Predetermination of Amendments for the Enhanced Phytoremediation of Fly Ash Dumps

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The phytostabilization of the slag and fly ash dumps was studied in an experimental block consisting of variants fertilized with different organic fertilizing agents, sewage sludge or biological sludge resulting from a slaughter house in the absence/presence of a bacterial organic stimulus. The bacterial organic stimulus was used during the sowing stage and subsequently through regular foliar applications on the grown crop. The used bacterial stimulus Biocomplex 900 is based on a marine brown algae extract provided by SC EKO GEA EAST SRL, Romania. In this paper, the germination degree of Lolium perenne seeds was studied at the same time with the coverage degree of sown areas. The plant development and the amount of biomass harvested were monitored. The slaughter house sludge used (0.5kg·m^{-2}) with the addition of Biocomplex 900 stimulus determined a rapid vegetation in the fertilized variant along with biomass harvests which was 2-3 times higher than in other variants.

Keywords: slag and fly ash dumps, organic fertilizers, algae extract, Lolium perenne

In general, the plants that grow in poor soils often develop interdependence with bacterial soil communities which are influenced by soil type, plant species and the applied agricultural technology. Changes in soil chemistry due to the treatment with fertilizing agents and/or amendment agents and changes in the plant chemistry/biochemistry can alter the crops [1]. Even if it is difficult to compare the results reported in the literature on this area and more difficult to conclude based on these results, the soil type clearly influences the amount of plants tissue: the roots or the aerial parts [2-4]. It has been reported that the changes that may occur in the rhizosphere. It is known that the presence of toxic metals can exhibit a long-term effect on ecosystems and a negative influence over phenomena associated with the soil biology [5-6]. Also, it is known that the presence of metals in soil reduces in situ the microbial biomass and shows a negative influence on the functionality of various ecosystem sections. In time, it can be observed the tolerance phenomena in soil bacteria [7-8]. The addition of organic fertilizer can increase the proliferation of microorganisms and nutrients uptake. The organic fertilizers added to a deposit of slag and fly ash caused a decrease in density of all old deposits. In the same time, the porosity and the water availability increased by the water retention capacity. The content of soluble carbon compounds has increased because of humic matter breaking phenomenon and also, the activity of some enzymes in the constitution of microbial consortium increased. The role of the organic matter is beneficial because it determines the positive change of soil physical properties, *e.g.*: the soil aggregate stability and the microbial activity attached to it [8-9]. Brown algae extracts used as stimulating agents for the microbial activity have increased the speed of the organic compounds oxidation in the soil and the formation of bioavailability components. Brown algae are known for their contribution to the development of soil reactions involving components of fertilizers compounds found in organic components of soil matrix [8].

The aim of this paper is to study the influence of a bacterial inoculums based on brown seaweed and the process of grassing with the *Lolium perenne* species, of slag and fly ash dumps, taking into consideration the following parameters: germination degree, coverage degree of the cultivated areas and the amount of biomass produced.

Experimental part

The study was conducted on variants of slag and fly ash topsoil, as fertilized variants with two types of sludge: sewage sludge and slaughterhouse sludge quantities of 0.5-2.5 kg·m^{-2} in the absence/presence of a bacterial seaweed stimulus provided by SC EKO GEA EAST SRL, Romania. These stimuli were added to ensure the bacterial needs for the transformation of unassimilated compounds into bioavailability compounds by plants. The experimental study was performed in four experimental blocks in pots with a useful capacity of 5.5 kg/pot. Each experimental block includes three variants. Each variant was studied in three replicates.

The Biocomplex 900 brown seaweed extract was used in this study as bacterial development stimulus, (table 1). The variants are shown in table 1.

The product in the form of brown liquid with a fish smell is not toxic to plants, animals and people. *Lolium perenne* species of plants was used for vegetation. The seeded quantity was 7.0 g/pot. During sowing with this plant the topsoil was wetted with a mixture of brown algae extract and water (ratio 1:50). Periodically, crops were foliar wetted with a wet mix based on seaweed extract. The crops are periodically controlled based on: a) characteristics of plants through: germination degree; surface coverage after seven weeks; and on the amount of resulted biomass; b) metals content of biomass.

Experimental soils variant analysis was done to determine the metal concentrations according to the Romanian standardized methodology, 5g soil samples analysis (in triplicate) was done to determine the metals concentrations. Metals were extracted from the soil

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No	Experimental variants/Slag and fly ash treatment											
	Sewage sludge fertilizer agent [kg·m^{-2}]						Slaughter house sludge fertilizer agent [kg·m^{-2}]					
1	Block 1 without seaweed extract			Block 2 with seaweed extract			Block 3 without seaweed extract			Block 4 with seaweed extract		
2	0.5	1.0	2.5	0.5	1.0	2.5	0.5	1.0	2.5	0.5	1.0	2.5
*3	*vn1*	*vn2*	*vn3*	*vns1*	*vns2*	*vns3*	*va1*	*va2*	*va3*	*vas1*	*vas2*	*vas3*

*Line 3 variants name; description: *v*=experimental variant; *n*=sewage sludge; *s*=seaweed extract; *a* = slaughter house sludge;

Table 1
EXPERIMENTAL VARIANTS MADE FOR SLAG AND FLY ASH CULTURE

Variants	*Heavy metals content in topsoil of experimental variants [mg·kg^{-1} D.M.]							
	Fe	Mn	Cd	Cr	Cu	Ni	Pb	Zn
Treated topsoil variants	2521.6 ± 32.2	155.0 ± 15.2	1.8 ±0.5	80.1 ±9.3	27.1 ±3.3	25.2 ±3.1	10.2 ±1.8	49.8 ±4.7
Reference values for metal content in soil under Ordin 766/1977	-	900	1	20	20	20	20	100

*Value are mean and standard deviation, n= 3 samples

Table 2
METAL CONCENTRATIONS IN POTS TOPSOIL OF EXPERIMENTAL VARIANTS

samples by heating with Aqua Regia for 2hrs, at reflux. After interrupting the heat, the system was left in stand-by for 16 h. Then the samples were diluted in a calibrated flask with deionized water to 50 mL. Plant sampling was done in agreement with the Romanian standardized methodology. Plant tissues were thoroughly washed with de ionized water to remove any soil particles attached to plant surfaces, 5g plant tissues were dried (105°C) to constant weight. Plant samples with precise weight are then brought to 550°C; to the residual materials 5mL of concentrated hydrochloric acid are added, samples are maintained 30 min on the dry sand bath. After filtering those in a paper filter with small porosity (type 640 de Mackerel-Nagel Germany), were taken to a calibrated flask (25 mL) with hydrochloric acid 1:1 solution. Plant and soil extracts analysis was done using Avanta GBC Atomic Absorption Spectrophotometer. The detection limit of the device for Cd, Cr, Cu, Fe, Mn, and Zn is of 0.05 mg/L, for Ni is of 0.10 mg/L, for Pb is of 0.20 mg/L.

Statistical data on changes in heavy metal plant bioaccumulation were analyzed. The linear correlation r of the data was performed using PAST software, which runs on standard Windows computers and is available free online [10-11].

Results and discussions

The metal content of soil experimental from the plots are presented in table 2.

The topsoil of experimental variants contains large quantities more than those imposed by Romanian law Ordin 766/1977

Table 3 shows the germination degree the cultivated area, coverage degree and the amount of green biomass for the variants fertilized with sewage sludge in the absence/presence of seaweed extract. The use of a small amount of sewage sludge (0.5 kg·m^{-2}) resulted in the *Lolium perenne* seeds germination in an area with 30% total sowed material. The resulted plants suffered morph-physiological changes (they became yellow and dry). Subsequently, the plants continued to dry gradually until the culture completely died. Fertilizer amount increasing by the sewage sludge added to the slag and fly ash (1.0-2.5kg·m^{-2}) determined a higher germination rate. The seeds sprout gradually reaching a germination degree of 40-50% in the third week from sowing. The surface coverage over a period of seven weeks after sowing was 80-90% for the *vn3* variant.

Crops seem to be healthy with a bright green color. Thus, sewage sludge amount increasing by 2.5 times produced the biomass increases by 1.5 times. The maximum amount of green biomass produced per fertilized experimental variant was 2.5 kg·m^{-2} and in the absence of the seaweed extract was 13.1g green biomass/pot. The seaweed organic stimulus added in the experimental variant fertilized with 0.5 kg·m^{-2} sewage sludge determined the

Parameters	Experimental variants/Slag and fly ash treatment					
	Sewage sludge without seaweed extract			Sewage sludge with seaweed extract		
Experimental variants	*vn1*	*vn2*	*vn3*	*vns1*	*vns2*	*vns3*
Germination degree [%]	30	40	50	15	60	70
Surface coverage [%]	Dry plants	90	80	60	90	90
*Biomass quantities [g/pot]	Dry plants	8.6±1.3	13.1±1.4	3.4±0,5	10.5±1.2	8.1±1.4

*Value are mean and standard deviation, n= 4 samples

Table 3
GERMINATION DEGREE, THE COVERAGE OF THE CULTIVATED AREA AND THE AMOUNT OF GREEN BIOMASS FOR SLAG AND FLY ASH EXPERIMENTAL VARIANTS FERTILIZED WITH SEWAGE SLUDGE

Parameters	Experimental variants/ Slag and fly ash treatment					
	Slaughter house sludge without seaweed extract			Slaughter house sludge with seaweed extract		
Experimental variants	*va1*	*va2*	*va3*	*vas1*	*vas2*	*vas3*
Germination degree [%]	10	30	5	80	40	10
Surface coverage [%]	60	50	60	90	70	35
*Harvest quantities [g/pot]	3.5±0.5	3.9±0.5	6.9±1.3	14.8±2.3	4.4±0.6	1.6±0.3

*Value are mean and standard deviation, n= 4 samples

Table 4
THE GERMINATION DEGREE, THE COVERAGE OF THE CULTIVATED AREA AND THE AMOUNT OF GREEN BIOMASS FROM SLAG AND FLY ASH VARIANTS FERTILIZED WITH SLAUGHTER HOUSE SLUDGE

formation of a vegetation layer during a period of seven weeks characterized by 60% coverage of sown area. Increasing the quantity of fertilizing agent with 1.0-2.5 kg·m^{-2} led to a boost of 90% to the covered area.

Crops seem to be healthy with a bright green color. The amount of harvested biomass in the fertilized variant with 0.5 kg·m^{-2} sewage sludge and seaweed extract was 3.4 g green biomass/pot. Duplication the amount of the fertilizing agent to 1kg·m^{-2} led to an increase up to 3 times of green biomass versus to the fertilized variant with sewage sludge of 0.5 kg·m^{-2}. This amount is increased with 20% versus to the amount obtained in a similar variant fertilized with 1.0 kg·m^{-2} of sewage sludge in the absence of a stimulus. Increasing the amount of sewage sludge to 2.5 kg·m^{-2} did not cause a similar increase of biomass harvested from this variant. The amount of biomass was similar to that harvested from the variant fertilized with 1kg·m^{-2} sewage sludge in the presence of a brown seaweed extract stimulus. The amount of green biomass resulted from the experimental variant treated with 2.5kg·m^{-2} sewage sludge in the presence of a brown seaweed extract was lower than the amount of biomass harvested from the one fertilized with sewage sludge and in the absence of seaweed extract. Table 4 shows the germination degree, the coverage in the cultivated area and the amount of biomass for crops fertilized with slaughter house sludge in the absence/presence of seaweed extract. The addition of slaughter house sludge produced the germination of *Lolium perenne* with 5-20% in the first phenophase. The plants have a yellow colour. The crop is presented as scattered cluster of plants. The plant germination from the slaughter house sludge without seaweed extract fertilized variant (table 4) occurred also in the following weeks.

Thus, at the end of the observation period (seven weeks of growth) the occupied area is 50–60%. The area occupied by the plant is about 20-30% lower than in similar cases of the experimental block treated with sewage sludge. The amount of harvested plants is 3.50-3.85g biomass/pot when using a quantity of slaughter house sludge of 0.5-1.0 kg·m^{-2}. The amount of biomass harvested from these options is 50% and 70%, which is lower than in the variants treated with sewage sludge. The maximum amount of biomass resulted was 6.9 g mass/pot using 2.5kg·m^{-2} of slaughter house sludge without seaweed extract. Adding the brown seaweed stimulus in a small amount (0.5kg·m^{-2}) of slaughter house sludge caused a very good and fast germination degree. Two weeks after seeding, 80% of the grains have emerged. The crop was healthy, green and the dense clusters of plants were formed. After seven weeks of growing, the plants have reached to cover 90% of sown area. In the studied variants, the amount of harvested biomass was 10% higher than in the variant treated with 2.5 kg·m^{-2} sewage sludge in the absence of an seaweed stimulus (the best option when treating ash with sewage sludge).

The germination degree after two weeks was lower in two variants: the one treated with slaughter house sludge of 1.0 kg·m^{-2} and respective, with 2.5 kg·m^{-2}. The increased slaughter house sludge quantity reduced dramatically the coverage degree. In the experimental variant fertilized with 1.0 kg·m^{-2} and treated with brown seaweed extract the area coverage reached 70% after seven weeks. The increased addition of slaughter house sludge to 2.5 kg·m^{-2} determined a 35% coverage of the cultivated area after seven weeks (i.e. half of the coverage being resulted in the experimental variant treated with 1.0 kg·m^{-2} slaughter house sludge and brown seaweed extract). The amount of green biomass obtained from the experimental variants of this device is reduced with the increase of fertilizing agent with the brown seaweed extract.

Table 5 and 6 shows metals content of plants tissues harvested from variants fertilized with slaughter house sludge/ sewage sludge in the absence/presence of seaweed extract. Spatial variability across the aerial part of plants is due to the different treatment of polluted soil and specifically plant metabolism.

Based on the data presented in table 5 resulted: 1) addition of algae extract caused an increase in the amount of metal vs. similar variant untreated with stimulus; in aerial tissue have not accumulated toxic metals Cr and Pb;

Metal	Experimental variants/ Slag and fly ash treatment. *Metals content of plants aerial tissue [mg·kg^{-1} D.M.]					
	Sewage sludge without seaweed extract			Sewage sludge with seaweed extract		
	vn1	*vn2*	*vn3*	*vns1*	*vns2*	*vns3*
Fe	*NH*	211.5±11.6	129.5±9.3	288.5±23.4	176.4±6.5	135.9±5.5
Mn	*NH*	36.8±2.8	41.2±2.3	37.7±2.4	39.9±3.4	47.6±3.5
Cu	*NH*	10.6±3.3	3.5±1.5	18.6±1.3	7.6±1.3	7.3±1.3
Cd	*NH*	0.26±0.1	0.3±0.1	1.2±0.1	0.3 ±0.1	0.2±0.1
Ni	*NH*	1.2±0.3	0.8±0.1	1.4±0.2	0.9±0.1	1.1±0.2
Zn	*NH*	38.5±2.3	28.7±1.7	35.2±1.9	34.8±2.3	39.8±2.4

NH-No harvest, *Value are mean and standard deviation, n= 4 samples

Table 5
METALS CONTENT OF AERIAL TISSUE OF PLANTS FROM VARIANTS FERTILIZED WITH SEWAGE SLUDGE

Metal	Experimental variants/ Slag and fly ash treatment *Metals content of plants aerial tissue [mg kg^{-1} D.M.]					
	Slaughter house sludge without seaweed extract			Slaughter house sludge with seaweed extract		
	va1	*va2*	*va3*	*vas1*	*vas 2*	*vas 3*
Fe	213.5±15.3	206.8±11.6	124.1±9.3	78.8±6.2	232.3±15.9	185.0±10.9
Mn	46.9±3.4	33.1±3.0	55.4±4.1	32.8±2.8	41.7±5.3	47.6±5.9
Cu	9.1±4.2	10.0±4.5	6.54±2.7	7.1±3.8	8.63±3.9	9.3±4.3
Cd	0.7±0.1	0.28±0.1	0.6± 0.1	3.2± 0.2	0.9± 0.1	0.9± 0.1
Ni	1.34±0.2	0.95±0.3	1.1±0.3	0.4±0.1	1.5±0.1	1.5±0.1
Zn	48.8±4.3	43.7±3.8	50.9±3.8	31.7±2.8	39.2±3.3	38.7±3.6

NH-No harvest, *Value are mean and standard deviation, n= 4 samples

Table 6
METALS CONTENT OF AERIAL TISSUE PLANTS FROM FERTILIZED VARIANTS WITH SLAUGHTER HOUSE SLUDGE

2) Cd content was below maximum allowable level (maximum content of 1.0 mg/kg relative feeding stuff with 12% moisture content [12]) by sewage sludge fertilization between 1.0-2.5 kg.m^{-2} and seaweed extract (Normal values <1 mg·kg^{-1} [13, 14]); 3) Cu content in dry vegetables leaves exceeded the normal value < 5 mg·kg^{-1} [14, 15]; 4) Zn concentration was below 20-50 mg·kg^{-1}, which has been reported as normal values for Zn in dry vegetables leaves [14, 15]; 5) the harvest from all variants should be carefully considered. However, given the relatively small amounts of toxic metals, grass will be able to recycle the compost.

Based on the data presented in table 6, it can be concluded: 1) in aerial tissue of plants have not accumulated toxic metals like Cr and Pb; 2) Cd content was below maximum allowable level (maximum content 1.0 mg/kg relative feeding stuff with moisture content 12% [12]) by all experimental variants fertilized with slaughter house sludge in absence/presence seaweed extract, except *vas1* experimental variants 0.5 [kg·m^{-2} D.M.] containing slaughter house sludge fertilizer with seaweed extract; [13, 14]; the addition of seaweed extract caused increasing amounts of Cu, Mn, Zn in aerial tissue vs. similar variants fertilized with slaughter house sludge without seaweed extract; 3) Cu concentration in dry vegetables leaves exceeded the allowance value <5 mg·kg^{-1} [14, 15]. 4) Zn concentration was below 20-50 mg·kg^{-1}, which has been reported as normal values for Zn in dry vegetables leaves [12, 13]; 5) the harvest from all experimental variants fertilized with sewage sludge in absence/presence seaweed extract should be carefully considered and assessed as toxic/non-toxic waste management. From the above analysis the biomass with relatively small amounts of metals, will be able to recycle the compost properly mixed with other materials.

Conclusions

For the rapid grassing of inert dumps it was necessary to ensure: 1.bioavailability amount of nutrients for plant growth, which were provided by the use of organic fertilizer; 2.stimulation of biogenesis in the rhizosphere to promote nutrients bioavailability. Best results regarding the vegetation of slag and fly ash dumps were obtained either for variants fertilized with large amounts of sewage sludge (2.5kg·m^{-2}) without the addition of stimulus or for variants fertilized with reduced amounts of sludge slaughter house (0.5kg·m^{-2}) with addition of stimulus. In the presence of large quantities of sewage sludge, it was not required to add a stimulating bacterial activity in the soil because the agent ensures the biogenesis quality and quantity in the inert soil. Using a bacterial inoculums based on brown seaweed sludge did not allow the stimulating plant growth conditions, probably because sewage sludge associated with seaweed extract led to antagonistic effects that inhibit optimal development of *plants.* Using small quantities of slaughter house sludge with a stimulating bacterial activity ensures the biogenesis quality and quantity introduced into inert soil and creates optimal conditions for plant development. Experimental variants fertilized with slaughter house and bacterial inoculums ensures a synergistic formation of active biogenesis which allows bioavailability nutrient for plants and a culture of healthy plants with abundant *Lolium perenne* species. The harvest from all experimental variants fertilized with bio sludge in absence/presence seaweed extract should be carefully considered and assessed as toxic/non-toxic waste management. From the above analysis the biomass with relatively small amounts of metals, will be able to recycle the compost properly mixed with other materials.

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