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# EFFECTS OF CADMIUM AND SALINITY-SODICITY ON ACID AND ALKALINE PHOSPHATASE ACTIVITY WITH REFERENCE TO ECOLOGICAL IMPORTANCE OF SOIL POLLUTION

# ODDZIAŁYWANIE KADMU ORAZ ZASOLENIA NA AKTYWNOŚĆ FOSFATAZY KWAŚNEJ I ZASADOWEJ W ODNIESIENIU DO EKOLOGICZNEGO ZNACZENIA SKAŻENIA GLEBY

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**Streszczenie.** Celem pracy było określenie zmian aktywności fosfatazy kwaśnej i zasadowej w glebie, wywołanych obecnością kadmu, w warunkach zróżnicowanego zasolenia. Doświadczenie przeprowadzono w warunkach laboratoryjnych, na próbkach gliny lekkiej, pobranych z poziomu ornopróchnicznego czarnych ziem Równiny Gumienieckiej. Zawartość węgla organicznego w glebie wynosiła 1,09%, azotu ogółem 0,14%, a pH w 1 M KCl 6,81. Do części ziemistych materiału glebowego wprowadzono, w różnych kombinacjach, wodne roztwory azotanu(V) kadmu i chlorku sodu. Ilość dodanego kadmu do gleby wynosiła 0, 1, 5 i 25 mg $Cd^{2+} \cdot kg^{-1}$, a ilość NaCl 0 i 0,5% wagowych. W 1., 7., 14., 28., 56. i 112. dniu doświadczenia oznaczono spektrofotometrycznie aktywność fosfataz. Otrzymane wyniki przeliczono w stosunku do aktywności enzymów w glebie kontrolnej (przyjmując ją za 100%) i podano jako procent inhibicji. Wyniki zmian aktywności fosfataz przedstawiono na wykresach ekologicznych stref zagrożenia. Aktywność fosfatazy kwaśnej i zasadowej uległa istotnym zmianom po wprowadzeniu do gleby kadmu oraz chlorku sodu. Zarówno kadm, jak i zasolenie gleby spowodowały inhibicję aktywności fosfataz glebowych, która zwiększała się wraz ze wzrostem stężenia metalu w glebie. Obecność chlorku sodu pogłębiała negatywne oddziaływanie kadmu na aktywność fosfatazową gleby. Zaobserwowana inaktywacja fosfataz wywołana dodatkiem kadmu w ilości 25 mg $\cdot kg^{-1}$ znajdowała się na wykresach stref ekologicznego zagrożenia w obszarze wartości krytycznych, co może świadczyć o zaburzeniach w metabolizmie związków fosforu w glebie.

**Key words:** alkaline and acid phosphatase, cadmium, salinity-sodicity, soil.
**Słowa kluczowe:** fosfataza kwaśna, fosfataza zasadowa, gleba, kadm, zasolenie.

## INTRODUCTION

Environmental pollution by heavy metals poses a health hazard for living organisms – plants, animals and humans (Wyszkowski and Wyszkowska 2009). Cadmium (Cd) is one of the most hazardous heavy metals in the environment (Xu et al. 2013). Irrational sewage irrigation and sludge application in agriculture, atmospheric deposition of industrial dusts, and the utilization of Cd-containing phosphate rock powder have led to increase Cd in the soil environment (Naidu et al. 1997).

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Cadmium is the most ubiquitous metal influencing soil biota (Hassan et al. 2013). At high concentrations, Cd is extremely toxic to soil and aquatic organisms while at low levels, it adversely affects microbial physiology (Sokolova 2004). The availability of Cd varies with the nature of Cd applied, the soil type and the environmental conditions (Dar 1995). Other soil factors that influence on the availability of Cd are: soil pH, cation exchange capacity, organic matter content, Fe and Mn oxides concentration, temperature, and salinity (Khan--Mohammadi and Nourbakhsh 2011). Excessive amounts of cadmium and other heavy metals, disrupt the homeostasis of soil by interfering with the control mechanisms on the level of genes, thus inhibiting the activity of microbial enzymatic proteins. They cause damage to metabolic pathways, often resulting in the apoptosis of cells (Wyszkowska et al. 2013).

Saline soils contain a high amount of soluble salts, primarily $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$ salt of $Cl^{-}$, $NO_3^{-}$, $SO_4^{2-}$ and $CO_3^{3-}$ (Siddikee et al. 2011). Salts in soil have a negative impact on soil physical, chemical, and biological properties and can ultimately deteriorate soil quality in both ecological and agricultural aspects. Salinity detrimentally affects microbial communities and their activity in soils, which are important in recycling of nutrients in soil, increase the fertility and maintain ecological functions (Rietz and Haynes 2003, Telesiński 2012).

Phosphatases are the enzymes that release inorganic phosphate from organic moiety and complex inorganic materials. It is said that they are essential in phosphorus cycle, even though their role in other various physical factors cannot be ignored (Banerjee et al. 2012). Phosphorus is vital to energy production and it is important in enumerable metabolic pathways in organisms (Rasol and Reshi 2010). Cellular signalling events cascades with phosphorylation and dephosphorylation, are associated with enzymes called phosphatases [EC 3.1.3.x]. Soil receives various phosphatases from living organisms that play important roles in the solubilization of inorganic phosphates (Acosta-Mortinez and Tabatabai 2000). Enzymatic activity of soil samples is a critical index of soil fertility because enzymes participate in nutrient cycles (Dick et al. 2000). Determination of the activity of acid and alkaline phosphatases could aid long-term monitoring of the phosphorus status of soil and changes in soil abundance due to anthropogenic factors (Lemanowicz et al. 2013).

Rating negative effects of xenobiotics on the environment is difficult, and to determine the severity and duration of adverse changes in the soil ecosystem may suffer from many problems (Wienhold et al. 2004). Domsch et al. (1983) suggested the concept according to which all changes in the metabolism of the components of soil, caused by chemical agents, may be compared with changes caused by microorganisms under natural stress conditions. All changes of process lasting over 60 days with the severity of 50%, compared to the control soil, are considered to be dangerous, others are regarded as acceptable or negligible.

The aim of this study was to determine activity of acid and alkaline phosphatase in soil treated with different doses of cadmium and sodium chloride and to identify if observed changes may be considered as negligible, tolerable or critical for soil ecosystem.

## MATERIAL AND METHODS

Research was carried out on soil samples collected from arable and humus horizon of Gumieniecka Plain (the North-West Poland, around Szczecin). Granulometric composition and physicochemical properties of soil used in the research are presented in the Table 1.

Table 1. Characteristics of soil used in the experiment
Tabela 1. Charakterystyka gleby użytej w doświadczeniu

| Granulometric fraction<br>Frakcja granulometryczna | | | Granulometric group<br>Grupa granulometryczna | $C_{org}$ | $N_t$ | pH | |
|---|---|---|---|---|---|---|---|
| 2.00 ≥ d > 0.05 mm | 0.05 ≥ d > 0.002 mm | d ≤ 0.002 mm | | | | $H_2O$ | KCl |
| 47% | 48% | 5% | sandy loam<br>piasek gliniasty | 1.09% | 0.14% | 7.09 | 6.81 |

Soil samples collected from the field were adjusted to air-dry state and sieved through a sieve with mesh of 2 mm diameter. Sallow components were divided into half kilogram samples and adjusted to 60% capillary water capacity. In the experiment changeable factors were:

– doses of Cd: 0, 1, 5 and 25 mg $Cd^{2+} \cdot kg^{-1}$ (as $Cd(NO_3)_2$);
– doses of NaCl: 0 and 0.5% of soil weight.

Soil samples were precisely stirred and stored in hermetic polyethylene bags at the temperature of 20°C and in a dark place. On day 1, 7, 14, 28, 56, 112 activity of alkaline [EC 3.1.3.1] and acid [EC 3.1.3.2] phosphatase activity was assayed spectrophotometrically according to the method of Tabatabai and Bremner (1969) in Margesin (1996) modification. Analyses were carried out using spectrophotometer UV-1800 produced by Shimadzu.

All analyses were done in three repetitions. Results of the research were analyzed statistically using two-factor analysis of variance in the complete randomization system. First factor was concentration of cadmium and second of NaCl. Analyses were carried out irrespectively on each 6 days of the experiment. To evaluate the significance of differences Tukey's test was used at the significance level of $\alpha = 0.05$. using Statistica 9.0 (StatSoft) and Excel 2010 (Microsoft).

Real values of the activity were calculated in reference of enzymatic activity in the control soil (assuming it as 100%) and specified as percent of inhibition. Changes of phosphatases activity were shown as environmental danger zones graphs suggested by Domsch et al. (1983). In the graphs, three ecological danger zones were marked: values negligible – with slight influence on enzymatic activity, values tolerable – with considerable influence on enzymatic activity but not cause inactivation of catalytic activity of enzymes, values critical – with the most negative impact on soil ecosystem which may cause degradation.

## RESULTS AND DISCUSSION

Activity of acid phosphatase in soil during the experiment fluctuated between 1.05 and 1.27 mmol p-NP · (kg d.w. soil · h)$^{-1}$. While activity of alkaline phosphatase ranged from 1.79 to 1.88 mmol p-NP · (kg d.w. soil · h)$^{-1}$. Addition of Cd and NaCl caused significant decrease of analyzed enzymes activity (Table 2, 3).

Table 2. Acid phosphatase activity in soil treated with Cd and NaCl (mmol p-NP · (kg d.w. soil · h)$^{-1}$)
Tabela 2. Aktywność fosfatazy kwaśnej w glebie z dodatkiem Cd i NaCl (mmol p-NP · (kg d.w. soil · h)$^{-1}$)

| NaCl content<br>Zawartość NaCl<br>(A) | Amount of $Cd^{2+}$ [mg · kg$^{-1}$] – Ilość $Cd^{2+}$ [mg · kg$^{-1}$]<br>(B) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 5 | 25 | $\bar{x}$ |
| Day 1 – Dzień 1. | | | | | |
| 0 | 1.27 | 1.02 | 0.55 | 0.20 | 0.76 |
| 0.5% | 0.63 | 0.46 | 0.37 | 0.15 | 0.40 |
| $\bar{x}$ | 0.95 | 0.74 | 0.46 | 0.17 | 0.58 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.021 B = 0.027<br>A × B = 0.045 B × A = 0.048 | | | | |
| Day 7 – Dzień 7. | | | | | |
| 0 | 1.08 | 0.94 | 0.66 | 0.25 | 0.73 |
| 0.5% | 0.64 | 0.54 | 0.46 | 0.15 | 0.44 |
| $\bar{x}$ | 0.86 | 0.74 | 0.56 | 0.20 | 0.58 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.015 B = 0.020<br>A × B = 0.031 B × A = 0.035 | | | | |
| Day 14 – Dzień 14. | | | | | |
| 0 | 1.05 | 0.95 | 0.72 | 0.28 | 0.75 |
| 0.5% | 0.73 | 0.44 | 0.43 | 0.21 | 0.45 |
| $\bar{x}$ | 0.89 | 0.69 | 0.57 | 0.24 | 0.60 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.045 B = 0.052<br>A × B = 0.092 B × A = 0.104 | | | | |
| Day 28 – Dzień 28. | | | | | |
| 0 | 1.14 | 0.85 | 0.63 | 0.33 | 0.74 |
| 0.5% | 0.75 | 0.49 | 0.57 | 0.12 | 0.51 |
| $\bar{x}$ | 0.94 | 0.67 | 0.60 | 0.22 | 0.62 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.032 B = 0.044<br>A × B = 0.082 B × A = 0.092 | | | | |
| Day 56 – Dzień 56. | | | | | |
| 0 | 1.11 | 1.01 | 0.73 | 0.27 | 0.78 |
| 0.5% | 0.55 | 0.87 | 0.70 | 0.25 | 0.59 |
| $\bar{x}$ | 0.88 | 0.94 | 0.72 | 0.26 | 0.69 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.035 B = 0.046<br>A × B = 0.082 B × A = 0.094 | | | | |
| Day 112 – Dzień 112. | | | | | |
| 0 | 1.21 | 1.02 | 0.87 | 0.42 | 0.88 |
| 0.5% | 0.90 | 0.83 | 0.69 | 0.19 | 0.65 |
| $\bar{x}$ | 1.05 | 0.92 | 0.78 | 0.30 | 0.77 |
| $LSD_{0.05}$<br>$NIR_{0,05}$ | A = 0.024 B = 0.031<br>A × B = 0.067 B × A = 0.078 | | | | |

Table 3. Alkaline phosphatase activity in soil treated with Cd and NaCl (mmol p-NP · (kg d.w. soil · h)$^{-1}$)
Tabela 3. Aktywność fosfatazy zasadowej w glebie z dodatkiem Cd i NaCl (mmol p-NP · (kg d.w. soil · h)$^{-1}$)

| NaCl content Zawartość NaCl (A) | Amount of $Cd^{2+}$ [mg · kg$^{-1}$] – Ilość $Cd^{2+}$ [mg · kg$^{-1}$] (B) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 5 | 25 | $\bar{x}$ |
| Day 1 – Dzień 1. | | | | | |
| 0 | 1.87 | 1.94 | 1.36 | 0.39 | 1.39 |
| 0.5% NaCl | 1.44 | 1.43 | 1.27 | 0.23 | 1.09 |
| $\bar{x}$ | 1.65 | 1.68 | 1.31 | 0.31 | 1.24 |
| $LSD_{0.05}$ | | | A = 0.181 | B = 0.211 | |
| $NIR_{0,05}$ | | | A × B = 0.329 | B × A = 0.413 | |
| Day 7 – Dzień 7. | | | | | |
| 0 | 1.88 | 1.86 | 1.29 | 0.47 | 1.37 |
| 0.5% NaCl | 1.39 | 1.36 | 1.21 | 0.17 | 1.04 |
| $\bar{x}$ | 1.63 | 1.61 | 1.25 | 0.32 | 1.20 |
| $LSD_{0.05}$ | | | A = 0.092 | B = 0.138 | |
| $NIR_{0,05}$ | | | A × B = 0.242 | B × A = 0.293 | |
| Day 14 – Dzień 14. | | | | | |
| 0 | 1.87 | 1.95 | 1.06 | 0.42 | 1.32 |
| 0.5% NaCl | 1.34 | 1.35 | 0.95 | 0.18 | 0.95 |
| $\bar{x}$ | 1.60 | 1.65 | 1.00 | 0.30 | 1.13 |
| $LSD_{0.05}$ | | | A = 0.110 | B = 0.191 | |
| $NIR_{0,05}$ | | | A × B = 0.278 | B × A = 0.312 | |
| Day 28 – Dzień 28. | | | | | |
| 0 | 1.82 | 1.75 | 1.14 | 0.56 | 1.32 |
| 0.5% NaCl | 1.25 | 1.27 | 0.97 | 0.34 | 1.11 |
| $\bar{x}$ | 1.53 | 1.51 | 1.05 | 0.45 | 1.21 |
| $LSD_{0.05}$ | | | A = 0.082 | B = 0.124 | |
| $NIR_{0,05}$ | | | A × B = 0.243 | B × A = 0.289 | |
| Day 56 – Dzień 56. | | | | | |
| 0 | 1.79 | 1.73 | 1.26 | 0.93 | 1.43 |
| 0.5% NaCl | 1.46 | 1.43 | 1.07 | 0.48 | 1.11 |
| $\bar{x}$ | 1.62 | 1.56 | 1.16 | 0.72 | 1.27 |
| $LSD_{0.05}$ | | | A = 0.043 | B = 0.054 | |
| $NIR_{0,05}$ | | | A × B = 0.103 | B × A = 0.117 | |
| Day 112 – Dzień 112. | | | | | |
| 0 | 1.81 | 1.74 | 1.39 | 1.22 | 1.54 |
| 0.5% NaCl | 1.59 | 1.70 | 1.29 | 0.42 | 1.25 |
| $\bar{x}$ | 1.70 | 1.72 | 1.34 | 0.82 | 1.39 |
| $LSD_{0.05}$ | | | A = 0.039 | B = 0.051 | |
| $NIR_{0,05}$ | | | A × B = 0.088 | B × A = 0.103 | |

Sodium chloride applied in amount of 0.5% decreased activity of assayed enzymes during the whole experiment. The level of inhibition of acid phosphatase was higher than alkaline phosphatase and reached 50% (on the day 1 and 56) while alkaline phosphatase activity did not exceed 33% (on the day 28). Other researchers also indicated, that under laboratory conditions, salinity negatively influenced enzyme activity in soil, although the level of inhibition varied depending on the kind of analyzed enzyme and soil type (Frankenberger and Bingham 1982, Ahmad and Khan 1988, Rietz and Haynes 2003, Telesiński 2012). Inhibition of enzyme activity in saline soils could be due to the osmotic dehydration of the microbial cells that liberate intracellular enzymes, which become vulnerable to the attack by soil proteases, with a consequent decrease in enzyme activity. The salting-out effect modifies the ionic conformation of the protein-enzyme active site, and specific ionic toxicity causes nutritional imbalance for microbial growth and subsequent enzyme synthesis (Frankenberger and Bingham 1982). Omar et al. (1994) and Siddikee et al. (2011) also indicated the effects of soil salinity on the carbon of microbial biomass and on enzyme activity. Garcia and Hernandez (1996) and Ghollarata and Raiesi (2007) showed that increase of soil salinity inhibited the phosphatase activities. However, in our study, the level of inactivation of both phosphatases in the soil with the addition of NaCl, was in the range of negligible or acceptable (Figs. 1A, 2A).

Application of cadmium salts into the soil also caused inhibition of phosphatase activity on each day of analyses. It was observed that inhibition enhance with increase of metal content in soil (Figs. 1B–D, 2B–D). Cadmium, in doses of 1 and 5 mg · $kg^{-1}$, inactivated both enzymes in ignored or tolerated range. The highest inhibition occurred at dose of 25 mg · $kg^{-1}$ where acid phosphatase activity decrease reached 84% (on the day 1) and alkaline phosphatase to 79% (on the day 1). Only acid phosphatese activity indicated in the last day of the experiment was inhibited the critical level. Inhibition of phosphatases in soil contaminated with cadmium was also observed by many researchers (Zheng et al. 1999, Landi et al. 2000, Wyszkowski and Wyszkowska 2009, Hassan et al. 2013). Cd, as one of the most toxic heavy metals, can occupy the active site of enzymes by binding to the mercapto, amino, and carboxyl groups of enzyme molecules to form more stable complexes, resulting in a competitive inhibition of substrates (Bruins et al. 2000). For example, the binding of Cd to the active site of phosphatase would reduce phosphatase activity and block metabolism. In addition, Cd can inhibit the growth and reproduction of soil microorganisms; reduce enzyme synthesis and secretion by microorganisms, and consequently decrease the soil enzyme activity. An indirect effect is also possible because changes in the community structure can modify the enzyme activity (Xu et al. 2013).

In saline soil with 0.5% of NaCl, effect of cadmium salt on acid and alkaline phosphatase activity was higher than in non-saline soil. Similar reaction, as for non-saline soil, was observed in soil with 1 i 5 mg · $kg^{-1}$ of Cd where phosphatase activity reduction was in the range of tolerated and ignored (Figs. 1B–C, 2B–C). In the soil samples with 25 mg · $kg^{-1}$ of Cd or NaCl, in the final period of the experiment, a serious acid and alkaline phosphatase inactivation was observed and it was located in the area of critical values which might indicate disturbances in the metabolism of phosphorus compounds in soil (Figs. 1D, 2D).

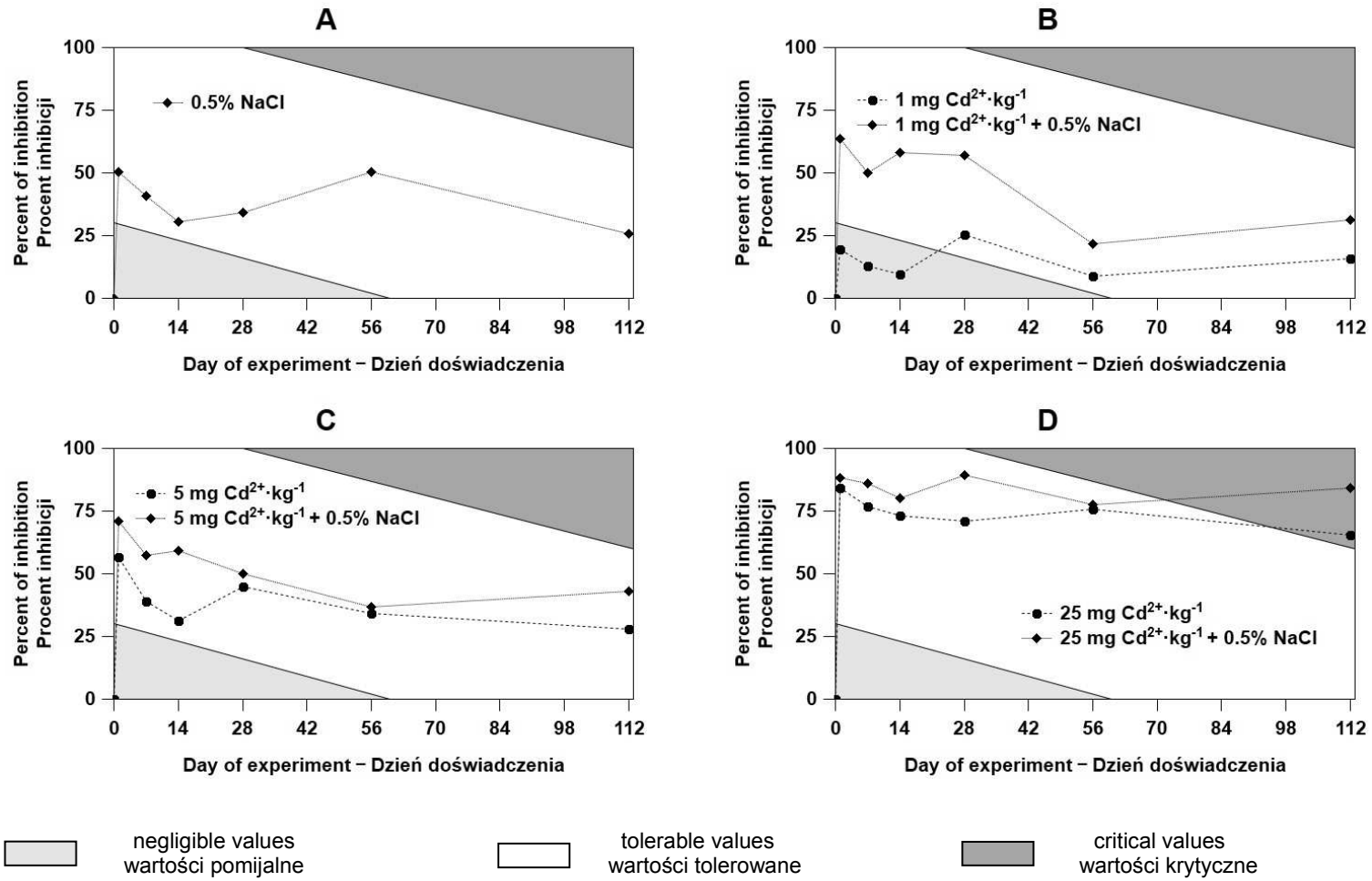


Fig. 1. The range of acid phosphatase inhibition in soil contaminated with NaCl (A) and different amounts of cadmium (B–D) with reference to ecological importance of soil pollution

Rys. 1. Wielkość inhibicji fosfatazy kwaśnej w glebie z dodatkiem NaCl (A) i różnych dawek kadmu (B–D) w odniesieniu do ekologicznego znaczenia skażenia gleby

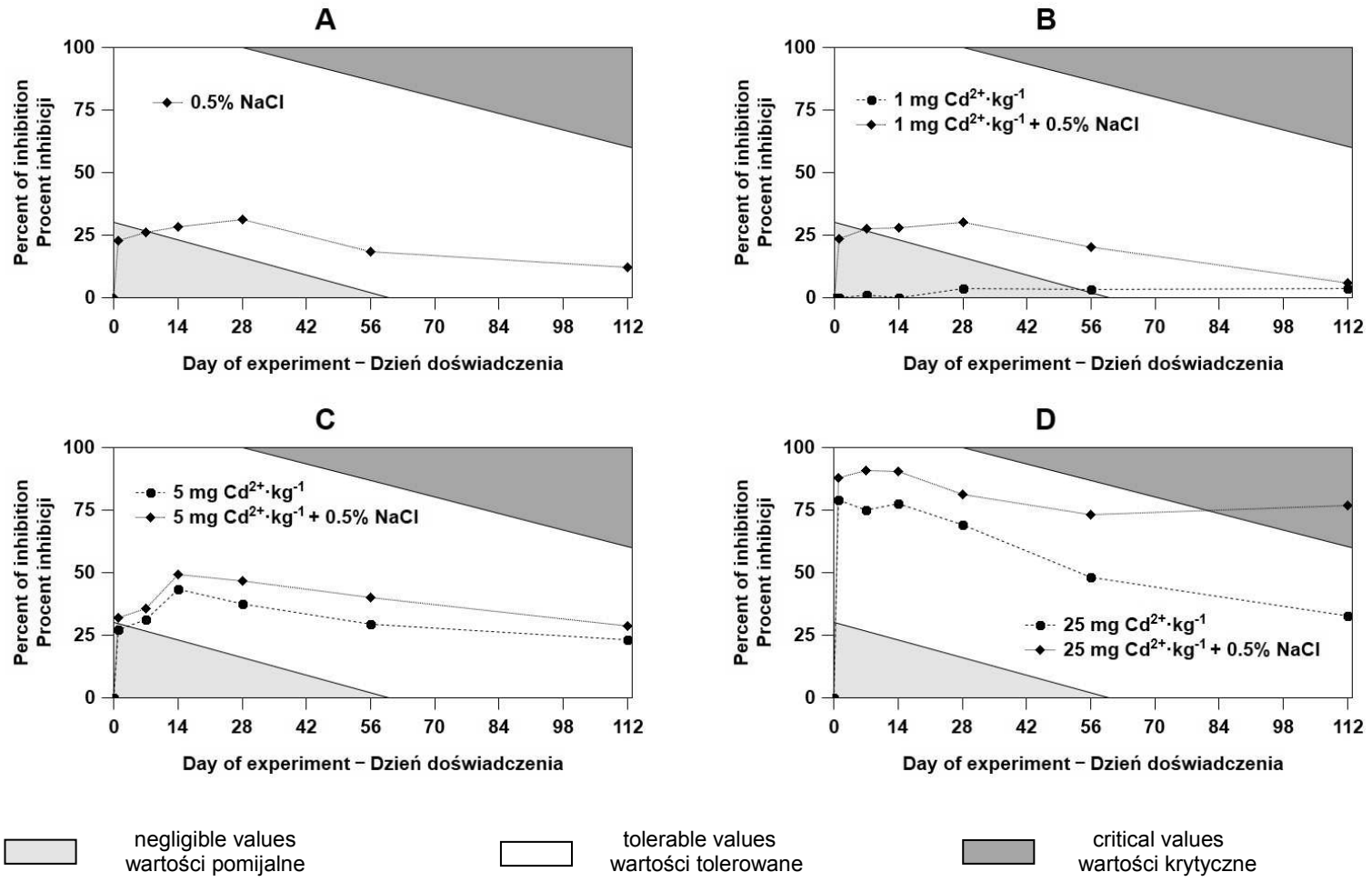


Fig. 2. The range of alkaline phosphatase inhibition in soil contaminated with NaCl (A) and different amounts of cadmium (B–D) with reference to ecological importance of soil pollution

Rys. 2. Wielkość inhibicji fosfatazy zasadowej w glebie z dodatkiem NaCl (A) i różnych dawek kadmu (B–D) w odniesieniu do ekologicznego znaczenia skażenia gleby

## CONCLUSIONS

1. The activity of acid and alkaline phosphatase changed significantly after addition of cadmium and sodium chloride to the soil.
2. Both, cadmium and salinity of the soil, caused inhibition of phosphatase activity, which increased with enhancing concentration of metals in the soil.
3. Sodium chloride intensified the negative effect of cadmium on phosphatase activity in the soil.
4. Observed inactivation of phosphatases caused by 25 mg · $kg^{-1}$ of Cd ranged, on the graphs of ecological danger zones, in the area of critical values, which may indicate that cadmium disturbs the metabolism of phosphorus compounds in soil.

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**Abstract.** The aim of the study was to determine the changes of the acid and alkaline phosphatase activity in the soil caused by the presence of cadmium and NaCl. The experiment was carried out in the laboratory condition, on soil samples taken from the arable-humus horizon of Gumieniecka Plain black earths. Granulometric composition of this soil was sandy loam. The organic carbon content was 1.09%, total nitrogen content was 0.14% and pH in 1 M KCl 6.81. Various concentrations of $Cd(NO_3)_2$ and NaCl were introduced to soil samples. The amount of cadmium added to the soil was 0, 1, 5 and 25 mg $Cd^{2+} \cdot kg^{-1}$ , and the amount of NaCl was 0 and 0.5% of soil weight. On day 1, 7, 14, 28 , 56 and 112 alkaline and acid phosphatase activity was determined spectrophotometrically. The obtained results are converted with respect to the enzyme activity in the soil controls (assuming it to be 100%) and given as percent of inhibition. The results were shown as environmental danger zones graphs. The activity of acid and alkaline phosphatase changed significantly after addition of cadmium and sodium chloride to the soil. Both, cadmium and salinity of the soil, caused inhibition of phosphatase activity, which increased with enhancing concentration of metals in the soil. Sodium chloride intensified the negative effect of cadmium on phosphatase activity in the soil. Observed inactivation of phosphatases caused by 25 mg $Cd^{2+} \cdot kg^{-1}$ of Cd ranged, on the graphs of ecological danger zones, in the area of critical values, which may indicate that cadmium disturbs the metabolism of phosphorus compounds in soil.