Enzymological and Physicochemical Evaluation of the Effects of Soil Management Practices

ALINA DORA SAMUEL[1], DELIA MIRELA TIT[2]*, CARMEN ELENA MELINTE (FRUNZULICA)[3,4], CIPRIAN IOVAN[2]*, LAVINIA PURZA[2], MANUEL GITEA[5], SIMONA BUNGAU[2]

[1]University of Oradea, Faculty of Sciences, 1 Universitatii Str., 410087, Oradea, Romania

[2]University of Oradea, Faculty of Medicine and Pharmacy, 29 Nicolae Jiga Str., 410028, Oradea, Romania

[3] University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd. 060474, Bucharest, Romania

[4]Politehnica University of Bucharest, Faculty of Applied Chemistry and Materials Science, Analytical Chemistry and Environmental Engineering Department, 1-7 Gheorghe P. Polizu Str., 011061, Bucharest, Romania

[5]University of Oradea, Faculty of Environmental Protection, 26 Gen. Gh. Magheru Blvd., 410048, Oradea, Romania

Soil enzymes activities have been considered as sensitive indicators of alteration soil quality by management. In order to obtain new data on the soil enzymological effects of soil management practices, we have determined some enzymatic activities in a preluvosoil submitted to a complex tillage and crop rotation experiment at the Agricultural Research and Development Station in Oradea (Bihor County). Actual and potential dehydrogenase, acid phosphatase and catalase activities were investigated in a split plot experiment where tillage (no-till and conventional tillage) was the main plot and crop rotation (Wheat/Maize, W/M; Soybean/Maize, S/M; Oats-Clover/maize, O-C/M) was the subplot. Soil samples were taken at 0-20, 20-40 and 40-60 cm. Each activity in both non-tilled and conventionally tilled soil under maize crop decreased with increasing sampling depth. It was found that no-till, in comparison with conventional tillage, resulted in insignificantly higher ($p>0.10$) soil enzymatic activities in the 0-20 cm layer and insignificantly (at least at $p>0.10$) lower activities in the deeper layers, excepting actual and potential dehydrogenase activities in the 40-60 cm layer, in which these activities were significantly lower($0.02>p>0.01$). Based on the absolute values of the enzymatic activities, the enzymatic indicator of soil quality (EISQ) was calculated. The EISQ values ranged between 0.201 and 0.974 indicating the presence of high enzymatic activities in the upper layer and a moderate intensity of the enzymatic activities in the deeper layers. A significant correlation between soil enzyme activities and physical and chemical indicators was established.

Keywords: soil enzymes, management practice, soil quality indicator

Historically, much attention has been focused on impacts of agriculture on soil erosion and depletion of organic matter. More recently additional attention has been focused on long-term impacts of agriculture on soil biology and biochemical parameters in soils. Biologically mediated processes in soils are central to the ecological function of soils. Soil biotic activity is the driving force in the degradation and conversion of exogenous plant material and anthropogenic depositions, transformations of organic matter, and evolution and maintenance of soil structure [1]. To study biological processes in soils various parameters have been used.

Soil enzymes serve several important functions. They are intimately involved in the cycling of nutrients, effect fertilizer use efficiency, reflect the microbiological activity in soil and act as indicators of soil change [2]. Soil enzymes not only play an active role in influencing soil fertility as a results of their involvement in the cycle of nutrients, which are required for plant growth, but also are sensitive biological indicators for soil quality evaluation besides sensitively reflecting changes in soil environment [3]. Obviously, along with other soil components, enzymes are also influenced by the environmental conditions, so that all the qualities and properties of the soil and the environment quality are reflected in the production of vegetables or fruits and by their nutritional content [4-9]. At the same time, a strong impact on the composition and quality of agricultural soil is caused by inappropriate discharges of domestic waste, animal husbandry, industrial waste (especially chemical, medical and pharmaceutical, petroleum) [10-20], and waste water etc. [21-25].

All soils contain a group of enzymes that determine soil metabolic processes which in turn, will depend on its physical, chemical, microbiological and biochemical properties. The enzyme levels in soil system vary in amounts due to the fact that each soil type has different amounts of organic matter, composition and activity of its living organisms and intensity of biological processes [26]. Therefore, soil enzymes, as an index of soil quality, can reflect changes in soil quality caused by time or other conditions [27]. Enzyme activities in the soil are not only closely related to the factors such as soil type, soil structure, organic matter and pH but also to the kinds of crops which are grown [28].

Management practices (e.g. crop rotation, tillage and application of fertilizers) may have diverse effects on various enzymes [29]. Although the type of tillage may be the dominant factor in creating changes in soil properties, other management and climatic factors also interact with tillage to accentuate or lessen the tillage effects. Crop rotation interacts with tillage to affect soil biological, physical and chemical properties because of the different management variables associated with each crop such as fertilizer and timing of fertilizer applications. Crop rotation also affects several soil properties as a result of the amount and kind of plant residues produced. In general, the effect of crop rotation on soil biological, chemical and physical

* email: mirela_tit@yahoo.com, Phone +40743963812;
dr.iovan@biostandard.ro, Phone: +40744660376

All the authors have equal contribution at this original article.

properties is more evident when coupled with no-tillage than with plowed treatments [30].

Some authors [31] reviewed the current status of work related to the potential uses of soil enzyme measurements and provide examples of ways various soil enzymes may be used to provide practical benefits to agriculture. We have continued to research ways that directly relate the enzyme activity of the soil to soil quality and overall soil sustainability with the goal being a development of useful technologies that are more biologically than chemically and physically based.

Experimental part

Materials and methods

The ploughed layer of the studied soil is of mellow loam texture, it has a *p*H value of 5.5, medium humus (2.32%) and P (22 ppm) contents, but it is rich in K (83 ppm).

The experiment started in 1992. The experimental field occupying 3.84 ha was divided into plots and subplots for comparative study of tillage and crop rotation. Tillage (no-till and conventional tillage) was the main plot and crop rotation (Wheat/Maize, W/M; Soybean/Maize, S/M; Oats-Clover/Maize, O-C/M) was the subplot. The plots were annually NP-fertilized at rates of 120 Kg of N/ha and 90 Kg of P/ha. The plots (and subplots) were installed in three repetitions.

In November 2015, soil was sampled from the 0-20, 20-40 and 40-60 cm depths of the subplots at the end of the maize crop. The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve, and finally used for enzymological analyses.

Actual and potential dehydrogenase activities were determined according to the methods described in [32]. The reaction mixtures consisted of 3.0 g soil, 0.5 mL TTC (2,3,5-triphenyltetrazolium chloride) and 1.5 mL distilled water or 1.5 mL glucose solution, respectively for potential dehydrogenase. All reaction mixtures were incubated at 37°C for 24 h. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically [33], at 485 nm. Dehydrogenase activities were expressed in mg of triphenylformazan (TPF) produced (from 2,3,5-triphenyltetrazolium chloride, TTC) by 10 g soil in 24 h.

Catalase activity was determined using the permanganometric method [32]. The reaction mixtures consisted of 3.0 g soil, 2 mL H_2O_2 3% and 10 mL phosphate buffer. It suffered incubation at 37°C for 1 h. Catalase activity was recorded as mg H_2O_2 decomposed by 1 g of soil in 1 hour.

Disodium phenylphosphate serve as phosphate substrate. One activity was measured: acid phosphatase activity in reaction mixtures to which acetate buffer (*p*H 5.0) was added. The buffer solutions were prepared as recommended by [34]. The reaction mixtures consisted of 2.5 g soil, 2 mL toluene (antiseptic), buffer solution and 10 mL 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the controls. All reaction mixtures were incubated at 37°C for 2 h. After incubation, the phenol released from the substrate under the action of phosphatase was determined spectrophotometrically (at 614 nm) based on the color reaction between phenol and 2.6-dibromoquinone-4-chloroimide. Phosphatase activity was expressed in mg phenol/g soil/2 hours.

The activity values were submitted to statistical evaluation by the two-way-t-test [35].

Results and discussions

Results of the enzymological analyses are presented in table 1, and those of the statistical evaluation are summarized in table 2.

Variation of soil enzymatic activities in dependence of sampling depth

It is obvious from table 1 that each enzymatic activity in both non-tilled and conventionally tilled plots under maize crop of all rotations decreased with increasing sampling depth. In addition, table 2 shows that the mean values of each of the four activities in both non-tilled and conventionally tilled plots also decreased with increasing soil depth.

The effect of tillage practices on the enzymatic activities in soil

Each of the four enzymatic activities determined was insignificantly higher ($p>0.10$) in the upper (0-20 cm) layer of the non-tilled subplots than in the same layer of the conventionally tilled plots. The reverse was true in the deeper (20-40 and 40-60 cm) layers, excepting, actual and potential dehydrogenase activities which were significantly higher ($0.02>p>0.01$) in the 40-60 cm in the conventionally tilled than of the non-tilled subplots.

Table 1

ENZYME ACTIVITIES IN A PRELUVOSOIL UNDER DIFFERENT TILLAGE AND CROP ROTATIONS SYSTEMS

Crop rotations*	Soil enzymatic activity**									
	Dehydrogenase [mg TPF/10 g soil/24 hours]				Acid phosphatase [mg phenol/ g soil/2 hours]		Catalase [mg H_2O_2/ g soil/hour]		EISQ***	
	Actual		Potential							
	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
Depth 0-20 cm										
W/M	7.27	6.91	21.92	20.35	4.57	4.02	3.51	3.37	0.843	0.818
S/M	8.88	7.84	21.89	21.12	5.52	4.45	3.87	3.81	0.960	0.888
O-C/M	9.18	8.78	22.98	17.44	4.90	4.78	4.20	4.94	0.971	0.974
Depth 20-40 cm										
W/M	4.77	5.04	13.42	14.52	3.66	3.83	2.93	2.24	0.616	0.639
S/M	5.44	6.84	13.81	14.03	3.36	3.79	3.33	3.48	0.670	0.735
O-C/M	6.94	7.31	12.42	13.21	3.82	3.90	3.56	3.63	0.708	0.758
Depth 40-60 cm										
W/M	1.12	1.75	3.35	4.04	1.60	1.23	1.46	1.64	0.201	0.403
S/M	1.92	2.40	4.65	5.29	1.17	1.23	1.71	1.76	0.257	0.433
O-C/M	1.26	2.24	5.28	5.60	1.43	1.53	1.93	1.97	0.271	0.309

**W – Wheat; M – Maize; S – Soybean; O-C – Oats-Clover; **N.t. – No-till; C.t. – Conventional tillage;*
****EISQ – Enzymatic indicators of soil quality.*

Table 2

SIGNIFICANCE OF THE DIFFERENT BETWEEN ENZYMATIC ACTIVITIES IN A PRELUVOSOIL SUBMITTED TO DIFFERENT TILLAGE AND CROP ROTATION SYSTEMS

Management practices	Soil enzymatic activity*	Soil depth [cm]	Mean activity values in management practices			Significance of the differences
			a	b	a-b	
No-till (a) vs. conventional tillage (b)	ADA	0-20	8.44	7.05	1.39	p>0.10
		20-40	5.71	6.39	-0.68	p>0.10
		40-60	1.43	2.13	-0.70	0.02>p>0.01
	PDA	0-20	22.26	19.63	2.63	p>0.10
		20-40	13.21	13.92	-0.71	0.10>p>0.05
		40-60	4.42	4.97	-0.55	0.02>p>0.01
	AcPA	0-20	4.99	4.41	0.58	p>0.10
		20-40	3.61	3.84	-0.23	p>0.10
		40-60	1.22	1.33	-0.11	p>0.10
	CA	0-20	3.86	4.04	-0.18	p>0.10
		20-40	3.27	3.11	0.16	p>0.10
		40-60	1.70	1.79	-0.09	0.10>p>0.05
The same crop in the three rotations						
W/M (a) vs. S/M (b)	ADA	0-60	4.47	5.49	-1.02	0.01>p>0.002
	PDA		12.93	13.79	-0.86	0.05>p>0.02
	AcPA		3.06	3.25	-0.19	0.10>p>0.05
	CA		2.52	5.98	-3.49	0.05>p>0.02
W/M (a) vs. O-C/M (b)	ADA		4.47	5.61	-1.14	0.05>p>0.02
	PDA		12.93	12.87	0.06	0.002>p>0.001
	AcPA		3.06	3.39	-0.33	0.02>p>0.01
	CA		2.52	3.37	-0.85	001>p>0.002
S/M (a) vs. O-C/M (b)	ADA		5.49	5.61	-0.12	0.05>p>0.02
	PDA		13.79	12.87	0.92	0.05>p>0.02
	AcPA		3.25	3.39	-0.14	0.01>p>0.002
	CA		5.97	3.37	2.61	0.10>p>0.05

**W – Wheat; M – Maize; S – Soybean; O-C – Oats-Clover.*

Our observation of higher soil enzyme activities under N.t. than C.t. is in agreement with other studies. For example, [36] who observed the 46% increase of acid phosphataseactivity due to N.t. in the surface layer showed that this enzyme is sensitive to disturbance. In addition, [37] indicated that tillage is the critical factor in sequestering C and microbial activities. Disturbance had a greater impact than the type of C inputs from crop rotations for maintaining or improving soil microbial biomass and activity. The increase in soil enzyme activities may be the result of soil physical and chemical changes so there is a direct expression on microbial biomass and soil enzyme activities. One argument, which can explain the increase in soil enzyme activities due to tillage, is that no-tillage can improve the microbial habitat [38]. Other studies showed that no-tillage system compared with conventional tillage increased size of macro aggregates [39]. The formation and stabilization of macro aggregates under N.t. soil represent an important mechanism for the protection and maintenance of soil organic matter than be lost under C.t. practices. Thus, macro aggregates provide an important microhabitat for microbial activity [40].

The effect of crop rotations on the enzymatic activities in soil

For evaluation of this effect, the results obtained in the three soil layers analyzed were considered together.

Soil enzyme activities as affected by the same crop in the three rotations. Actual and potential dehydrogenase and catalase activities were significantly higher (at least at $p<0.05$), while acid phosphatase activity was insignificantly higher ($0.10>p>0.05$) in the W/M crop rotation than in the S/M rotation. The soil under W/M rotation, was more enzyme active than in the O-C/M rotation. The difference between the two rotations was significant higher ($p<0.05$) in the case of each activity. In the soil of maize in which the previous crop was soybean, dehydrogenase and acid phosphatase activities were significantly higher ($p<0.05$ and $p<0.01$, respectively), while catalase activity was insignificantly higher ($0.10>p>0.05$) than in maize in which the previous crop was oats-clover.

As compared with the data in the literature [41], we may consider that crop rotations have significantly higher levels of microbial biomass and soil enzyme activities. Continuous monoculturing of a single crop species typically results in reduction of crop yields in comparison to the same species in rotation and these reductions usually are not associated with fertility. It has been suggested that alleopathic toxins derived from decomposing plant residues may inhibit yields, so there is increasing evidence that the *rotation effect* is due to the suppression of deleterious microorganism that build up under continuous cropping.

Enzymatic indicators of soil quality.

In order to establish a hierarchy of plots in all crop rotation and considering that all four enzymatic activities have equal importance, we have calculated the enzymatic indicators of soil quality (EISQ) [42].

The maximum individual value, calculated from the composition of the reaction mixtures are: 21.56 mg phenol (phosphatase activities), 60 mg splitted H_2O_2 (catalase activity), and 13.45 mg formazan (dehydrogenase activity). We mention that the enzymatic indicator may have values ranging between 0 (when no real activity of any of the studied enzymes detected) and 1 (when all the activities have real individual values equal to the maximum theoretic values).

The enzymatic potential of soils defined by the values of the quality enzymatic indicators is represented in figures 1-3.

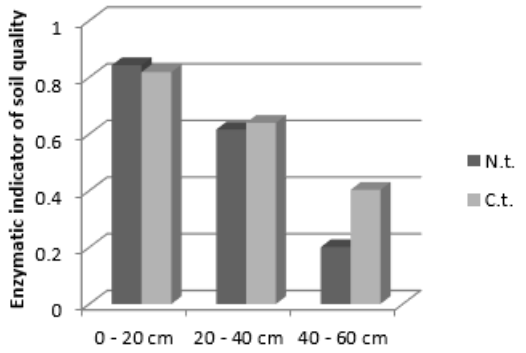

Fig. 1. The enzymatic potential of preluvosoil in W/M rotation

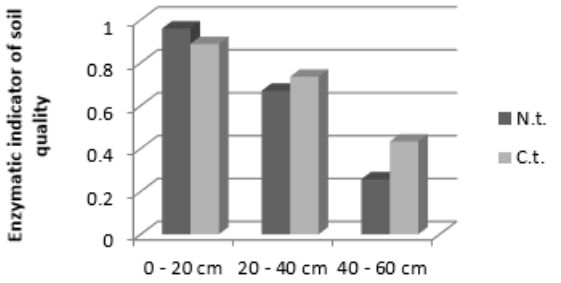

Fig. 2. The enzymatic potential of preluvosoil in S/M rotation

All three figures illustrate that only in the 0-20 and 20-40 cm layers, soil exceeds the 0.61 value of the EISQ, and the value of soil from 40-60 cm is lower than 0.43 of the EISQ. The enzymatic indicators of the analyzed soil quality offers an overall image on the intensity of the enzymatic activity, of the general biological activity. Based on the results and in comparison with data in the specialty literature [43, 44]

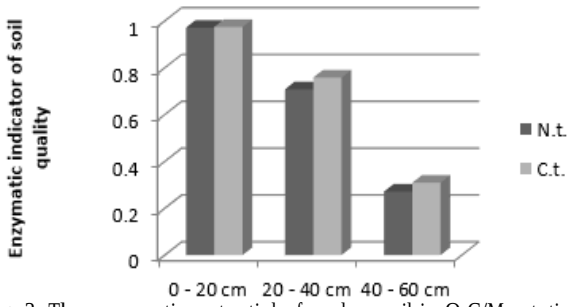

Fig. 3. The enzymatic potential of preluvosoil in O-C/M rotation

we may consider that the analyzed soil have an appreciable biological potential. Only the soil in the 40-60 cm has lower values of the enzymatic quality indicators, which set the basis for this appreciation.

Relationships between enzymatic activities and physical and chemical properties

Physical and chemical parameters determined are presented in table 3 and 4.

The correlation coefficient (r) across tillage, W/M crop rotation and depths was used to quantify the strength of relationships existing among enzymes, and of enzymes versus the physical and chemical properties (table 3 and 4). For establishing the relationships, the results obtained in the three soil layers analyzed were considered together.

It is evident from table 5 that the activities of all four enzymes were significantly inter correlated which suggests that tillage and crop rotations systems have similar effects on the activities of those enzymes involved in intracellular metabolism and in P cycling in soil.

Table 3
PHYSICAL PROPERTIES IN A PRELUVOSOIL UNDER DIFFERENT TILLAGE AND CROP ROTATION (W/M) SYSTEMS SOIL DEPTH[cm]

Soil depth [cm]	Physical properties*					
	Soil density [g/cm^3]		Resistance to penetration [kg/cm^2]		Coefficient of filtration [mm/h]	
	N.t	C.t	N.t.	C.t	N.t.	C.t.
0-20	1.40	1.39	28.36	28.16	11.83	6.83
20-40	1.49	1.40	41.70	35.36	11.73	5.37
40-60	1.54	1.46	50.66	44.13	5.26	5.43

Table 4
CHEMICAL PROPERTIES IN A PRELUVOSOIL UNDER DIFFERENT TILLAGE AND CROP ROTATION (W/M) SYSTEMS

Soil depth [cm]	Chemical properties*							
	pH		$N\text{-}NO_3$ [mg N/kg soil]		$N\text{-}NH_4$ [mg N/kg soil]		P_2O_5 [mg P_2O_5/100g soil]	
	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
0-20	5.36	5.43	0.43	0.34	1.32	0.69	12.33	10.86
20-40	5.27	5.53	0.40	0.19	1.27	0.38	11.96	8.70
40-60	5.37	5.53	0.31	0.27	0.61	0.40	11.20	9.56

**$N\text{-}NO_3$ – Nitrate content; $N\text{-}NH_H$ – Ammonium content; P_2O_5 – Mobile phosphorus content.*

Table 5
SIMPLE CORRELATIONS (r) BETWEEN SOIL ENZYME ACTIVITIES, PHYSICAL AND CHEMICAL PROPERTIES IN 0-60 cm

Variables	Actual dehydrogenase (ADA)	Potential dehydrogenase (PDA)	Acid phosphatase (AcPA)	Catalase (CA)
ADA	-	-	-	-
PDA	0.998*	-	-	-
AcPA	0.970*	0.966*	-	-
CA	0.956*	0.954*	0.912*	-
Physical properties				
SD	-0.813*	-0.800	-0.753	-0.655
RP	-0.954*	-0.950*	-0.880*	-0.869*
CF	0.837*	0.854*	-0.695	-0.804
Chemical properties				
pH	-0.920*	-0.913*	-0.870*	-0.940*
$N\text{-}NO_3$	0.988*	0.994*	0.968*	0.931*
$N\text{-}NH_4$	0.929*	0.941*	0.823*	0.908*
P_2O_5	0.985*	0.977*	0.959*	0.972*

**Significantly at $p \leq 0.05$*

The enzymes showed similar sensitivity to the same physico-chemical variable. One of the most important soil properties is pH. Soil pH affects the activity of enzymes due the *p*H sensitivity of amino acid functional groups that alter conformational and chemical changes of amino acids essential for binding and catalysis. The pH can also affect availability of nutrients, controls the composition and diversity of the microbial community, alters the equilibrium solid phase and impacts plant response. In our study, a remarkable feature is that all enzyme activities were significantly negatively and strongly correlated with pH. Other physico-chemical variables such as RP, $N\text{-}NO_3$, $N\text{-}NH_4$, and P_2O_5 also exhibited strong and positive significant relationship with enzymes.

Decisively, an assessment is needed of the multiplicity of physical, chemical and biological factors that control biogeochemical processes, along with their variations in time and space. Indeed, management practices influence correlation among enzymes and, between enzymes and physico-chemical factors.

Conclusions

Stable plant production is dependent on soil structure, organic matter, and nutrient cycling which is a function of chemical, physical and biological properties.

Many soil enzymes, including those in this study, can exist in viable microbial cells and as free enzymes completed by soil mineral and humic substances. Thus, enzyme activity represents the cumulative effect of past management practices on soil biology. Management practices that include crop rotations with legumes and minimal disturbance by tillage can maintain soil biological activities and organic matter for very long periods of time.

Physical and chemical properties have been extensively used to measure soil quality. However, these properties usually change on decades, which is too long for management practices. In contrast, soil properties based on biological and biochemical activities, such as soil enzymes, have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality. Therefore, soil enzyme activities have been suggested as suitable indicators of soil quality because of their intimate relationship with soil biology, ease of measurement and rapid response to change in soil management.

References

1.DICK, W.A., Soil Sci. Soc. Am. J., 48, 1984, p. 569.
2.DICK, W.A., CHENG, L., WANG, P., Soil Biol. Biochem., 32, 2000, p.1915.
3.YANG, L., ZHANG, Y., LI, F., Advances in Citrus Nutrition, Springer, SRIVASTAVA, A.K., (Ed.), Netherlands, 2012, p.143-156.
4.BUNGAU, S., BALDEA, I., COPOLOVICI, L., Rev. Chim.(Bucharest), **54**, no. 3, 2003, p.213.
5.COPOLOVICI, D., BUNGAU, S., BOSCENCU, R., TIT, D.M., COPOLOVICI, L., Rev. Chim.(Bucharest), **68**, no. 3, 2017, p. 507.
6.PALLAG, A., BUNGAU, S. G., TIT, D.M., JURCA, T., SIRBU, V., HONIGES, A., HORHOGEA, C., Rev. Chim.(Bucharest), **67**, no. 3, 2016, p. 530.
7.VICAS, S., BANDICI, L., TEUSDEA, A., TURCIN, V., POPA, D., BANDICI, G., CyTA – J. Food, 2017, p. 1. http://www.tandfonline.com/doi/full/10.1080/19476337.2017.1317667
8.SOCACI, S., SOCACIU, C., MURESAN, C., FARCAS, A., TOFANA, M., VICA[a], S.I., PINTEA, A., Phytochem. Analysis, **25**, nr. 2, 2014, p. 161.
9.MASU, S., NICORESCU, V., POPA, M., J. Environ. Prot. Ecol., **16**, nr. 3, 2015, p. 968.
10.BUNGAU, S., BUNGAU, C., TIT, D.M., J. Environ. Prot. Ecol., **16**, nr. 1, 2015, p.56.
11.TIT, D.M., BUNGAU, S., NISTOR CSEPPENTO, C., COPOLOVICI, D.M., BUHAS, C., J. Environ. Prot. Ecol., **17**, nr. 4, 2016, p.1425.
12.POPESCU, D.E., BUNGAU, C., PRADA, M., DOMUTA, C., BUNGAU, S., TIT, D.M., J. Environ. Prot. Ecol., **17**, nr. 3, 2016, p. 1011.
13.BUNGAU, S., SUCIU, R., BUMBU, A., CIOCA, G., TIT, D.M., J. Environ. Prot. Ecol., **16**, nr. 3, 2015, p. 980.
14.BALINT, R., NECHIFOR, G., AJMONE-MARSAN, F., Environ. Sci.-Processes & Impacts, **16**, nr. 2, 2014, p. 211.
15.BALINT, R., ORBECI, C., NECHIFOR, G., PLESCA, M., AJMONE-MARSAN, F., Rev. Chim.(Bucharest), **64**, no. 11, 2013, p. 1218.
16.DIACONU, I., GIRDEA, R., CRISTEA, C., NECHIFOR, G., RUSE, E., TOTU, E.E., Rom. Biotech. Lett., **15**, nr. 6, 2010, p. 5702.
17.DUMITREL, G.A., GLEVITZKY, M., POPA, M., CHIRILA, D., PALEA, A., J. Environ. Prot. Ecol., **18**, nr. 1, 2017, p. 55.
18.VICA, M., POPA, M., DUMITREL, G.-A., GLEVITZKY, M., TODORAN, A., J. Environ. Prot. Ecol., **15**, nr. 1, 2014, p. 64.
19.MASU, S., DRAGOMIR, N., POPA, M., J. Environ. Prot. Ecol., **14**, nr. 3, 2013, p. 1901.
20.POPA, M., GLEVITZKY, M., POPA, D., VARVARA, S., DUMITREL, G.-A., J. Environ. Prot. Ecol., **13**, nr. 4, 2012, p. 2123.
21.DUMITREL, G.-A., GLEVITZKY, M., POPA, M., VICA, M. L., J. Environ. Prot. Ecol., **16**, nr. 3, 2015, p. 850.
22.POPA, M., VINTAN, D., ROXANA, B., POPA, D., J. Environ. Prot. Ecol., **15**, nr. 1, 2014, p. 53.
23.POPA, M., GLEVITZKY, M.., POPA, D.M., DUMITREL, G.-A., J. Environ. Prot. Ecol., **15**, nr. 4, 2014, p. 1543.
24.GLEVITZKY, M., VICA, M, POPA, M., AXINTE, R., VARVARA, S., J. Environ. Prot. Ecol., **12**, nr. 3, 2011, p.1110.
25.ORBECI, C., NECHIFOR, G., STANESCU, R., Environ. Eng. Manag. J., **13**, nr. 9, 2014, p. 2153.
26.YANG, W.Q., WANG, K.Y., Chin. J. Appl. Environ. Biol., **8**, nr. 5, 2002, p.564.
27.CAO, H., SUN, H., YANG, H., SUN, B., ZHAO, Q., Chin. J. Appl. Environ. Biol., **9**, nr. 1, 2003, p. 105.
28.DICK, R.P., SANDOR, J.A., EASH, N.S., Agric., Ecosyst., Environ., 50, 1994, p.123.
29.DICK, R.P., RASMUSSEN, P.E., KERLE, E.A., Biol. Fertil. Soils, 6, 1988, p.156.
30.DICK, W.A., DANIEL, T.C., Effects of Conservation Tillage on Groundwater Quality- Nitrates and Pesticides, Lewis Publishers, LOGAN, T.J., DAVIDSON, J.M., BAKER, J.L., OVERCASH, M.R., (Eds.), Chelsea, 1987, p.125-147.
31.DICK, W.A., TABATABAI, M.A., Soil Microbiology Ecology: Applications in Agricultural and Environmental Management, Marcel Dekker, METTING F.B., (Ed.), New York, 1992, p.95-127.
32.DRAGAN-BULARDA, M., Microbiologie generala – lucrari practice. Universitatea Babes-Bolyai, Cluj-Napoca, 2000.
33.BUNGAU, S., COPOLOVICI, D., COPOLOVICI, L., Instrumental Analytical Methods / Metode instrumentale de analiza, Italian Academic Publishing, 2015.
34.ÖHLINGER, R., Methods in Soil Biology, Springer, SCHINER, F., OHLINGER, R., KANDELER, E., MARGESIN, R., (Eds.), Berlin, 1996, p. 241-243.
35.SACHS, L., Angewandte Statistik Anwerdung Statisticher Methoden, Springer, SACHS, L., (Ed.), Berlin, 2002, p.189-195.
36.BALOTA, E.L., COLOZZI-FILHO, A., ANDRADE, D.S., DICK, R.P., Soil Tillage Res., **77**, 2004a, p.137.
37.BALOTA, E.L., KANASHIRO, M., COLOZZI-FILHO, A., ANDRADE, D.S., DICK, R.P., Braz. J. Microbiol., 35, 2004b, p. 300.
38.SAMUEL, A.D., DOMUA, C., CIOBANU, C., 'ANDOR, M., Rom. Agric. Res., 25, 2008, p.61.
39.CASTRO FILHO, C., LOURENCO, A., GUIMARAES, M., FONSECA, I.C.B., Brazil. SoilTill. Res., 65, 2002, p. 45.
40.DICK, R.P., Agric., Ecosyst., Environ., 40,1992, p. 25.
41.DENG, S.P., TABATABAI, M.A., Biol. Fertil. Soil, 24, 1997, p. 141.
42.MUNTEAN, V., CRISAN, R., PASCA, D., KISS, S., DRAGAN-BULARDA, M., Int. J. Salt Lake Res., **5**, nr. 1, 1996, p. 35.
43.BALDRIAN, P., Plant Soil Environ., **55**, nr. 9, 2009, p. 370.
44.ACOSTA-MARTINEZ, V., TABATABAI, M.A., Biol. Fertil. Soils, 31, 2000, p. 85.

Manuscript received: 15.04.2017