

Studies to Limit Bioaccumulation of Heavy Metals in Biomass Grown on Lignite Fly Ash Deposits

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*The bioaccumulation of Cr, Cu, Fe, Mn, Ni, Pb and Zn, in clover harvest is significantly reduced when using municipal stabilized sludge (biosolids) fertilizer mixed with indigenous volcanic tuff. This study investigates an ecological vegetation method for lignite fly ash deposits. Furthermore, adding biosolids provides nutrients and the tuff secure the water retention: they gradually released from the plant need. The coverage of selected plant *Trifolium pratense* was evaluated in the following variants: fly ash in absence/presence of biosolids fertilizer and volcanic tuff. The vitality and abundance of plant was significantly higher when using biosolids and tuff vs. other variants.*

Keywords: bioaccumulation metals, clover harvest, lignite fly ash, biosolids, volcanic tuff

Big towns face serious problems concerning the large quantity of waste coming from:

- burning coal in thermo-electric power stations, resulting fly ash deposits, which do not contain the necessary nutrients for growing plants, based on bio-available C, N and P have a variable content of heavy metals;

- municipal sludge, with important quantities of bio-available nutrients for plants, but not recommended for the fertilization of agricultural fields.

In both situations, the now formed waste deposits occupy important areas of field surrounding towns, as areas that alter the landscape and are exposed to spreading some pollutants due to the action of climatic factors: water, wind, temperature etc., and constitute the subject of concern for many studies regarding the installation of a healthy, stable and protective vegetable layer [1-6].

The choice of the plant species to be used in the phytostabilization studies has to depend upon the strategy of installation and the maintenance of the vegetable layer but also the uptake of metals from the fly ash layer [7-10]. *Trifolium pratense* is the species of plant used. It is defined as an optional metallophile plant [11]. *Trifolium spp.* accumulates less metal in the aerial part, so it is more suitable for the phyto-management of the ash deposits [12]. Studies and investigations are performed in order to determine a strategy of formation of a vegetable layer of *Trifolium spp.* on the polluted soils with heavy metals. From the performed studies regarding the formation of the vegetable layer with *Trifolium spp.* on the polluted fields with heavy metals, it results as a main objective the nitrogen fixation in the soil and less the state of bioaccumulation level of metals in the aerial tissue [8].

The experiments in greenhouses and on experimental fields have shown that healthy vegetation is developing on dumps covered with layers of ash and then seeded. If in the first years a nematocide effect of the ash is revealed, after 12 years of treatment with lignite coal ash, the presence of a healthy fauna, based on nematodes for the treated soil is found. Also, a reduced bio-availability of heavy metals is found and a reduced phytotoxicity in comparison with the grown plants. The use of flying ash needs to be mentioned, resulted from the burned coal in the thermoelectric power stations, with the purpose of reducing

metals mobility and phytotoxicity from the polluted soils [12-13]. The effect of biosolids in the vegetation strategy for semi-arid fields and bioaccumulation of metals in different parts of the plant is that of growing the biomass quantity up to 2 times in comparison with the control [13-14]. In order to improve the stable phyto-technologies for the ash dumps, agricultural amendments are used [15-18].

A factor used to compare the bioaccumulation of metal in plants is the metal transfer factor - TF. TF refers to the level of accumulated metal in different parts of the plant reported to the quantity of metal from the soil [8, 19].

Another comparison factor for the bioaccumulated metals in the plant is the translocation factor, TC, that refers to the level of accumulated metal in the aerial part of the plant reported to the quantity of metal from the root [20-21].

According to the values of the two factors, TF and TC a conclusion may emerge that there are differences regarding the quantity of metals accessed by the root and then moved in the aerial part of the plant. The quantity of metals that exists in the biomass of young or mature plants may represent a risk for the beings that transit or for the ones already installed in the ecosystems formed on grassy dumps [22]. The level of grassing for the ash dumps through the formed culture of clover may be evaluated by the Scale for Vegetation Analysis Braun-Blanket [23]. Depending on the characteristics of the biomass, it can have different uses: food for animals, addition to compost, etc. [24].

The present study refers to:

- the determination of metal concentration from the polluted fly ash deposits;

- concentration of metals in the roots and aerial parts of *Trifolium pratense* species;

- the determination of the transfer factor, TF, which refers to the accumulation of metal in roots versus the quantity of metal from the soil and the translocation factor, TC, or the level of accumulated metal in the aerial part of the plant reported to the quantity of metal from the root;

- the evaluation of the studied plants tolerance in the presence of oxidative stress generated by the presence of metals.

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	Metal content from biosolids [mg/kg DM]							
	Fe	Mn	Cu	Cd	Cr _{tot}	Zn	Ni	Pb
Biosolids	2353.7	314.9	425.9	20.9	134.7	351.0	14.7	175.2

Table 1
HEAVY METAL CONTENT FROM THE
BIOSOLIDS

Experimental part

The study was performed on an experimental block consisting of 18 experimental pots, 0.075 m² each. The experimental variants of the study were as follows: 1) C– fly ash variant with no added organic fertilizer, as control, 2) CB– fly ash-variant fertilized with organic fertilizer, biosolids, 3) CBZ1– fly ash-mixed with biosolids and indigenous volcanic tuff. The volcanic tuff quantity was of 5 t/ha dose, 4) CBZ11– fly ash-mixed with biosolids and indigenous volcanic tuff of 2.5 t/ha dose, 5) CBZ2– fly ash-mixed with biosolids and modified tuff of 5 t/ha dose, 6) CBZ22– fly ash-mixed with biosolids and modified tuff of 2.5 t/ha dose. Fly ash comes from the ash deposits. The experimental variants were fertilized with biosolids (municipal sludge) 15 t of D.M./ha. Biosolids metal content is presented in table 1.

Biosolids, formed from the anaerobic mesophile fermented municipal sludge, had when applied a humidity of 79.2%, an organic matter content of 3.4% total nitrogen, 0.93% phosphorous, and pH= 6.5.

Tuff is an indigenous volcanic rock with zeolite content (approx. 70% clinoptilolite from Mârşid quarry), with a 0.2–2 mm coarseness (Z1). The modified volcanic tuff (called tuff-Aln) was prepared in the (ECOIND) laboratories [25]. This is dispersed as 80% D.M. aqueous suspension (Z2).

Fly ash and soil samples analysis was done to determine the total Fe, Mn, Cd, Cr, Cu, Ni, Pb, and Zn concentrations according to the analysis method: the heavy metals were extracted from the soil samples by heating with Aqua Regia for 2h, at reflux. After interrupting the heat, the system was left in stand-by for 16 h. Then the samples were diluted in a flask with distilled water to exactly 50 mL. Plant tissues were thoroughly washed with distilled water to remove any soil particles attached to plant surfaces. The tissues were dried (105°C) to a 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, were taken to a calibrated flask with hydrochloric acid 1:1 solution. Plant and soil extracts analysis was done using a spectrophotometer, Varian Spectra AAS.

The selected plant was *Trifolium pratense*. The sowing was done in April 2011 with the amount of 20g/ pot.

The transfer factor, TF, which refers to the level of metal accumulation in different parts of the plant reported to the quantity of metal from the soil. In this case, the TF - transfer factor from the soil into the roots is given by the ratio Q_r/Q_{soil} where Q_{soil} is the quantity of metal presented in the soil, [mg/kg D.M.] and Q_r , the quantity of metal accumulated in the root tissue of the plant, [mg/kg D.M.].

2. The translocation factor, TC, which refers to the level of metal accumulation in plants, aerial part, reported to the quantity of metal stored in the root as Q_s/Q_r ratio, where Q_s is the quantity of metal accumulated in the aerial part tissue of the plant, [mg/kg D.M.], and Q_r the quantity of metal accumulated in the root tissue of the plant, [mg/kg D.M.].

The plants are analyzed concerning the state of the selected species culture by:

Predominance - abundance index

Predominance expresses the degree of soil covering with plants, due to aerial parts projection on soil. The abundance is an expression of plants density but also of favorable environmental conditions and offers important ecological data, such as the role and potency of species to use space, light, air, humidity, and food etc., the soil capacity to retain water from precipitations, average rainfall quantity form the period of time etc.

Because the abundance is in direct relation with predominance, a predominance-abundance index was proposed by Braun-Blanquet, taken into consideration the following Cover-Abundance Scale:

- +) few individuals and a very small degree of covering: < 5%;
- 1 level- more individuals, but with small covering degree: < 5%;
- 2 level-small abundance and limited covering degree: 5 - 25%;
- 3 level-abundant individuals and high degree of covering: 25-50%;
- 4 level-abundant individuals and good degree of covering: 50-75%;
- 5 level-abundant individuals and very good degree of covering: 75–100%.

Vitality index

Vitality represents the plants' feature to complete their vital cycle going through all the growth and development phases, from germination to fruition. This feature shows the adaptation level of a species to the ecological conditions from a phytocenosis. In order to evaluate the vitality, many assessment scales may be used, the most common is the Vitality scale by Braun-Blanquet with the following categories (stages):

- 1st level - developed plants, that regularly and completely end their life cycle;
- 2nd level - less developed plants that multiply mostly vegetative and do not always end their life cycle;
- 3rd level - plants that vegetate, but weakly multiply and do not completely end their vegetative cycle;
- 4th level - poorly developed plants that do not multiply in any way.

Results and discussions

The soil characteristics are presented in table 2 from the experimental variants of cultures from the plots.

The quantity of metals from the untreated ash varies in this order: Fe>Mn>Cr > Zn> Ni> Cu>Pb.

The addition of biosolids or biosolids and tuff does not determine significant modifications of the quantity of metals from the treated fly ash. The concentration of metals from the experimental variants of fertilized fly ash with biosolids and with a mixture of biosolids with different doses of indigenous volcanic tuff, modified and unmodified, varies in a similar way with that from the untreated ash.

The quantity of metals accumulated in the roots of the plants grown in pot with untreated fly ash varies in the following order: Fe>Cr >Mn ≡ Pb> Zn> Cu>Ni.

Nr. crt	Experiment variants	Heavy metals content in topsoil [mg/kg D.M.]						
		Fe	Mn	Cu	Cr	Ni	Pb	Zn
1	C	1953.3	163.0	21.8	121.8	32.4	10.1	74.7
2	CB	1935.1	165.9	15.8	98.4	32.4	12.6	69.5
3	CBZ1	1909.3	153.4	17.0	87.6	30.5	10.4	63.5
4	CBZ11	1898.2	153.6	18.2	70.5	31.8	10.6	67.7
5	CBZ2	1843.7	148.7	17.1	72.1	32.8	13.6	69.2
6	CBZ22	1844.4	149.2	17.2	77.8	29.9	13.5	69.8

Table 2
METAL CONCENTRATIONS IN
POTS TOPSOIL OF
EXPERIMENTAL VARIANTS

Nr crt	Experiment variants	Heavy metals content in plant root [mg/kg D.M.]						
		Fe	Mn	Cu	Cr	Ni	Pb	Zn
1	C	603.7	87.7	13.7	110.0	3.7	87.5	41.3
2	CB	630.0	78.9	11.6	76.4	1.5	62.2	50.0
3	CBZ1	421.8	65.8	15.0	84.6	3.9	78.1	62.5
4	CBZ11	593.7	64.7	20.8	72.3	4.2	78.2	104.2
5	CBZ2	293.9	62.9	10.5	64.7	3.1	26.3	72.3
6	CBZ22	392.8	61.0	10.8	43.8	3.5	28.9	94.6

Table 3
THE ROOT HEAVY METALS CONTENT

The analysis of roots heavy metals content is presented in table 3.

The control of metal concentration from the aerial part biomass can be achieved by using a suitable quantity of biosolids that will secure a harvest, and at the same time the quantity of biosolids used in order to avoid big accumulations of metals in the tissue [6]. The treatment of fly ash with biosolids determined the reduction of metal accumulation in roots with 29-30% for Pb and Cr and with 10-15% for Cu and Mn in comparison to the accumulation of the same metals in the roots of plants grown on untreated fly ash. But plants in this phenophase have the tendency to accumulate metals in the aerial parts. Therefore, biosolids determine the growth of metal bioaccumulation in the aerial part of plants. Thus a major quantity will move from the roots in other parts of the plant, as shown in figure 1.

The treatment of fly ash with biosolids and tuff determined the reduction of the accumulation in roots with 60-70% for Pb and Cr and with 20-30% for Cu and Mn in comparison to the accumulation of the same metals in the roots of plants grown on untreated ash, so the translocation of metals from the root to other parts of the plant diminished. When using the treatment with biosolids and tuff, the quantity of Fe accumulated in the plant roots can be reduced with up to 50%. The quantity of Zn accumulated in the roots increases especially in the case of combined treatments. To be noted, in all studied cases low accumulations of Ni were found in the roots <5 mg/kg D.M.

Trifolium spp. from the dicotyledonous class blocks much metal in its root releasing in the first phenophase certain quantities in the aerial part of the plant where they accumulate. In the case of Ni, the accumulation in the roots is small and the transport or the bioaccumulation in the aerial part is very high.

The reports from literature are correlated with the obtained experimental data [8].

In figure 1 the variations of metal accumulation in the aerial part are presented, for the vegetative cycle and growth period: Shoot S1 3-4 cm, Shoot S2 7-9 cm, and Shoot S3, fructification stage. In stage 2, Shoot S2, the addition of biosolids determined an increase of bioaccumulation in the aerial part for Pb and Cu with 46% or 58% in comparison with bioaccumulation from plants grown on untreated fly ash variants. The addition of tuff determined, in this stage, a reduction efficiency of translocation of metal, as well as bioaccumulation in the aerial tissue for Fe, Cu and Cr with 50-60%, and for Pb with 40-50% in comparison with bioaccumulations of metals in plants grown on untreated fly ash substrate. The reduction efficiency depends on the nature and the added quantity of tuff. Higher efficiency is found when using some reduced doses of tuff in the bio-solids mixture, in the variant of modified volcanic tuff. In stage Shoot 3 the bioaccumulations from the aerial part are similar with the ones from the previous stage. Still to be noted the behavior of plants grown with bio-solids addition mixed with reduced doses of tuff variant of modified tuff, when a major limitation of bioaccumulation of Pb is found.

The quantity of metals present in the mature plants harvested from this experimental variant of Pb < 0.5 mg/kg D.M., Cu, Cr, Ni < 2 mg/kg D.M., Mn and Zn < 30 mg/kg D.M. and Fe < 45 mg/kg D.M. does not represent a risk for the beings that transit or for those installed in the ecosystems formed on grassy fly ash deposits.

These phenomena are shown through the variation of the transfer factor TF, which is presented in table 4. In table 5, the variation of the translocation factor TC is presented for the toxic metals Cr, Pb, Cu from treated and untreated ash. TC refers to the level of accumulation of metal in plants

aerial part reported to the quantity of metal accumulated in root.

In the first stage Shoot S1, large quantities of Cr are transported in the aerial part, TC has high values. The deposit of Cr in the aerial part is 10 times higher than in the root. In the next stages TC diminishes 10^3 times. This time the deposit of Cr from the aerial part is 10 times more reduced than in the root. In the case of Pb the stored quantity in the aerial part is up to 6 times higher than in the first stage in comparison with the quantity stored in the root

The treatment of fly ash in the experimental variant fly ash with biosolids and modified tuff 1% wt., determined a reduction of the translocation of Pb from the root in the aerial part of over 3,5-4 times unlike the case of plants grown on Fly ash variant. In maturity stages TC has sub-unitary values proving the fact that the access of Pb in the root and aerial part is significantly diminished. In the case of Cu in the first phenophase the applied treatments determine an increase of the quantity of metal from the

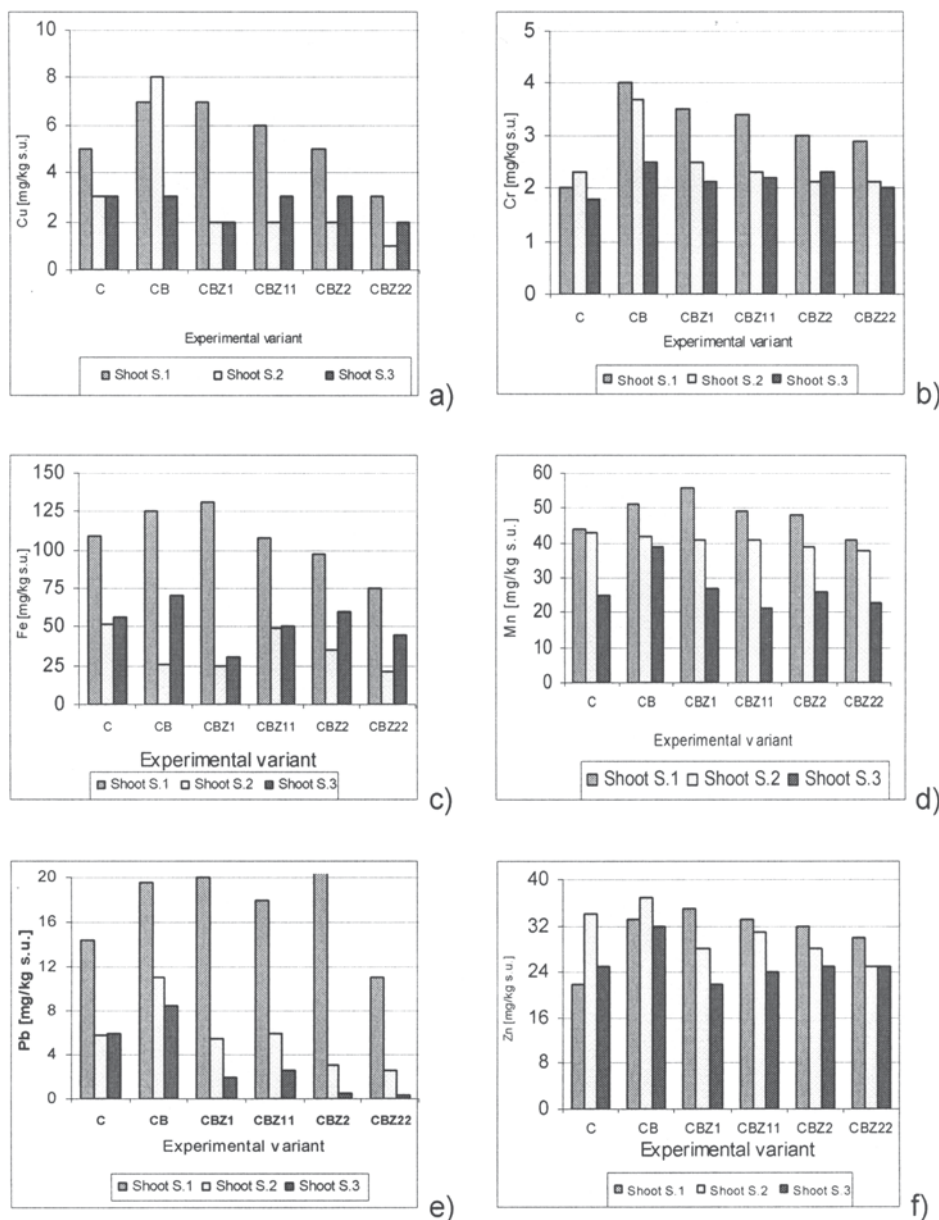


Fig. 1. Metal concentrations accumulated in the aerial tissues of *Trifolium pratense* species, Shoot S1 3-4 cm, Shoot S2 7-9 cm, and Shoot S3, fructification stage: a) Fe content; b) Mn content; c) Cu content; d) Cr content; e) Cr_{tot} content; f) Zn content; g) Ni content; h) Pb content

Nr. crt	Experimental variants	Transfer factor (TF)						
		Fe	Mn	Cu	Cr	Ni	Pb	Zn
1	C	0.31	0.54	0.66	0.90	0.11	8.7	0.55
2	CB	0.33	0.48	0.75	0.78	0.05	4.9	0.71
3	CBZ1	0.22	0.43	0.88	0.96	0.13	7.5	0.94
4	CBZ11	0.31	0.42	1.16	1.02	0.12	7.5	1.52
5	CBZ2	0.16	0.42	0.61	0.90	0.09	1.9	1.04
6	CBZ22	0.21	0.41	0.62	0.56	0.11	2.1	1.35

Table 4
TRANSFER FACTOR, TF

Nr crt	Experiment variants	Translocation factor (TC)								
		Shoot S1			Shoot S2			Shoot S3		
		Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb	Cu
1	C	-	6.07	0.36	-	0.60	0.15	-	0.60	0.13
2	CB	19.0	3.20	0.60	0.03	0.70	0.51	0.03	0.70	0.18
3	CBZ1	24.3	3.90	2.00	0.03	0.20	0.12	0.02	0.20	0.12
4	CBZ11	21.3	4.31	3.20	0.03	0.23	0.11	0.03	0.23	0.18
5	CBZ2	21.7	1.25	2.00	0.03	0.04	0.12	0.03	0.04	0.14
6	CBZ22	15.2	1.73	3.10	0.03	0.03	0.09	0.03	0.03	0.11

Table 5
TRANSLOCATION FACTOR, TC

Crt. No.	Experiment variants	Predominance- abundancy indices	Vitality index,
		Cover-Abundance Scale Braun-Blanquet	Vitality scale Braun-Blanquet
1	C	1 level-	4 level –
2	CB	3 level -	2 level –
3	CBZ1	3 level -	2 level –
4	CBZ11	3 level -	2 level
5	CBZ2	4 level 75%;	1 level –
6	CBZ22	4 level	1 level - regular bases

Table 6
ANALYSIS OF ECOLOGICAL ABUNDANCE AND
VITALITY, FOR *TRIFOLIUM SPP.* AFTER
BRAUN-BLANQUET
COVER - ABUNDANCE SCALE

root in the aerial part $TC > 1$. In the mature stages, the diminishing TC is of tens of times.

In the case of Fe and Mn the TC values are sub unitary for the analyzed stages.

Plants cultivated on the untreated fly ash variant have an unhealthier aspect, are smaller and rarer. The addition of biosolids determines cultures with a healthy aspect in the first phenophases, showing suffering because of lack of water from the summer period. The most vigorous cultures are obtained in pots that contain tuff. A major good influence can be observed in the case of the addition of modified tuff at minimum doses, as it is presented from the physiological analysis of the plant presented in table 6.

The obtained results show that it is recommended for the growth of aerial biomass quantity a soil treatment with mesophilic fermented urban sludge. The optional addition of modified volcanic tuff maintains a healthy culture, with no accumulation in the aerial parts and no risks for segments of the food chain. The addition of modified indigenous volcanic tuff maintains a healthy culture, which tolerates a total content of metals: Fe, M, Cr, Cu, Ni, Pb and Zn of over 2.3 g/kg D.M. The percentage of occupied cultivated area with clover is between 50-75%, the plants are well developed and fructified, thus completing the cycle of life.

Conclusions

Fly ash deposits fertilized with bio-solids contain the required nutrients for the seed germination and for the development of a clover culture. The addition of modified indigenous volcanic tuff maintains a healthy culture, which tolerates a total content of metals of fly ash: Fe, M, Cr, Cu, Ni, Pb, and Zn of over 2.3 g/kg D.M.

The transfer factor TF from soil into the plants root, the translocation factor TC from root to aerial part of the plant and the effective bioaccumulation quantity in the harvested biomass proves the restriction of metal access for Fe, Mn, Cu, Cr, and Ni; most efficient for Pb when adding mixed bio-solids with minimal doses of modified indigenous volcanic tuff. The quantity of metals present in the mature plants biomass, harvested from this experimental variant (Pb < 0.5 mg/kg D.M., Cu, Cr, Ni < 2 mg/kg D.M., Mn and Zn < 30 mg/kg D.M. and Fe < 45 mg/kg D.M.) does not represent a risk for the ecosystems that can be installed on the grassy fly ash deposits.

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