PHYTOTOXICITY OF INDUSTRIAL CONTAMINATED SOILS

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Abstract: Soil is the principal component of the terestrial ecosystem on which is exercited the most of the industrial pollution. Even if soil have an increased biodegradation potential, the intensive antropogenic activities cause serious imbalances in biodiversity maintaining which ensure this process. Fate and bioavailability of contaminants in soil is often assessed through bioassays, biomonitoring and ecotoxicological testing of soil quality in order to identify negative processes. In this context, the aim of the study was to investigate the phytotoxicity of some soil samples contaminated with heavy metals (mining area Certejul de Sus, Hunedoara county) and with petroleum products (extraction area Poieni - Teleorman and deposit area Constanta). Soil toxicity was assessed using the microbiotest Phytotoxkit with *Sorghum saccharatum, Lepidium sativum* and *Sinapis alba*, where seeds germination and roots growth inhibition were set as endpoints. Soil samples contaminated with heavy metals (As, Cu, Ni, Zn, Co, Cd, Cr) showed inhibition of germination in the range of 0 to 16.66% (insignificant compared to control) and roots growth inhibition in the range of 0.34 to 31.58%, indicating a moderate toxicity. Soil sample contaminated with petroleum products showed a different toxicity due to different sampling sections. The soil sampling from the extraction area highlited a severe phytotoxicity on all plants (inhibition of germination in range of 45.5% - 90% and roots grouth inhibition in 49.63% - 92.62%), toxicity was caused by significant loading of petroleum products (> 2500 mg / kg d.m). The soil sample from Constanta deposit showed no phytotoxicity on mustard and cress plants, but a low effect was observed on roots growth in case of sorghum plants (9.27%). Our experiments emphased the utility of the use of phytotoxicity microbiotest which can provide relevant informations about soil toxicity necessary in environmental risk assessment. The method protocol is easily to understand and apply, economical regarding the space use, maintenance and obtaining relevant results in a short time and easier to interpret.

Key words: soil, phytotoxicity, heavy metals, petroleum products

INTRODUCTION

Soil is a non-renewable resource with many essential functions: food, storage, filtration and biotransformation of many substances including water, carbon and nitrogen; because represents the principal support of human activities their quality is permanently affected.

Nowdays, only nine European Members States have specific legislation for soil protection. Generally, soil protection is based on chemicals limiting in these compartments or in the materials applied on them. Physico-chemical monitoring is not enough because not all risks are assessed. The presence of unknown chemicals which not allow the analytically detection lead to gaps in risks estimation. Given these, European Commision have decided to extend existing legislative plan to ecotoxicological monitoring using soil toxicity bioassays. A new soil protection policy "Soil Thematic Strategy" (COM2006/231) and a new Directive on soil (COM 2006/232) are initiated.

International trend is the development of routinely bioassays applied in complex researches of the soil pollutants effects and their bioavailability, mixtures toxicity, species sensitivity and biodiversity, factors which influencing the ecotoxicity and temporal aspects of soil contamination. In Czech Republic,

in order to protect the soil, exist a national procedure which recommend the use of tests battery to evaluate the waste toxicity, involving invertebrate and plants. This country is planning a new Directive on the sediment toxicity assessment to be used in agriculture, which involves using of some tests battery with enchytraide, springtails, nitrifying bacteria and higher plants (van Gestel, 2012; Hofman et al., 2012).

Plants represents the primary producers and the main source of food for animals and humans. Contaminants from water, soil or air reach in plants organs by internal absorption (through membrane cell) but also by external absorption (eg. deposits on leaves and stems) (Frederic, 1978).. Is note that the level of pollutant toxicity in plants depend of biotic and abiotic factors such as: species, plant organs, soil structure, presence of other compounds which can modified the bioavailability, test conditions and exposure time.

Phytotoxicity involves the total toxicity assessment of contaminated soil / water samples on terrestrial or aquatic plants. The existing methods differ through: plant species, test sample, test purposes, beneficiary and laboratory availability. The parameters which can be monitored during the exposure periods of toxic samples on plants are: plants multiplication, mortality, size changes, seed germination, fresh weight, dry weight, deformation of fiziological structures, respiration, chlorophyll content, leaves removing and harvest radament. Generally the phytotoxicity tests are used by herbicides producers (in order to ensure plants safety which are not the herbicide target), plant protection organizations (for alleopathy studies), international associations (U.S. EPA, OECD, responsible for chemicals release on market) and environmental remediation companies (in phytoremediation studies) (Fochtman, 2000).

Terrestrial toxicity tests leaded on higher plants, according to EPA, OECD, FDA or ISO methodology, involve acute an chronic effects monitoring using the following species: dicotyledons, *Sinapis alba, Brassica alba* (mustard), *Lactuca sativa* (lettuce), *Phaseolus aureus* (beans), *Lycopersicon esculentum* (tomato*), Cucumis sativum* (cucumber), *Daucus carota* (carrot); and monocotyledons, *Zea mays* (peas*), Oryza staiva* (rice), *Hordeum vulgare* (barley), *Allium cepa* (onion), other cereal species (Poaceae) (Persoone, 2007).

In order to reduce the testing efforts of phytotoxicity some microbioteste (Phytotoxkit) were developed for rapid elongation of roots, which allow the comparative toxicity assessement in only 3 days of exposure. Usually the test plants (such as *Sorghum saccharatum, Lepidium sativum, Sinapis alba*) have a rapid seed germination and a early growth.

The main occurred soil contaminants are heavy metals and petroleum products resulted after industrial activities (mining and petroleum industry).

Heavy metals are significantly and frequently occured in all environmental compartments due to their persistence. Once reached in the system, they have a stable behavior and remain for long periods or they can be mobilized under the influence of weather conditions, presence of other chemical compounds or absorpted by plants. Although the metals are essentials for physiological processes, when they exceed admisible limits can cause adverse acute and chronic effects. Metals removed in natural environment, have a high potential for bioaccumulation in aquatic or terrestrial organisms (plants and animals) which generally affecting the early stages of development. Metals concentrations migrate from soil / water / sediment from a food chain to another, from plants to animals and finally in humans, causing chronic accumulation and severe diseases especially for childrens. Considering Dudova (2004) and Frederic (1978), all heavy metals showed severe and moderate effects on seeds germination, plant growth and development and a huge bioacummulation potential.

Contaminated soils with petroleum products can be remediated using plants which can degrade them through root exudates capable of carbon and energy replacement, facilitating the bioacumulation of metals in roots and stems (Eve Riser-Roberts, 1998). A recent study showed that the first significant effects on plants are observed at 10 mg / kg d.m. petroleum products. Plant tolerance is higher in soils with low acidity and high organic matter concentration. This aspect confirm that the PAHs absorption is influenced by organic and inorganic fractions of soils which reduce bioavailability and finally, the

toxicity. A prolonged exposure time (> 15 days) cause a lower toxicity due to a decrease bioavailability (caused by evaporation or degradation and soil microbial tolerance to persistent pollutants) (Klimkowicz et al., 2012; Marcin, 2012).

The goal of this paper was to investigate the toxicity of some soil samples contaminated with heavy metals and oil products on higher plants (monocotyledonous and dicotyledonous) in order to identify the impact on plants growth and development**,** using Phytotoxkit microbiotest.

MATERIAL AND METHOD

Phytoxicity test

The Phytotoxkit microbiotest (MicroBioTest Inc. Belgium) follows the ISO11269-1 methodology. The end points of test are based on seeds germination and the roots growth inhibition after 3 days of plants seeds exposure to contaminant soils in comparison with a reference soil.

Toxicity test use flat and shallow transparent test plates composed of two compartiments, the lower one contains soil saturated till the water holding capacity. Seeds of the selected tests plants are positioned at equal distance near the middle ridge of the test plate on a black filter paper placed on top of the hydrated soil. The test plates was placed vertically in a holder and incubated at 25^{o}C. The incubation period depends on the time of seeds germination and the roots growth speed, which are species - dependent. A the end of the incubation period a digital picture of plates is taken. The analyses and the length mesurements was perform using ImageTool Programme. The assays was carried out in 3 replicates for each of the 3 test plants.

The selected plants were *Sorghum saccharatum* (SOS 140611), *Lepidium sativum* (LES 290310) and *Sinapis alba* (SIA 051011). Control tests were conducted using reference OECD soil (85% sand, 10% kaoline and 5% peat) and boric acid (250 mg/kg control soil) (Phytotoxkit Standard Operational Procedure, 2012).

Samples

The phytotoxicity tests were performed for heavy metals and petroleum products contaminated soils.

Heavy metals contaminated soil samples were collected from an old gold extraction area - Certejul de Sus (Macris Valley) at 18 km from Deva, Hunedoara district, Romania. Four soil samples were collected from 0-10 cm (S1/1 and S2/1) and 30-40 cm depth (S1/2 and S2/2). In table no. 1 are sumarised the metals concentration (EN ISO 11969 and ISO 11047) and literature data concerning the metal bioaccumulation in plants.

Table 1

Metal concentrations Certejul de Sus

Metals	Heavy metals concentrations [mg/kg d.m.]				Metal accumulation in plants (ppm) *
	S1/1 (0-10cm)	S1/2 (30-40cm)	S2/1 (0-10cm)	S2/2 (30-40cm)	
Arsenic	454	345	1102	1810	<1-50
Cadmium	13.1	9.9	30.5	51	0.1-50
Cooper	24.9	24.5	19.8	24.1	5-1500
Cromium	4.67	5.63	1.26	2	<1-700
Nickel	4.86	12.6	2.69	2.25	<5-500
Lead	117	72.8	66.5	137	<7-7000
Zinc	239	186	231	144	<25-5800
Cobalt	8.77	6.25	2.42	1.63	<5-300

*(Frederick W., 1978); d.m - dry matter.

The samples showed significant concentrations of heavy metals, the highest values were detected for arsenic, lead and zinc. The most contaminated sample was S2, where was found significant arsenic, cadmium, copper, chromium and lead concentrations at 30-40 cm depth. The S1 sample showed no significant changes in metals concentration.

The soil samples contaminated with petroleum products were collected from an extraction area (PP Poieni – Teleorman) and petroleum products deposit (PP Constanta) at 10 cm depth. The petroleum products were detected using SR 7877/2 method. The analytical analyses showed a great pollution in Poieni (2505.5 mg/kg d.m) and 181 mg/kg d.m in Constanta.

Contaminated soil samples were analyzed in terms of microbiological load, in order to estimate the biodegradation soil potential (table no. 2). The soil presented a significant microbial load (10^4-10^7 TNG/g d.m. mezophifilic bacteria at 22^{o}C), which may indicate a great potential for organic matter biodegradation.

Table 2

Microbial load of soil samples

Mezophilic bacteria at 22^{o}C [UFC / g d.m)	
S1/1 (0-10cm)	$19x10^6$
S1/2 (30-40cm)	$17x10^5$
S2/1 (0-10cm)	$23x10^4$
S2/2 (30-40cm)	$21x10^4$
PP Constanta	$2x10^7$
PP Poieni	$13x10^5$

Soil sample treatment

Each 1 kg soil sample was air-dryed and sieved and the water holding capacity (WHC) were calculated. In picture no. 1 and 2 the soil sample preparation stages before test are presented.

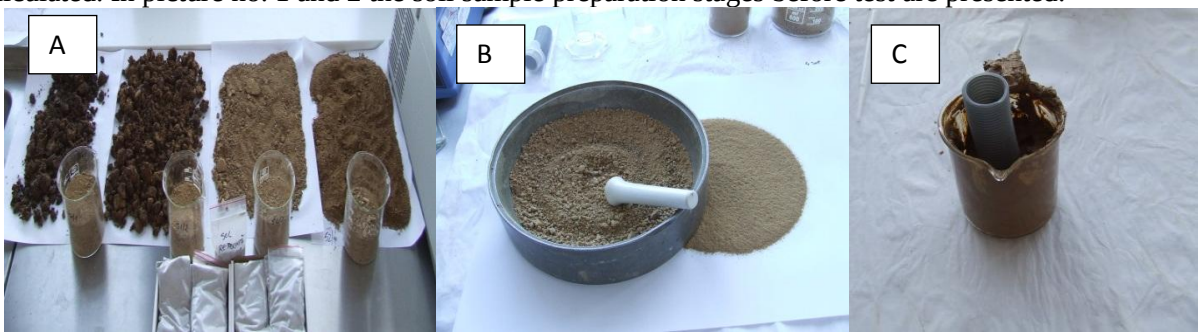

Fig. 1 Soil treatment (A - drying, B - sieving, C – water saturation)

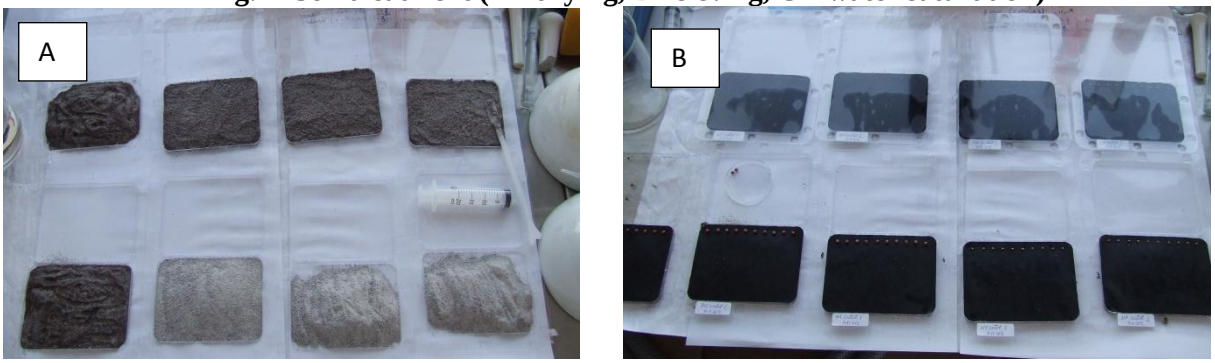

Fig. 2 Soil flattening (A) and seeds placing (B) in test plates

RESULTS AND DISCUSSIONS

Table 3 and 4 present the seeds germination and roots growth effects of contaminated soils on each selected plant.

Table 3

Seeds germination

Samples code	Plant species / Seeds germination inhibition (%)		
	Sinapis alba	***Lepidium sativum***	***Soghum saccharatum***
S1/1 (0-10cm)	16.66	13.33	0
S1/2 (30-40cm)	0	0	0
S2/1 (0-10cm)	0	0	0
S2/2 (30-40cm)	0	0	0
PP Constanta	0	3.33	0
PP Poieni	50	90	45.5
Reference (Boric acid)	0	0	0

Table 4

Roots growth inhibition

Samples code	Plant species / Roots growth inhibition (%)		
	Sinapis alba	***Lepidium sativum***	***Soghum saccharatum***
S1/1 (0-10cm)	12.33	30.38	0.34
S1/2 (30-40cm)	1.13	10.19	1.9
S2/1 (0-10cm)	12.73	22.84	23.39
S2/2 (30-40cm)	9.91	31.58	25.86
PP Constanta	-26.85	-4.69	9.27
PP Poieni	56.21	92.62	49.63
Reference (Boric acid)	29.41	45.63	29.44

The figures 3 ÷ 7 presents the experimental results concerning the tested soil samples phtotoxicity. Soil samples contaminated with heavy metals showed seeds germination inhibition ranging from 0 to 16.66% and roots growth inhibition in range of 0.34 to 31.58%,that indicate moderate toxicity. The most toxic sample was S2/2 (30-40 cm). The *Sinapis alba* had the lowest degree of roots growth (9.91%) inhibition and *Lepidium sativum* was the most affected (31.58%). No inhibition of seeds germination was observed. Also, in case of S1/1 and S2/1 samples (0-10 cm) was observed a roots growth inhibition by 30% (for sample S1/1 - *Lepidium sativum*) and in terms of germination, there was a inhibition of < 20%, not significant compared to control test. Plants sensitivity in heavy metal intoxication was: *Lepidium sativum*> *Sorghum saccharatum* > *Sinapis alba*.

Soil samples contaminated with petroleum products showed a different toxicity due to different sampling sections. Sample taken from petrolum extraction area had the most severe phytotoxicity (inhibition of germination / roots growth were 45.5% / 49.63% *Sorghum saccharatum*, 50% / 56.21% *Sinapis alba* and 90 % / 92.62% *Lepidium sativum*), the toxicity being caused by significant load of petroleum products (> 2500 mg / kg d.m).

Soil samples taken from petroleum storage area had no phytotoxicity for *Sinapis alba* and *Lepidium sativum* plants, but had a lowest inhibition of roots growth for *Sorghum saccharatum* plants (9.27%). Preliminary chemical analysis of petroleum products performed after three days of testing emphased a concentration decrease from 181 mg / kg d.m to 123 mg / kg d.m. These suggests that the

pollutant biodegradation of soil under the microbial load (mesophilic bacteria at 22°C, $2x10^7$ TNG/g d.m) are stimulated by incubation temperature and absorption processes in plant roots.

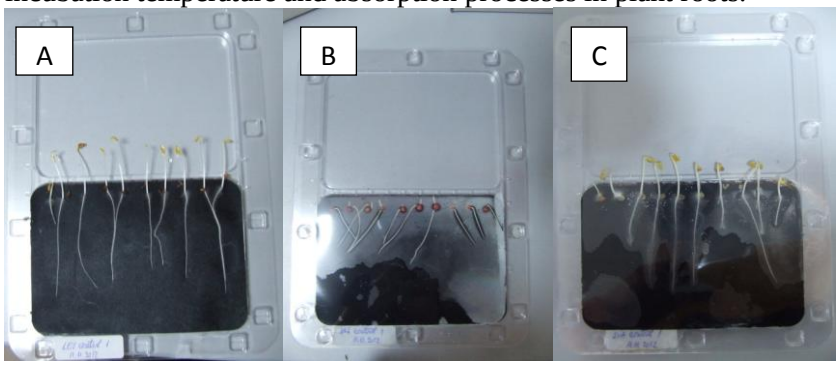

Fig. 3 Control plates after 3 days of incubation (A - *Lepidium sativum*, B – *Sorghum saccharatum*, C – *Sinapis alba*)

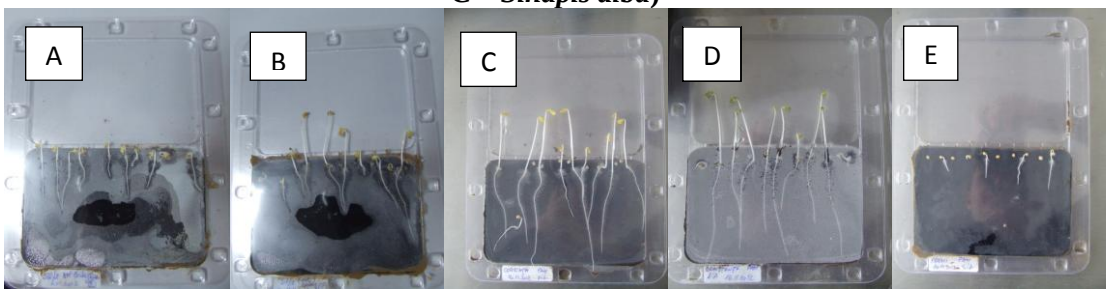

Fig. 4 Test plates after 3 days of incubation for *Sinapis alba* (A – S2/2; B - S1/1; C, D - Constanta, E - Poieni)

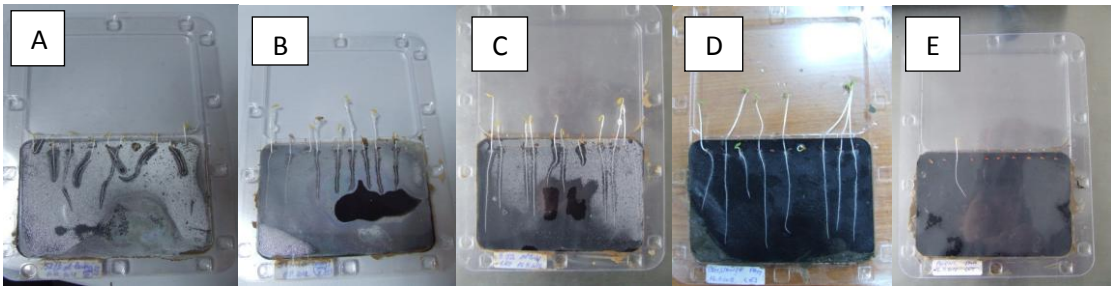

Fig. 5 Test plates after 3 days of incubation for *Lepidium sativum* (A – S2/2, B - S1/1, C- S1/2, D-Constanta, E-Poieni)

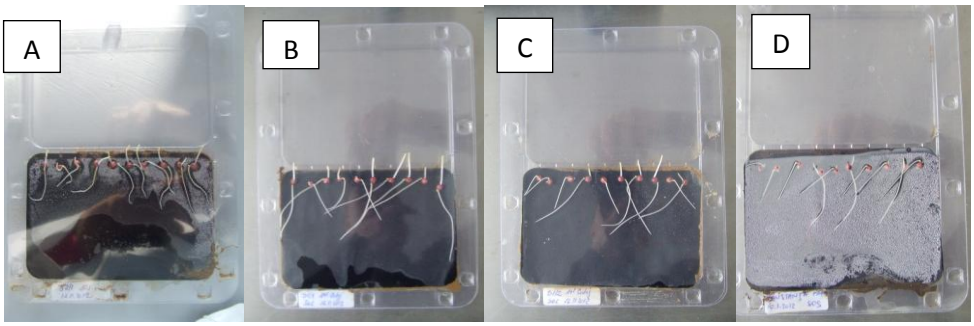

Fig. 6 Test plates after 3 days of incubation for *Sorghum saccharatum* (A – S2/1, B - S1/1, C- S1/2, D - Constanta)

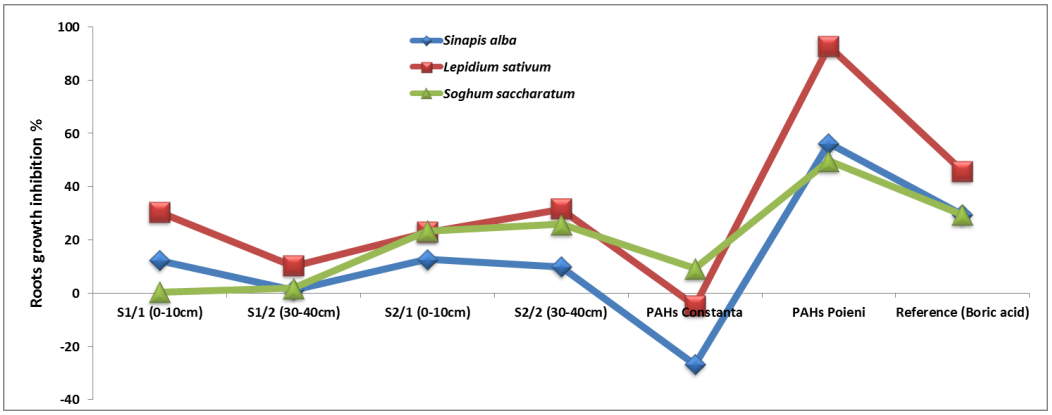

Fig. 7 Roots growth inhibition – heavy metlas soil samples (S1/1, S1/2, S2/1, S2/2) and petroleum product soil samples (Constanta and Poieni)

In control test the germination seeds was 100% for all selected plant species. Length average of plant roots in control test was 33.74 mm for *Sinapis alba*, 49.09 mm for *Lepidium sativum* and 35.02 mm for *Sorghum saccharatum*.

In the reference test with boric acid was obtained the following values for inhibition of roots growth: 29.49% for *Sinapis alba*, 45.63% for *Lepidium sativum* and 29.44% for *Sorghum saccharatum*. The control data values range in range of certificated of the phytotoxicity microbiotest. No inhibition of seeds germination was obtained.

CONCLUSIONS

The present paper presented the phytotoxicity of some soil samples contaminated with heavy metals and petroleum products. Soil toxicity was assessed using the microbiotest Phytotoxkit with *Sorghum saccharatum, Lepidium sativum* and *Sinapis alba*, where seeds germination and roots growth inhibition were set as endpoints.

Contaminated soil samples showed different germination inhibitions in range of 0 to 90% and roots growth inhibitions in range of 0.34 to 92.62%, indicating a moderate toxicity (in case of heavy metal contamination) and severe toxicity (in case of sample with > 2500 mg petrolem products / kg d.m).

Phytotoxicity experiments highlighted the utility of the microbiotests which can provide relevant rapid informations about soil toxicity useful in environmental risk assessment. The method protocol is easily to understand and apply, economical regarding the space use, maintenance and obtaining relevant results in a short time and easier to interpret.

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