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CHANGES IN THE ACTIVITY OF PHOSPHATASE AND THE CONTENT OF PHOSPHORUS IN SALT-AFFECTED SOILS GRASSLAND HABITAT NATURA 2000

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Abstract. The subject of this study was the humus horizons in the salt-affected soils of Natura 2000 (Ciechocinek, Poland). In the adequately prepared soil, there were determined: pH in CaCl₂, total organic carbon (TOC), exchangeable cations (Ca²⁺, Mg²⁺, Na⁺, K⁺), salinity (EC_{1.5}), the content of total (TP) and available phosphorus (AP), the activity of alkaline (AlP) and acid (AcP) phosphatases. TOC affected the degree of saturation of the sorption complex with basic cations, as confirmed by correlation analysis. In the analysed soil samples a series of quantitative cations of basic character is as follows: Ca²⁺>Na⁺>K⁺>Mg²⁺. Increased salinity has modified the qualitative and quantitative composition of the soil solution. Correlation analysis confirmed the significant relationship between the conduction of the electrolytic soil and the content of sodium and potassium cations. The highest value of EC_{1.5} was found in the soil sampled near the ditch (sites 12, 13, 16). According to PN-R-04023 (1996), this soil classifies as class V with a very low content of P available but the availability factor for phosphorus value ranged from 2.773 to 5.252% indicating that soil P was sufficient for plant growth in this habitat. Significant positive correlations were found between salinity, alkaline phosphatase and exchangeable K⁺, Na⁺. Significant negative correlations were found between EC_{1.5} with P available and the availability factor for this nutrient (AF). The positive significant correlations among soil alkaline phosphatase and some physicochemical properties suggested that salinization had effects on these variables. Alkaline phosphatase may be used as indicators of soil quality in salinized grassland habitat Natura 2000.

Keywords: base cations, electrical conductivity, phosphatase, phosphorus, salt-affected soils

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INTRODUCTION

The main measure implemented to protect the environment on a European Union (EU) scale was the establishment of the European Ecological Network Natura 2000 in order to preserve valuable natural or semi-natural areas (Bartula *et al.* 2011; Grodzinska-Jurczak and Cent 2011). Natura 2000 network already covers about 18% of EU territory. Concerted effort to preserve the natural heritage of Europe on the basis of a uniform law aims at optimizing costs and increasing beneficial results for the environment (Wamelink *et al.* 2013). The EU rules, which form the basis for the creation of the Natura 2000 network, were incorporated into the Polish legislation system with the publication of the Nature Conservation Act of 16 April 2004. Therefore, under the EU Habitats Directive (Council Directive 92/43EWG) providing proper status of Natura 2000 habitats is one of the most important tasks of nature conservation in Poland.

The salinity of soil can be caused by either natural (sea waters and mineral) or anthropogenic factors (waste and post-industrial waste, mineral fertilizers). Salinization affects around 3.8 million ha in Europe. Most affected are Campania in Italy, the Ebro Valley in Spain, and the Great Alföld in Hungary, but also areas in Greece, Portugal, France, Slovakia and Austria (CEC 2006). Naturally saline soils cover only a small area of Poland. They can be found on areas which are reinforced by highly mineralized groundwater. They are accompanied by rare inland halophytes. Halophytes are a group of plants which grows on areas rich in readily soluble salts. According Siddikee *et al.* (2011), saline soils contain a high amount of soluble salts, primarily cations Ca^{2+} , Mg^{2+} , Na^+ and K^+ , salt of SO_4^{-2} , Cl^- , NO_3^- and CO_3^{-3} . Sorption properties and exchangeable cations content play an important role in the process of leaching of nutrient components from the soil and determine the effectiveness of fertilization, what is of importance in plant nutrition processes (Bartkowiak and Długosz 2010). The level of saturation of the sorptive complex with base cations determines the fertility of soils and their resistance to chemical degradation (Paluszek 2014). According to Grigore *et al.* (2012), saline soils are poor in nutrients, but it is generally accepted that salt stress is the most important restrictive factor for plant growth in this type of habitats. In the last century a lot of natural salt meadows decreased their area as a result of e.g. site drainage, drying up of saline springs, change of water relations due to the lack of strict protection of the Natura 2000 sites. Soil monitoring on the protected areas should be a part of strategic scope of research. Saline and sodic landscapes can impact carbon and nutrient cycling, and organic matter decomposition. Available phosphorus is only a very small fraction of total P in the terrestrial ecosystems (less than 6%). Many researchers have reported that the fractions and dynamics of P in soil also depend upon various soil characteristics such as pH (Liang *et al.* 2010; Lemanowicz 2013), organic matter concentrations (Xiao *et al.* 2012; Lemanowicz and Bartkowiak 2013).

High salinity suppresses the phosphorus uptake by plant roots and reduces the available phosphorus by sorption processes. One of the indices of changes in the content of phosphorus in soil can be the measurement of the activity of soil phosphatases, especially phosphomonoesterases which take part in the process of mineralization of organic phosphorus (Lemanowicz and Krzyżaniak 2015).

The study was conducted in the naturally salt-affected grassland in the areas of Natura 2000 in central Poland. The primary objective of this study was to determine the distribution of the content of total and available phosphorus, availability factor of this macroelement and the activities of phosphomonoesterase of natural saline soil against its selected chemical properties.

MATERIAL AND METHODS

Study area and soil sampling

The soil was sampled in Ciechocinek (Poland) in the area of halophyte natural reserve, which was founded in 1963 on the area of 1.88 ha ($52^{\circ}52'N$; $18^{\circ}46'E$; the Kuyavian-Pomeranian Province, central Poland) (Fig. 1).

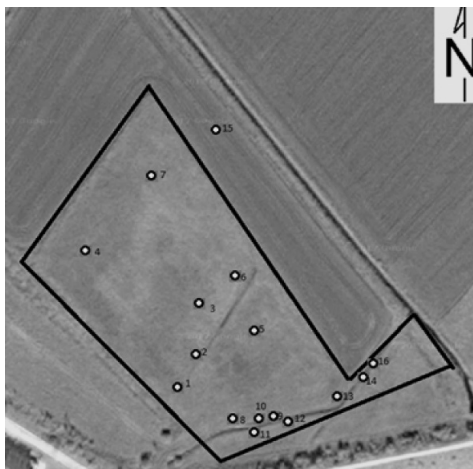


Fig. 1. Localization of the study area

The local climate is a typical temperate climate, with annual rainfall of about 500 mm. The annual mean temperature of the study area is $8.1^{\circ}C$. The reserve is situated on the complex of Vistula meadows and cropland. This habitat is inscribed on the list in Annex I of the Habitats Directive. Vertical seepage of mineral water causes salinity of these areas. The water is in contact with the Zechstein rocks salt structures up to groundwater level. As a result of the drainage of the valley (in the 50s and 60s of the 20th century) the water level fell by

more than 1 m. This led to the almost complete disappearance of the salt areas. Today, it is a refuge habitat of rare species of halophytes in Poland, particularly *Aster tripolium* and *Salicornia europaea* which, apart from Ciechocinek, do not grow in inland areas impacted by mineral brines. Through the halophyte reserve passes a shallow ditch draining storm water or the brine directly flowing from the neighbouring graduation towers (unique in Europe, wooden structures from the 19th century, built to thicken the brine in salt production process). Since the beginning of the 21st century, an extensive use of the land reserve began (mowing and grazing) and a ditch valve to discharge storm water from the graduation towers was installed. Halophilous vegetation began to spread in the reserve and small patches of *Salicornia europaea* were first seen outside the ditch.

A total of sixteen soil samples were collected (designated as site from 1 to 7, site from 11 to 14 and site 16 nearest to the drainage ditch; site 15 in agricultural area, respectively) during the summer of 2014. In agricultural area (site 15) winter wheat was cultivated, NPK fertilization was applied at the following rates: 154 kg ha⁻¹ N, (NH₄NO₃), 38.37 kg ha⁻¹ P (as (NH₄)₃PO₄) and 99.6 kg ha⁻¹ K (as KCl, 60% of K). Triplicate soil samples were collected from the 0–20-cm depth in each site. According to the international soil classification WRB (IUSS Working Group WRB 2015), most of them can be referred to as Fluvic Gleysols (Salic, Sodic).

Physiochemical parameters of the soil

Chemical analyses were performed on air-dried and sieved samples (<2 mm). Each sample was analysed in triplicate. In the adequately prepared soil samples the following were assayed: pH in 0.01 M CaCl₂ measured potentiometrically, total organic carbon (TOC) was determined with the TOC FORMACTS™ analyser Primacs provided by Skalar, the exchangeable cations of Ca, Mg, Na, K in the extract of BaCl₂ (PN-EN ISO 11260: 2011) using the method of atomic absorption spectrometry and emission spectrometry applying the spectrophotometer Philips PU 9100, electrical conductivity (EC_{1:5}) was determined in deionized water in a 1:5 soil: water extract. The content of total phosphorus (TP) was determined as described by Mehta *et al.* (1954), by treating soil with concentrated HCl and then with 0.5 M NaOH. After mixing, the extracts were mineralized with a respective mixture of concentrated acids: nitric acid (V), perchloric acid (VII) and sulphuric acid (VI) at the ratio of 10:1:4. Available phosphorus (AP) with the Egner-Riehm method – DL (PN-R-04023: 1996). The phosphorus content was calorimetrically defined by measuring the intensity of molybdenum blue and SnCl₂ reaction. The content of AP for soil is based on the available P status of the soil, which has been classified depending on the P concentration. The availability factor (AF%) for phosphorus, as suggested by Xiao *et al.* (2012), was applied for this purpose. It is expressed as follows: AF%=(AP/TP)*100.

Biochemical analyses

Prior to enzyme activity analyses, soil samples were thawed at 4°C until soil enzymatic analysis was performed within 2 weeks of sampling and soil moisture was determined after drying at 105°C for 48 h.

The activity of alkaline (AIP) [E.C. 3.1.3.1] and acid (AcP) [E.C. 3.1.3.2] phosphatase with the Tabatabai and Bremner method (1969). It is based on the colorimetric assaying of released substrate: p-nitrophenylphosphate after the incubation of soil with of MUB (Modified Universal Buffer) at pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase samples for 1 h at the temperature of 37°C.

Statistical analyses

Classical statistics was used to study tendencies (mean, median) and the variability (standard deviation SD, coefficient of variation, minimum and maximum) of the sample population. $CV\% = (SD/Mean) * 100$. Coefficient of variation (CV) was used to reflect the degree of discrete distribution of different TOC, TP, AP exchangeable cations contents and activity of phosphatase, and to indicate indirectly the activeness of the selected element in the examined environment. Values where 0–15%, 16–35%, and >36% indicate low, moderate, or high variability, respectively (Wilding 1985). The descriptive statistical analysis, such as Pearson's correlation coefficients were calculated using STATISTICA 7.0. All effects were considered significant at the $p < 0.05$ and $p < 0.001$ level.

Principal component analysis (PCA) was applied using data for soil phosphatase activities, content phosphorus and soil physical-chemical properties. The first two principal components (PC1 and PC2) were selected for further interpretation of the results. Hierarchical cluster analysis (CA) with Ward's method (1963) was used to identify the similarity groups between the sampling 16 sites.

All analytical measurements were performed with three replications. Arithmetic mean values are shown in tables.

RESULTS AND DISCUSSION

Soil pH, electrical conductivity and total organic carbon

Soil pH in 0.01M CaCl₂ range is limited and varies from 4.45 to 7.40 (Table 1) with a mean value of 6.33 ± 1.12 (Table 3). Soils from sites 1 to 7 were acidic (Table 1), and sites from 8 to 16 were slightly alkaline. This parameter is extremely important as it influences the surface charge magnitude of soil colloids, which is reflected in the cations exchangeable capacity. Then in the soil the balance between the content of exchangeable cations and the amount of

soil minerals weathering products in soil solution is established (Crabtree 1986). Electrical conductivity ($EC_{1.5}$) ranges from 1.89 dS m^{-1} to 15.18 dS m^{-1} and has arithmetical mean of $6.67 \pm 3.71 \text{ dS m}^{-1}$ (Table 3). In the study conducted by Piernik and Hulisz (2011), the soil salinity levels of natural saline were between $1.58\text{--}38.5 \text{ dS m}^{-1}$. Electrical conductivity is a soil parameter that indicates indirectly the total concentration of soluble salts and is a direct measurement of salinity (Mahmood *et al.* 2013). $EC_{1.5}$ was slight and very strong in most of the samples ($<2 \text{ dS m}^{-1}$) and has only one low value (1.89 dS m^{-1}) (Table 1).

TABLE 1. PH IN CaCl_2 , SALINITY ($EC_{1.5}$) AND EXCHANGEABLE CATIONS IN THE SURFACE SAMPLES

Sampling Sites	Clay %	pH CaCl_2	$EC_{1.5}$ dS m	Ca^{2+}	Mg^{2+}	Na^+	K^+
				mmol(+) kg^{-1}			
1	1.05	5.21	2.47	4.26	0.263	0.339	0.253
2	0.89	6.39	2.54	5.74	0.261	0.450	0.145
3	2.06	5.23	2.31	3.57	0.219	1.059	0.150
4	3.25	4.66	1.89	3.11	0.196	1.431	0.194
5	8.41	5.14	2.59	13.30	0.183	2.283	0.247
6	7.72	4.45	2.11	8.38	0.004	0.517	0.318
7	13.08	5.29	2.68	15.57	0.108	1.395	0.217
8	4.62	7.19	9.25	14.55	0.104	13.87	0.370
9	5.59	7.35	10.17	16.39	0.915	14.12	1.025
10	4.65	7.36	10.87	13.96	0.701	3.654	0.901
11	15.35	7.40	9.89	19.50	0.187	3.901	1.002
12	3.06	7.39	14.48	6.92	0.100	14.41	0.626
13	4.39	7.14	13.62	22.74	0.245	1.633	0.772
14	10.78	7.30	4.75	9.19	0.179	14.12	0.687
15	8.73	6.56	2.01	19.27	0.026	0.557	0.681
16	5.62	7.31	15.18	16.49	0.586	16.11	0.957

The total organic carbon content in the soil ranged from 3.910 to 7.780 g kg^{-1} (Table 2) (with a mean value of 6.204 ± 1.413) (Table 3), and the lowest content was recorded in the soil collected from site from 1 to 7 (Table 2). According to the literature, organic matter as an ingredient of soil (derived from various sources) increases its sorption capacity, the content of base cations and the degree of saturation of the sorption complex with basic cations, decreases hydrolytic acidity and increases soil pH (Caravaca *et al.* 1999; Frouz *et al.* 2006; Bielińska and Mocek 2010). The analysis of correlation confirmed a significant correlation between organic carbon and pH of the soil ($r=0.975$, $p<0.01$) and the content of cations of sodium ($r=0.696$, $p<0.05$) and potassium ($r=0.761$, $p<0.05$) (Table 6). Similar results were obtained by Ross *et al.* (1991, 2008) and Peinemann *et al.* (2000) in their research.

TABLE 2. THE CONTENT OF TOTAL ORGANIC CARBON (TOC), TOTAL PHOSPHORUS (TP), AVAILABLE PHOSPHORUS (AP), ACTIVITY OF ALKALINE (ALP) AND ACID (ACP) PHOSPHATASE AND THE AVAILABILITY FACTOR (AF) AND ENZYMATIC INDEX OF SOIL PH (ALP/ACP)

Sampling Sites	TOC g kg ⁻¹	TP g kg ⁻¹	AP g kg ⁻¹	AF %	ALP		AlP/AcP
					mM	pNP kg ⁻¹ h ⁻¹	
1	4.61	0.519	0.0152	2.928	0.677	1.429	0.47
2	6.35	0.480	0.0191	3.680	0.795	1.209	0.66
3	4.89	0.538	0.0163	3.030	0.601	1.198	0.50
4	4.25	0.522	0.0178	3.410	0.612	1.288	0.48
5	4.65	0.546	0.0164	3.004	0.632	1.294	0.49
6	3.91	0.492	0.0159	3.232	0.526	1.419	0.37
7	4.80	0.429	0.0152	3.543	0.601	1.308	0.46
8	7.39	0.319	0.0103	3.229	1.058	1.395	0.76
9	7.41	0.348	0.0098	2.816	1.171	1.426	0.82
10	7.33	0.348	0.0108	3.103	1.094	1.419	0.77
11	7.27	0.307	0.0094	3.062	1.144	1.528	0.75
12	7.78	0.312	0.0087	2.788	1.209	1.556	0.78
13	6.41	0.382	0.0107	2.801	0.958	1.358	0.71
14	7.21	0.369	0.0119	3.225	1.137	1.496	0.76
15	7.60	0.377	0.0198	5.252	1.098	1.567	0.70
16	7.41	0.357	0.0099	2.773	1.207	1.389	0.77

TABLE 3. STATISTICAL PARAMETERS OF THE SELECTED SOIL PROPERTIES

Parameters	(n)	Min	Max	Mean	Median	SD	CV %
Clay	16	0.89	15.35	6.20	5.12	4.20	67.75
pH CaCl ₂	16	4.45	7.40	6.33	6.85	1.12	17.76
EC	16	1.89	15.18	6.67	3.71	5.05	75.78
Ca ²⁺	16	3.11	22.74	12.05	13.63	6.24	51.77
Mg ²⁺	16	0.004	0.915	0.267	0.191	0.251	93.95
Na ⁺	16	0.339	16.11	5.615	1.958	6.304	112.2
K ⁺	16	0.145	1.025	0.534	0.498	0.330	61.78
TOC	16	3.910	7.780	6.204	6.810	1.413	22.77
TP	16	0.307	0.546	0.415	0.379	0.086	20.93
AP	16	0.009	0.020	0.014	0.013	0.003	27.67
AlP	16	0.526	1.209	0.907	1.008	0.260	28.69
AcP	16	1.198	1.567	1.392	1.407	0.112	8.095
AF	16	2.773	5.252	3.242	3.164	0.600	18.51
AlP/AcP	16	0.370	0.820	0.640	0.705	0.149	23.41

SD – standard deviation, CV [%] – coefficient of variation

TABLE 4. RELATIONSHIP BETWEEN SELECTED SOIL PROPERTIES
($P < 0.05$, $P < 0.001$ *)

Variables		Equation	<i>r</i>	<i>r</i> ²
Dependent	Independent			
Total organic carbon	Alkaline phosphatase	$y = 5.2902x + 1.403$	0.974	0.950
Alkaline phosphatase	Available phosphorus	$y = -56.710x + 1.677$	-0.691	0.447
pH CaCl ₂	Available phosphorus	$y = -231.595x + 9.479$	-0.779	0.607
pH CaCl ₂	Alkaline phosphatase	$y = 4.2177x + 2.508$	0.957	0.952
pH CaCl ₂	Total organic carbon	$y = 0.777x + 1.515$	0.975*	0.952
Na ⁺	Total organic carbon	$y = 3.107x - 13.665$	0.696	0.484
K ⁺	Total organic carbon	$y = 0.177x - 0.568$	0.761	0.579
Na ⁺	Available phosphorus	$y = -1216x + 22.136$	-0.725	0.525
K ⁺	Available phosphorus	$y = -60.34x + 1.380$	-0.709	0.502
Ca ²⁺	Alkaline phosphatase	$y = 12.19x + 0.994$	0.508	0.258
Na ⁺	Alkaline phosphatase	$y = 17.57x - 10.334$	0.726	0.527
K ⁺	Alkaline phosphatase	$y = 1.081x - 0.446$	0.852	0.727
EC _{1:5}	Available phosphorus	$y = -1191x + 22.85$	-0.884	0.782
EC _{1:5}	Alkaline phosphatase	$y = 14.793x - 6.749$	0.761	0.579
EC _{1:5}	Na ⁺	$y = 0.523x + 3.737$	0.651	0.425
EC _{1:5}	K ⁺	$y = 11.51x + 0.526$	0.750	0.563
EC _{1:5}	AF	$y = -4.287x + 20.577$	-0.508	0.258
AIP/AcP	Available phosphorus	$y = -26.68x + 1.002$	-0.668	0.446
AIP/AcP	pH CaCl ₂	$y = 0.131x - 0.188$	0.981	0.964
AIP/AcP	Na ⁺	$y = 0.016x + 0.547$	0.694	0.482
AIP/AcP	K ⁺	$y = 0.358x + 0.448$	0.789	0.623

TABLE 5. VALUES OF THE THREE EXTRACTED FACTOR LOADINGS FOR
13 ELEMENTS

Parameters	Component matrix	
	PC1	PC2
TOC	0.929*	0.205
TP	-0.934*	-0.209
AP	-0.837*	0.411
pH	0.957*	0.082
AIP	0.965*	0.173
AcP	0.616	0.470
Ca ²⁺	0.582	0.327
Mg ²⁺	0.482	-0.487
Na ⁺	0.751*	-0.264
K ⁺	0.894*	0.060
EC	0.868*	-0.305
AF	-0.229	0.918*
AIP/AcP	0.941*	0.091
Variation (%)	63.82	14.45
Eigenvalue	8.295	1.878

* statistically significant

The exchangeable cations

In the analysed soil samples a series of quantitative cations of basic character is as follows: $\text{Ca}^{2+} > \text{Na}^+ > \text{K}^+ > \text{Mg}^{2+}$. Also in the studies of Lemanowicz and Krzyżaniak (2015) in salt-affected Mollic Gleysols in direct surroundings of the graduation towers in Inowrocław (Poland), and Kobierski et al.'s (2011) research concerning soil of the Drawskie Lakeland, calcium was the dominant cation in the sorption complex. Whereas in the research of Piernik and Hulisz (2011) in natural saline grasslands in the villages and meadows in the valley of river, cations were arranged in the following order: $\text{Na}^+ > \text{Ca}^{2+} > \text{K}^+ > \text{Mg}^{2+}$. The difference in exchangeable bases between not saline soils and saline soils are mainly in cation ratios. Not saline soils are: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$. The analysis of the contents of individual cations in sorption complex showed a clear dominance of calcium ions. The contents of this cation ranged from 3.11 to 22.74 mmol(+) kg⁻¹ (Table 3). Large amounts of calcium cations are characteristic of alkaline soils. In the analysed samples such a relationship was also observed. Second after calcium in terms of the number of occurrence in the analysed soil sorption complex were sodium ions. The concentration of Na⁺ was in a wide range of from 0.339 to 16.11 mmol(+) kg⁻¹ and average 5.615 mmol(+) kg⁻¹. Please note that sodium cations are one of the most easily leached cations from the soil by rain water and their large quantities in surface profiles of soil levels are only observed when there is a continuous supply of sodium compounds into the soil from specific sources (Czerwiński 1996; Kwasowski 1996; Ochman and Jezierski 2011). Increased content of ions in the analysed samples from 8 to 14 and the sample no 16 were caused by the close proximity of the ditch-powered water discharged from the nearby graduation towers. The high content of sodium ions in water extracts of soils indicates a violation of the natural system of base cations in the soil solution, which for most soils of a temperate climate are dominated by calcium, then by magnesium and potassium. This allows one to conclude that the increased salinity significantly modifies the quantitative and qualitative composition of soil solutions. But according to Rengasamy and Olson (1991), soils high in organic matter are generally resistant to Na adsorption and rarely display sodic behaviour, largely due to increased hydrophobicity caused by the presence of hydrophobic organic compounds. The calculated correlation coefficients confirmed a significant correlation between Na⁺ ions ($r=0.651$, $p<0.05$) and K⁺ ($r=0.750$, $p<0.05$) and the conduction of electrolyte (Table 4). Similar results were obtained by Czerwiński (1996) and Ochman *et al.* (2011) in the analysed soils fed by post-floatation waters and by Krzyżaniak and Lemanowicz (2013) in saline soils of the Kujawy region. In the analysed samples, similarly to the soils studied by Piernik and Hulisz (2011), the lowest Mg²⁺ ions were observed, its content was in a narrow range of from 0.004 to 0.915 mmol(+) kg⁻¹.

The content of phosphorus

The total phosphorus content was in the range of 0.307–0.546 g kg⁻¹ (mean 0.415 g kg⁻¹ ± 0.086), while the soil available phosphorus content ranged from 0.009–0.020 g kg⁻¹ (mean value 0.014 g kg⁻¹ ± 0.003) (Table 3). According to PN-R-04023 (1996), this soil classifies as V class with a very low content of AP. According to Lemanowicz and Krzyżaniak (2015), a constant supply of a large amount of cations and anions from the structures can, as a result, lead to permanent changes in the chemical properties of soils, making direct surroundings of the graduation towers. In fact, aerosol production is a natural process deriving from the interaction of wind stress graduation towers. Soil available phosphorus content had significant negative correlation with pH CaCl₂ value ($r = -0.779$, $p < 0.05$) (Table 4). There is a considerable evidence in the literature (Lakhdar *et al.* 2009; Krzyżaniak and Lemanowicz 2013) that pH can affect forms and dynamics of this nutrient in soil. At pH > 7 phosphorus is precipitated by Ca²⁺ and Mg²⁺.

Based on the present results of the content of total and available phosphorus, there was calculated availability factor AF(%) for phosphorus. In the soil investigated, the AF value ranged from 2.773% to 5.252% (Table 3). When the value of the AF ratio is higher than 2% it indicates that soil P was sufficient for plant growth in this environment. According to Qadir and Schubert (2002) and Pan *et al.* (2013), most saline soils are adequately supplied with phosphorus. Concentration of Na⁺ ions may result in more soluble Na₃PO₄ formed. In this study, the concentration of Na⁺ ions increased with an increase in salinity (positive correlation), which leads to increased AP content in soils. The highest value of the AF (5.252%) was obtained in the soil of the site 15 (arable soil) (Table 2). It could be due to the inflow of total organic carbon (TOC), which stimulates the activity of phosphatases, which speeds up the P cycling.

Soil alkaline and acid phosphatase

The activities of both alkaline and acid phosphatase are closely related to soil pH, with acid phosphatase dominating in acid soils, and alkaline phosphatase in alkaline soils (Wang *et al.* 2011). According to Zhang *et al.* (2014), soil pH also affects the activity of enzymes due to the pH sensitivity of amino acid functional groups that alter the conformational and chemical changes of amino acids essential for binding and catalysis. According to Wilding's (1985) classification, the CV values of soil alkaline phosphatase activities was relatively low (8.095%), whereas those of acid phosphatase activities (49–53%) were at the medium level.

The reduction of enzyme activity in saline soils could be due to the osmotic dehydration of the microbial cells that liberate intracellular enzymes. Soil alkaline phosphatase activity had the same (28.69% – moderate) variability as the available phosphorus (27.67%) (CVs Table 3).

Correlation analysis among phosphatase activities soil and chemical properties indicated several significant trends (Table 4). Correlation analysis showed that there was significant correlation between the total organic carbon and the activities of soil alkaline phosphatase ($r=0.974$, $p<0.05$), indicating that an increase in soil enzymatic activity is a consequence of increased OM (organic matter) (Siddikee *et al.* 2011). Comparing soils sites from 1 to 7 showed much lower enzyme activities than in soils of sites from 8 to 16 (maximum alkaline and acid phosphatase activities were observed in soils from the sites where halophytic vegetation was found) (Table 2). According to Rao *et al.* (1996), organic matter has ameliorative influence on biological activity in salt-affected soils and inhibition of alkaline and acid phosphatase activities in soil sites from 1 to 7 might be due to the lower bacterial population and activity under the limited carbonaceous substrates. The results indicated that pH CaCl_2 value was positively correlated with alkaline phosphatase activity at an extremely significant level ($r=0.975$, $p<0.05$) but not with acid phosphatase activity. In the study of Wang *et al.* (2011) alkaline phosphatase activity was more than twice as high as acid phosphatase activity, mainly because soil pH was in the range of 7.2–7.7. Our result agrees with earlier reports (Siddikee *et al.* 2011) that alkaline phosphatase activity was not predominant in neutral or alkaline soils. Zhang *et al.* (2014) states that with the soil pH varying from 7.38 to 10.00, AIP activities decreased exponentially. According to Tripathi *et al.* (2007), phosphatase activity responded more to pH than to the differences in soil salinity. Soil $\text{EC}_{1.5}$ had a significant positive correlation with alkaline phosphatase activity ($r=0.761$, $p<0.05$). The influence of soil $\text{EC}_{1.5}$ on enzyme activities might be that the level of soil salt had some impacts on enzyme configuration and formation of its activity centre. Changes in salinity also have the potential to impact extracellular enzyme activity by influencing molecular stability and protein confirmation states (Lakhdar *et al.* 2009). Soil available phosphorus content had a significant ($r=-0.884$, $p<0.05$) negative correlation with the $\text{EC}_{1.5}$ value. In saline soils the availability of phosphorus depends on the length of the roots of the plants and the negative effect of chlorides on the absorption of P by the roots. Alkaline reaction is usually modified by the phosphorus balance, which in those soils undergoes retrograde processes transforming into sparingly soluble phosphates.

The AIP/AcP ratio of the soil profiles falls within the range of 0.370–0.820 (Table 3). In most cases the values exceed 0.50 (site 2 and sites from 8 to 16) (Table 2), which in the case of the soil analysed was confirmed with the potentiometer method of pH in CaCl_2 measurement, which is connected with the fact that phosphomonoesterases are the enzymes which are one of the most sensitive indicator of change of soil pH (Dick *et al.* 2000, Krzyżaniak and Lemanowicz 2013).

A significant statistical negative correlation between the content of available phosphorus and the activity of alkaline phosphatase in soil was recorded ($r=-0.691$; $p<0.05$). Negative correlation between alkaline phosphatase activi-

ty and available phosphorus content showed that alkaline phosphatase activity is higher in the soil deficient in soil phosphorus. No significant correlations between available P and acid phosphatase activities were found, which suggests that the AIP was the adequate parameter defining the soils analysed, unlike the activity of acid phosphatase.

Soil alkaline phosphatase activities showed significant positive correlations with exchangeable Na^+ ($r=0.726$, $p<0.05$) and Ca^{2+} ($r=0.508$, $p<0.05$) content.

Soil enzymes are an indicator of soil biological activity because, according to Koper *et al.* (2008), Siddikee *et al.* (2011), Lemanowicz (2013), Guan *et al.* (2014), enzymes are the products of vital activity, and their activities may reflect the states of soil biological metabolism and material transformation. Changes in salinity and alkalinity in the soil could impact the activities of soil phosphatase and the content of phosphorus.

A principal component analysis (PCA) was carried out taking into account the following thirteen parameters: pH CaCl_2 , conductivity ($\text{EC}_{1:5}$), Ca^{2+} , Mg^{2+} , Na^+ , K^+ , TOC, TP, AP, AIP, AcP, AF and AIP/AcP. The contribution of the two first axes to the total variability is 78.37% (Table 5) for all the soil samples: 63.82% for the first axis (PC1) and 14.45% for the second (PC2) (Fig. 2).

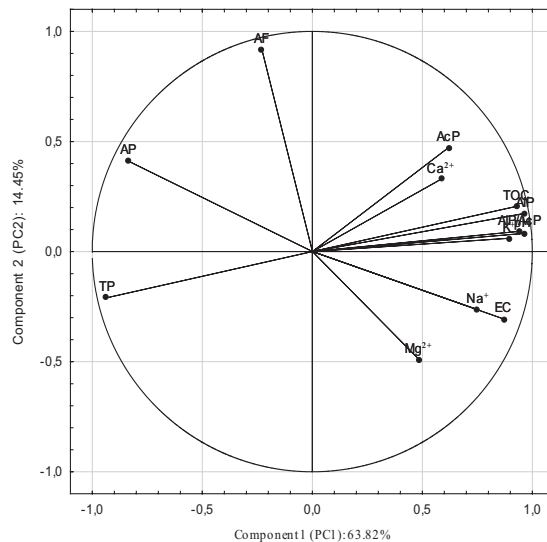


Fig. 2. Configuration of variables in the system of the first two axes of principal components PC1 and PC2 of the content of total organic carbon (TOC), total (TP) and available (AP) phosphorus, the activity alkaline (AIP) and acid (AcP) phosphatase and physical-chemical properties in soil (the exchangeable cations of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , pH CaCl_2 , EC)

PC1 is essentially characterized by 9 parameters (seven strongly correlated in the positive direction and two in the negative direction). Both TOC, pH, AIP and AIP/AcP content had a major positive effect on PC1 (>0.900), while soil

TP and AP content had a negative effect on PC1 (>0.900) (Table 5). Moreover, K and $EC_{1.5}$ had a major positive effect on PC1 (>0.800). This association strongly suggests that these variables have a similar source. The distribution of these elements is mainly controlled by natural parent materials. The meaning of PC1 also agrees with the correlation coefficient between these variables. Alkaline phosphatase was located on the same site and near pH, $EC_{1.5}$, TOC indicating that mainly the activity of this enzyme varies in terms of this soil property (as indicated by the length and direction of its vector). The non-significant correlation of acid phosphatase with the 1st PC implies that the enzyme is not associated with the total organic carbon and phosphorus. Factor 2 (PC2) is dominated by AF (available factor) accounting for 14.45% of the total variance. The obtained results demonstrate that statistical procedures towards classifying the content of phosphorus, activity phosphatase as groups in terms of relationship with soil some properties and identifying their probable origin in soil.

Cluster analysis (CA) is a statistical technique for classifying different soil samples by the relationships among measured variables representing soil properties. CA rendered a dendrogram as shown in Fig. 3A, grouping all 16 sampling sites into three statistically significant clusters.

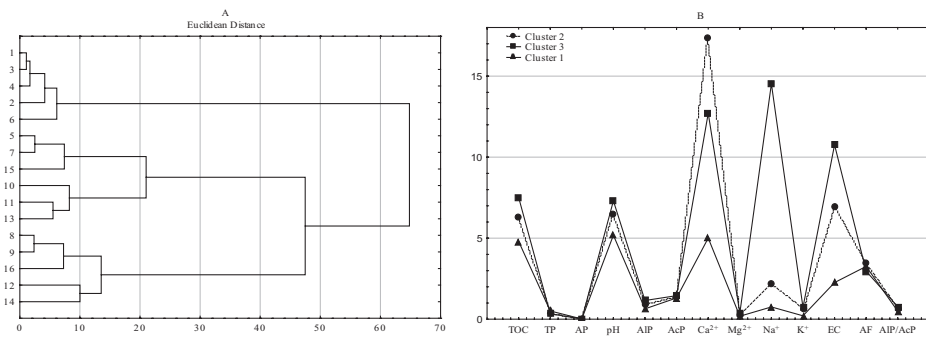


Fig. 3 A and B. Similarity dendrogram for sampling sites (1–16) (A) and a graph *k*-means for the study sites (B).

The most relevant in this study was Cluster 3, formed by most sampling sites nearest to the drainage ditch (cases 8, 9, 12, 14, 16). These sampling sites were characterized by their higher sodium ions concentrations, electrical conductivity ($EC_{1.5}$) and pH in $CaCl_2$ (Fig. 3B), which might be related to the specific location of major tributaries of the saline. Five soil samples (cases 1, 2, 3, 4, 6) were included in Cluster 1 due to its lower alkaline phosphatase activity (AIP), total organic content and lower EC, pH and the exchangeable cations (Fig. 3B).

CONCLUSIONS

1. In the analysed soil samples a series of quantitative cations of basic character is as follows: $\text{Ca}^{2+} > \text{Na}^+ > \text{K}^+ > \text{Mg}^{2+}$. Increased salinity has modified the qualitative and quantitative composition of the soil solution.

2. Correlation analysis confirmed the significant relationship between the conduction of the electrolytic soil and the content of sodium and potassium cations.

3. According to PN-R-04023 (1996), this soil classifies as class V with a very low content of AP but the availability factor for phosphorus (AF) value ranged from 2.773 to 5.252% indicating that soil P was sufficient for plant growth in this habitat.

4. The positive significant correlations among soil alkaline phosphatase and some physicochemical properties suggested that salinization had effects on these variables. Alkaline phosphatase may be used as indicators of soil quality in salinized grassland habitat Natura 2000.

5. Salinization on soil the content phosphorus and the activity phosphatases in grasslands habitat Natura 2000 in Ciechocinek (Poland) need further evaluation, which could provide rational management measures to promote their long-term sustainability.

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