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Influence of salt content on enzymatic activities and halophytes distribution in Cojocna zone, Romania

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SUMMARY. Salty soil samples from three zones of Cojocna (Cluj county, Romania) have been investigated physico-chemically and enzymologically. Rizosphere of some halophytes were investigated in order to assess saline stress on bacterial enzyme activities and halophytes adaptation mechanisms to enhanced salt content. Several physico-chemical parameters of salty soil were determinated: pH, conductivity and different mineral ion contents. Changeful bacterial enzymatic activities and mineral ion composition were detected according to soil salt content. Reduced values of all enzymatic activities were detected. Based on the studied enzyme activities the enzymatic indicators of soil quality (EISQ) were calculated. The EISQ values were low, between EISQ = 0.201 and 0.236. In the sudied salty area, only a few halophyte species were observed and identified, the most common are: *Salicornia herbacea, Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda.* All three salty zones were classified as sodic and saline-sodic soils.

Key words: Cojocna, enzymatic activities, halophyte, salty soil.

Introduction

Soil enzymes have a very active contribution in soils, being involved in biogeochemical cycles, energy metabolism, pollution removal and other main biogeochemical processes of soil ecosystem (Caldwell, 2005). Soil microorganisms,

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along with their activities, have a fundamental contribution to the biotic community and the ecosystem functioning in natural terrestrial habitats. All types of soil contain a bulk of enzymes which triggers the soil productivity, physical and chemical properties, and health. They play a main role in the decomposition of organic matter (Sinsabaugh *et al.*, 1991). The amount of enzymes varies according to the type, constituents, organic matter content and biota of soil and heir biological functions. It is known that microorganisms contribute to soil enzyme composition, but this composition is completed by animals and plants. Soil enzyme activity constitutes a main indicator for soil quality, especially regarding different types of pollution (Yang *et al.*, 2016). High-salinity in soil, sometimes caused by saline groundwater, impedes the germination and growth of plants (Zhang *et al.*, 2015; Guo and Liu, 2014). Beside high salinity, lack of macronutrients also characterize the saline soils and causes their limitation.

Soil enzymes catalyze the organic matter decomposition, and their activity is in tight relation with the physical and chemical properties of soil (Kussainova *et al.*, 2013), structural composition of microbiota (Nielsen *et al.*, 2014) or with the vegetation (Mierzwa-Hersztek *et al.*, 2016). Both the composition of microbiota and the enzymatic activity are main qualitative indicators of soil quality, but the generation and functioning mechanisms of soil bacterial community are yet to be fully understood (Xun *et al.*, 2015).

This study aims to assess the salty soil enzymatic activity, the micro- and macro elements in it, which underlie the functioning of saline soil ecosystems. Such parameters have been prior used to assess the status and productivity of soil in natural ecosystems.

Materials and methods

Study site and soil sampling. The field site is located in the Transylvanian Plateau, East of Cluj-Napoca city at an elevation about 330 m. Cojocna zone is representative for the salty areas in the Transylvanian Plateau. The climate is temperate-continental. The prevalent halophytes are *Salicornia herbacea, Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda*, with a coverage of approximately 50% in Cojocna areas.

Soil samples were collected in 2016 from Cojocna. The three collection sites were: 1) N46.74304 E23.84265, 2) N46.74319 E23.84290 and 3) N46.74328 E23.84295. The soil samples were passed in a portable storage box and transported into the laboratory. The soil samples used for measuring enzymatic activity and physico-chemical properties were air-dried and then temporarily stored at 4 ºC, for further analyses.

Physical and chemical properties of soils. After removing the grass and other external objects, soil samples were air-dried, ground and sieved (<2 mm) prior to determination of available nutrients and soil characteristics. The salinity, electrical conductivity (EC), pH and redox potential (Eh) of the soils were potentiometrically measured in aqueous fraction (1:5) (ISO- 10390). Mineral N (NO_3^-N and NH_4-N), available phosphorus (PO_4), available potassium (K), sodium (Na) and other micro- and macro-elements of the soil were determined by ion chromatography (Ion Chromatograph IC 1500 DIONEX).

In order to determine the elements above were used: Ultra-pure water (0.055 μS/cm; 18.2 MΩ/cm), free of oxidants, purified using the Ultra Clear TWF UV system (SG GmbH, Germany); Elution solution for cations: 20 mM methanesulfonic acid (99.0%) (Fluka, Germany); Eluent solution for anions: 4.5 mM Na_2CO_3/0.8 mM $NaHCO_3$ (Dionex, USA). The analyze procedure followed strictly the conditions mentioned in the IC 1500 DIONEX Chromatograph Operating Manual as well as the specific standard procedures (US-EPA 1993; ASTM 1999; Jackson, 2000; US-EPA 2007).

Enzymatic activities in soil samples. Activities of the following four enzymes in soil were measured: phosphatase, catalase, actual and potential dehydrogenase (Alef and Nannipieri, 1995; Carpa *et al.*, 2014).

Dehydrogenase activity (actual and potential) was determined after 24 h incubation of the soil samples at 37 ºC with TTC solution, and expressed by the amount of the formed 2,3,5-triphenylformazan (mg formazan/g soil). Enzymatic activity of dehydrogenases was determined by spectrophotometry using an Able Jasco V530 spectrophotometer at 440 nm wavelength.

Phosphatase activity was determined after 24 h incubation of the soil samples at 37 ºC with phenyl phosphate disodic solution, and it is expressed in mg phenol/g soil. Phosphatase activity was determined by using an Able Jasco V530 spectrophotometer at 620 nm wavelength.

Catalase activity was determined after 1 h incubation of the soil samples at room temperature with H_2O_2 solution. The residual H_2O_2 is determined by titration with $KMnO_4$ in the presence of H_2SO_4. Catalase activity was expressed in mg split H_2O_2/g soil.

The analytical data serves as the base for calculating the enzymatic indicator of the soil quality (EISQ) (Muntean *et al.*, 1996).

Results and discussions

Halophytes in Cojocna zone. The vegetation growing in salty zones may be very diverse in some part of world, while in other regions they are dominated by a few halophytic species (Isacch *et al.*, 2006). Halophytes, the vegetation of saline habitats, are a specialized plant group, characterized by the possession of great osmotic tolerance (Chaudhary *et al.*, 2015). *Salicornia herbacea, Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda* are dominant species in the salty zone of Cojocna (Fig. 1).

Figure 1. Saline soil in Cojocna zone. *Limonium gmelinii* and *Artemisia santonica*

Physico-chemical analyses of soil samples. The categories in which soils affected by salt are divided are as follows: saline soils, saline-sodic soils and sodic soils (Table 1) (Brady and Weil, 2002; Eynard *et al*., 2006). This division takes into account the electrical conductivity (EC), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR).

Table 1.

Classification of salt-affected soils, according to the electrical conductivity (EC), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) values (Eynard *et al*., 2006).

Type of soil	EC (dS/m)	ESP (%)	SAR ($mmol^{0.5}/L^{0.5}$)
Non saline and non sodic soil	< 4	< 15	< 13
Saline soil	> 4	< 15	< 13
Saline-sodic soil	> 4	> 15	> 13
Sodic soil	< 4	> 15	> 13

The analyzed soils according to EC, ESP and SAR are presented in the following table (Table 2). Sodium adsorption ratio (SAR) was calculated online (http://turf.okstate.edu/water-quality/sar-calculator) and for ESP using the formula (http://www.iadclexicon.org/exchangeable-sodium-percentage-esp/):

$$EPS = \frac{[100(-0.0126 + 0.01475 \times SAR)]}{[1 + (-0.0126 + 0.01475 \times SAR)]}$$

Table 2.

Classification of analyzed soils, according to to the electrical conductivity (EC), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) values

Sample	EC (dS/m)	ESP (%)	SAR ($mmol^{0.5}/L^{0.5}$)	Type of soil
1	0.927	36.06649	39.1	Sodic soil
2	3.140	48.42104	64.5	Sodic soil
3	4.450	54.63361	82.5	Saline-sodic soil

Physico-chemical parameters detected in salty soil from Cojocna are presented in Table 3.

Table 3.

Physico-chemical parameters of salty soils from Cojocna

Sample	pH	Conductivity (µS/cm)	Na	Cl	NH_4	NO_3 (mg/kg)	K	Mg	Ca	SO_4
1	8.84	927	951.6	912.6	0.0	18.3	59.0	5.4	35.0	89.4
2	9.21	3140	2240.8	3566.7	0.2	26.2	188.0	24.0	51.4	105.7
3	9.09	4450	3785.2	6049.4	0.4	53.4	247.7	40.8	92.2	229.0

Sodium chlorides and sulphates, calcium, kallium and magnesium are the most commonly present soluble salts. Nitrates also may be present or not. Sodium and chloride are the principal ions encountered in highly saline soils, but the present calcium and magnesium serve as nutritional elements for plants.

Saline soils have poor macronutrient availability and high soil salinity (Zhang *et al*., 2015). The high content of Na and Cl in all three samples indicates the presence of salt (NaCl) in soil, from salt deposits in the geological substrate (Fig. 2a). The high salt content along with alkaline pH affects plant growth by inhibiting nutrient absorption (K, Mg, Ca) at the root level (Fig. 2b,d). The vast majority of nutrients are absorbed by plants in a neutral, slightly alkaline pH environment.

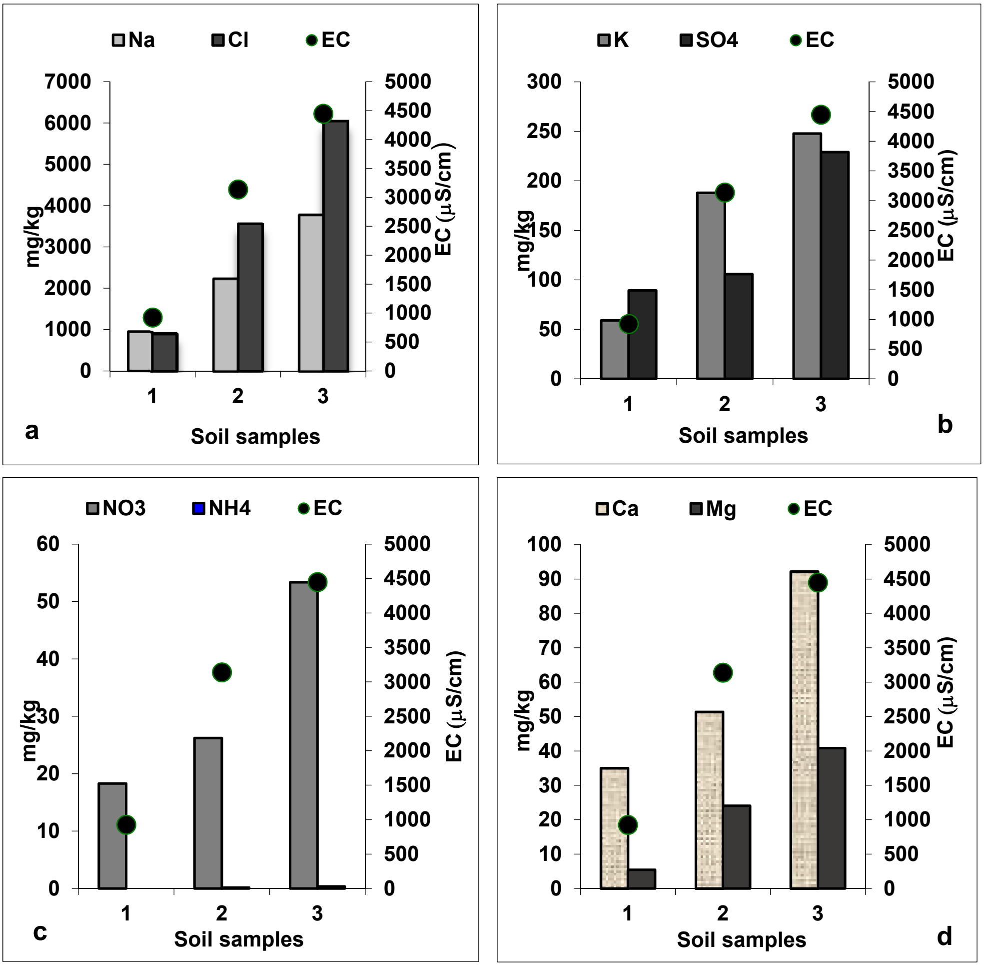

Figure 2. Micro- and macroelements content of soil samples
a=Na, Cl; b=K, PO_4; c=NH_4, NO_2, NO_3; d=Mg, Ca, SO_4.

Available nitrogen is taken up by plant roots in the form of (NO_3^-) and (NH_4^+) (Fig. 2c). The available forms of nitrogen are very water soluble and move rapidly through the soil profile with rainfall and irrigation. Changing and soluble ammonium is found in the upper horizon of soils, typically in small and relatively constant amounts, below 10 mg N-NH_4/kg (Vintila *et al.*, 1984). The nitrate content is less than 60 mg/kg in all analyzed samples, which is a normal value. At persistent amounts of more than 100 mg N-NO_3/kg, the phenomenon of nitrate pollution of the soil, plant and groundwater can occur (Lăcătuşu *et al.*, 2000).

Enzymatic activities in soil. The activity of enzymes is dependent on the physico-chemical properties of the soil. In salinized soils the soil enzyme activity is heavily hindered, which indicate a high level of stress in those soils (Chaudhary *et al*., 2015). This activity is directly related to the active biomass in soil, and both are precise indicators for soil quality (Stepniewska *et al*., 2009). The activities of enzymes in halophytic soil are depicted in Table 4.

Table 4.

Enzymatic activities in soil samples from Cojocna

Samples	Enzymatic activities				EISQ
	Dehidrogenases activities (mg formazan/g soil)		Phosphatase activity (mg fenol/g soil)	Catalase activity (split H_2O_2/g soil)	
	actual	potential			
1	0.301	1.016	0.248	49.98	0.236
2	0.101	0.439	0.249	44.88	0.201
3	0.216	0.338	0.140	46.07	0.204

Actual and potential dehydrogenases activities. The whole amount of oxidative activity performed by the microbial consortia in soil is assessed by dehydrogenase activity, so it represent a reliable indicator of microbial activity in soil (Kussainova *et al.,* 2013).

Dehydrogenase activity is growing by increasing the organic matter in the soil. Actual dehydrogenase activity reaches maximum values of 0.301 mg formazan/g. soil in the Sample 1. The minimum value was 0.101 mg formazan/g. soil recorded for the Sample 2. The potential dehydrogenase activity (PDA) presented much higher values for all zones studied due to the carbon source (glucose) added (Fig. 3).

The dehydrogenase activity is the result of the action of living microorganisms and the proliferation capacity. There is a strong relationship between the number of microorganism in the soils and dehydrogenase activity.

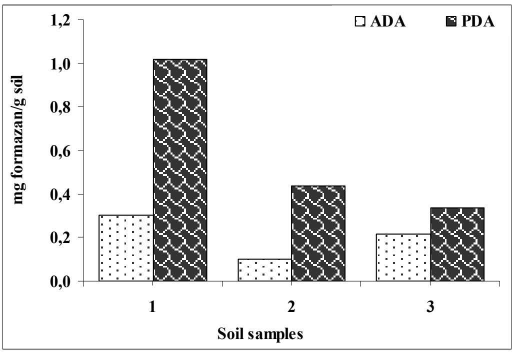

Figure 3. Actual and potential dehydrogenases activities(ADA and PDA) in soil samples from Cojocna

Phosphatase and catalase activities. Plants need free, available phosphate, which is released mainly by phosphatases, from the decomposition of organic matter. These enzymes can be classified in rhizosphere phosphatases and nonrhizosphere soil phosphatases, the first ones having a more intense activity (Chen *et al.,* 2012). The solubility of organic P compound is another factor influencing the activity of these enzymes (Zhu *et al*., 2017). The spatial distribution of phosphatases in soils influences this enzyme activity (Story and Brigmon, 2017). Phosphatase activity from Cojocna salty zones is quite uniform in samples 1 and 2. Slightly lower values came from the Sample 3, populated with *Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda*, where the maximum value was 0.14 mg phenol/g. soil (Fig. 4a).

Dehydrogenase and phosphatase are considered enzymes which play key roles in the metabolic functions of bacteria in their habitats (Huang *et al*., 2009). Thus, these enzymes can provide usefull information regarding the efects of environmental changes (Zheng *et al*., 2017).

The intracellular enzyme catalase is present in all aerobic bacteria and most of the facultative anaerobes, but is not present in obligate anaerobes (Alef and Nannipieri, 1995). The number of aerobic microorganisms in soil and fertility of the soil are in conjunction with catalase activity, enzyme activity which indicate the aerobic microbial activity (Trasar-Cepeda *et al*., 1999). Catalase activity is a very stable activity in soil and decreases with depths, similar to the content of organic carbon (Alef and Nannipieri, 1995).

Catalase activity from Cojocna salty zones is quite uniform in all samples. Slightly higher values came from the Sample 1, populated *Artemisia santonica* and *Limonium gmelinii* where the maximum value was 49.98 mg H_2O_2/g. soil (Fig. 4b). Catalase activity is very stable in soil and has a significant corelation with the organic carbon content and the depth of soil (Alef and Nannipieri 1995).

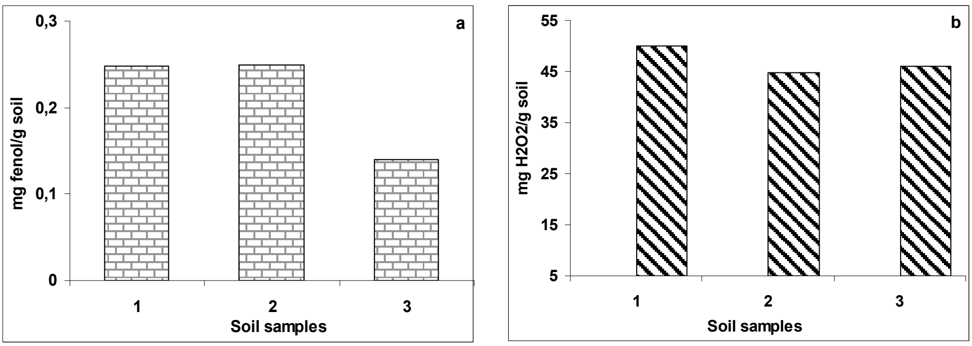

Figure 4. Phosphatase (a) and catalase (b) activities in soil samples from Cojocna

Enzymatic Indicator of Soil Quality (EISQ). Assessing the enzyme activities in soils constitute a research instrument for evaluating the functional diversity of microbiota and the biochemical processes in these habitats. Theoretically, the enzymatic indicator can have values between 0 (when there is no activity in any sample) and 1 (when all the real individual values are equal to the maximal theoretical individual one). The absolute values of enzyme activities in the studied salty soils are presented in Fig. 5. It can be noticed that the Cojocna salty soils have a lower enzymatic indicator. The maximum values were found in Samples 1 (0.236). The quality of these salty soils are lowest, and it is known that the quality of a soil is better the higher EISQ gets.

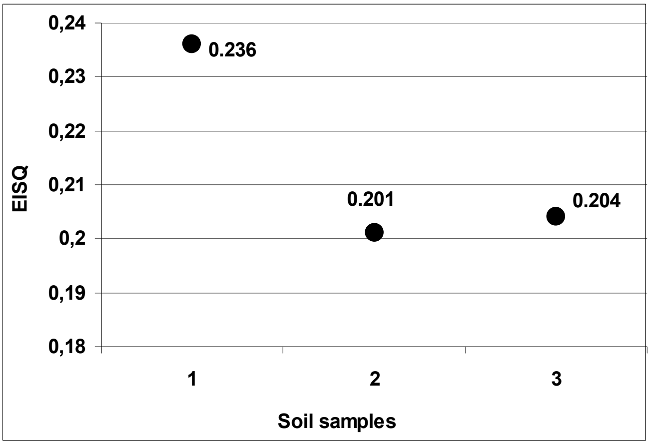

Figure 5. EISQ in soil samples from Cojocna

Generally, the enzyme potential of soil directly or indirectly reflects the activity of microbiota, the influence of different physical, chemical anthropogenic factors and even of intensity of different enzyme activities in the soil. Therefore, the function of an ecosystem can not be understood without the active implication of enzyme processes (Drăgan-Bularda *et al.*, 2004).

Conclusions

Several halophytes were identified on salty soils from Cojocna, the prevalent being: *Salicornia herbacea, Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda.*

Soil samples from the rhizosphere of the prevalent halophytes were physico-chemically analysed. The pH ranges between 8.84 and 9.21. Out of all the samples, the one populated with *Puccinellia distans*, *Artemisia santonica, Limonium gmelinii* and *Salsola soda* (sample 3) showed high values of the salts composition. All the soils were included to sodic and saline-sodic soils.

All the studied enzyme activities (actual and potential dehydrogenases, phosphatase, catalase) were detected in salty soil samples from Cojocna. Their intensity varied considerably, according to the location and the halophilic plants from whose rhizosphere the soil was sampled.

Based on the studied enzyme activity the enzymatic indicator of soil quality was calculated, which was low, between 0.201 and 0.236. As can be observed, the enzymatic indicator of soil quality reached low levels, meaning that the soils are not hosting an abundant bacterial population. This is due to the environmental conditions,especially to the high salinity, soils that form an infertile, unfavorable for life environment.

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