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Original paper

The assessment of the soil mineralization processes along the traffic corridors from urban and rural areas in Romania

I. PĂCEŞILĂ[1,2], E. RADU[1], C. C. BÎRSAN[1], M. CONSTANTIN[1,2]

[1] Institute of Biology Bucharest, Spl. Independentei, 296, 060031, Bucharest, Romania
[2] The Research Institute of the University of Bucharest, ICUB, Bucharest, Romania

Abstract The soils of the green areas located near the traffic corridors accumulate different pollutants, especially heavy metals, which have an important impact on the microbial decomposition processes. The extracellular enzymes can be used as an indicator of the metabolic activity of the microorganism communities. Four extracellular enzymes - α and β glucosidase, alkaline phosphatase and alanine aminopeptidase - were assessed in green areas close to the traffic zones in several urban, peri-urban and rural localities in Romania. The data showed that the highest values of enzymatic activities were registered in rural areas. At the same time, the spatial dynamics of the studied enzymes revealed an important variability between the three types of sampled areas, without a clear pattern of their distribution.

Keywords soil mineralisation, extracellular enzymatic activity

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*Corresponding author: I. PĂCEȘILĂ, Institute of Biology Bucharest, Spl. Independentei, 296, 060031, Bucharest, Romania.
E-mail: ioan.pacesila@gmail.com

Introduction

The extracellular enzymatic activity it is well acknowledged to play an important role in the mineralization of the detrital organic matter (DOM) and in the nutrient recycling process in soils, contributing to the ecosystems functioning. The extracellular enzymes are released in soils by numerous microorganisms (especially by bacteria and fungi), but also by the roots of some plants. They hydrolyze the large organic polymeric molecules (a major component of DOM) to simpler molecules that can be used by microorganisms or plants as a source of nutrients and energy. Due to their action, these enzymes are involved in the main biogeochemical cycles of the biogenic elements like C, N, S, etc. Thus, the evaluation of the extracellular enzymes activity could conduct to a better understanding of the DOM composition, a major component in the soil formation. The quantitative and qualitative alterations of DOM influence the composition and distribution of microorganism communities and, therefore, the specific of the biochemical processes carried out at the soil level - processes of a great importance for defining the characteristics of different types of soil (IGALAVITHANA & al. [1]; DATTA & al. [2]; ZAHIR & al. [3]; SHERENE [4]).

The intensity of the enzymatic activity in soils varies with the pressure of the environmental - physical, chemical and biological factors, of natural or anthropogenic origin. In the urban and rural inhabited areas, the presence of increasing quantities of heavy metals in soils (Ni, Cd, Cu etc.) is often recognized as a major factor that influences the activity of the extracellular enzymes. Another factor that can impact the decomposition processes in soil is represented by the pollution with fertilizers (especially of organic nature) used in the agricultural treatments. Most frequently, these fertilizers stimulate the enzymatic activity in soil and, thus, modify the rates of the biogeochemical processes at this level (KIM [5]; PETRISOR & LAZAR [6]; IGALAVITHANA et al. [1]).

The composition of the vegetation that covers the soils also influences the extracellular enzymatic processes in respect to the quantity and quality of the detrital organic matter produced. The enzymatic activity in soil is more intense in its upper layer, often related to a higher microbial biomass and detrital organic matter amount of this substrate. In the rhizosphere, the intense activity of many enzymes is sustained by a higher amount of the cellular debris and root excretes released in the environment (DICK & KANDELER [7]).

Regarding the urban and suburban areas, the soil undergoes different biochemical changes due to anthropogenic activities: agriculture, forestry, industrial activities, infrastructure and support activities etc. (MOREL & al. [8]). Thus, the urban soils are characterized by a higher heterogeneity and are frequently contaminated with important quantities of pollutants such as heavy metals and hydrocarbons, which represent a real thread for the human population, as well as for the plant and animal communities.

The purpose of the present study is to analyze and highlight the dynamics of the extracellular enzyme intensity in vegetated soils from territories along different traffic corridors in urban, peri-urban and rural localities in Romania.

Materials and methods

The samples were taken from the first 5 cm below the surface layer of the soil, between April and May 2016, from several urban and rural localities in the NE, E and SE part of Romania. All the 60 sampled points were placed in central or peripheral areas of the localities, in the proximity of trafficked roads, in parks or in the green areas of the blocks of flats. The sample size in different counties followed the density of the air quality monitoring stations in the chosen locations, according to the National Network of the Air Quality Monitoring in Romania (www.calitateaer.ro [9])), in order to asses possible pressures induced by the airborne pollution across the heterogeneity of our study area (ŞTEFĂNUŢ & al. [10]).

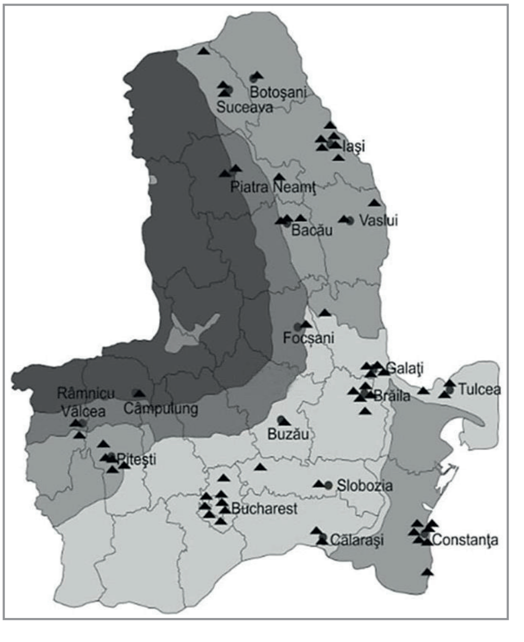

Figure 1. The study area and the sampling stations

The enzymatic activity was assessed using the substrate consumption method (OBST [11]). Four enzymes involved in soil mineralization processes (BANERJE et al. [12]; ZHANG et al. [13]; NIEMI et al. [14]) have been investigated: α glucosidase (α GLC) and β glucosidase (GLC) associated with the C cycle, the alkaline phosphatase (AP) involved in the P cycle and the alanine aminopeptidase (AMP) active in the N cycle (Tab. 1). The intensity of the enzymatic activity was expressed in units of nmol substrate per gram per hour or per day (24 h). All the processed data used in the cluster multivariate analysis were log transformed.

The statistical analyzes were performed using the PAST program ver. 2.17c (HAMMER & al. [15]) and following PAST Reference manual methodology.

Table 1. The enzymatic activities assessed in the urban, peri-urban and rural localities from the NE, E and SE part of Romania

Enzymatic activity	Substrate	Measurement unit (nmol $g^{-1}h^{-1}$)
α Glucosidase (EC 3.2.1.20)	p - nitrophenyl - α - D - glucopyranoside	p-nitrophenol
β Glucosidase (EC 3.2.1.21)	p - nitrophenyl - β - D - glucopyranoside	p-nitrophenol
Alkaline phosphatase (EC 3.1.3.1)	4 - nitrophenyl - phosphate	p-nitrophenol
Alanine aminopeptidase (EC 3.2.1)	L - alanine - 4 - nitroanilide - hydrochloride	p-nitroanilline

Table 2. The intensity ranges of the assessed enzymatic activities across the study sites

Enzymatic activity	nmol substrate $g^{-1}d^{-1}$
α Glucosidase (α GLC)	469,5 ± 457,5
β Glucosidase (β GLC)	1033,5 ± 997,5
Alkaline phosphatase (ALP)	1100 ± 1037
Alanine aminopeptidase (AMP)	1574 511

Results and discussion

By analyzing **the spatial distribution of the extracellular enzymatic activity** in the considered study areas, the highest intensity was mainly recorded for alanine aminopeptidase and the lowest, for α-amylase, with large variations for each enzyme (Tab.2).

In the rural areas, the enzymatic activity often appeared to be higher than in the urban areas (Fig. 2). The exception was observed for AMP that registered a higher average intensity for the peri-urban area.

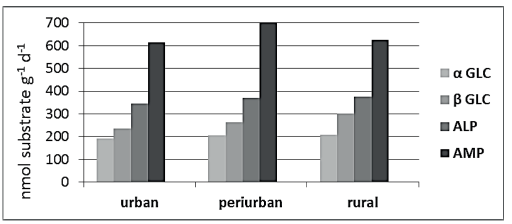

Figure 2. The average activity of the extracellular enzymes studied in the urban, periurban and rural areas

The data suggest the presence of some less favorable conditions for the MOD mineralization processes in the urban, highly trafficked areas. The accumulation of the polluting substances, especially heavy metals from the traffic emissions, can induce a significant impact in the environment on the microorganism communities, mainly decreasing their extracellular enzymatic activity (KHAN et al. [16]). However, this was not a tendency for all the analyzed sites; many of them in the urban area presented a higher extracellular enzymatic activity than in the rural areas. The dynamic of mineralization processes is influenced by many local factors, but the quantity and quality of the substrate are playing a major role (BURNS [17] DICK & KANDELER [7]). In our study, we aimed to sample a high variability of soil quality across the selected locations. On the other hand, some categories of microorganisms are known to develop different mechanisms of resistance to the heavy metals stress (AZARBAD & al [18], HOOSTAL & al. [19]). As well, the rate of absorption of the heavy metals by the vegetal layer modulate their impact on soils (TANGAHU et al. [20]), and subsequently on the bacterial extracellular enzymatic activity.

α GLC, registered the most intense activity in an urban park from Bucharest (751 nmol p-nitrophenol $g^{-1}d^{-1}$, Titan Park) and the lowest in a different type of urban site, in Iași, when we sampled the green area located in the proximity of the trafficked N. Iorga street (12 nmol p-nitrofenol $g^{-1}d^{-1}$).

β GLC activity was the most intense in Bosia village from Iași county (2031 nmol p-nitrophenol $g^{-1}d^{-1}$), at a sampling site placed near the main road, while its lowest value was assessed in an urban location, in Brăila - Brăila county (32 nmol p-nitrophenol $g^{-1}d^{-1}$), at the green area close to the central Independenţei Square.

AP phosphatase enzymatic activity varied between 2137 nmol p-nitrophenol $g^{-1}d^{-1}$ in Măgurele - Ilfov county (at a station located in the garden of a block of flats located to the outskirts of the city) and 63 nmol p-nitrophenol $g^{-1}d^{-1}$, in Botoşani - Botoşani county (for a sample taken as well from the garden of a block of flats, from M. Eminescu street).

AMP registered its highest value in Iași (3085 p-nitrofenol $g^{-1}d^{-1}$), in the rural area from the outskirts of the city, near M. Sadoveanu alley. Its minimum intensity (63 nmol p-nitrophenol $g^{-1}d^{-1}$) was assessed at the same station where AP recorded its minimum intensity, namely in Botoşani, in the garden of a block of flats.

In addition, because the intensity of soil level enzymatic processes can be influenced by soil physico-chemical characteristics (BŁOŃSKA et al [21]), it is also important to consider the types of soil identified in the studied areas. According to the data from the literature (STANILA & DUMITRU [22]) the ones from the category chernisols (predominantly chernozem), cambisols (predominantly distriambosols) and luvisols (predominantly luvosols) are prevalent. From table 3 it can be observed that, in general, α and β GLC were more intense in the stations located in regions where chernisols are dominant, while AP and ALP recorded higher values in the regions where cambisols predominate. In general, chernisols are recognized to have a high content of organic matter (VYSLOUŽILOVÁ et al [23]), but the concentration and quality of organic matter may widely vary in the case of all soil types, depending on the specificity of the area (ZARNEA [24]). However, the extracellular enzymatic activity across the sampling sites of different soil types did not show a high variation, suggesting that, most likely, the metabolic activity of the heterotrophic microorganism communities involved in the geochemical circuits at C, N and P, was not significantly influenced by the type of soil present in the sampling area.

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Table 3. The intensity of enzymatic activity assessed on different soil types

Enzymatic activity average value (nmol substrate h^{-1} mL^{-1})	**α GLC**	**β GLC**	**ALP**	**AMP**
chernisols	215,16	284,67	355,16	641,25
cambisols	206,53	269,61	351,87	646,46
luvisols	205,98	277,95	351,62	623,12

For a better understanding of the spatial variability of each bacterial extracellular enzymatic activity in soil across the study zones - urban, peri-urban, rural areas, we further performed the cluster analysis following Ward's method (Fig. 3).

Regarding the intensity of the substrate consumption performed by the four studied enzymes, we observed a similarity between α GLC and β GLC activities (Fig. 3). This could be the result of the amount of plant debris in the analyzed soils, the two enzymes being involved in the degradation of the vegetal glucidic substrates such as cellulose and starch. The activities of these two enzymes also showed the smallest spatial variability in the three types of studied areas. At the same time, we should mention that each enzyme revealed a specific dynamic per site type.

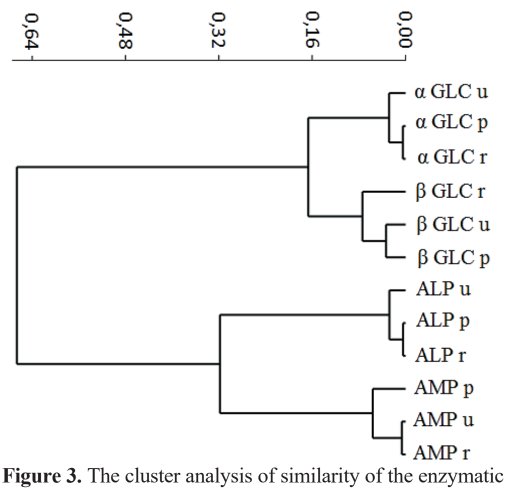

Figure 3. The cluster analysis of similarity of the enzymatic activities evaluated across the tree types of sampled locations (u – urban, p – peri-urban, r – rural) (Ward's method, Coph. Corr.: 0,696)

APL and α GLC enzymatic activity are the most similar in rural and peri-urban locations. Instead, the variability of β GLC enzymatic activities was the lowest between urban and peri-urban areas, and AMP, between the urban and rural sites.

The differences between the intensity of the extracellular enzymatic activities evaluated at different locations could be explained by the variation of the pressures exerted by the natural or anthropic environmental factors (KIM [4], DICK & KANDELER [8]).

The extracellular enzymes are considered to be very sensitive to changes in the ecosystems state, including pollution. Consequently, they can be used as early indicators of soil quality and health (LI & al. [25]) based on their property of quick response to changes in the environment, in comparison to other parameters (RAO & al. [26]). Due to this property, the study of the extracellular enzymatic activity of the bacterial communities can be used to improve the understanding of the dynamic and role of soils in the evolution of different types of ecosystems.

Conclusions

The intensity of the assessed enzymatic activities registered important variations across the sampling stations in the surveyed area, a fact that could be explained by considering the high variability of soil quality between the selected locations. The highest intensity values were recorded in most of the cases for the alanine aminopeptidase and the lowest for the α-glucosidase enzymatic activity. Generally, considering the entire data set, the enzymes analyzed from the rural soil substrate often showed a higher intensity in comparison with the ones analyzed from the urban areas samples. The pollution from the sites along the trafficked roads or from the central areas of the cities could explain these differences. α GLC and β GLC revealed the highest spatial similarity in terms of their substrate consumption across the sampled sites. Each enzyme presented a specific dynamics in all the three types of studied locations (urban, peri-urban and rural areas).

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References

1. A.D. IGALAVITHANA, S. E. LEE., Y. H LEE, D. C. TSANG, J. RINKLEBE, E. E. KWON., Y. S. OK, Heavy metal immobilization and microbial community abundance byvegetable waste and pine cone biochar of agricultural soils, *Chemosphere* 174: 593–603 (2017).
2. R. DATTA, S. ANAND, A. MOULICK, D. BARANIYA, S. I. PATHAN, K. REJSEK, V. VARNOVA, M. SHARMA, D. SHARMA, A. KELKAR, How enzymes are adsorbed on soil solid phase and factors limiting its activity: A Review, *Int. Agrophys*, 31: 287–302 (2017).
3. Z. A. ZAHIR, M. A. R. MALIK, M. ARSHAD, Soil enzymes research: review, *Online Journal of Biological Science*, 1(5): 299–307 (2001).
4. T. SHERENE, Role of soil enzymes in nutrient transformation: A review, *Bio Bulletin*, 3(1): 109– 131 (2017).
5. H. KIM, A review of factors that regulate extracellular enzyme activity in wetland soils, *Korean Journal of Microbiology*, 51(2): 97-107 (2019).
6. I. G. PETRISOR, I. LAZAR, Emerging Contaminants–The Problem, Examples and Bioremediation AlternativesPart I, *Romanian Biotechnological Letters*, 11: 2693-2701 (2006).
7. R. DICK, E. KANDELER, Enzymes in Soil, Encyclopedia of Soils in the Environment, *Elsevier, Oxford*, 448-456 (2005).
8. J. L. MOREL, C. CHENU, K. LORENZ, Ecosystem services provided by soils of urban, industrial, traffic mining, and military (SUITMAs), *J. Soils Sediments*, 15:1659-1666 (2015).
9. http://www.calitateaer.ro/public/home-page/?__locale=ro, accessed on February 2016.
10. S. ȘTEFĂNUȚ, A. MANOLE, C.M. ION, M. CONSTANTIN, C. BANCIU, M. ONETE, M. MANU, I. VICOL, M. M. MOLDOVEANU, S. MAICAN, I. COBZARU, G. R. NICOARĂ, I. L. FLORESCU, D. E. MOGÎLDEA, M. D. PURICE, D. C. NICOLAE, D. R. CATANĂ, G. TEODOSIU, A.C. DUMITRACHE, M. G. MARIA, C. VÂTCĂ, M. OANȚĂ, K. ÖLLERER, Developing a novel warning-informative system as a tool for environmental decision-making based on biomonitoring, *Ecological Indicators*, 89: 480-487 (2016).
11. OBST U., Test instructions for measuring the microbial metabolic activity in water sample. Anal Chem, 321:166, 168 (1985).
12. A. BANERJEE, S. SANYAL, S. SEN, Soil phosphatase activity of agricultural land: A possible index of soil fertility, *Agricultural Science Research Journal*, 2: 412–419 (2012)
13. L. ZHANG, W. CHEN, M. BURGER, L. YANG, P. GONG, Z. WU, Changes in soil carbon and enzyme activity as a result of different long-term fertilization regimes in a greenhouse field, *PLOS ONE*, 10(2):e0118371 (2015).
14. R. M. NIEMI., I. HEISKANEN, S. SAARNIO, Weak effects of biocharamendment on soil enzyme activities in mesocosms in bare or *Phleum pratense* soil, *Boreal Environ .Res.*, 20: 324–33 (2015).
15. Ř. HAMMER, D.A.T. HARPER, P.D. RYAN, PAST: Paleontological statistics software package for education and data analysis, *Palaeontologia electronica*, 4(1): p. 9 (2001).
16. S. KHAN, Q. CAO, A. E-L. HESHAM, Y. XIA, J. Z. HE, Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb, *Journal of Environment Sciences*, 19: 834–840 (2007).
17. R. G. BURNS, Enzyme activity in soil: location and a possible role inmicrobial ecology, *Soil Biology & Biochemistry*, 14: 423 – 427 (1982).
18. H. AZARBAD, C. A. VAN GESTEL, M. NIKLIŃSKA, R. LASKOWSKI, W. F. RÖLING, N. M. VAN STRAALEN, Resilience of soil microbial communities to metals and additional Stressors: DNA-based approaches for assessing “Stress-on-Stress” responses, *Int J Mol Sci*, 17 :933 (2016).
19. M. J. HOOSTAL, M. G. BIDART-BOUZAT, J. L. BOUZAT, Local adaptation of microbial communities to heavy metal stress in polluted sediments of Lake Erie, *FEMS Microbiol Ecol*, 65: 156-168 (2008).
20. B. V. TANGAHU, S. ABDULLAH, H. BASRI, M. IDRIS, N. ANUAR, M. MUKHLISIN, A review on heavy metals (As, Pb, and HG) uptake by plants throug phytoremediation, *Int. J. of Chemical Engeneering*, 1-31 (2011).
21. E. BŁOŃSKA, J. LASOTA, M. ZWYDAK, The relationship between soil properties, enzyme activity and land use, *Forest Research Papaper*, 78(1):39–44 (2017).
22. A. L. STĂNILĂ, M. DUMITRU, Soils Zones in Romania and Pedogenetic Processes, *Agriculture and Agricultural Science Procedia*, 10: 135-139 (2016).
23. B. VYSLOUŽILOVÁ, D. ERTLEN, D. SCHWARTZ, L. ŠEFRNA, Chernozem. From concept toclassification: a review, *AUC Geographica*, 51: 85–95 (2016).
24. G. ZARNEA, Tratat de microbiologie generală, Vol. V, *eds. Of Romanian Academy*, *Bucharest*, pp. 583 - 611 (1994).
25. T. LI, L. MENG, U. HERMAN, Z. LU, J. CRITTENDEN, A Survey of Soil Enzyme Activities along Major Roads in Beijing: The Implications for

Traffic Corridor Green Space Management, *Int. J. of Environmental Research and Public Health*, 12: 12475–12488 (2015).

26. RAO C. S., GROVER M., KUNDU S., DESAI S., Soil Enzymes, Encyclopedia of Soil Science, Third Edition 2100-2107. (2017).