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## SEASONAL CHANGES OF ARYLSULPHATASE ACTIVITY AND SULPHATE CONTENT IN ANTHROPOGENIC SOIL\*

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### ABSTRACT

The paper studies the effects of soil salinization caused by the waste from soda production deposited in soils in and around the CIECH Soda Polska S.A. plant on the soil sulphur and sulphate content and arylsulphatase activity. Soil samples were collected in April and October 2014 at two depths from 8 representative sites and from the control. The pHKCl values ranged from 7.35 to 7.98 and showed alkaline reaction of all the soils researched at both depths and on both sampling dates. Soil samples differed in electrical conductivity ( $EC_{1.5}$ ), which varied from 0.133 to 11.26 mS  $cm^{-1}$ . The factory's impact has led to lasting changes in the activity of arylsulphatase and in the sulphur content. At the sites where soil was determined to represent average and high salinity, on both soil sampling dates, the activity of this hydrolase tended to be the lowest. Except for the soil sampled from the control site, all the other soil samples showed a considerable content of sulphates ( $>50$  mg  $kg^{-1}$ ), which points to strong anthropogenic pollution. The soil remediation works conducted in and around the CIECH factory were not found to affect the content of total and sulphate sulphur or the activity of hydrolase. Values of the soil resistance (RS) and resilience (RL) indices were calculated. The low value of soil resistance to AR activity at site 8 (RS = 0.050 in 0-20 cm and 0.039 in 20-40 cm) together with the high  $EC_{1.5}$  value, a very high content of sulphates ( $>50$ ) and a growing population of *Salicornia europaea* L., demonstrates the soil environment's capacity of adapting to anthropogenic salinization. The results show that the activity of arylsulphatase expressed with the indices of soil resistance accurately represents the response of soil properties to soil salinization.

**Keywords:** arylsulphatase, soda industry, soil resilience, soil resistance, sulphur.

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## INTRODUCTION

Soil salinity depends on the content of easily soluble salts, including chloride ( $\text{Cl}^-$ ), sulphate ( $\text{SO}_4^{2-}$ ) and nitrate ( $\text{NO}_3^-$ ) anions as well as sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) cations (SHI, WANG 2005). Under salinity stress, accumulation of surplus  $\text{Na}^+$  and  $\text{Cl}^-$  deteriorates the acquisition and homeostasis of essential nutrients (FAGERIA et al. 2008), making it more difficult for water and nutrients to move through the root membrane (VOLKMAR et al. 1998) and decreasing their concentrations in leaves (KHAN et al. 2010). Oxidative stress limits the growth and development of microorganisms and plants, and when prolonged it may disturb plant metabolism and result in cell death. The importance of sulphur in salinity tolerance is confirmed further by the regulation of different enzymes of S-assimilation under salinity stress. Studies have shown that plant sulphur assimilation enzymes increase, decrease or remain unaffected by salinity stress (KOPRIVOVA et al. 2008).

Measures of enzymatic activity in saline soils can be also used for evaluating the degree of degradation of salinated soils and a soil restoration potential. Despite the wealth of data on effects of plant and soil salinization on physicochemical soil properties, very few studies have dealt with the influence of salinization on the activity of soil enzymes participating in the sulphur cycling in soil.

The aim of the present paper has been to determine the effect of soil salinization on changes in the activity of arylsulphatase (EC 3.1.6.1.), an enzyme involved in sulphur transformation processes, in soil sampled from sites located in and around the CIECH Soda Polska S.A plant in Inowrocław.

## MATERIAL AND METHODS

### Experimental design

The research was conducted in the Notec River valley, in an area adjacent to the plant CIECH Soda Polska S.A in Inowrocław, the Kujawsko-Pomorskie Province (Poland). Inowrocław lies at the altitude 52°45' N and longitude 18°14' E. Thus, the town is characterized by having the lowland climate of moderate altitudes.

The soil material was sampled in April and October 2014 from the premises of the plant CIECH Soda Polska S.A. in Inowrocław, from its vicinity and from an arable field (control).

Site 1 – at the edge of the factory's ponds; the soil had been flooded with spent soda sludge until the year 2000 and then left to undergo natural succession. Currently, the area is planned to be included in a remediation program. Site 2 – in the vicinity of the pond, similarly to site 1, it had been

flooded with spent soda sludge. Site 3 – in the vicinity of the factory's clarifying-cooling tank with the permeable bottom and a drainage system. The waste includes carbonate salts. Site 4 – in the vicinity of the pond used for ashy water storage. Site 5 – a sampling location not far from the sedimentation tank, an area under restoration. Site 6 – in the vicinity of the pond where technical and agrotechnical soil remediation measures had been implemented. The site is overgrown with grasses. Site 7 – an arable field in the vicinity of the plant, cropped with winter cereals. Site 8 – an area on the border of the soda plant, sewage treatment plant and a waste storage site, a former arable field now overgrown with halophytes, mostly common glasswort (*Salicornia europaea* L.). Site 9 – the control sampling area, which was an arable field cropped with winter cereals and located in Inowrocław. Two soil samples were taken at each site, from the depth of 0-20 cm and 20-40 cm.

### Activity of arylsulphatase and physicochemical soil properties

The following parameters were determined in properly prepared material: pH in 1M KCl measured potentiometrically, electroconductivity ( $EC_{1:5}$ ) – with the conductometric method, total carbon ( $C_{tot}$ ) and total nitrogen (TN) – in a TOC FORMACTSTM analyser Primacs (provided by Skalar). The content of total sulphur ( $S_{tot}$ ) and sulphate sulphur ( $SO_4^{2-}$ ) was assayed according to the BARDSLEY-LANCASTER method (1960), modified by COMN-IUNG. The activity of arylsulphatase AR (EC 3.1.6.1) was determined according to the TABATABAI and BREMNER method (1970). All analytical measurements were performed with three replications.

### Data analyses

The index we propose for resistance (RS) was derived from the formula suggested by ORWIN and WARDLE (2004):

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

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

The RL index values are calculated from the following formula:

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

where  $|D_0|$  is the difference between control  $C_0$  and disturbed soil ( $P_0$ ) in soil taken in April ( $t_0$ ),  $|D_x|$  stands for a difference between the control and the polluted soil after time  $t_x$  (soil sampled in October).

## Statistical analysis

The results were submitted to analysis of variance, and the significance of differences between means was verified with the Tukey's test at the significance level of  $p < 0.05$ . The calculations involved the use of ANOVA supported by Microsoft Excel. As for descriptive statistical analysis, the Pearson's correlation coefficients were calculated using Statistica 7.0 (StatSoft Inc.).

## RESULTS AND DISCUSSION

Considering the grain size composition, the analyzed soils were light ones. The soil content of clay ranged from 4.2 to 14.76%. The soil clay fraction 0.002 mm in size determines the soil sorption capacity, soil buffering and water storage capacity. The  $\text{pH}_{\text{KCl}}$  values ranged from 7.35 to 7.98 and showed alkaline reaction in all the soil samples at both depths and on both sampling dates. The farmland soil had neutral and alkaline reaction:  $\text{pH}_{\text{KCl}}$  7.20 to 7.27 (0-20 cm and 20-40 deep, respectively) – Table 1. The analysis of variance did not reveal a significant effect of either the sampling site or the sampling depth on the measured pH values. Higher pH values were noted in the soils sampled from the 20-40 cm horizons (Table 1). The electrical conductivity in the soils in the vicinity of CIECH Soda Polska S.A. plant ranged from  $0.822 \text{ mS cm}^{-1}$  to  $89.59 \text{ mS cm}^{-1}$ .  $\text{EC}_{1:5}$  values below  $0.8 \text{ mS cm}^{-1}$  implicating non-salinated soil were determined only in the control sample at the 20-40 cm layer. Slightly salinated soils ( $0.8\text{-}1.6 \text{ mS cm}^{-1}$ ) originated from sites 5 and 6, from the 0-20 cm layer, and from the control, from the 20-40 layer, as well as from site 3. Average salinity ( $\text{EC}_{1:5} = 1.6\text{-}3.2$ ) was identified in the soil from site 5 and from the control, the 20-40 cm layer, as well as from site 6, the 0-20 cm layer. The soil from site 1 reached the highest  $\text{EC}_{1:5}$  value in both horizons (45.46 in horizon 0-20 cm as well as 89.59 in horizon 20-40 cm). The high soil salinity in site 8 was reflected by the structure of the plant cover in the vicinity of this site (halophytes, e.g. *Salicornia europaea* L.) (PIERNIK et al. 2015). There was a notable effect of the soda factory on the adjacent areas (sites 7, 8 and 9), expressed by the high pH and  $\text{EC}_{1:5}$  values in soil. The changes were due to salt penetrating to shallow groundwater, hence the salinization of very fertile soils in the adjacent areas (CIEŚLA et al. 1981). Other causes of soil salinization, related to the impact of the source of salts, are winds which can blow dry waste from the sedimentation tank's surface, and emissions of lime particulates during production. According to PIERNIK et al. (2015), pollution with waste from the soda industry can be seen as a relatively permanent process, and since there is much waste stored in the sedimentation tanks, the impact of the CIECH plant will be felt for many years to come.

The content of  $\text{C}_{\text{tot}}$  did not depend on either the soil sampling site or its depth; it ranged from  $19.12 \text{ g kg}^{-1}$  to  $108.6 \text{ g kg}^{-1}$  in the soil sampled from the

Table 1  
Some physicochemical soil properties

Sampling site	Percent of fraction <0.002		C <sub>tot</sub> (g kg <sup>-1</sup> )	N <sub>org</sub> (g kg <sup>-1</sup> )		pH KCl	EC <sub>1:5</sub> (mS cm <sup>-1</sup> )				
	0-20	20-40		0-20	20-40						
	depth (cm)										
Sites without plant cultivation	1	14.76	11.96	108.6	93.47	1.910	0.280	7.98	7.78	45.46	89.59
	2	4.23	4.2	83.47	102.7	1.270	1.400	7.80	7.81	12.84	24.18
	3	7.93	7.95	121.4	109.0	2.000	0.440	7.59	7.82	1.127	0.953
Sites with plant cultivation	4	7.86	7.17	86.56	83.29	2.830	2.860	7.67	7.69	6.527	9.327
	5	5.64	5.62	90.96	96.03	3.290	3.970	7.66	7.75	0.822	2.553
	6	7.35	5.86	104.7	107.0	0.820	1.300	7.90	7.95	1.949	12.27
Area close to the plant	7	4.84	4.76	19.60	19.12	1.770	1.880	7.70	7.73	6.556	5.498
	8	6.62	6.60	69.79	57.95	4.360	3.560	7.35	7.38	29.38	28.02
Control	9	8.19	9.80	17.93	18.40	1.960	2.010	7.27	7.20	1.105	0.704

factory's premises and from its vicinity. The lowest  $C_{\text{tot}}$  was recorded in the soil sampled from the control ( $C_{\text{tot}}$  17.93-18.40 g kg<sup>-1</sup>) – Table 1. The content of total nitrogen (TN) did not depend on a soil sampling depth or its site. The highest TN content (4.36 g kg<sup>-1</sup> 0-20 cm deep and 3.56 g kg<sup>-1</sup> 20-40 cm deep) was reported in the soils sampled from site 8, adjacent to the plant.

Long-term storage of post-production waste interferes with the total and sulphate sulphur content and AR activity of soil. Considering the threshold sulphur content in the soil surface layer for heavy soils (>35% fraction < 0.02 mm), such as the soils from the premises of the CIECH Soda Polska S.A plant and its vicinity, it was found that only the soil sampled in April from the control site in the 0-20 cm layer contained a natural amount (<0.25 g kg<sup>-1</sup>) of total sulphur, implicating low pollution, while the soils from sites 1 and 3 from the depth of 20-40 cm revealed high levels (1.01-2.0 g kg<sup>-1</sup>) of this element. The other soils, irrespective of the sampling date and site (area of the plant, places which have been restored or adjacent to the plant as well as the soil from the control sampled in autumn) showed an average (0.26-1.0 g kg<sup>-1</sup>) content of  $S_{\text{tot}}$ . As well as being the sulphur form available to plants, sulphate ( $\text{SO}_4^{2-}$ ) is one of the key soluble anions in brine (SCHERER 2009). Except for the soils sampled from the control, all the other soils demonstrated a very high content of sulphates (>50 mg kg<sup>-1</sup>), which suggests strong anthropogenic pollution. The soil sampled from the control, at both dates and from both depths of the soil profile, showed a natural content of sulphates. Adsorption of  $\text{SO}_4^{2-}$  is pH-dependent and intensifies at low soil pH (PRIETZEL et al. 2001). The results of investigations by DĄBKOWSKA-NASKRĘT and BARTKOWIAK (2018) in soil near the plant CIECH Soda Polska S.A. showed that cations responsible for salinity measured by electrical conductivity were calcium and, to a lesser extent, sodium. Sulphur may precipitate in the form of  $\text{SO}_4^{2-}$  as calcium, magnesium or sodium sulphate and coprecipitate with  $\text{CaCO}_3$  (SCHERER 2009). The salts are primarily composed of carbonates, chlorides, sulphates and bicarbonates of calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ) and sodium ( $\text{Na}^+$ ) (MANCHANDA, GARG 2008).

The arylsulphatase activity (AR) depended on the depth and sampling site (Table 2) and varied within a wide range: from 0.125 mM pNP g<sup>-1</sup> h<sup>-1</sup> to 5.419 mM pNP g<sup>-1</sup> h<sup>-1</sup>. An especially high AR activity (5.41 mM pNP g<sup>-1</sup> h<sup>-1</sup> in April and 3.950 mM pNP g<sup>-1</sup> h<sup>-1</sup> in October) appeared in the soil from site 1 sampled at the 0-20 cm deep. As for the soil sampled at the 20-40 cm deep from the same site, the AR activity was 95% lower. Regarding the 0-20 cm depth, at both soil sampling dates, high AR activity was reported in soil from sites 9 and 7 (Table 2). The lowest AR activity at both sampling dates and both depths was identified in the soils sampled from site 8, from 87% to 93% lower than in the control. Although the microbial activity is severely suppressed, it is evident that substantial microbial activity persisted under saline conditions (YUAN et al. 2007). No significant effect of soil pH, EC,  $C_{\text{org}}$ , and TN on the activity of arylsulphatase was reported. However, it can be noted that at the sites where the soil was determined to be of average (site 1,

Table 2

Arylsulphatase activity (AR) and the total ( $S_{\text{tot}}$ ) and sulphate sulphur ( $SO_4^{2-}$ ) content

Sampling site	Arylsulphatase (mM pNP g <sup>-1</sup> h <sup>-1</sup> )		$S_{\text{tot}}$ (g kg <sup>-1</sup> )		$SO_4^{2-}$ (mg kg <sup>-1</sup> )		
	soil depth (cm)						
	0-20	20-40	0-20	20-40	0-20	20-40	
April							
Sites without plant cultivation	1	5.419a	0.254d	1.307a	1.380a	161.7a	388.8a
	2	0.125d	0.706c	0.617b	0.288e	94.05f	62.15f
	3	0.800c	1.114bc	0.664b	1.369a	108.4d	94.40de
	4	0.806c	0.739c	0.554bc	0.545de	103.8e	102.9cd
Sites with plant cultivation	5	0.629c	0.830bc	0.621b	0.833b	109.4c	171.9b
	6	0.312cd	0.149d	0.728b	0.627c	99.20f	88.55e
Area close to the plant	7	1.666b	1.118b	0.445bc	0.563cd	171.5a	140.5c
	8	0.188d	0.130d	0.684b	0.676c	120.1b	112.6c
Control	9	1.963b	1.752a	0.233d	0.370e	10.65g	10.20g
October							
Site without plant cultivation	1	3.950a	0.207f	1.124a	1.388a	150.3a	346.8a
	2	0.093g	0.624e	0.592d	0.259g	100.2c	77.57g
	3	0.724d	1.019c	0.702c	1.247b	109.0bc	84.86f
	4	0.276f	0.169gf	0.649c	0.604d	95.52c	84.05f
Sites with plant cultivation	5	0.544e	0.798d	0.493ef	0.791c	106.7c	177.3b
	6	0.775d	0.678e	0.509e	0.527e	98.49c	103.3e
Site close to the plant	7	1.728c	1.170b	0.439f	0.592d	157.0a	136.2c
	8	0.256f	0.206f	0.768b	0.648d	112.3b	93.53e
Control	9	2.056b	1.546a	0.292g	0.417f	10.69e	9.505h

In columns, homogeneous groups are followed by the same latter.

depth 20-40 cm) and high salinity (site 8) at both sampling dates, the activity of this hydrolase was the lowest. The results suggest that its activity was limited by soil-accumulated ions and alkaline cations. SIDDIKIE et al. (2010) claim that decreased activity of enzymes in permanently or temporarily salinated soils is due to a change in the osmotic potential triggered by a toxic effect of specific ions and modification of the centre of an active enzyme, which is a protein. ERGASHEVA and EGAMBERDIEVA (2014), on the other hand, found that acid phosphomonoestrerase, phosphodiesterase and galactosidase were not affected by an increase in soil salinity; neither did they correlate with the soil's electrical conductivity, Cl or Na. According to SHI and WANG (2005), the activity of an enzyme depends on both its nature and the composition of the salt. Dehydrogenases were more inhibited by salinity, while hydrolases showed much lower inhibition. In general, inhibition of the activ-

ity of soil enzymes by salt solutions decreased in the following order, as compared with the same horizon ECe:  $\text{NaCl} > \text{CaCl}_2 > \text{Na}_2\text{SO}_4$  (SHI, WANG 2005). In the present study, we recorded high values of the coefficients of positive correlations between the activity of the analyzed hydrolase and the content of  $S_{\text{tot}}$  ( $r = 0.949$ ,  $p \leq 0.05$ ) as well as sulphates ( $r = 0.968$ ,  $p \leq 0.05$ ), suggesting that the activity of this enzyme was stimulated in the presence of a high sulphur content in soil. Arylsulphatase catalyzes the release of  $\text{SO}_4^{2-}$  from sulphate esters and the highest sulphate content in soil usually inhibited its activity (SAVIOZZI et al. 2006). RAO et al. (2000) conclude that phosphatase immobilized in loams, i.e. organic and organic-mineral complexes, showed catalytic features totally different from those of the free enzyme. The available contradictory findings provide ample justification for undertaking further investigations into the effects of salinity on sulphur transformation and enzymes involved in this process, in the context of the structure, function and diversity of anthropogenic soils.

The AR activity was different; the values of the indices are presented separately. Both resistance and resilience are expressed by values between -1 and +1. The value +1 shows that the disturbing factor had no effect, maximum resistance and resilience (ORWIN, WARDLE 2004).

An effect of a soil sampling site on the RS index value was noted. The highest RS index values for arylsulphatase activity in the soils under study were recorded, for both depths, in site 7, i.e. in the soil located in the vicinity of the plant (RS was 0.737 for the 0-20 cm depth and 0.469 for the 20-40 cm depth) – Table 3. The resistance of soil in this site may be due to long-term cultivation of cereals, which supported the recovering soil. The resistance of arylsulphatase activity depended on a soil depth, e.g. at site 2 the RS value for the 20-40 cm depth was 87% higher than the RS value for the 0-20 cm depth. In sites 1, 2, 3 and 5 and 8, the RS of AR activity were higher at the lower depth than in the 0-20 cm depth. Low values of the coefficient of soil resistance to AR activity in site 8 (0.050 in the 0-20 cm depth and 0.039 in 20-40 cm) and in site 6 (0.086 in the 0-20 cm depth and 0.044 in 20-40 cm) implicate that the impact of soil salinization was nearly omnipresent (Table 3). Site 8 with its highest EC value (11.26) and a very high content of sulphates (>50) suggests the capacity of the soil environment to adapt to anthropogenic salinization, which is also evidenced by the occurrence of *Salicornia europaea* L. at the same that location. The results show that arylsulphatase activity expressed through indices of soil resistance accurately represents the response of soil properties to soil salinization.

The RL for arylsulphatase was the lowest in soil from the vicinity of the plant, i.e. sites 1, 2, 3 and 8 from the depth of 20-40 cm (0.056, 0.063, 0.095 and 0.095, respectively), which implies the long-term influence of soil salinization. The highest RL value was calculated for soil from site 1 from the depth of 0-20 cm (0.292) and site 6 from the depth of 20-40 cm (0.297). The closer the RL value to 1, the higher the soil's capacity to counteract external factors (LIPIŃSKA et al. 2014).

Table 3

Values of soil resistance and resilience to arylsulphatase activity

Site		ARS Arylsulphatase activity			
		resistance in soil (RS)		resilience of soil (RL)	
		sampling depth (cm)			
		0-20	20-40	0-20	20-40
Sites without plant cultivation	1	-0.276	0.078	0.292	0.056
	2	0.033	0.252	-0.033	0.063
	3	0.256	0.466	-0.068	0.095
	4	0.258	0.267	-0.212	-0.152
Sites with plant cultivation	5	0.191	0.310	-0.063	0.104
	6	0.086	0.044	0.126	0.297
Area close to the plant	7	0.737	0.469	-0.050	0.255
	8	0.050	0.039	-0.007	0.095

Table 4

Relationship between the parameters investigated

Treatment	pH <sub>KCl</sub>	EC <sub>1:5</sub>	C <sub>tot</sub>	TN	AR	S <sub>tot</sub>	SO <sub>4</sub> <sup>2-</sup>
pH KCl	1						
EC <sub>1:5</sub>	<b>-0.527</b>	1					
C <sub>tot</sub>	<b>-0.652</b>	<b>-0.004</b>	1				
TN	<b>0.579</b>	0.126	<b>-0.637</b>	1			
AR-arylsulphatase	-0.096	0.090	0.223	0.229	1		
S <sub>tot</sub>	-0.116	0.093	0.242	-0.247	<b>0.949</b>	1	
SO <sub>4</sub> <sup>2-</sup>	-0.111	0.086	0.245	0.245	<b>0.968</b>	<b>0.987</b>	1

Correlation coefficients (*r*) given in boldface are significant at  $p \leq 0.05$ .

## CONCLUSIONS

The persistent impact of the CIECH Soda Matwy plant has caused lasting changes in the soil's electrical conductivity (EC<sub>1:5</sub>), total carbon (C<sub>org</sub>) and total nitrogen (TN) in and around the soda plant. Salt penetration from the post-production waste storage site to shallow groundwater resulted in the salinization of the adjacent soil. The activity of soil arylsulphatase participating in sulphur transformations in soil underwent seasonal changes and depended on the content of total sulphur and sulphate sulphur. The low value of the resistance of soil to AR activity in site 8, with a high EC<sub>1:5</sub> value and a very high content of sulphates as well as growing *Salicornia europaea* L., demonstrates the capacity of the soil environment to adapt to anthropogenic salinization.

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