

## Enzymatic activity of the Kuyavia Mollic Gleysols (Poland) against their chemical properties

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### ABSTRACT

The research results have shown that the enzyme pH index (0.49–0.83) confirmed the neutral or alkaline nature of the soils. Neither the changes in the content of available phosphorus nor in the activity of dehydrogenases, catalase, alkaline and acid phosphatase in soil were due to the factors triggering soil salinity; they were a result of the naturally high content of carbon of organic compounds, which was statistically verified with the analysis of correlation between the parameters. There were recorded highly significant values of the coefficients of correlation between the content of available phosphorus in soil and the activity of alkaline ( $r = 0.96$ ;  $P < 0.05$ ) and acid phosphatase ( $r = 0.91$ ;  $P < 0.05$ ) as well as dehydrogenase ( $r = 0.90$ ;  $P < 0.05$ ). To sum up, one can state that Mollic Gleysols in Inowrocław are the soils undergoing seasonal salinity; however, a high content of ions responsible for salinity is balanced with a high content of organic carbon, humus, phosphorus and calcium directly affecting the fertility of the soils analyzed. The activity of the enzymes depended on the natural content of carbon of organic compounds and not on the factors affecting the soil salinity, which points to the potential of such tests for soil environment monitoring.

**Keywords:** phosphatases; dehydrogenases; catalase; salinity; cations; soil

The soils in the Inowrocław Plain, including the soils of the Health Resort Park in Inowrocław, are one of the greatest Mollic Gleysols complexes in central Poland. The functional values of Mollic Gleysols, their high productivity and fertility make the areas exposed to an intensive agricultural cultivation. However, it is not the only factor which could have a negative effect on the soils. In the Inowrocław Plain the soda industry developed at a large scale, degrading the soil environment by salinity in the adjacent areas. A specific anthropogenic factor found in the Health Resort Park in Inowrocław, connected with brine mining and graduation towers are locally affecting the soils. A permanent supply of a large amount of cations

and anions can, as a result, lead to permanent or seasonal changes in the chemical properties of soils making up the direct neighbourhood of the graduation towers (Krzyżaniak 2011, Krzyżaniak and Długosz 2012), and thus affect their fertility. Salinity is the important environmental stress factor that affects the plant growth and nutrition of plant (Wang et al. 2012). Soil enzymes play the basic role in the ecosystems acting as catalysers of chemical reactions. They lead to the decomposition of organic matter, the nutrients circulation, stimulate the transition of biogenic elements into forms easily available to plants, and so the activity of enzymes can serve as ‘soil fertility index’ (Brzezińska 2011). According to Shi et al. (2008),

natural or anthropogenic salinity can change the soil fertility as a result of the changes caused due to biochemical processes. An excessive content of easily-soluble inorganic salts in soil has a negative effect on the changes in the conformation of enzymatic protein which, as a result, results in a decrease in its catalytic activity (Rietz et al. 2001, Telesiński et al. 2008, Siddikee et al. 2011).

The present research aimed at evaluating the potential of applying enzymatic tests to diagnose the level and the amount of salinity of the soil environment in the Health Resort Park in Inowrocław.

## MATERIAL AND METHODS

The research area is made up of an almost 60 ha of the Health Resort Park in Inowrocław, located in the Kujawsko-Pomorskie province, the Inowrocław county, the commune of Inowrocław (in centre Poland). The location of the Health Resort Park in Inowrocław is determined by the latitude (52°78'N) and longitude (18°24'E); with the lowland climate of moderate altitudes.

From the park area in summer (June) 2010, 27 representative soil samples were taken from the topsoil (0–20 cm) (Figure 1). The area receives between 400 mm and 500 mm rainfall per annum (mainly during summer months), while the temperature ranges between –9°C and 20°C. The soils in the research area were classified based on the physicochemical properties, the grain size composition and the profile structure as Mollic Gleysols (WRB 2006). The surface horizon of the health resort park soils was dominated by sand fraction (2.0–0.05 mm) ranging from 64% to 88% and the colloidal clay – from 5.0% to 16.0%. The ratio of the values of those two fractions must be due to the surface horizons of Mollic Gleysols becoming sand-like, as described earlier (Krzyżaniak-Sitarz 2008).

In the adequately prepared soil samples the following were assayed: pH in H<sub>2</sub>O and pH in 1 mol/L KCl measured potentiometrically, hydrolytic acidity (Hh) with the Kappen method (Soil Survey Investigation 1996), total organic carbon (TOC) was determined with the TOC analyser Primacs provided by Scalar, the content of calcium carbonate with the Scheibler method, the exchangeable cations of Ca, Mg, Na, K in the extract of 1 mol/L NH<sub>4</sub>Cl with pH 8.2 using the method of atomic absorption spectrometry and emission spectrometry applying the spectrophotometer Philips PU

9100 (PN-ISO 11260, Poland, 2011), the water-soluble forms of Ca, Mg, Na, K with the method of atomic absorption spectrometry and emission spectrometry using the spectrophotometer Philips PU 9100 (PN-ISO 11260, 2011), salinity with the conductometric method (EC), the content of chlorides with the argentometric method, the content of sulphates with the nephelometric method, the content of hydrogencarbonates using the acid-base titration method (Reeuwijk 2002), the content of available phosphorus (P<sub>E-R</sub>) with the Egner-Riehm method – DL (PN-R-04023, 1996), the activity of dehydrogenases (DEH) [E.C. 1.1.1] in soil with the Thalmann method (Thalman 1968), the activity of catalase (CAT) [E.C. 1.11.1.6] in soil with the Johnson and Temple (1964), the activity of alkaline (ALP) [E.C. 3.1.3.1] and acid (AcP) [E.C. 3.1.3.2] phosphatase with the Tabatabai and Bremner method (Tabatabai and Bremner 1969).

All the assays were made in three reps; the paper demonstrates the arithmetic means of the results. Besides the results of the analyses of the features investigated were exposed to the analysis of simple correlation ( $P < 0.05$ ) which determined the degree of the dependence between respective features. The analysis of the correlation was made using Statistica for Windows Pl software (Poland).

## RESULTS AND DISCUSSION

The content of carbon of organic (TOC) compounds ranged from 1.08% to 3.85%, which can be converted into the value of humus accounting for 1.85–6.63% (Table 1). The values of those parameters do not show a visible spatial variation due to specific factors, however, they are rather a

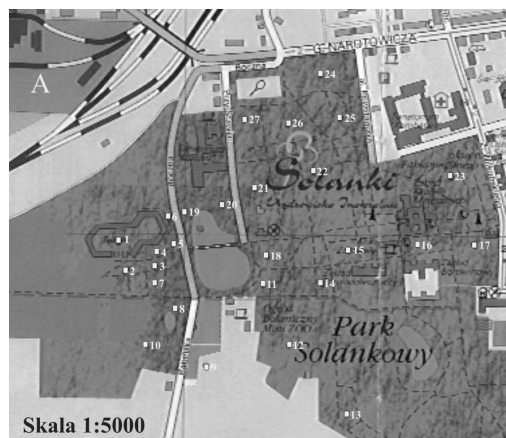


Figure 1. Location of sampling sites in the Inowrocław Health Resort Park

result of their natural variation, unlike the value of pH in 1 mol/L KCl and in H<sub>2</sub>O depending on the location of the sampling site.

The highest pH values showing a neutral to alkaline reaction was recorded for the level adjacent to graduation towers (samples number 1–8, 18, 19) pH<sub>KCl</sub> 6.63–7.69, pH<sub>H<sub>2</sub>O</sub> 7.37–7.99. The other area analysed, with little exceptions, demonstrated the reaction from acid to neutral pH<sub>KCl</sub> 5.03–7.07, pH<sub>H<sub>2</sub>O</sub> 6.11–7.53 (Table 1). Hydrolytic activity was closely connected with the reaction measurement. The sites showing alkaline reaction demonstrated undetectable concentration of hydrogen cations, while the samples from the other area showed the Hh

values falling within 3.9–47.3 mmol<sub>+</sub>/kg (Table 1). In the sorption complex of the surface horizon calcium dominated 58.39–584.06 mmol<sub>+</sub>/kg, the second biggest was sodium cation 2.88–54.34 mmol<sub>+</sub>/kg, followed by magnesium 3.80–17.9 mmol<sub>+</sub>/kg and potassium 1.2–6.9 mmol<sub>+</sub>/kg (Table 1).

The analysis of water-soluble cations in the surface horizon of soil showed that the highest concentrations were assumed by ions Na<sup>+</sup> 0.67–38.76 mmol<sub>+</sub>/kg, followed by Ca<sup>2+</sup> 0.13–10.19 mmol<sub>+</sub>/kg, K<sup>+</sup> 0.60–5.8 mmol<sub>+</sub>/kg, Mg<sup>2+</sup> 0.30–1.7 mmol<sub>+</sub>/kg (Table 2).

The content of anions in soil solution was as follows: Cl<sup>-</sup> 2.00–41.20 mmol<sub>+</sub>/kg, HCO<sub>3</sub><sup>-</sup> 0.00–

Table 1. Chemical parameters and exchangeable cations in the surface samples

Sample No.	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Hh	CEC	TOC (%)	P <sub>E-R</sub> (mg/kg)	pH <sub>H<sub>2</sub>O</sub>	pH <sub>KCl</sub>
	(mmol <sub>+</sub> /kg)									
1	387.4	6.8	42.45	4.4	0.00	441.0	1.60	46.85	7.99	7.05
2	292.4	12.5	28.22	6.8	0.00	339.9	2.22	80.62	7.37	6.63
3	338.1	10.5	23.82	6.6	0.00	379.0	2.11	126.3	7.55	7.19
4	219.5	10.2	47.55	4.7	0.00	281.9	1.93	95.72	7.53	7.44
5	236.1	9.6	52.61	5.2	0.00	303.5	1.21	75.21	7.99	7.69
6	241.1	6.5	54.34	4.9	0.00	306.8	3.85	175.2	7.96	7.64
7	235.1	12.2	14.7	4.4	0.00	266.4	2.07	87.79	7.60	7.09
8	269.6	10.2	4.94	5.8	3.9	294.4	1.86	26.99	7.65	6.85
9	125.1	8.3	3.44	4.8	18.8	160.4	1.67	46.76	6.83	5.85
10	197.2	9.8	2.88	3.2	0.00	213.1	1.69	51.30	7.42	7.06
11	361.5	14.6	20.9	6.2	0.00	403.2	2.45	195.1	7.61	7.40
12	584.0	8.3	15.95	6.9	47.3	662.4	1.78	65.16	6.04	5.57
13	377.6	8.3	13.43	4.9	13.1	417.3	1.43	70.96	6.97	6.23
14	234.1	17.9	16.08	6.8	5.3	280.2	3.21	222.3	7.53	6.73
15	161.8	6.0	15.69	4.2	7.9	195.6	1.34	43.73	7.08	6.33
16	157.2	6.8	11.47	3.8	17.9	197.2	1.40	35.26	6.49	5.98
17	149.7	7.5	15.61	3.4	0.00	176.2	1.56	139.7	7.46	7.24
18	241.3	30.4	24.65	3.7	0.00	300.0	2.61	181.7	7.54	7.26
19	339.1	11.3	38.64	4.3	0.00	393.3	1.83	63.70	7.74	7.42
20	171.8	10.5	11.48	3.2	0.00	197.0	1.85	61.32	7.22	6.69
21	89.03	6.0	5.39	1.2	27.1	128.7	1.27	31.57	6.20	5.20
22	58.39	6.1	5.59	3.5	28.4	102.0	1.41	27.63	6.11	5.03
23	422.9	12.9	5.28	2.4	0.00	443.5	2.13	201.6	7.73	7.41
24	216.1	7.9	4.72	2.0	0.00	230.7	1.58	36.57	7.28	7.07
25	164.0	6.9	5.16	3.6	6.1	185.8	2.13	62.54	7.24	6.50
26	103.2	5.7	5.17	3.5	14.9	132.5	1.08	19.64	6.60	6.07
27	130.7	3.8	4.86	2.7	21.4	163.5	1.20	82.64	6.39	5.63
Mean	240.8	9.91	18.33	4.33	7.85	281.3	1.86	87.18	7.22	6.67
SD	118.3	5.141	15.81	1.506	12.05	126.0	0.622	59.96	0.58	0.75

SD – standard deviation

8.38 mmol<sub>+</sub>/kg, SO<sub>4</sub><sup>-</sup> 0.02–2.46 mmol<sub>+</sub>/kg (Table 2). A few parameters such as exchangeable Na, water-soluble Na and chloride ions demonstrated a clear relationship between the distance from the graduation tower and the concentration of respective ions. The highest values of the concentration were reported in the samples close to the graduation tower, however, the greater the distance, the lower the concentration of those ions, which was confirmed by the values of electrolytic conductivity. The EC values divided the area analysed also into two parts; the first one is the area located within the graduation tower where the EC values ranged from 0.79 to 3.77 mS/cm and the other area with the EC values within the range of

0.25–1.00 mS/cm (Table 2). Higher EC values were reported earlier by Rietz et al. (2001) and Saviozzi et al. (2011), which points to a strong salinity of the soils. The content of available phosphorus in soil fell within the range from 19.64 to 222.3 mg P/kg (87.2 mg P/kg, on average) (Table 1). The soil investigated, according to the criteria provided in PN-R-04023 (1996), can be classified as class I with a very high content of P<sub>E-R</sub>.

The activity of dehydrogenases in horizon A of Mollic Gleysols varied a lot (0.182–0.584 mg TPF/kg/h) (Table 3). However, no dependence between the location of soil sampling sites and the activity of that enzyme was found.

Table 2. Water-soluble forms of cations and anions in the surface samples

Sample No.	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	EC (mS/cm)	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
	(mmol <sub>+</sub> /kg)					(mmol <sub>+</sub> /kg)		
1	0.24	0.8	15.00	5.8	1.84	13.20	8.38	0.10
2	0.25	0.8	6.07	3.1	0.87	5.00	3.38	0.19
3	0.95	1.1	3.28	1.9	0.79	10.10	1.62	0.13
4	10.19	1.7	31.58	0.7	3.34	36.20	2.50	0.98
5	9.02	1.4	38.76	0.7	3.77	41.20	2.50	1.33
6	0.29	0.9	33.01	0.6	2.90	26.10	6.62	1.20
7	0.73	1.1	1.84	3.4	0.75	2.50	5.00	0.09
8	1.17	1.1	2.74	2.6	0.87	3.70	4.12	0.26
9	0.13	0.6	0.90	2.6	0.29	3.10	1.62	0.09
10	0.60	1.0	0.67	2.3	0.59	2.00	3.38	0.13
11	4.63	1.6	3.12	1.5	0.95	2.70	6.62	0.26
12	0.75	0.7	0.98	2.1	0.35	3.40	0.38	0.16
13	1.11	0.8	0.79	2.5	0.41	2.60	2.12	0.13
14	3.60	1.5	2.47	1.6	0.84	3.10	4.62	0.27
15	1.68	0.8	1.25	1.1	0.45	3.50	1.25	0.15
16	0.33	0.7	0.75	1.5	0.35	2.50	0.88	0.13
17	1.97	1.0	4.09	1.3	1.22	9.90	1.25	0.59
18	1.22	1.3	10.79	1.9	1.76	7.90	5.38	2.46
19	4.81	0.9	17.37	2.7	2.52	22.00	6.62	1.69
20	2.66	0.7	1.35	1.1	0.64	3.50	1.25	0.02
21	1.11	0.5	1.37	1.8	0.31	2.60	0.38	0.04
22	0.52	0.4	1.46	1.0	0.25	3.60	0.38	0.07
23	5.02	0.8	0.68	1.2	1.00	2.70	6.25	0.86
24	2.24	1.0	1.22	1.6	0.73	3.40	2.50	0.16
25	2.10	0.5	0.97	1.6	0.64	2.70	4.12	0.13
26	0.86	0.3	1.27	1.4	0.33	2.70	2.12	0.07
27	0.76	0.3	0.69	1.1	0.28	2.80	0.00	0.10
Mean	2.18	0.9	6.94	1.87	1.07	8.32	3.15	0.43
SD	2.57	0.372	10.88	1.075	0.979	10.60	2.32	0.60

SD – standard deviation

The activity of soil catalase fell within a very wide range from 0.003 to 0.990 H<sub>2</sub>O<sub>2</sub>/g/h (Table 3). It is an anti-oxidation enzyme, participating in the defence of the plants from abiotic and biotic factors triggering oxidation stress among plants, e.g. growing in saline soils (Jaspers and Kangasjärvi 2010, Wu et al. 2012). A lack of significant relationships between the activity of catalase and the content of chloride and sodium ions in soil points to an inconsiderable salinity of the surface horizons of the Health Resort Centre soils.

The activity of alkaline and acid phosphatases fell within a wide range; 1.327–3.399 mmol pNP/kg/h

as well as 1.603–4.378 mmol pNP/kg/h, respectively (Table 3). The activity of acid phosphatase was higher than the alkaline by an average of about 26%. The results do not coincide with the earlier reports (Dick and Tabatabai 1984) on the activity of alkaline phosphatase dominating in neutral or alkaline soil reaction. Similar results were recorded by Siddikee et al. (2011) who claim that a decreased activity of enzymes (alkaline phosphatase) in the permanently or seasonally saline soils can be due to a change in the osmotic potential caused by a toxic effect of specific ions and the modification of the centre of the active enzyme being a protein Zahran (1997).

Table 3. Activity of the dehydrogenases (DEH), catalase (KAT), alkaline (AIP) and acid phosphatases (AcP) and ratio of alkaline to acid phosphatase AIP/AcP (enzyme pH index)

Sample No.	DEH (mg TPF/kg/h)	CAT (H <sub>2</sub> O <sub>2</sub> /kg/h)	AIP	AcP	AIP/AcP
			(mmol pNP/kg/h)		
1	0.343	0.000139	1.824	2.639	0.69
2	0.443	0.000128	2.136	3.624	0.59
3	0.486	0.000115	2.535	3.714	0.68
4	0.481	0.000990	2.345	3.716	0.63
5	0.429	0.000930	2.029	3.363	0.60
6	0.521	0.000108	2.617	3.972	0.66
7	0.471	0.000860	2.161	3.482	0.62
8	0.182	0.000032	1.363	1.851	0.74
9	0.328	0.000036	1.830	2.589	0.71
10	0.351	0.000003	1.909	3.150	0.61
11	0.584	0.000950	3.061	4.346	0.70
12	0.408	0.000990	2.079	3.028	0.69
13	0.412	0.000850	2.073	3.329	0.62
14	0.571	0.000910	3.399	4.378	0.78
15	0.308	0.000930	1.727	2.532	0.68
16	0.272	0.000750	1.481	2.396	0.62
17	0.519	0.000650	2.534	3.721	0.68
18	0.544	0.000920	2.703	4.190	0.64
19	0.386	0.000105	2.053	3.159	0.65
20	0.376	0.000710	1.896	2.871	0.66
21	0.266	0.000057	1.554	2.421	0.64
22	0.221	0.000054	1.362	2.124	0.64
23	0.543	0.000048	2.742	4.351	0.63
24	0.281	0.000028	1.702	2.487	0.68
25	0.366	0.000055	1.917	2.878	0.67
26	0.198	0.000048	1.327	1.603	0.83
27	0.457	0.000051	1.824	3.699	0.49
Mean	0.398	0.000423	2.340	3.170	0.66
SD	0.115	0.000414	0.522	0.774	0.06

SD – standard deviation

Table 4. Person's correlation coefficients ( $n = 27$ )

Parameters	$P_{E-R}$	AIP	AcP	DEH	CAT	$pH_{H_2O}$	$pH_{KCl}$	Hh	TOC
$P_{E-R}$	1	0.96	0.91	0.90	ns	0.48	0.53	0.39	0.75
AIP	–	1	0.93	0.94	0.41	0.52	0.57	0.41	0.73
AcP	–	–	1	0.98	ns	0.48	0.55	0.40	0.63
DEH	–	–	–	1	0.43	0.48	0.56	0.39	0.63
CAT	–	–	–	–	1	ns	ns	ns	ns
$pH_{H_2O}$	–	–	–	–	–	1	0.94	0.90	0.50
$pH_{KCl}$	–	–	–	–	–	–	1	0.88	0.48
Hh	–	–	–	–	–	–	–	1	ns
TOC	–	–	–	–	–	–	–	–	1

Significant at  $P < 0.05$ ; ns – not significant.  $P_{E-R}$  – available phosphorus; AIP – alkaline; AcP – acid phosphatase; DEH – dehydrogenases; CAT – catalase; DEH – dehydrogenases, Hh – hydrolytic acidity, TOC – total organic carbon

Based on the present results of the activity of alkaline and acid phosphatases, the enzymatic index of soil pH (AIP/AcP) was calculated (Dick et al. 2000). In the Phaeozems investigated, the AIP/AcP value ranged from 0.49 to 0.83 (Table 3). When the value of the AIP/AcP ratio is higher than 0.50, one deals with the neutral or alkaline soil reaction, which was confirmed for the soil analysed with the potentiometric  $pH_{KCl}$  measurement method (Lemanowicz 2011). The enzyme pH index can be used as an alternative method to determine the soil reaction.

Both the changes in the content of available phosphorus and the activities of dehydrogenases, catalase, alkaline and acid phosphatases in soil were not caused by factors causing soil salinity but a result of a natural content of carbon of organic compounds, which was proven by the statistical analysis of correlation of the parameters investigated (Table 4). Different results were reported by Saviozzi et al. (2011) investigating the soils with considerably higher EC values, however with a lower content of total organic carbon. With that in mind, the measurement of the enzymatic activity allows for monitoring the effect of salinity on the changes in soil parameters (Telesiński et al. 2008, Siddikee et al. 2011). However, a significant relationship was found between the content of carbon of organic compounds and the enzymatic activity of Mollic Gleysols ( $r = 0.93–0.94$ ;  $P < 0.05$ ). A significant correlation between the activity of the enzymes and organic matter was also noted by Siddikee et al. (2011). An increase in the enzymatic activity was a result of an increased content of organic matter as well as the microbiological soil activity, which enhances its structural stability, density and thus

increases the biomass of soil microorganisms. It is connected with the abundance of easily accessible energy-supplying substance in soil, namely TOC, which determines the development of soil microorganisms being one of the sources of enzymes Silva and Fay (2012). High significant values of the coefficients of correlation were recorded between the content of available phosphorus and the activity of alkaline phosphatase ( $r = 0.96$ ;  $P < 0.05$ ) as well as acid phosphatase ( $r = 0.91$ ;  $P < 0.05$ ) in soil (Table 4). Lemanowicz (2011), Bartkowiak and Lemanowicz (2012) also report on a strict interaction between the activity of phosphatases and the content of available phosphorus, which results from a long-term occurrence of extracellular enzymes in the bonds with soil colloids in soil. A positive significant value of the coefficient of correlation between the activity of dehydrogenases and the content of available phosphorus ( $r = 0.90$ ;  $P < 0.05$ ) was also noted in soil.

The analyses demonstrated a significant relationship between the content of exchangeable and water-soluble magnesium and the activity of dehydrogenases, catalase, alkaline and acid phosphatase of the Mollic Gleysols of the Health Resort Park in Inowrocław (Table 4). According to Wyszowska and Wyszowski (2003), magnesium even at low concentrations modifies the biological soil properties, increasing the number of most soil microorganisms and thus stimulating the activity of urease and acid and alkaline phosphatase. Magnesium is also considered a modifier-activator of the enzymatic reactions, present in the environment, affecting the speed, mostly phosphatases and phosphokinase.

Mollic Gleysols in Inowrocław are the soils exposed to seasonal salinity; a high content of ions

responsible for salinity is balanced with a high content of carbon, humus, phosphorus and calcium directly affecting the fertility of the soil analyzed.

The activity of enzymes in the Mollic Gleysols investigated depended on the natural content of carbon of organic compounds and not on the factors affecting the salinity of soils, which points to the potential of those tests to soil environment monitoring.

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