Lotus corniculatus Crop Growth of in Crude Oil Contaminated Soil. Part 2 Biomass Metals Bioaccumulation

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Abstract
Phytoremediation involves the ability of plants to remove pollutants and is a promise on low costs and efficient processes for cleaning oil polluted soil. Studies for phytoremediation of soils polluted with petroleum products were critical and were based on monitoring strategies implemented efficiency. These strategies are based on the necessity of treating polluted soil and plant cultivation. Treatment was performed with recycled materials, sewage sludge as fertilizer and fly ash as amendment. The studies took on the characteristics of qualitative and quantitative of *Lotus corniculatus* crops, plants tolerant to conditions for phytoremediation strategy implemented on polluted soils by 80.5±3.9 $g \cdot kg^{-1}$ D.M. The use of sewage sludge mixed with fly ash resulted in formation of a layer covering the surface with vegetable grown by 85-94% in July and by 67-83% in August. In *Lotus corniculatus* crops have not been registered bioaccumulation of toxic metals according to legislation from Romania.

Keywords: harvest, *Lotus corniculatus*, metals bioaccumulation, phytoremediation, recycling

1. Introduction

The principles of phytoremediation with plants also apply for land polluted with organic pollutants from category of petroleum products. These petroleum products (name Total Petroleum Hydrocarbons, TPH) have different bio-available degrees of pollutants for environment because before or later come to be metabolized by living organisms in the soil. For the optimization of phytoremediation processes soil fertilization and amendment was required. For example, fly ash amendment determined the modification of electrical conductivity (EC), the degree volatilization of the salts of N and the bioavailability of P from PO_4^{-3} [1, 2]. Furthermore water holding capacity improves; soil texture improves; soil buffering capacity improves, bulk density of soil amend;; increase the soil aeration degree, change degree percolation and water retention degree in the fly ash treated areas. Fly ash amendment determined pH adjusting, crust formation alter; feed the soil with micro-nutrients *i.e*. Fe, Zn, Cu, Mo, B, etc. and macro-nutrients *i.e*. K, P, Ca, etc. Also replaces the soil ameliorants (fertilizers, lime, etc.) and can be used for insecticide purposes. The fly ash amendment decreases the metal mobility and availability in polluted soil [1-4]. The research results reported for the phyto-toxicity have shown that optimal doses of fly ash, 10%-30% on the weight of soil, were most effective for seed germination and for

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optimum development of a crop of plants [5]. The content of heavy metals, including Cd, Pb, Ni, in crops from fly ash treated soils was similar to metal content of harvested crops from untreated fly ash soil. In contrast, tissue concentrations of some trace elements, including, Cu, Cr, Mg, Mn and Zn, decreased in plants grown in some fly ash amended soils [3]. Using the quantities of fly ash of 40, 80 and 120 $t\cdot ha^{-1}$ on a sandy soil and loamy-sandy soil in which increased amount of Si, P and K has not caused an excessive takeover of metals [1, 6, 7]. It is recommended 100 $t\cdot ha^{-1}$ fly ash for normal agricultural land and 200 $t\cdot ha^{-1}$ on problematical land [8]. The content of heavy metals in rice crops from the normal soil does not differentiate compared to crop is on 200 $t\cdot ha^{-1}$ of fly ash treated soil [9]. In general treating soils with fertilizers and fly ash has no effect of bioaccumulation of As, Ba, B, Cd, Co, Cr, Cu, Pb, Hg, Mn, Ni, Ag or Zn in leaf tissues. Thus, fly ash amendment may be a suitable management option for culture on soils, since fly ash improved soil [2, 10-12]. Of how it establishes the optimal harvest time of plant in the first year of vegetation also depends the durability of economic use of perennial legumes. Thereby, in the first year, first harvest will be made at the beginning of appearance of first flower buttons. The next harvests will make every 35-38 days. If all harvesting in the first year of vegetation is realized early, strongly decreases the longevity of legumes, due to decrease of the number of shoots per unit area, the appearance of gaps in culture and increasing the degree of weed. Thereby, in the normal conditions of cultivation, without irrigation, when is realized 2-3 production per year, mowed at the optimum intervals of harvest (every 35-38 days), the longevity of *Lotus corniculatus* culture is 5-7 years [13]. The paper studied the characteristics of biomass from research of phytoremediation of polluted land with petroleum products (TPH), fields treated with sewage sludge and fly ash:

1. The amount harvested from two successive harvests;
2. Biomass quality respectively the amounts of metals bio-accumulate in aerial part harvested by two successive plants harvest.

2. Materials and methods

The experimental variants studied are of TPH soil polluted with 80.5±3.9 $g\cdot kg^{-1}$D.M. untreated or treated with sewage sludge fertilizer in the presence/absence of an amendment on the basis of fly ash from power plant. The fertilized variants were sown with *Lotus corniculatus*. The experimental study block included experimental variants of TPH soil contaminated with an amount of 80.5±3.9 $g\cdot kg^{-1}$ D.M. untreated/treated with sewage sludge in the amount of 250 g per vegetation pot (25 $t\cdot ha^{-1}$) and fly ash different amount (5-50 $t\cdot ha^{-1}$). The experimental variants were: P- non-cultivated polluted soil; P0– cultivated polluted soil fertilized with sewage sludge; P1 cultivated polluted soil, fertilized with sewage sludge in mixture with 500 g fly ash per vegetation pot; P2–cultivated polluted soil, fertilized with sewage sludge in mixture with 250 g fly ash per vegetation pot; and P3-cultivated polluted soil, fertilized with sewage sludge in mixture with 50 g fly ash/vegetation pot. At intervals prescribed by the specific norms of the culture was harvested the green mass. The successive harvesting was performed in the second year of culture, in months of July and August. Metal analysis was done for the aerial parts obtained in the 2nd year of culture. The metal analysis of aerial part of plant was presented by Mâșu (2013) [14]. Plant and soil extracts analysis was realized using an atomic absorption spectrophotometer, Varian Spectra AAS. The detection limit of the device is 0.04 $mg\cdot L^{-1}$.

3. Results and discussion

In table 1 are presented the total amounts of harvested green mass: harvest I and harvest II, the amount of roots harvested in August and the green biomass quantities of aerial part and roots quantities ratio. Were harvested 65-80 g green mass/vegetation pot from experimental variants fertilized with 250 g sewage sludge per vegetation pot and addition of 50-500 g fly ash per vegetation pot. The amount of harvested roots it was between 15.0-29.7 g per vegetation pot. The aerial part and roots ratio was 2.6-4.3 indicating that in polluted soil the development of rhizosphere was major. In Figure 1 is observed that in August is reduced the surface covered with plants, probably due to excessive heat.

Table 1. Total amounts of harvested green mass (harvest I amount and harvest II amount), the amount of roots harvested in August and report of the harvested quantities of aerial part/roots part

No	Parameters	Experimental variants (minimum-maximum)		
		P 1	P 2	P 3
1	Aerial part harvest I amount and harvest II amount (g/vegetation pot)	65-70	70-80	68-77
2	Roots part (g/ vegetation pot)	15.0-20.4	17.3-25.2	17.2-29.7
3	Quantities report of aerial part/roots	3.4-4.3	3.2-4.0	2.6-4.0

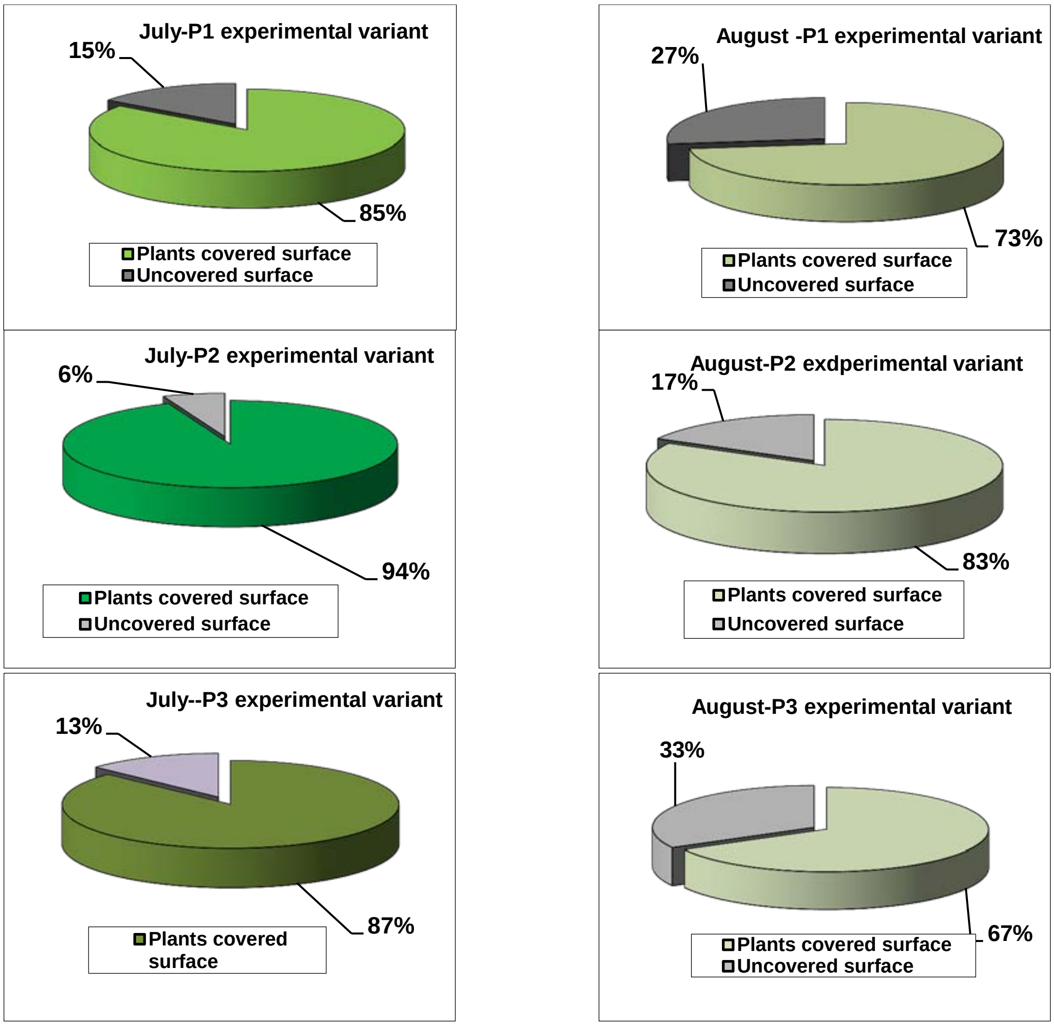

Figure 1. Comparative presentation of the degree of coverage with vegetation on experimental variants cultivated with *Lotus corniculatus* (P1 cultivated polluted soil, fertilized with sewage sludge in mixture with 500g fly ash per vegetation pot; P2–cultivated polluted soil, fertilized with sewage sludge in mixture with 250g fly ash per vegetation pot; P3-cultivated polluted soil, fertilized with sewage sludge in mixture with 50g fly ash/vegetation pot)

From aerial tissue of plants were determined the heavy metals: Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. It is mentioned that in aerial tissue were not detected metals Cd, Cr and Pb. In tables 2 and 3 are presented the amounts of heavy metals bio-accumulated by green mass harvested in July, harvest I and in August harvest II from studied experimental variants. It is observed from Tables 2 and 3 that:

1. In harvests of *Lotus corniculatus* have not been registered bioaccumulation of toxic metals, for which legislation in Romania presents admissible limits *i.e.* Cd, Cr and Pb;
2. In plants tissue from harvest II it was amount bio-accumulate with 72.5% reduced of Fe than in plants tissue aerial part from harvest I;
3. Amounts of Cu, Mn, Ni and Zn bio- accumulate in aerial tissue were not excessive. The amount of Cu from plants was between 5.25-8.57 mg·kg^{-1} D.M., the amount of Mn between 25.29-59.8 mg·kg^{-1} D.M., the amount of Ni between 2.8-6.86 mg·kg^{-1} D.M., and the amount of Zn s between 41.1-75.2 mg·kg^{-1} D.M.

Table 2. The means amounts of heavy metals in plants tissue bio-accumulated by green mass harvested from variants cultivated with *Lotus corniculatus* in July, harvest I

No	Metals	The means amounts of heavy metals in plants tissue bio-accumulated/experimental variants (mg·kg^{-1} D.M.)		
		P 1	P 2	P 3
1	Cu	8.44	8.57	7.21
2	Fe	358.78	257.80	138.70
3	Mn	59.80	58.50	41.25
4	Ni	3.25	3.34	3.36
5	Zn	50.90	65.67	48.90

Table 3. The means amounts of heavy metals in plants tissue bio-accumulate by green mass harvested from variants cultivated with *Lotus corniculatus* in August, harvest II

No	Metals	The means amounts of heavy metals in plants tissue bio-accumulated/experimental variants (mg·kg^{-1} D.M.)		
		P 1	P 2	P 3
1	Cu	5.25	8.37	6.42
2	Fe	111.12	51.44	44.50
3	Mn	31.94	25.29	31.10
4	Ni	2.80	6.86	3.76
5	Zn	41.10	75.20	55.40

In harvests of *Lotus corniculatus* have not been registered bioaccumulation of toxic metals, for which legislation in Romania (Order 74/2011 for the approve the sanitary veterinary norm concerning undesirable substances from products for forages, act of The National Sanitary Veterinary and Food Safety act publish in The Official Journal no. 876 from December 12, 2011), presents admissible limits *i.e.* Cd, Cr and Pb. Maximum Romanian limit for organic matter with 12% humidity is 1 mg·kg^{-1} D.M. for Cd and 100 mg·kg^{-1} D.M. for Pb. The legislation of Romania has no norms for metals such as Fe, Mn, Ni, and Cu [15].

4. Conclusions

The amount of *Lotus corniculatus* harvested in August was lower than the amount harvested in July on similar experimental variations. The addition of fly ash mixed with an agent fertilizer, sewage sludge caused the formation of a healthier vegetation cover of *Lotus corniculatus* culture and with a capacity to covering the cultivated surface by 63-90 % higher in July and 60-77 % in August. In aerial part of plants of *Lotus corniculatus* have not been registered bioaccumulation of toxic metals, for which legislation in Romania (Order 74/2011) presents admissible limits *i.e.* Cd, Cr and Pb. The amounts of metals bio-accumulated in tissues were reduced. The amount of Cu from plants was between 5.25-8.57 mg·kg^{-1} D.M., the amount of Mn between 25.29-59.8 mg·kg^{-1} D.M., the amount of Ni between 2.8-6.86 mg·kg^{-1} D.M., and the amount of Zn s between 41.1-75.2 mg·kg^{-1} D.M. *Lotus corniculatus* can be used to phytoremediation of polluted soils with petroleum products in conditions of fertilization with sewage sludge in mixture with fly ash in optimal amounts. The resulting biomass can be used as cellulosic material addition to different composting biodegradable waste.

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