic plate divided in two compartments with glasslike cover and having for dimensions 21.00 x 15.50 x 0.80 cm.

Table 2

Leachate heavy metals concentrations

Heavy metal	*Concentration (mg L^{-1})*	*Maximum concentration allowable by law (mg L^{-1})*
Chromium	1.579	1
Copper	0.314	0.1
Cadmium	0.161	0.2
Zink	0.925	0.5
Lead	1.007	0.2
Iron	1.952	-
Nickel	0.180	0.5

Figure 1. Phytotoxicity test kit.

In one of the compartments will place a particular type of soil named OECD reference soil, purchased from MicroBioTests Inc., Belgium. It is a substrate using frequently in a standard toxicity tests with a known composition shown in Table 3. This soil not contains heavy metal ions.

Table 3

OECD reference soil composition

Soil compounds	*Quantity (%)*
Dry quartz sand	85
Kaolin	10
Peat moss *Sphagnum*	5
Calcium carbonate ($CaCO_3$)	To obtain a pH of 7-7.5

Thus, in the bottom compartment of kit, was placed the reference soil, with a spatula, so that all corners are covered. The soil was saturated with leachate at different concentrations as follows: 6.25%, 12.5%, 25%, 50% and 100%, each dilution needing another kit, then covered with dark filter paper on which were placed 10 plant seeds at a distance approximately 1 cm to each other. After have been closed with glasslike cover, were put at thermostat for 3 days at 25 ± 3°C temperature. The results were biometrical interpreted, visually by the number of seeds germinated and measured roots and shoots elongation with software Image Tool 3.0.

Based on phytotoxicity tests and for keeping the processes under control at laboratory scale, was passed to the second stage of the experiment, phytoremediation, in which has been sampled soil, from the near neighborhood of Botoşani old landfill, having the same characteristics as in landfill, presented in Table 4.

Table 4

Characteristics of soil using in phytoremediation

Parameters	*Values*
pH	7.34
Moisture (%)	58.30
Total carbon (%)	4.92
Total Kjeldahl nitrogen (%)	0.07
Chromium (mg Kg^{-1} dry matter)	0.02
Copper (mg Kg^{-1} dry matter)	0.04
Lead (mg Kg^{-1} dry matter)	0.17
Nickel (mg Kg^{-1} dry matter)	0.01
Zink (mg Kg^{-1} dry matter)	2.14

For increasing phytoremediation process rate, this was achieved by adapting and inoculation of bacteria that exist naturally in soil microbiota, having the role to facilitate the bioavailability of pollutants in soil, named plant growing promoting bacteria. These strains of bacteria were isolated from the leachate contaminated soil, originated from Botoşani old landfill.

Approximately 1 g of wet soil sample were serially diluted using 25 mM phosphate buffer and inoculated through flooding over on nutrient agar (Difco) solid medium, adding from 1 to 5 mL leachate. The plates were incubated for 48 hours at 37°C. Different colonies of bacteria were harvested and purified on the nutrient agar medium with 5 mL leachate. During this time were gradually added higher leachate quantities, finally obtaining leachate resistant bacteria colonies, with higher resistance at toxicity. Colonies were grown on nutrient broth liquid medium for 48h at 37°C. In the end was obtained a bacterial suspension used for inoculating seeds of rape and white mustard. Selected seeds, with high germination ability, were purchased from the Bank of Vegetable Genetic Resource from Suceava. These initially were sterilized at surface tegument in sodium hypochlorite solution 1% for 30 seconds and rinsed in sterile water, whereupon inoculated by soaking bacterial suspension for 1 hour and planted. In plastic pots, each containing about 300 g of soil were sown 10 inoculated bacteria plant seeds, about 2.5-3 cm between them. Soil contamination was made in a controlled mode by saturation with leachate at different concentrations: 6.25%, 12.5%, 25%, 50% and 100%, each dilution and plant species needing another pot. One of the pots was not contaminated with leachate, used only distilled water, this representing references value. In order to maintain the constant soil moisture, once a week it was saturated with leachate on the concentration corresponding pot, simulating field conditions. To highlight the efficiency of inoculation bacteria in this experiment, were duplicated the pots, which were sown non inoculated bacteria seeds.

The plants were grown during 60 days, at a temperature about of 25 ± 3°C, relative humidity 70±5% and natural light 12/12 day/night with solar intensity at 3000 lux±5%.

The main operation for samples preparation and analyzing, were performed as: after 5 days, the phytotoxicity tests kit, were examined in order to identify seeds which have germinated and were photographed and measured root length and shoot length by Image Tool soft.

After 45 days growing, the rape and white mustard plants was carefully removed. With distilled water, the roots were rinsed for removing the possible soil remainings and after that were measured shoot length and fresh weight for the two plant species.

For identifying heavy metal ions concentrations in plants, were dried during 24 hours, in the oven, at a temperature of 60°C, dry tissues of plant were grinding for mineralization process by acid digestion. Thus obtained solution was elemental analyzed by Atomic Absorption Spectrometry (AAS).

Results and Discussion. Vigor index calculating for each seedlings of plant studied, at each leachate concentrations was possible after visual observation sand measured root and shoot length by Image Tool., and finally was applied equation no. 1 (Abdul-Baki & Anderson 1973).

In Tables 5 and 6, depending on the degree of seeds germination of rape and white mustard, and according to root length and shoot length, vigor index was calculated for each plant and leachate concentration. Vigor index is a numerical value higher than one. The higher this value is higher, the plant is more resistant to applied pollutant and influence on growth and development is minimal. As can be seen, in the above tables, for both rape and white mustard, at high concentrations of the pollutant applied, decreased vigor index:

$$\text{Vigor index} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination } (\%) \quad (1)$$

Table 5

Biometric parameters after *Brassica napus* seeds germination according to different leachate concentrations

Brassica napus	*Leachate concentrations*					
	0%	*6.25%*	*12.5%*	*25%*	*50%*	*100%*
Root length (mm)	93.6	92.4	87.3	78.1	36.4	2.1
Shoot length (mm)	3.4	2.9	1.7	0.7	0	0
Germination (%)	100%	100%	90%	80%	80%	60%
Vigor index	9700	9530	8010	6304	2912	126

Table 6

Biometric parameters after *Sinapis alba* seeds germination according to different leachate concentrations

Sinapis alba	*Leachate concentrations*					
	0%	*6.25%*	*12.5%*	*25%*	*50%*	*100%*
Root length (mm)	89.1	85.4	52.9	35	27.2	0
Shoot length (mm)	22.5	21.8	17.5	13.7	5.6	0
Germination (%)	100%	100%	90%	80%	80%	0%
Vigor index	11160	10720	6336	3896	2624	0

As can be observed, in Figures 2 and 3, with increasing leachate concentration, decreased seeds germination ability. At 100% leachate concentration, rapa seeds just beginning to germinate, while white mustard seeds not germinated at the same leachate concentration. But white mustard seedlings have better developed shoot length, while rapa seedlings have better developed root length, therefore a more efficient root system badly needed in the decontamination of polluted soils.

In the Figures 4 to 8, were plotted the quantities for each of the 6 heavy metal ions, uptake in rapa plants, after the experiments, depending on the leachate concentration with which has been saturated the soil.

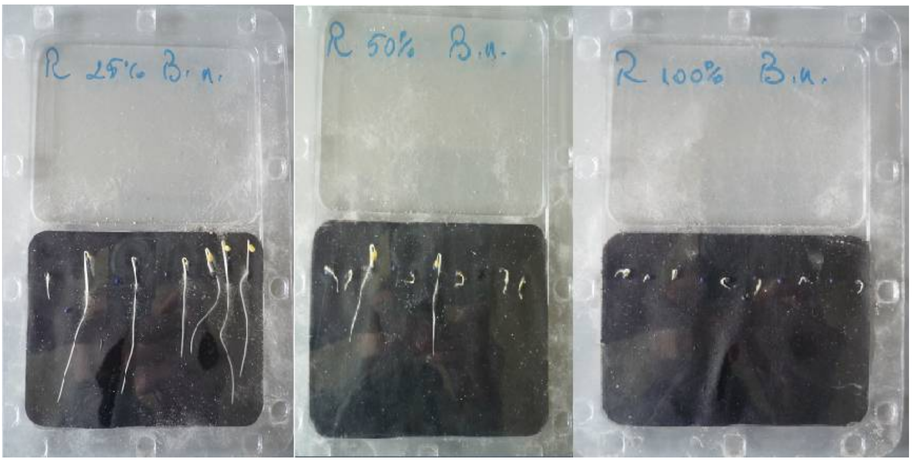

Figure 2. *Brassica napus* seeds germinated at different leachate concentrations.

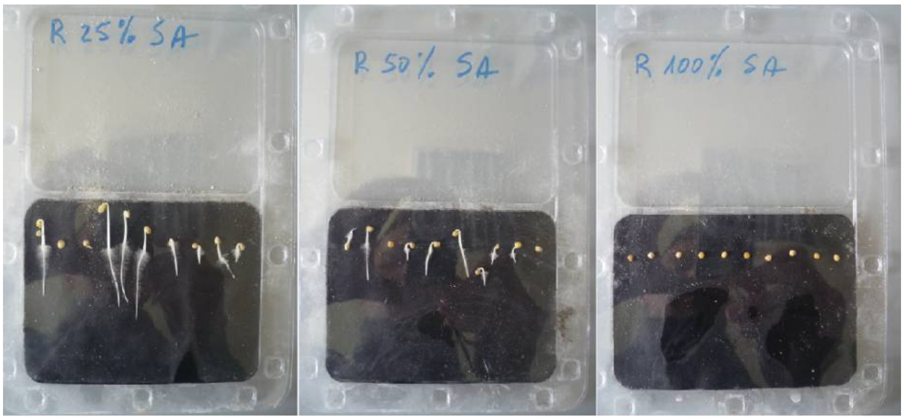

Figure 3. *Sinapis alba* seeds germinated at different leachate concentrations.

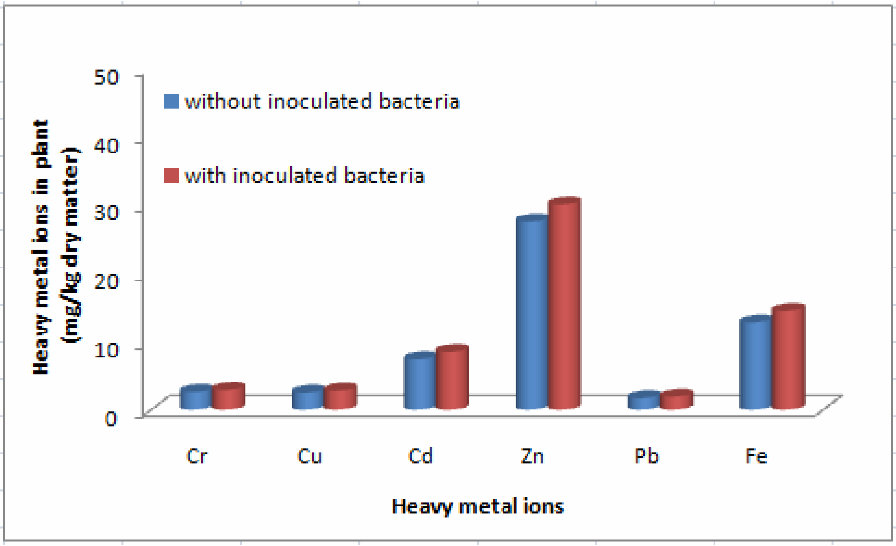

Figure 4. Uptake of heavy metals in *Brassica napus* tissues at 6.25% leachate concentration.

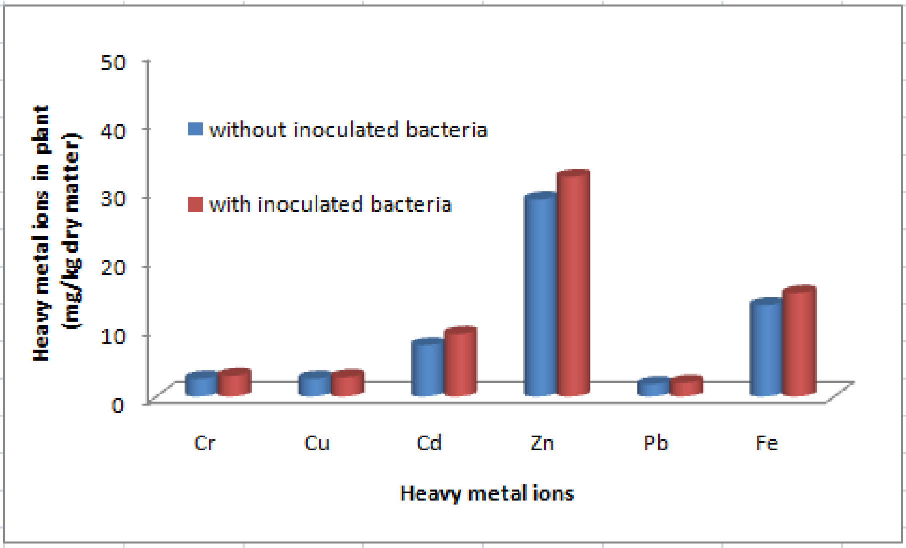

Figure 5. Uptake of heavy metals in *Brassica napus* tissues at 12.5% leachate concentration.

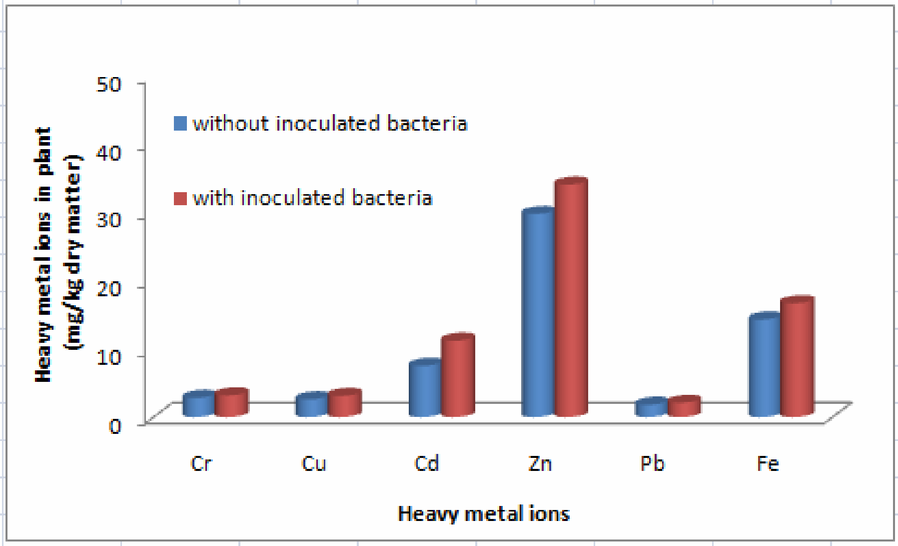

Figure 6. Uptake of heavy metals in *Brassica napus* tissues at 25% leachate concentration.

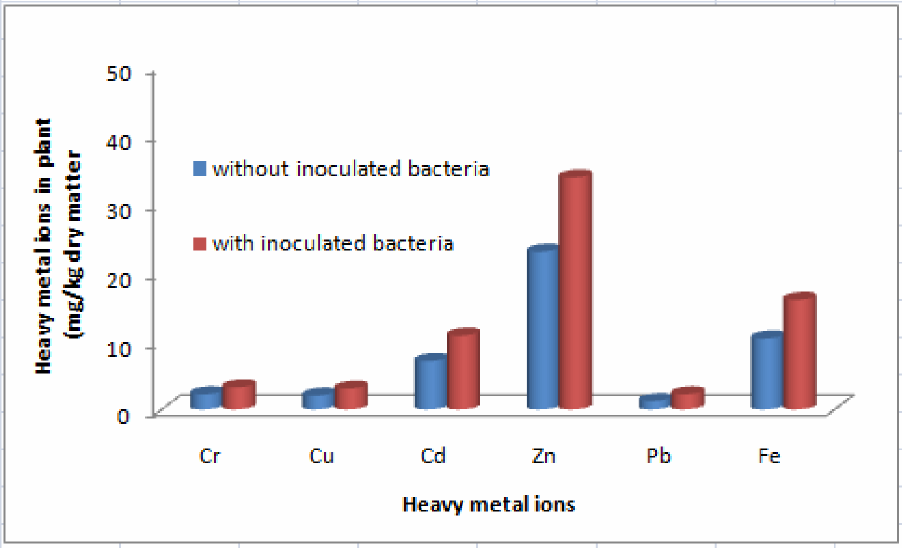

Figure 7. Uptake of heavy metals in *Brassica napus* tissues at 50% leachate concentration.

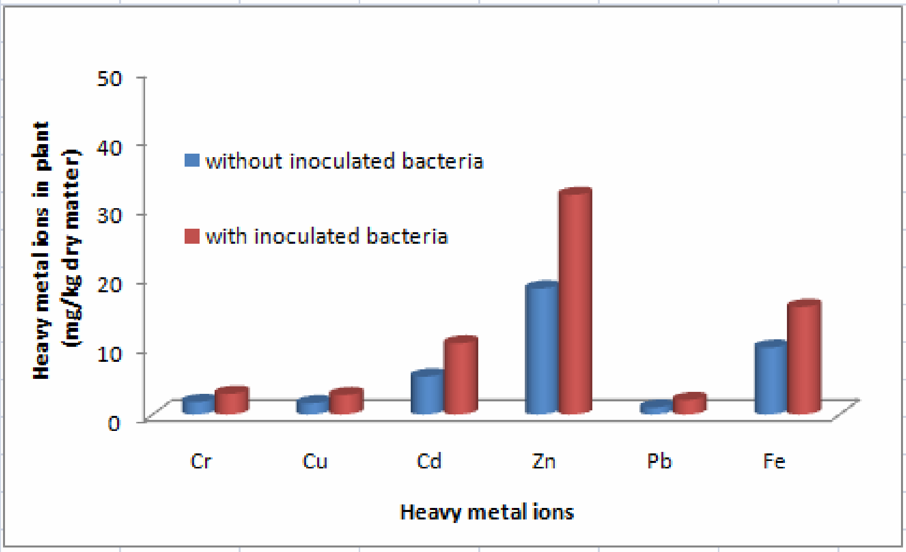

Figure 8. Uptake of heavy metals in *Brassica napus* tissues at 100% leachate concentration.

Normally, without inoculated adapted bacteria from rizosphere, the bioaccumulation process of heavy metal ions, respectively decontamination of soils polluted with leachate, have better yields in the event that leachate which polluted soil has lower concentrations. This is because at lower leachate concentrations in soil, that has a more reduced toxicity, which allows rapa plants to develop optimally and also to accumulate in their own tissues, whole quantity of the metal ions which has been dispersed. If the leachate concentration which has polluted the soil is higher, is observed process stagnation in bioaccumulation and even decrease it.

In pots that were inoculated adapted bacteria, who had the natural habitat soils polluted with leachate, it is observed that at increased leachate concentrations in soil, decontamination efficiency is much higher, because bacteria use an excellent source of carbon and nitrogen from leachate in own metabolism. This reduces the toxicity and creating bioavailability products in soil, which are then easily absorbed by plant radicular system.

Of the heavy metal ions included in the study, zink and iron were the best accumulated by rapa plants, which are commonly found in soil trace elements used in the physiological processes of plants and bacteria. Also among the ions of heavy metals accumulated in significant proportions is included cadmium, despite its high toxicity. Rest of the elements: lead, chromium and copper were accumulated in moderate amounts, lead having the lowest rate of accumulation in plant tissues.

For white mustard plants, in the Figures 9 to 13, were plotted the heavy metal ions quantity, uptake in their tissues according to each concentrations of leachate studied.

In contrast with rapa plants, white mustard heavy metal ions accumulated in proper tissues slightly lower amounts. This was due to vigor index reduced values at high leachate concentrations in soil.

In the absence of inoculation adapted bacteria, the bioaccumulation process of heavy metal ions from leachate contaminated soils is greater at low concentrations of pollutants in the soil, while at high concentrations of the pollutant decreases, due to toxicity that inhibits plant growth and their physiological activities.

Whereas, in the presence of inoculated bacteria, decontamination of soil polluted with leachate is improved substantially. Thus, besides zink and iron, another element, namely chromium is well tolerated and therefore accumulated by white mustard plants, copper instead having the lowest rate of retention in white mustard plants.

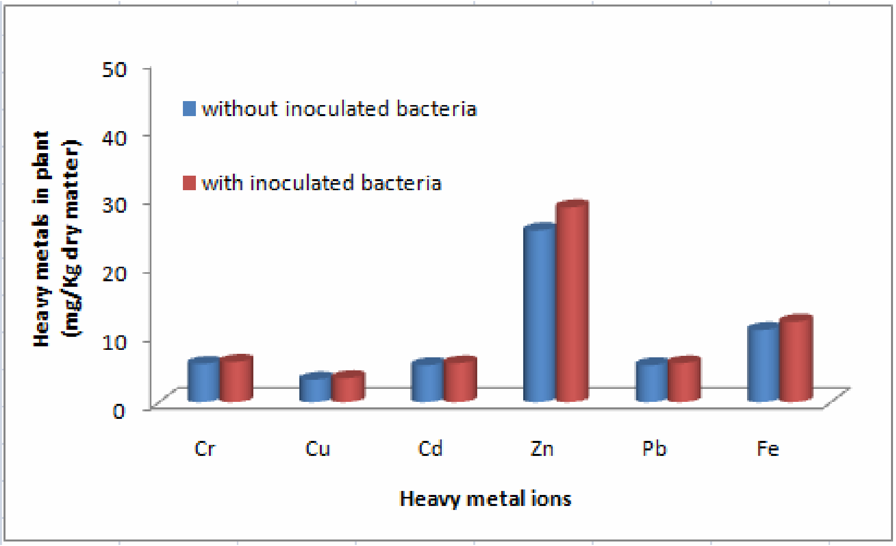

Figure 9. Uptake of heavy metals in *Sinapis alba* tissues at 6.25% leachate concentration.

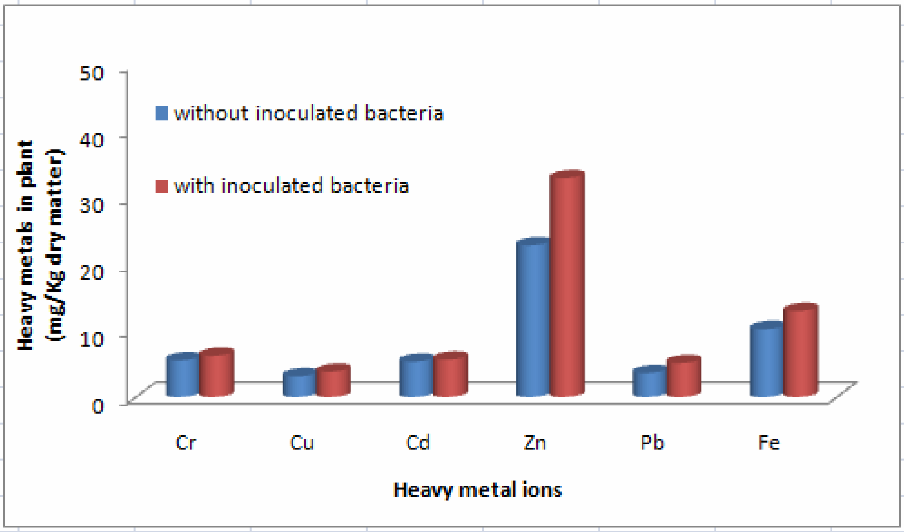

Figure 10. Uptake of heavy metals in *Sinapis alba* tissues at 12.25% leachate concentration.

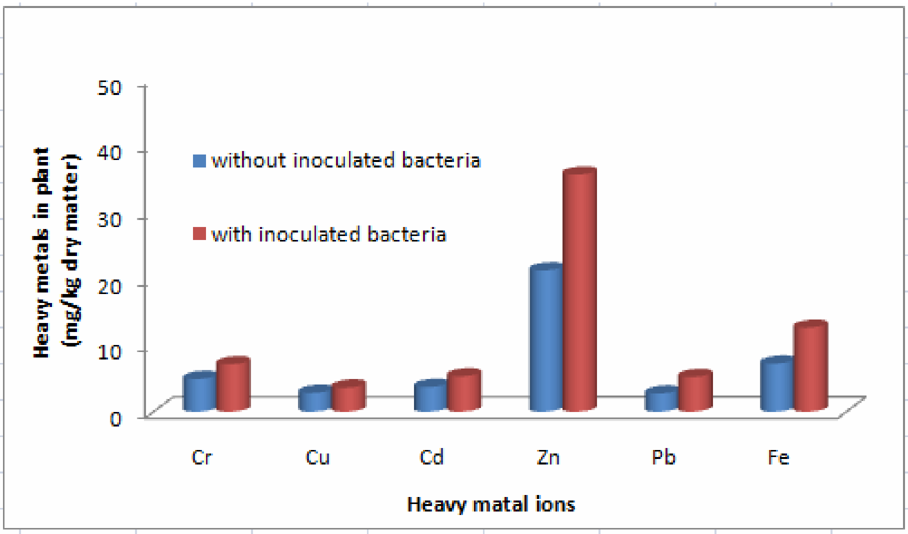

Figure 11. Uptake of heavy metals in *Sinapis alba* tissues at 25% leachate concentration.

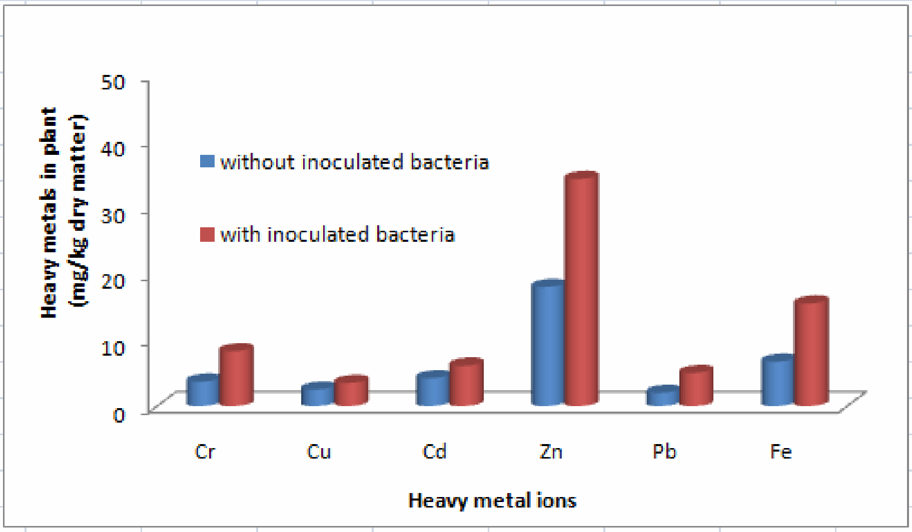

Figure 12. Uptake of heavy metals in *Sinapis alba* tissues at 50% leachate concentration.

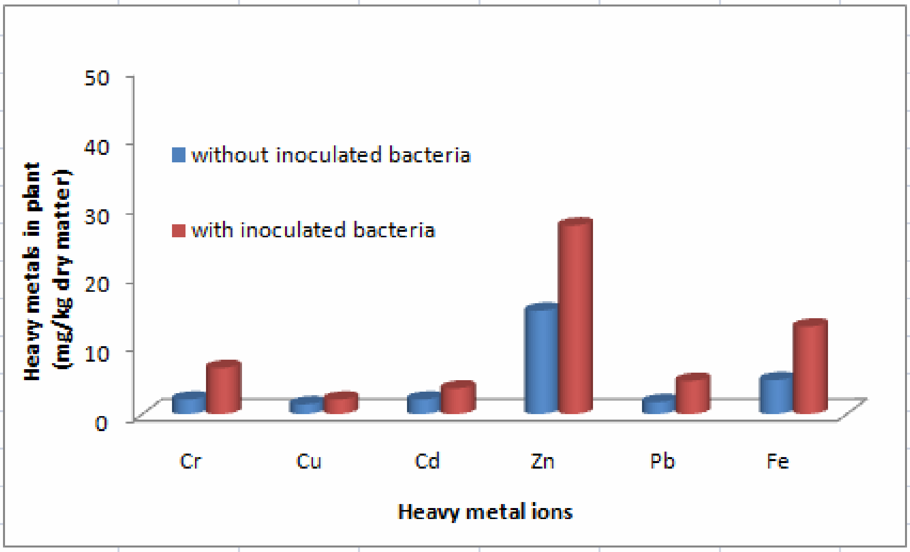

Figure 13. Uptake of heavy metals in *Sinapis alba* tissues at 100% leachate concentration.

Conclusions. Following the experiments was determined the toxicity of leachate sampled from Botoşani old landfill, on the germination capacity of two plant species: rape (*Brassica napus*) and white mustard (*Sinapis alba*). Such was determined, vigor index for each plant species at five different leachate concentrations (6.25%, 12.5%, 25%, 50% and 100%) and could see that with increasing leachate concentration, decreased plant vigor index, more accurate seeds germination capacity. So as to at 100% leachate concentration, seed germination of the two types of plants did not occur.

Then was studied at laboratory scale ability of rape and white mustard in decontamination of soil polluted with leachate, in the presence of bacteria adapted and inoculated from rizosphere and in their absence, especially bioaccumulation the most frequent of heavy metal ions chromium, cooper, cadmium, zink, lead, iron. The best yields were obtained when adapted bacteria were inoculated from rhizosphere, even at high leachate concentrations in soil (50%, 100%). In their absence, the decontamination efficiency decreases at higher leachate concentrations in soil, the process has been active up to 25% leachate concentrations. Thus, in the case of rapa in the presence of rizosphere growth promoting bacteria could be observed increased bioaccumulation capacity, especially for zink, iron and cadmium ions, while for white mustard the bioaccumulation capacity in their tissues is more elevated for zink, iron and chromium ions. The other elements studied were accumulated in moderate amounts.

It can be concluded that the decontamination of soils polluted by landfill leachate is possible using the phytoremediation method, being able to get an improvement of decontamination performance by inoculating some rizosphere bacteria which have been previously adapted, but it is a long time process that is performed by successive crops.

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*** European Directives 2006/12/CE about waste.

*** European Directives 99/31/EC about solid waste landfilling.

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