ENZYME ACTIVITIES IN TECHNOGENIC SOIL RESULTING FROM THE RECULTIVATION OF THE BAUXITE MINE FROM PĂDUREA CRAIULUI (ROMANIA)

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Abstract. *Actual and potential dehydrogenase, catalase, urease, acid and alkaline phosphatase activities and nonenzymatic catalytic activities were determined in the 0–10, 10–20, 20–30 and 30–40 cm layers of the soil from bauxite mine spoils (Pădurea Craiului Mountains) submitted to biological recultivation. It was found that each activity decreased with increasing sampling depth. In the recultivated soil, higher enzymatic activities were recorded than in the native soil. Both of them were less enzyme-active than the forest soil. Soil enzyme activities were strongly intercorrelated whereas the nonenzymatic catalytic activity was weakly correlated with enzymatic activities.*

Rezumat. *Activitățile enzimatice (dehidrogenazică actuală şi potențială, catalazică, ureazică, fosfatazică acidă şi alcalină) şi activitatea catalitică neenzimatică au fost determinate la patru adâncimi: 0-10, 10-20, 20-30, 30-40, întrun sol dintr-o mină de bauxită din Munții Pădurea Craiului supus recultivării biologice. Activitățile enzimatice şi neenzimatică studiate au scăzut cu adâncimea. S-a constatat că activitățile enzimatice au fost mai ridicate în solul recultivat comparativ cu solul nativ dar mai mici decât în solul de pădure. S-au putut stabili corelații pozitive între activitățile enzimatice studiate.*

INTRODUCTION

Technogenic soils are soils that form during the technical and biological recultivation of overburdens, tailings and other spoils and wastes resulting from mining and other industrial activities. At the same time, all these wastes constitute a dangerous source of environmental pollution (Harris et.al., 1989; Kiss et.al., 1991).

The evolution of technogenic soils is the process of transforming all these wastes into agricultural or forest soils or into soils used for other purposes (parks etc). Simultaneously, this process is accompanied by reduction or elimination of the polluting effects of wastes on the environment (Persson, 1988).

The practical importance of this process is growing because the development of mining and other industries leads to increasing amounts of wastes and, therefore, the recultivation of wastelands becomes more and more a major economic necessity (Ross et.al., 1992).

The evolution of technogenic soils, which reflects the efficiency of recultivation, is studied using many physical, chemical and biological methods (Stroo et.al., 1985). Enzymological methods have also been applied and it has been

found that the level of enzymatic activity is a good indicator of the degree of evolution of technogenic soils.

The present paper deals with the enzymatic potential in the profiles (0–40 cm) of spoil plots in the bauxite mine from Pădurea Craiului Mountains (Romania).

MATERIAL AND METHODS

In August 2007, soil was sampled from the 0–10, 10–20, 20–30 and 30–40 cm depths of the three plots in a soil from bauxite mine spoils (Pădurea Craiului Mountains).

The soil samples were collected from native soil (P1), recultivated soil (P2) and from the nearby forest soil (P3). The recultivation with ryegrass (*Lolium multiflorum L.*) and meadow clover (*Trifolium pratense L.*) was installed in 2006.

The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve and, finally, used for enzymological analyses.

Enzymological analyses

Two enzymatic activities (actual and potential dehydrogenase) were determined according to the methods described in (Kiss et. al., 1985). Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced from 2,3,5-triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 hours.

Catalase activity has been determined using the permanganometric method. The same technique was used for the determination of nonenzymatic catalytic activity, but the soil samples have been thermically inactivated by autoclaving (Kiss et. al., 1985). Catalase and nonenzymatic catalytic activities are expressed as mg of H_2O_2 decomposed by 1g of soil in 1 hour. Urease activity is determined according to the method described in (Kiss et. al., 1989) and is expressed in mg of NH_4/100g soil/24 hours. For the determination of phosphatase activities, disodium phenylphosphate served as enzyme substrate. Two activities are measured: acid phosphatase activity in reaction mixtures to which acetate buffer (pH 5.0) was added, and alkaline phosphatase activity in reaction mixtures treated with borax buffer (pH 9.4). The buffer solutions were prepared as recommended by (Ohlinger, 1996). Phosphatase activities are expressed in mg phenol/g soil/2 hours.

The activity values were submitted to statistical evaluation by the two *t* test (Sachs, 2002) and the correlations between the enzymatic activities were determined according to the methods described in (Samuel et. al., 1999).

RESULTS AND DISCUSSIONS

Results of the enzymological analyses are presented in *Table 1*, and those of the statistical evaluation are summarised in *Table 2*.

Variation of soil enzymatic activities in dependence of sampling depth

It is evident from *Table 1* that each activity decreased with sampling depth in all plots.

Enzymological data

For enzymological evaluation of the three plots, the results obtained in the four soil layers analysed were considered together.

Significant ($p<0.05$ to $p<0.001$) and unsignificant ($p>0.05$ to $p>0.10$) differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and the type of the plot.

The difference between the two plots: native and recultivated was significant (at least at $p<0.05$), while catalase and alkaline phosphatase were unsignificantly higher ($p>0.10$ and $p>0.05$, respectively) in the recultivated soil.

One can see from *Table 2*, that the enzymatic activities were always higher in the 0 – 40 cm layer of the forest soil in comparison with the native soil. In the forest soil, the activities were significantly higher (at least at $p<0.02$), excepting alkaline phosphatase activity which was only unsignificantly higher ($p>0.05$) than in the native soil.

Comparision of plots P2 and P3 reveals that each of the six enzymatic activities determined was significantly higher (at least at $p<0.05$) in the forest than in the recultivated soil, excepting actual and potential dehydrogenase activities which were unsignificantly higher ($p>0.10$).

Statistical data

Simple correlation (r) between enzymatic activities in the 0–40 cm layer (*Table 3*) showed that soil enzyme activities were strongly intercorrelated ($r=0.434$ to $r=0.939$), whereas nonenzymatic catalytic activtity was weakly correlated ($r=0.250$ to $r=0.372$) with enzymatic activities.

CONCLUSIONS

Our results are in good agreement with the literature data (Drăgan-Bularda et.al., 1987; Fresquez et.al., 1982; Lindemann et.al., 1984) and constitute novelties for the enzymological characterisation of a soil from bauxite mine spoils.

The literature reviewed (Uzbeck, 1991; Wigfull et.al., 1987) shows that application of enzymological methods makes it possible to indicate the degree of evolution of technogenic soils, the transformation of overburdens and other spoils and wastes into agricultural and forest soils, the efficiency of the recultivation measures applied.

In comparison with microbiological parameters, the enzymes are more synthetic indicators of the evolution of technogenic soils because they reflect a) due to their accumulation in form of humic complexes, the past of technogenic soils, and b) due to their catalytic activity, which plays a key role in nutrient cycles, the present biological status of these soils.

Table 1

Enzymatic activities in soil from bauxite mine spoils (Pădurea Craiului Mountains)

Soil enzymatic activity*	Soil depth (cm)	Plots		
		Native (P1)	Recultivated (P2)	Forest (P3)
ADA	0-10	7.42	9.09	9.80
	10-20	7.00	8.12	9.08
	20-30	6.44	7.56	8.96
	30-40	3.64	6.72	6.16
ADP	0-10	9.52	11.48	14.96
	10-20	7.00	10.92	14.28
	20-30	5.60	9.80	12.43
	30-40	4.06	8.40	7.84
AC	0-10	10.85	10.90	12.60
	10-20	10.10	10.20	11.80
	20-30	9.35	9.70	11.60
	30-40	7.20	9.70	10.60
CAn	0-10	13.70	14.00	14.20
	10-20	12.30	13.50	13.90
	20-30	12.25	13.30	13.50
	30-40	11.85	12.90	13.00
UA	0-10	6.37	9.83	12.99
	10-20	5.83	7.99	10.82
	20-30	5.29	6.66	8.33
	30-40	4.79	5.99	7.99
AcPA	0-10	0.188	0.281	0.352
	10-20	0.179	0.269	0.294
	20-30	0.141	0.183	0.209
	30-40	0.125	0.150	0.195
AlkPA	0-10	0.126	0.133	0.173
	10-20	0.117	0.125	0.160
	20-30	0.098	0.105	0.155
	30-40	0.070	0.090	0.141

*ADA – Actual dehydrogenase activity.
PDA – Potential dehydrogenase activity.
CA – Catalase activity.
CAn – Nonenzymatic catalytic activity.
UA – Urease activity.
AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.

Table 2

Significance of the differences between enzymatic and nonenzymatic catalytic activities in soil from bauxite mine spoils (Pădurea Craiului Mountains)

Area	Soil enzymatic activity*	Soil depth (cm)	Mean activity values			Significance of the differences
			a	b	a - b	
Native soil (a) versus recultivated soil (b)	ADA	0 – 40	6.12	7.87	-1.75	0.05>p>0.02
	ADP	0 – 40	6.54	10.15	-3.61	0.01>p>0.002
	AC	0 – 40	9.37	10.12	-0.75	p>0.10
	CAn	0 – 40	12.52	13.42	-0.90	0.02>p>0.01
	UA	0 – 40	5.57	7.61	-2.04	0.05>p>0.02
	AcPA	0 – 40	0.158	0.220	-0.06	0.05>p>0.02
	AlkPA	0 – 40	0.102	0.113	-0.01	0.10>p>0.05
Native soil (a) versus forest soil (b)	ADA	0 – 40	6.12	8.50	-2.38	0.0001>p
	ADP	0 – 40	6.54	12.37	-5.83	0.01>p>0.002
	AC	0 – 40	9.37	11.65	-2.28	0.02>p>0.01
	CAn	0 – 40	12.52	13.65	-1.13	0.02>p>0.01
	UA	0 – 40	5.57	10.03	-4.46	0.02>p>0.01
	AcPA	0 – 40	0.158	0.262	-0.10	0.01>p>0.002
	AlkPA	0 – 40	0.102	0.113	-0.01	0.10>p>0.05
Recultivated soil (a) versus forest soil (b)	ADA	0 – 40	7.87	8.50	-0.63	p>0.10
	ADP	0 – 40	10.15	12.37	-2.22	p>0.10
	AC	0 – 40	10.12	11.65	-1.53	0.01>p>0.002
	CAn	0 – 40	13.42	13.65	-0.23	0.05>p>0.02
	UA	0 – 40	7.61	10.03	-2.42	0.01>p>0.002
	AcPA	0 – 40	0.220	0.262	-0.04	0.05>p>0.02
	AlkPA	0 – 40	0.113	0.113	0.00	0.05>p>0.02

*ADA – Actual dehydrogenase activity.
PDA – Potential dehydrogenase activity.
CA – Catalase activity.
CAn – Nonenzymatic catalytic activity.
UA – Urease activity.
AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.

Table 3

Simple correlations (r) between soil enzyme activities in soil from bauxite mine spoils

Variables*	ADA	PDA	CA	CAn	UA	AcPA	AlkPA
ADA	-	-	-	-	-	-	-
PDA	0.931	-	-	-	-	-	-
CA	0910	0.434	-	-	-	-	-
CAn	0.882	0.932	0.851	-	-	-	-
UA	0.814	0.902	0.842	0.372	-	-	-
AcPA	0.793	0.877	0.806	0.250	0.939	-	-
AlkPA	0.809	0.842	0.860	0.269	0.785	0.789	-

*ADA – Actual dehydrogenase activity.
PDA – Potential dehydrogenase activity.
CA – Catalase activity.
CAn – Nonenzymatic catalytic activity.
UA – Urease activity.
AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.

REFERENCES

1. **Drăgan-Bularda M., Blaga G., Kiss S., Pasca D., Gherasim V., Vulcan R.**, **1987**, *Effect of long-term fertilization on the enzyme activities in a technogenic soil resulted from the recultivation of iron strip mine spoils*. Studia Universitatis Babes – Bolyai, Biologia, 32(2), 47–52.
2. **Fresquez P.R., Lindemann W.C., 1982** - *Soil and rhizosphere microorganisms in amended coal mine spoils.* Soil Science Society of American Journal, 46, 751–755.
3. **Harris J.A., Birch P., 1989** - *Soil microbial activity in opencast coal mine restorations*. Soil Use and Management, 5, 155–160.
4. **Kiss S., Drăgan-Bularda M., Pasca D., 1985** - *Enzymological study of the evolution of technogenic soils*. Evolution and Adaptation (Cluj), 2, 229–278.
5. **Kiss S., Drăgan-Bularda M., Pasca D., 1989** - *Enzymology of the recultivation of technogenic soils*. Advances in Agronomy, 42, 229–278.
6. **Kiss S., Drăgan-Bularda M., Pasca D., 1991** - *Enzymology of the evolution of some technogenic soils*, Evolution and Adaptation (Cluj), 4, 125–132.
7. **Lindemann W.C., Lindsey D.L., Fresquez P.R., 1984** - *Amendment of mine spoil to increase the number and activity of microorganisms.* Soil Science Society of American Journal, 48, 574–578.
8. **Öhlinger R., 1996** - *Phosphomonoesterase activity with the substrate phenylphosphate.* In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (eds.) *Methods in Soil Biology*, p. 210–213, Springer, Berlin.
9. **Persson T.J., Funke B.R., 1988** - *Microbiology of stored topsoil al North Dakota stripminig sites*. Arid Soil Research and Rehabilitation, 2, 235–250.
10. **Ross D.J., Speir T.W., Cowling J.C., Feltham C.W., 1992** - *Soil restoration under pasture after lignite mining: management effects on soil biochemical properties and their relationships with herbage yields,* Plant and Soil, 140, 85–97.
11. **Sachs L., 2002** - *Der Statistik Test*. In: Sachs, L. (ed.), *Angewandte Statistik Anwerdung statisticher Methoden*, p. 189–195, Springer, Berlin.
12. **Samuel A.D., Kiss S., 1999** - *The effects of soil management practices on the enzymatic activities in a brown luvic soil*. Studia Universitatis Babes – Bolyai, 44(1-2), 189–197.
13. **Stroo H.F., Jencks E.M., 1985** - *Effect of sewage sludge on microbial activity in an old, abandoned minesoil*. Journal of Environmental Quality, 14, 301–304.
14. **Uzbeck I.K., 1991** - *Particularities of the enzymatic activity in recultivated soils*. Pochvovedenie, 3, 91– 96.
15. **Wigfull S., Birch P., 1987** - *The microbiology of landfill soils.* Land and Minerals Surveying, 5, 349–353.