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CHANGES IN OXIDOREDUCTASES ACTIVITY IN ANTHROPOGENIC SALTY SOILS

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Abstract. To study the effects of anthropogenic salinity-sodicity on dehydrogenase (DHA) and catalase (CAT) activity in soil, samples were collected from the Ciech Soda Polska S.A. Plant in Inowrocław. The soils closest to the plant were assayed to determine pH, electrical conductivity ($EC_{1:5}$), and enzymes activity. The soil resistance (RS) and resilience (RL) indices were computed. The soil was sampled in July and October 2015 from the plant area not covered by the recultivation treatments (locations no 1, 2, 3, 4) and the locations where the agrotechnical soil recultivation was performed (5, 6). The successive soil sampling locations (7, 8) were located in the vicinity of the plant, while the control (point 9) – beyond the impact of the plant. Soil was sampled from the depth of 0–20 cm and 20–40 cm. To compare the activity of the oxydoreductases sampled from various locations, indices were calculated to facilitate estimation of both soil processes degradation and recultivation.

It has been observed that the highest value of pH_{KCl} and electrical conductivity increased the CAT and inhibited the DH activity. RS values for the dehydrogenase activity closed to 0 for the soil from stands in the vicinity of the plant, meaning the effect of saline on soil not only in places of stored post-soda sludge, but also in the area near the Ciech Soda Polska S.A. The highest value of soil catalase RL in location no 5, 0–20 cm deep, from recultivation area suggests a correct recultivation.

Keywords: dehydrogenase, catalase, resistance in soil, resilience of soil

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INTRODUCTION

Land degradation by salts is a major threat to sustainable crop production in many arid and semi-arid regions of the world (Bossio *et al*. 2007). In Poland, except for the soils in the coastline of the Baltic Sea, natural salty soils, characteristic for dry climate, do not occur. An increased soil salinity is therefore related to anthropogenic factors and occurs in the areas where soil formations have a direct contact with salt or salinated waters (Hulisz 2007, Siddikee *et al.* 2011). The effects of exaggerated salts in the soil solution involve reduced water uptake due to low osmotic potential, high pH, and ion competition limiting nutrient uptake (Mavi and Marschner 2012). Such conditions reduced plant growth and have a negative influence on the size and activity of soil microbial biomass and biochemical processes of soil organic matter (Tripathi *et al.* 2006, Yuan *et al.* 2007). Some microorganisms react to low osmotic potential by accumulating osmolytes to retain water, while sensitive microbes disappear (Hagemann 2011). The osmotic effects and ionic toxicity caused by salt stress generate oxidative stress and inhibit the system which is to re-establish homeostasis to control stress damage repair and detoxification (Hernández and Almansa 2002). The oxidative stress is characterized by the overproduction of highly active oxygen species (AOS) predominantly represented by superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$). The enzymatic defence of organisms include antioxidant enzymes, e.g. catalase (CAT; EC 1.11.1.6). Dehydrogenases (DH; EC 1.11.1.) are enzymes which exist in all living organisms, which is applicable as an index of changes in soil quality. Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles. Dehydrogenase activity can be used as a sensitive marker of soil degradation and soil microbial activity (Garcia *et al.* 1997).

An excessive content of easily-soluble inorganic salts in soil has a negative effect on the changes in the conformation of enzymatic protein, which results in a decrease in its catalytic activity (Telesiński *et al.* 2008, Siddikee *et al.* 2011). Therefore, to observe the change in enzymes activities, our research involved recalculating two indices to facilitate the estimation of both degradation and rehabilitation processes in soils (Orwin and Wardle 2004).

The aim of the present research has been to evaluate the effect of salinity on soil activity of catalase and dehydrogenase.

MATERIALS AND METHODS

The research was performed in the Noteć River valley, within the area adjacent from the plant of Ciech Soda Polska S.A. in Inowrocław, the Kujawsko-Pomorskie Province (Central Poland). The city's location is determined by altitude (52°45'N) and longitude (18°14'E), the lowland climate of moderate altitudes. In the Ciech Soda, during the production process used the Solvay ammonia method related to high precipitation onerous to the environment. According to Koś and Miakota (1988), an average chemical composition of such waste is dominated by $CaCO_3$ (40%), and the most important components include $Ca(OH)_2$ (18%), $CaCl_2$ (13%) as well as NaCl (7%).

The research material was made up by soil sampled in July and October 2015 in the area of the plant of Ciech Soda Polska S.A. in Inowrocław, in the area in its vicinity and in the arable field (which constituted the control).

Stand no 1 – at the edge of the ponds; it was flooded with post-soda sludge by 2000 and left to the effect of natural succession. Currently, the area is planned to be included in the recultivation program.

Stand no 2 – in the vicinity of the pond, similarly as stand no 1, it was flooded with post-soda sludge.

Stand no 3 – close to the clarifying-cooling tank, with a leaking bottom, provided with drainage. Precipitating waste includes carbonate salts.

Stand no 4 – in the vicinity of the pond used for the storage of ashy waters.

Stand no 5 – a sampling location not far from the sedimentation tank, the area under recultivation.

Stand no 6 – close to the pond where the technical and agrotechnical recultivation process was completed. The stand is grown with grasses.

Stand no 7 – an arable field in the vicinity of the plant, grown with winter cereal crops.

Stand no 8 – an area on the border of the soda plant, sewage treatment plant and rubbish. A former arable field. The occurrence of halophytes, mostly common glasswort (*Salicornia europaea* L).

Stand no 9 – a control sampling location. An arable field with winter cereal crop in Osiedle Bydgoskie Street in Inowrocław.

From each research stand, two soil samples were taken from the depth of 0–20 cm and 20–40 cm.

Soil properties and enzymes analysis

In the adequately prepared material, the following were determined: pH in 1M KCl measured potentiometrically (ISO 10390), electroconductivity ($EC_{1:5}$) with the conductometric method. The activity of selected oxidoreductase enzymes: the activity of dehydrogenases (DH) [E.C. 1.1.1] in soil with the Thalmann method (1968), the activity of catalase (CAT) [E.C. 1.11.1.6] in soil with the Johnson and Temple method (1964).

Data analysis

The index that we propose for resistance (RS) were derived from the formulas suggested by Orwin and Wardle (2004):

$$RS(t_0)=1-\frac{2|D_0|}{(C_0+|D_0|)} \quad (1)$$

Where $|D_0|$ is the difference between the control (C_0) and the disturbed soil (P_0) sampled in July (t_0).

Values of RL index are calculated from the following formula:

$$RL \text{ at } t_x=\frac{2|D_0|}{(C_0+|D_x|)}-1 \quad (2)$$

Where $|D_0|$ is the difference between the control (C_0) and the disturbed soil (P_0) sampled in July (t_0), $|D_x|$ means the difference between control and polluted soil sampled in October (t_x).

The results were exposed to the analysis of variance and the significance of differences between means was verified with the Tukey test at the significance level of $p<0.05$. The calculations involved the use of ANOVA based on Microsoft Excel.

RESULTS

The highest pH (pH_{KCl} 8.10–10.65) value showing alkaline reaction was recorded for soil from the area without reculivation stand no 1 at both depths, at both times of sampling. A high pH value was also found in soil from recultivated locations (stand no 6). In those locations there was also found an increased pH value with soil depth. The area in the vicinity of Ciech Soda and the farmland analysed, demonstrated a neutral reaction pH_{KCl} 6.65–7.207 (Table 1). Under Polish conditions the most frequent cause of a strongly alkaline reaction are secondary reactions of dominating NaCl in soil environment. Displacing other cations from soil colloids by sodium and having leached at least some anions Cl^- non-adsorbed by colloids, Na_2CO_3 and $NaHCO_3$ (alkaline-hydrolysing salts) get formed in the soil solution (Hulisz 2007).

$EC_{1:5}$ values ranged from 0.92 to 53.6 $mS \cdot cm^{-1}$ in the area without recultivation and in locations with recultivation the EC values ranged from 1.03 to 4.58 $mS \cdot cm^{-1}$ (Table 2). In the recultivation area in the stand no 6, the highest values of $EC_{1:5}$ (36.60 $mS \cdot cm^{-1}$ and 35.1 $mS \cdot cm^{-1}$) were reported only in 20–40 cm depth. Higher EC values were noted in the soils from the area in the vicinity of Ciech Soda PL, especially in stand no 8 in the area near to the Ciech Soda PL.

Table 1. Exchangeable acidity in the soils investigated

Location (I factor)		pH in KCl			
		08.07.2015		16.10.2015	
		Sampling depth (II factor)			
		0–20 cm	20–40 cm	0–20 cm	20–40 cm
1	area without recultivation	8.10	10.65	7.60	9.20
2		7.65	7.50	7.60	7.50
3		7.65	7.50	7.55	7.45
4		7.45	7.45	6.95	6.90
5	area with recultivation	7.25	7.25	7.15	7.25
6		7.55	8.80	8.00	8.05
7	area near to Ciech Soda PL	7.00	6.85	6.80	6.85
8		7.00	6.85	7.10	7.20
9	Farmland – control	7.05	6.95	6.80	6.65
	$LSD_{0.05}$ Interaction		I 0.169 I/II 0.636	II 0.774 II/I 0.095	

Table 2. Electrical conductivity in the soils investigated

Location (I factor)		$EC_{1:5}$ Electrical conductivity $mS \cdot cm^{-1}$			
		08.07.2015		16.10.2015	
		Sampling depth (II factor)			
		0–20 cm	20–40 cm	0–20 cm	20–40 cm
1	area without recultivation	48.6	48.9	49.2	53.6
2		5.85	13.9	6.08	14.5
3		0.92	1.61	1.25	1.89
4		7.74	10.5	8.09	10.5
5	area with recultivation	1.03	1.52	1.42	1.96
6		3.66	36.6	4.58	35.1
7	area close to Ciech Soda PL	8.98	8.94	9.89	9.54
8		20.7	18.6	26.47	18.0
9	Farmland – control	0.48	0.40	0.55	0.58
	$LSD_{0.05}$ Interaction		I 0.513 I/II 2.530	II 3.126 II/I 4.421	

Soil salinity was caused by many years of incorrect storage of the so-called lime sludge in sediment tanks. An additional cause of soil salinity related to the impact of that source of salinity is the wind dried waste from the surface of sediment tanks and emissions of lime dust during production (Rytelewski *et al.* 1993). The effect of soda industry waste can be considered a relatively permanent process. The amount of waste stored in sedimentation tanks is so high that even with a complete inhibition of their accumulation, the waste will be spreading around for many years still (Hulisz 2007). The analysis of variance of the results obtained in our experiment showed that sampling location significantly influenced soil dehydrogenase and catalase activity.

The activity of dehydrogenase varied a lot (0.037–0.127 mg TPF·kg^{-1}·h^{-1}) in the samples from locations affected by the plant (Table 3). The highest activity of dehydrogenase reported in the control 0.276–0.480 mg TPF·kg^{-1}·h^{-1}. The activity of dehydrogenases in the soil sampled from the area affected by the Ciech Soda PL at the first date was, on average, 79–89% and in the second one – by 78–87% lower, as compared with the control. There were found the lowest activity of dehydrogenases in the soils affected by the plant locations without recultivation (stand no 1) and in the soils sampled from the area in the vicinity of Ciech (stand no 8) at both dates of sampling.

Table 3. Soil dehydrogenases activity

		DHA Dehydrogenases activity mg TPF·g^{-1}·h^{-1}					
Location (I factor)		08.07.2015			16.10.2015		
		Sampling depth (II factor)					
		0–20 cm	20–40 cm	mean	0–20 cm	20–40 cm	mean
1	area without recultivation	0.056	0.051	0.054	0.038	0.038	0.038
2		0.127	0.065	0.096	0.073	0.055	0.064
3		0.056	0.083	0.070	0.041	0.040	0.041
4		0.059	0.075	0.067	0.061	0.069	0.065
5	area with recultivation	0.089	0.083	0.086	0.050	0.068	0.059
6		0.049	0.048	0.049	0.055	0.053	0.054
7	area in the vicinity of Ciech Soda PL	0.073	0.112	0.093	0.054	0.041	0.047
8		0.043	0.081	0.062	0.051	0.037	0.044
9	Farmland – control	0.435	0.480	0.458	0.276	0.324	0.300
$LSD_{0.05}$ Interaction			I n.s. I/II n.s.		II 0.076 II/I n.s.		

n.s. – not significant differences

Hinojosa *et al.* (2008) studied the effect of soil moisture pre-treatment on the status and health of microbiological enzymatic activities of soil as indicators of heavy metal-contaminated and reclaimed soils. DH activities were significantly greater for pre-treated rewetted and incubated soils. Higher DH activities were recorded in non-polluted area is (71.4 µg TPF g^{-1}·dw·h^{-1}), average activities reported in reclaimed areas (53.0 µg TPF g^{-1}·dw·h^{-1}) and least value reported in polluted area (2.9 µg TPF g^{-1}·dw·h^{-1}). Enzyme activities significantly decreased with increasing pollution: non-polluted soil showed the highest DH activity values, polluted soil – the lowest, and restored soil – intermediate values. However, rewetting generally increased enzyme activities of non-polluted and reclaimed. Polluted soils recorded much lower increases or even decreases in enzyme activities with rewetting. In soil affected by the Ciech Soda PL, the dehydrogenase activity was significantly inhibited in salinized soils, and the results did not show that the recultivation treatments essentially increased the activity of DH compared to stand from the area without recultivation.

The research results of Furtak and Gajda (2017) showed that ecological (without mineral fertilization and plant chemical protection) farming system beneficially influenced soil environment and soil were characterized by a higher DH activity. The research conducted by Siddikee *et al.* (2011) indicate that the soil chemical variables related with salinity-sodicity are relevant related with the sampling distance from the causes and are the stress factors, which greatly affect microbial and biochemical properties.

The activity of soil catalase fell within a very wide range from 0.023 to 0.332 mg H_2O_2 $g^{-1}\cdot h^{-1}$ in soil affected by the plant and it ranged from 0.185 to 0.389 H_2O_2 $g^{-1}\cdot h^{-1}$ for the control (Table 4). CAT is one of the most effective antioxidant enzymes that can degrade H_2O_2 into water and molecular oxygen in the peroxysomes where H_2O_2 is produced from β-oxidation of fatty acids and photorespiration (Fazeli *et al*. 2007). Wu *et al.* (2012) reported that CAT activity of shoot in two different cultivar wheat was increased under osmotic stress compared to control. In the soil from the vicinity of Ciech Soda PL there was noted the highest CAT activity in the soil sampled from stand no 1, with the highest value of exchangeable acidity as well as electrical conductivity, as well as the lowest DH activity. These results suggest that in the soil from that location, microorganisms and plants inducted more catalases to protect against oxidative damage caused by saline stress. A high CAT activity in the control suggests that the soil was affected by a factor, different than salinity, triggering the defences include antioxidant enzymes.

Table 4. Soil catalase activity

CAT Catalase activity mg H_2O_2 $g^{-1}\cdot h^{-1}$							
Location (I factor)		08.07.2015			16.10.2015		
		Depth of sampling (II factor)					
		0–20 cm	20–40 cm	mean	0–20 cm	20–40 cm	mean
1	area without recultivation	0.242	0.278	0.260	0.332	0.289	0.310
2		0.023	0.040	0.032	0.172	0.181	0.176
3		0.079	0.032	0.055	0.083	0.053	0.068
4		0.174	0.168	0.171	0.321	0.155	0.238
5	area with recultivation	0.068	0.055	0.062	0.295	0.257	0.276
6		0.079	0.060	0.069	0.121	0.208	0.165
7	area in the vicinity of Ciech Soda PL	0.145	0.236	0.190	0.200	0.251	0.225
8		0.047	0.077	0.062	0.255	0.172	0.214
9	Farmland – control	0.185	0.193	0.189	0.308	0.389	0.349
$LSD_{0.05}$				I n.s.	II 0.142		
Interaction				I/II n.s.	II/I n.s.		

n.s. – not significant differences

The value of soil dehydrogenase and catalase resistance and resilience were reported in Tables 5 and 6. The activities of DH and CAT were different; the value of the indices are presented separately. The value of both resistance and resilience bounded by -1 to +1. The value of +1 showing that the disturbance had no effect (maximal resistance and resilience) (Orwin and Wardle 2004).

Table 5. Value of soil dehydrogenase activity resistance and resilience

		DHA Dehydrogenases activity			
Location		Resistance in soil (RS)		Resilience of soil (RL)	
		Sampling depth			
		0–20 cm	20–40 cm	0–20 cm	20–40 cm
1	area without recultivation	0.069	0.056	0.229	0.200
2		0.171	0.073	0.205	0.213
3		0.069	0.095	0.235	0.166
4		0.073	0.085	0.272	0.227
5	area with recultivation	0.114	0.095	0.210	0.216
6		0.060	0.053	0.272	0.229
7	area in the vicinity of Ciech Soda PL	0.092	0.132	0.240	0.131
8		0.052	0.092	0.271	0.163

Table 6. Value of soil catalase activity resistance and resilience

		CAT Catalase activity			
Location		Resistance in soil (RS)		Resilience of soil (RL)	
		Sampling depth			
		0–20 cm	20–40 cm	0–20 cm	20–40 cm
1	area without recultivation	0.529	0.388	0.407	-0.081
2		0.066	0.116	0.087	-0.152
3		0.271	0.090	-0.360	-0.352
4		0.888	0.771	-0.083	-0.807
5	area with recultivation	0.225	0.166	0.800	0.022
6		0.271	0.184	-0.276	-0.153
7	area in the vicinity of Ciech Soda PL	0.644	0.636	-0.459	-0.525
8		0.146	0.249	0.445	-0.303

The RS value of 1 is meaning that the effect of analyzed contamination on soil appeared in 200%, 0 – in 100%. When the RS value is 1, soil is fully tolerant to a given contamination, which is zero effect of contamination appeared (Lipińska *et al.* 2014).

The values of soil dehydrogenase resistance suggested that the impact of saline stress in the soil affected by the plant, as compared to the control soils, was considerable. The lowest value of RS was found in the soil sampled from the depth of 0–20 cm from the area in the vicinity of Ciech Soda PL (stands no 1, 3, 6 and 8). That indicated a significant influence of salinity on soil dehydrogenase activity in this area. There were not found a considerable difference between the

RL value for DH activity in investigated soil which suggests similar resistance of their soils on salinity. The highest values of catalase resistance are observed in the soil from stand no 4, which suggested that the activity of soil catalase was the most tolerant to oxidative stress compared to soil from other stands.

Soil resilience is a response to the previous stresses (Orwin and Wardle 2004). The closer the RL value to 1, the higher the soil capacity to counteracting external factors (Lipińska *et al.* 2014). A low RL value suggests a long-term effect of salinity. The highest values of catalase resilience obtained in the soil from stand no 5 at the depth of 0–20 cm implied that the soil was distinguished higher resilience on soil saline. The way of recultivation in this area should be continued.

CONCLUSIONS

1. The soils got salinited due to a long-term storage of the so-called post-production lime sludge in sedimentation tanks. The effect of soda industry waste is seen from a high value of exchangeable acidity, electrical conductivity as well as the activity of oxydoreductases.
2. The activity of dehydrogenases, enzymes considered as an index of microbial activity in the soils exposed to the impact of soda plant was 78–89% lower, as compared with the control.
3. RS values for the dehydrogenase activity close to 0 for soil from stands in the vicinity of the plant meaning the effect of saline on soil not only in places where were storaged post-soda sludge but also in the area near the Ciech Soda Polska S.A.
4. There was recorded the highest catalase activity in the soil sampled from the stand without recultivation, where also the highest value of exchangeable acidity and electrical conductivity and the lowest DH activity were assayed.
5. The results imply a response of plant and microorganisms by the induction of more catalases to protect against oxidative damage caused by salinity stress. The highest value of index RL of soil catalase in stand from recultivation area suggests a correct recultivation way in this area.

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