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Impact of manganese, iron, and cobalt fractions on soil enzyme activities

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ABSTRACT

The goal of this study was to investigate the metal impact (Mn, Fe, Co) on enzymatic activities of soils cultivated using the simplified method, during spring, summer, and autumn. The distribution of studied metals between fractions was assesed according to the BCR method. Four fractions were evaluated: acid soluble and exchangeable (F1), reducible, which is bound to Fe/Mn oxides (F2), oxidizable, which is bound to organic matter (F3), and residual (F4). The highest Fe and Co percentage share was stated in fraction F4 (71.0 and 49.2%, respectively). The most of Mn gathered fraction F2 (50.8%). The lowest amount of Mn and Co was found in fraction F3 (13.6 and 17.7%, respectively) and for Fe, in fraction F1 (1.3%). In spring, the significant dependence was noted between F1/Mn/dehydrogenase, F3/Fe/dehydrogenase, F2/Co/dehydrogenase, and F4/Fe/protease. Such a relationship was found between F1/Mn/urease, F3/Fe/urease, F3/Co/urease, and F3/Co/phosphatase during autumn. During summer, F1/Fe caused an increase in phosphatase activity.

Keywords: enzyme activity, arable soil, metal fraction, metal, BCR method.

INTRODUCTION

Metals, as pollutants, are persistent, may be toxic and accumulate in the natural environment. Their toxicity towards living organisms is dependent on the exposure period and dose [Pande et al., 2022]. They affect soil microorganisms by limiting their number and activity [Abbas et al., 2021]. Manganese, iron and cobalt, are essential for the microorganisms [Farrag, 2017; Zeinert et al., 2018; Uzoh and Babalola, 2020]. At the same time, they become harmful if present in excessive amounts [Łopusiewicz et al., 2020; Zhang, 2022; Wu et al., 2022]. This is also related to the influence on the enzymatic activity of the soil, the source of which are, among others, soil microorganisms. The enzymatic activity of soils is influenced by abiotic, biotic and anthropogenic factors. Human activity related to fertilization and the use of plant protection products is the main source of metals in agricultural soils and contributes to increasing the content of metals [Furtak and Gałązka, 2019, Alengebawy et al., 2021, Kumar et al., 2023]. The largest amounts of studied metals are introduced into the soil with phosphate fertilizers produced on the basis of phosphate rocks. For example, ammonium phosphate contains 8–2000 mg·kg^{-1} of Mn [Górecka and Górecki, 2000]. Lime fertilizers, mineral fertilizers with microelements, livestock manure and wastewater are less significant sources of the investigated metals. Importantly, most of the metals of anthropogenic origin accumulate in the top layer of soil, i.e. in the area with the highest content of microorganisms, and therefore with the highest enzymatic activity. The activity of soil enzymes can be treated as an indicator of the biological health of the soil, because they play an important role in many life processes, such as nutrient cycling, mineralization, and nitrification [Rajeev et al., 2024]. Dehydrogenase is important for maintaining health and soil fertility because it is involved in the processes associated with microbial respiration. Phosphatase is responsible for the mineralization of organic phosphorus compounds, which is of fundamental importance for plants, because they only take up inorganic phosphorus. Urease is vital in soil due to its participation in the nitrogen cycle, one of the most essential nutrient cycles. It hydrolyzes urea, releasing ammonium ions, thus affecting the soil pH [Daunoras et al., 2024]. Protease is also involved in the N cycle. It hydrolyzes proteins, releasing nitrogen in a form usable for plants [Raju et al., 2017].

Manganese exists in eleven oxidation states, but only the Mn^{3+} and Mn^{2+} oxidative states are of biological importance [Röllin, 2011]. Manganese regulates high reactive oxygen species levels in bacteria. Excessive amounts of Mn may result in metabolism and respiration disorders of the microorganisms in soil. Also, they have a negative effect on stability and diversity of microbial community. The stability of microbial community in soil is decreasing at the content of Mn^{2+} greater than 300 mg/kg [Wu et al., 2022]. Iron is involved, among others, in C, N, S and P cycling, metabolism of hydrogen, glutamine and glutamate, photosynthesis, production of isoleucine and leucine, as well as in the electron transfers of phototrophy, chemolithotrophy, and respiration [Hemkemeyer et al., 2021]. This element occurs in microorganisms in two forms, Fe^{2+} and Fe^{3+}. In high concentrations, it contributes to the generation of reactive oxygen species, which cause the oxidation of lipids contained in cell walls and proteins, and also damage DNA [Sorokina et al. 2013]. Cobalt most often occurs in +2 and +3 oxidation states. Excessive Co^{2+} amount in the soil has a negative impact on plants and microorganisms. Its content in the soil is closely related to Fe, Mn and Al [Zaborowska et al., 2016]. This element is mainly found in iron minerals [Kabata-Pendias and Pendias, 2001]. Cobalt takes part in some metabolic processes, such as sugar metabolism, redox processes and nucleoprotein synthesis [Kosiorek and Wyszkowski, 2019]. It acts as a cofactor of vitamin B12 and other enzymes in animals, plants, bacteria, yeast and archaea. The toxicity of cobalt has been recognized in bacteria, fungi and plants. At micro- and millimolar amounts, it slows down citric acid cycle and cellular respiration [Łopusiewicz et al., 2020]. Its toxicity is also related to weakening enzymatic hydrolysis, acceleration or inhibition of cell division, inhibition of hydrolysis of biopolymers and disturbances in the course of redox reactions. With an excess of this element unstable enzymatic reaction products and radicals begin to form, and due to the increased osmotic pressure, the cell wall is destroyed [Kosiorek and Wyszkowski, 2019].

Metals, through their phytotoxic effects, can cause instability of plant cell membranes, reduce chlorophyll content, inhibit growth, and change enzyme activity [Romero-Freire et al., 2023]. Manganese is toxic, in general, when the content in the above-ground plant parts exceeds 150 $mg \cdot kg^{-1}$ dry mass. It is related to weakened uptake and transport of other elements, including magnesium, iron and phosphorus, inhibition of enzyme activity and photosynthesis [Li et al., 2019]. Iron can be harmful at a content above 500 $mg \cdot kg^{-1}$ dry mass in plant tissue. Its toxicity manifests itself in disturbances of the cell redox balance. This leads to physiological, metabolic and morphological changes in the plant [Lapaz et al., 2022]. The toxicity of cobalt towards plants is manifested, among others, by formation of hydroxyl radicals, reactive oxygen species, hydrogen peroxide radicals, increase in proline and malondialdehyde content, as well as changes in the activity of antioxidant enzymes [Khan et al., 2024].

The topic of the influence of metal fractions on the enzymatic activity of soils is poorly represented in the literature. The following research is an attempt to expand the knowledge about the influence of manganese, iron and cobalt on the enzymatic activity of soil. The novelty of studies in this area is the ability to determine what pool of metal is bioavailable or potentially bioavailable, and thus may influence the functioning of soil enzymes. This also allows determining, at least partially, why the same metal may sometimes have a positive and sometimes negative effect on the activity of individual enzymes, because the fractional composition of the metal may change over time, mainly depending on weather conditions. To the best of author's knowledge, there is no study in this area regarding arable soils, including those cultivated without plowing.

In this study, investigation aimed to determine whether fractions of manganese, iron, and cobalt caused changes in activities of dehydrogenase, protease, urease and alkaline phosphatase in the soils cultivated by simplified method. The aim of this research was to: 1) investigate the effect of sampling date on enzymatic activity and fractionation of Mn, Fe and Co; 2) identify which metal fractions affected the studied enzyme activities in seasons. It was hypothesized that the effect on enzyme activity should be most visible in the case of the acid soluble and exchangeable fraction, which is the most mobile and therefore the most bioavailable.

MATERIALS AND METHODS

Sample collection

Soil samples (*Albic Luvisols*) were collected from the topsoil (0–25 cm) of nine fields with a total area of approx 150 ha, around Radzie (53° 55’ 49.00” N; 22° 04’ 13.75” E) and Gawliki Wielkie (54° 01’ 19.83” N; 22° 05’ 59.95” E), Poland. Six subsamples (about 1 kg each) were taken diagonally from each field using Egner’s stick and mixed. They were dried at room temperature and sieved through a nylon sieve with a mesh diameter of 2 mm prior of analysis. The studied area is located in northeastern Poland and the soils mostly have a boulder clay origin. Soils were sampled three times in 2015, during the spring, summer and autumn.

Non-tillage cultivation was used in the study area for five years before soil sampling. In the Gawliki Wielkie area, winter wheat was grown, while in the Radzie area – broad bean. Applied fertilization, temperatures and monthly rainfall in the study area as well as sampling protocol were shown in the previous paper [Łukowski and Dec, 2021]. In short, the total rainfall in the April, July and October was 36.1, 70.1 and 14.9 mm, respectively. The average temperatures in these months were 6.8, 17.6, 6.1 °C, respectively.

Physico-chemical analysis

The Mn, Fe and Co contents in the fractions and their total content were evaluated by the flame atomic absorption spectrometry using Thermo Scientific iCE 3500. The soil for determining the total metal content (sample weight 1g) was digested using the Ethos Easy apparatus in aqua regia (9 mL HCl : 3 mL HNO_3). Mineralization parameters were as follows: temperature rise to 200 °C within 15 minutes (1800W) and hold for 15 minutes (1800W). The percentage of each fraction in the total element content was estimated. Reference material (NIST 2711A Montana II soil) was used for the quality control of the determination of the total metal content. Also, the metal recovery in the studied soil samples was estimated with the following formula: ((sum of F1–F4)/total metal content) × 100. Average recovery range was 100–136%, 135–151% and 112–144%, for Mn, Fe and Co, respectively.

Organic carbon in the studied samples was determined by oxidation with $K_2Cr_2O_7$ in the presence of H_2SO_4, Kjeldahl nitrogen with the FoodAlyt D5000, prior digestion in concentrated H_2SO_4 and granulometric composition by the sieve analysis.

Fractions of Mn, Fe, and Co in the studied soil samples were assayed with the ultrasound accelerated BCR method, which allows the extraction of four fractions [Leśniewska et al., 2014] . The extraction scheme is shown in Figure 1.

The activity of protease was determined according to Macura and Vágnerová [1969], with the usage of azo-casein. Alkaline phosphatase was evaluated in accordance with the method by Tabatabai and Bremner [1969]. Dehydrogenase activity was assayed spectrophotometrically [Thalmann, 1968] after 24h incubation using 1% triphenylotetrazole chloride (TTC) as a substrate, and urease activity was determined by Hoffmann and Teicher method [1961]. In the previous paper [Łukowski and Dec, 2021], a more detailed description of enzyme activity determination can be found.

Statistical procedure

All results (except for granulometric properties of soil) were submitted to one-way ANOVA with the use of Statistica 13.1 software. To define the significant differences among Mn, Fe, and Co fractions, soil physicochemical properties, and activity of soil enzymes, the least significant difference test was used ($P < 0.05$). To evaluate the correlations between study results, Pearson’s correlation coefficients ($P \leq 0.05$) were computed using Statistica 12.5.

RESULTS

Physicochemical properties of soil sample

The organic carbon content ranged from 0.12 to 7.15%, nitrogen from 0.14 to 0.52%, and the pH value was in the range 3.93–7.59. The average content of sand, silt and clay was 29.9, 25.3 and 20.0%, respectively. Significant correlations between the characteristics of studied soil samples and enzyme activities were not stated.

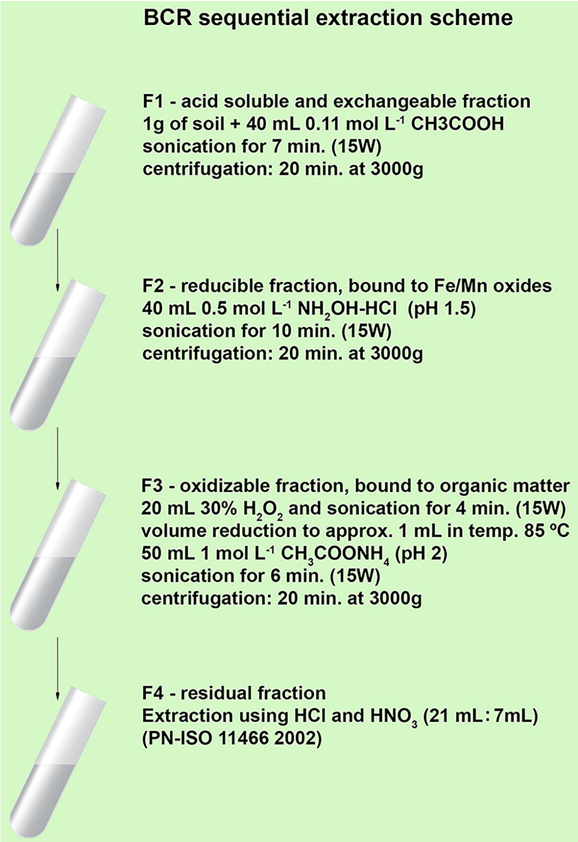

Figure 1. The ultrasound accelerated sequential extraction procedure for metal fractionation according to BCR (Community Bureau of Reference)

Content and fractional distribution of metals

On average, the content of total metal was in the range of 202–560, 1747–2037, 2.60–12.7 $mg \cdot kg^{-1}$ for Mn, Fe, Co, respectively, and was typical for uncontaminated agricultural soils. Fraction F2 contained the most of Mn (50.8% on average) and the least the fraction F3 (13.6%). Fractions F1 and F4 gathered 24.0 and 31.6% of total Mn, respectively. The highest percentage of Fe was stated in fraction F4 (71.0%) and the lowest in fraction F1 (1.3%). In fractions F2 and F3, 32.3 and 40.2% of total Fe were noted, respectively. The most of Co was stated in fraction F4 (49.2%). Its lowest percentage (17.7%) was observed in fraction F3. Fractions F1 and F2 constituted 24.4% and 36.2% of total Co, respectively. Only for the cobalt, significant differences in the fractional composition depending on the season were found (Figure 2). Fraction F3 gathered the most of Co in autumn, significantly more than in the spring. The percentage of F4/Co clearly decreased in autumn in relation to spring. The most significant dependences between the characteristics of investigated soils and fractional composition of metals were noted in the case of cobalt (Table 1). Sand content positively influenced F1/Co (0.445), while the clay content was negatively correlated with this fraction (-0.455). F4/Co was negatively correlated with sand content (-0.582) and positively with clay content (0.381). Clay content had a positive effect on F2/Mn (0.446), and pH had the same effect on F1/Mn (0.572). In the case of iron only one significant relationship, was found, between F2/Fe and clay (0.436).

Enzyme activities

Protease activity was in the range of 0.021–0.034 mg azocasein $kg^{-1} \cdot h^{-1}$, alkaline phosphatase 0.044–0.400 mg pNP $kg^{-1} \cdot h^{-1}$, dehydrogenase 0.0009–0.0220 mg TPF $kg^{-1} \cdot 24h^{-1}$, and urease 4.12–8.90 mg N $kg^{-1} \cdot h^{-1}$ (all enzymes activities were converted to dry weight). Table 2 contains the average enzyme activities for all samplings.Significant differences in activity depending on the season were noted only in the case of dehydrogenase and protease (Figure 3). Dehydrogenase activity was significantly higher during the spring as compared to the autumn. Protease activity was the highest in April, clearly higher than in July. The activities of phosphatase and urease were the highest in April.

Effect of metal fractions and soil properties on enzyme activities

The impact of metal fractions on enzyme activities was most visible in spring and autumn (Table 3). Protease activity was highly affected in April by F4/Fe (-0.819). Strong impact of F1/Fe (0.690) in July and F3/Co (-0.705) in October on phosphatase activity was observed. Dehydrogenase activity was correlated with F1/Mn (0.764), F3/Fe (-0.693) as well as F2/Co (0.815) during the spring. In the autumn, the activity of urease was correlated with F1/Mn (0.685), F3/Fe (-0.818) and F3/Co (-0.879).

DISCUSSION

Metal distribution between chemical frations

The bioavailability of metals changes as a result of physicochemical interactions with the soil components; therefore, their total content

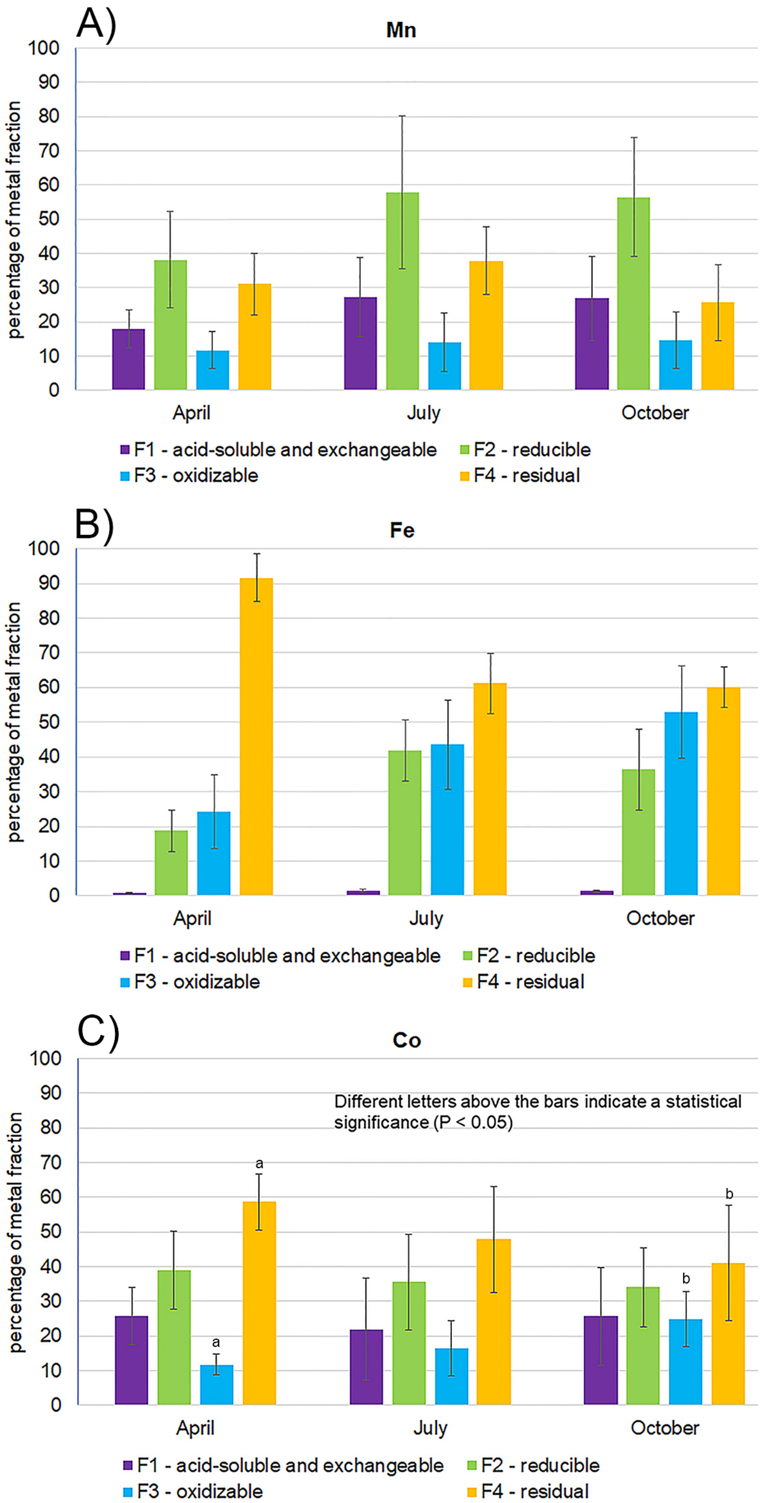

Figure 2. Changes of fractional composition of Mn (A), Fe (B) and Co (C) depending on season. Statistical significance ($P < 0.05$) is marked with different letters above the bars (mean ± standard deviation)

does not comprehensively reflect their harmfulness [Hu et al., 2021]. Determining the fractional composition of metals in soil using sequential extraction is a useful method for assessing their mobility and toxicity, also towards microorganisms [Boughattas et al., 2019; Malinowska and Jankowski, 2020]. It is unclear which metal fractions have the greatest impact on the life processes of microorganisms [Pan et al., 2020].

The research results indicate that the largest pool of Mn may become bioavailable in the occurrence of oxygen deficiency in the soil, i.e.

Table 1. Correlation coefficients between properties of investigated soils and Mn, Fe and Co fractions; sand (2–0.05 mm), silt (0.05–0.002 mm), clay(< 0.002 mm); n = 27

Parameter	Mn				Fe				Co			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
pH	0.572*	-0.038	-0.089	0.147	0.147	0.360	0.154	-0.369	0.011	-0.027	-0.232	-0.249
Organic C	0.182	0.023	0.047	-0.269	0.228	-0.082	0.255	-0.155	-0.105	-0.178	0.160	-0.364
Sand	0.271	-0.331	-0.074	-0.175	0.230	-0.162	0.180	-0.120	0.445*	-0.208	0.361	-0.582*
Silt	0.120	0.205	-0.292	-0.570*	-0.094	0.005	0.095	-0.179	0.239	0.042	0.273	-0.194
Clay	-0.097	0.446*	-0.053	0.195	-0.116	0.436*	0.108	-0.135	-0.455*	0.064	-0.132	0.381*

Note: F1, acid-soluble and exchangeable fraction; F2, reducible fraction; F3, oxidizable fraction; F4, residual fraction, * significant for P < 0.05.

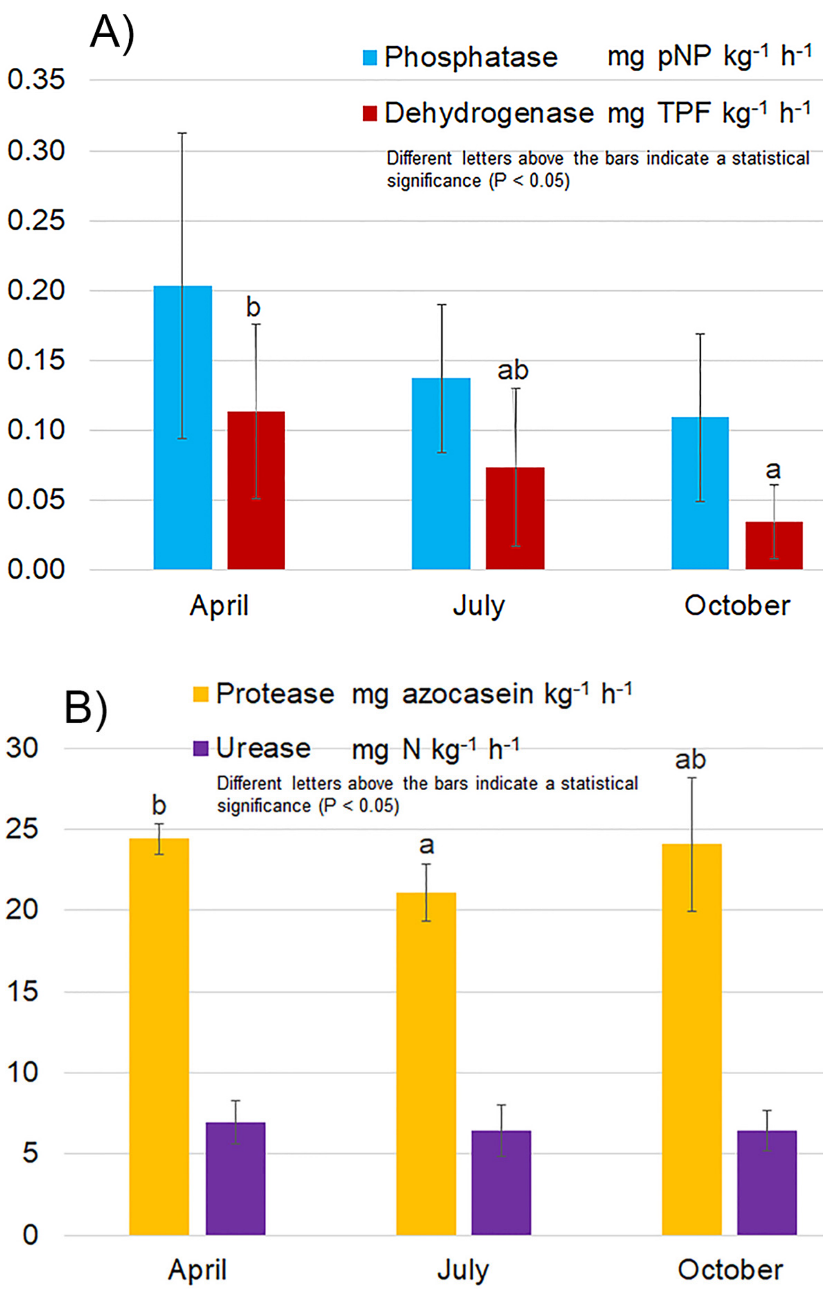

Figure 3. Changes of phosphatase and dehydrogenase activities (A) as well as protease and urease activities (B) depending on season. Statistical significance (P < 0.05) is marked with different letters above the bars (mean ± standard deviation)

Table 2. Enzyme activities in the investigated soils (mean ± standard deviation) [Łukowski and Dec, 2018]

Enzyme	Sampling site								
	1	2	3	4	5	6	7	8	9
Protease (mg kg^{-1} h^{-1})	0.024±0.003	0.022±0.002	0.021±0.002	0.024±0.001	0.022±0.002	0.021±0.002	0.027±0.006	0.022±0.002	0.024±0.002
Phospha-tase (mg kg^{-1} h^{-1})	0.124±0.033	0.231±0.023	0.134±0.040	0.130±0.029	0.231±0.123	0.100±0.038	0.197±0.111	0.149±0.085	0.052±0.008
Dehydrogenase (mg kg^{-1} h^{-1})	0.0046±0.0023	0.0047±0.0003	0.0091±0.0049	0.0142±0.0076	0.0097±0.0059	0.0076±0.0046	0.0080±0.0062	0.0068±0.0065	0.0017±0.0007
Urease (mg kg^{-1} h^{-1})	7.36±0.83	8.43±0.41	6.38±0.92	5.09±1.28	5.65±0.52	6.61±1.76	7.43±1.23	6.40±0.68	6.18±1.12

reducing conditions, because the highest percentage of Mn was recorded in the fraction associated with Fe/Mn oxides. However, the smallest part of Mn can be released into the soil solution and thus affect the enzymatic activity under oxidizing conditions. In the case of Fe, the largest share was found in the residual fraction, considered immobile, bound to silicates matrix. The release of metals from this fraction is possible as a result of the activity of plant roots and microorganisms [Cruz et al., 2022]. In fraction F1, the most available, there was the least amount of Fe. A similar pattern for Mn and Fe in the soil was stated by Sarpong et al. [2023]. The reducible and oxidizable fractions contained 69.01 and 0.88% Mn, respectively. The residual fraction comprised approximately 61% Fe, and the acid-soluble only 0.22% Fe. In the case of Mn, the authors also emphasized its highest mobility, and therefore bioavailability to plants and microorganisms, as the total share in the first three fractions was 92.4%. The results of the conducted research confirm such a high mobility of Mn, because the mobile pool of this metal was 88.4%. This may impact growth of

Table 3. Correlations between fractions of Mn, Fe, Co and pH, organic carbon, as well as enzyme activities, in sampling dates (n = 27)

Parameter	Mn				Fe				Co			
	Soil sampling in April											
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
pH	-0.081	0.198	-0.025	0.266	-0.078	0.546	0.324	-0.596	-0.157	-0.518	-0.175	0.155
Organic C	-0.327	0.429	-0.124	-0.509	0.908*	-0.206	0.354	-0.518	-0.032	-0.162	0.405	0.249
Protease	0.391	0.011	-0.406	0.249	0.125	0.317	0.286	-0.819*	-0.265	-0.218	-0.159	0.069
Phosphatase	-0.281	0.280	-0.059	-0.453	0.542	0.225	0.501	-0.554	0.134	-0.354	0.359	0.567
Dehydrogenase	0.764*	-0.341	-0.498	-0.296	-0.168	-0.545	-0.693*	0.393	0.619	0.815*	-0.108	-0.648
Urease	-0.278	-0.169	0.466	0.461	-0.121	0.557	0.281	-0.138	-0.528	-0.578	0.499	0.102
Soil sampling in July												
pH	0.310	-0.549	0.195	0.627	0.114	0.161	-0.445	-0.164	0.534	0.197	0.533	0.017
Organic C	-0.457	-0.171	0.449	-0.250	0.854*	-0.260	0.415	-0.453	0.506	-0.096	0.095	-0.427
Protease	0.277	-0.389	0.176	0.656	-0.216	0.017	-0.437	0.007	0.201	0.298	0.506	0.068
Phosphatase	-0.637	0.092	0.369	-0.340	0.690*	-0.299	0.372	-0.160	-0.160	-0.010	0.091	-0.229
Dehydrogenase	0.297	-0.340	0.003	0.628	-0.345	-0.039	-0.202	0.072	0.029	0.029	0.483	0.278
Urease	0.301	0.083	-0.341	-0.457	-0.270	-0.029	-0.147	0.591	-0.278	-0.495	-0.259	0.279
Soil sampling in October												
pH	0.805*	-0.204	-0.513	-0.109	-0.140	0.020	-0.230	0.475	-0.291	0.235	-0.332	-0.233
Organic C	0.400	-0.208	-0.042	-0.104	0.050	-0.271	0.040	0.388	-0.338	-0.214	-0.110	-0.459
Protease	0.277	-0.337	-0.329	0.299	0.229	-0.074	0.017	0.197	-0.189	0.108	-0.020	0.290
Phosphatase	0.038	0.296	0.053	0.532	-0.025	0.665	-0.618	-0.031	-0.549	0.458	-0.705*	0.643
Dehydrogenase	0.042	0.406	0.063	-0.428	-0.427	0.209	-0.275	-0.134	-0.206	0.317	-0.345	-0.490
Urease	0.685*	0.157	-0.552	0.236	-0.268	0.652	-0.818*	0.221	-0.438	0.248	-0.879*	0.024

Note: F1 – acid soluble and exchangeable fraction; F2 – reducible fraction; F3 – oxidizable fraction; F4 – residual fraction, * significant for P < 0.05.

microorganisms and metabolic processes, which in turn affects soil health as well as nutrient cycling [Khoshru, 2023].

As in the case of Fe, the largest part of Co was present in an unavailable form, and cobalt associated with organic matter constituted the smallest part of the total content of this metal. Malinowska and Jankowski [2020] observed a similar distribution. In the residual fraction and fraction bound to organic matter, they recorded 27.7 and 21.9% Co on average, respectively, in the control treatment. According to Iwegbue [2013], cobalt is present mainly in the residual form, and the least of this element is collected by the soluble and exchangeable fractions. The second largest pool of cobalt is bound to oxides. As stated by Banerjee and Bhattacharya [2021], the bioavailability of cobalt in the soil is regulated by the interaction of the soluble and exchangeable fractions with the fraction of iron oxides (crystalline and amorphous) and the fraction associated with organic matter. Li et al. [2001] found slight similarity in the distribution of Mn and Co in soil. In the case of both elements, the largest part was the residual form, 35.8 and 45.8%, respectively. The soluble and exchangeable fractions accumulated the least amount.

Significant differences in the fractional composition in the sampling dates were noted only in the case of cobalt. The gradual decrease in the percentage of fraction F4 indicated the redistribution of the studied element to fraction F3, bound to organic matter. This was confirmed by the gradual increase of the cobalt content in this fraction during the study. Such redistribution may occur due to the activity of soil microorganisms. The second factor that may cause it, i.e. a considerable decrease in soil reaction, did not occur in the conducted research.

Impact of soil physicochemical properties on metal fractions

The physicochemical properties of the studied soil had a small influence on the fractional composition of the metals. This impact was most visible in the case of cobalt. Sand and especially clay significantly influenced its content in fractions F1 and F4. The contents of Mn and Fe in fraction F2 were moderately influenced by the clay content. This is associated with the properties of the fine soil fraction. It is characterized by a high content of organic matter and Fe/Mn/Al oxides and hydroxides, negatively charged surfaces and a larger specific surface area, as compared to other soil fractions [Ren et al., 2023; Zhao et al., 2024]. Research results by many other authors indicate that metal species and concentrations are dependent on the soil granulometric fraction [Zhang et al., 2016; Zhang et al., 2021; Gong et al., 2023].

Metal fraction effect on soil enzymatic activity

Enzymatic activity is a reflection of biological processes in the soil, which in turn are related to its physicochemical properties and stress conditions; therefore, it can be treated as an indicator of the soil environment quality [Chowdhury, 2021]. Nowadays, soil biological activity is usually determined by measuring phosphatases, dehydrogenases, proteases and urease activities [Furtak and Gałązka, 2019].

The influence of the metal fractions on the enzymatic activity was not unequivocal. The fraction associated with organic matter most often had a negative and significant impact on enzyme activities. The strong relationship between F3/Fe and F3/Co and urease activity could be related to the gradual increase of iron and cobalt content in discussed fraction during the study. This negative impact appeared in autumn, when the percentage of both metals in fraction F3 was the highest among the mobile fractions. This effect might be manifested in two ways, because urease acts as an intracellular or extracellular enzyme, contained in the soil solution or associated with inorganic and organic colloids [Miśkowiec and Olech, 2020]. Reduced activity of urease, one of the most important enzymes in the soil, has its advantages. These include reducing nitrogen losses due to ammonia volatilization and leaching of nitrite ions, as well as reducing the toxicity of ammonia and accumulating nitrites formed after urea hydrolysis [Sherene, 2017]. The strong negative correlation between F3/Co and phosphatase activity recorded in autumn was also associated with an increase of cobalt content in fraction F3, which confirms the gradual decrease in phosphatase activity during the studies. The negative impact of Fe bound to organic matter on the dehydrogenase activity in spring, when its activity was the greatest, is difficult to explain, since the percentage of fraction F3 was then the lowest (22.4%). The acid soluble and exchangeable fraction was the second, which effect on the activity of the studied enzymes was noticeable. Study results showed that this fraction may contribute to the increase in the activity of dehydrogenase, phosphatase and urease in the soil. This was particularly visible in the case of F1/Mn and dehydrogenase, as a positive correlation was found in all three seasons, but this effect was statistically significant only in spring. The positive impact of fraction F1 on dehydrogenase activity must have been related to the effect on soil microorganisms, and not on the enzyme itself, because it is an intracellular enzyme. The highest dehydrogenase activity in April was probably caused by favorable environmental conditions for the development of microorganisms [Teimouri et al., 2019]. Research results have long confirmed that the enzymatic activity of soils is significantly influenced by such factors as moisture, soil temperature and average annual temperature [Yao et al., 2011; Hammerl et al., 2019; Daunoras et al., 2024; Pupin et al., 2024]. Also, these factors probably caused that dehydrogenase and protease activities varied significantly between seasons.

CONCLUSIONS

The research results show that the metal fraction associated with organic matter had the greatest impact on the activity of the studied enzymes. The seasonality of these relationships indicated that they were mainly based on changing weather conditions. It can also be concluded that the negative impact of the discussed fraction will be more pronounced in the soils with a high content of organic matter. Interestingly, the influence of the acid-soluble and exchangeable fraction, most easily bioavailable, on the enzymatic activity, was negligible. It was only noticeable in the case of manganese and iron. This was a positive impact, so it did not reduce soil productivity or fertility. A positive correlation between the metal content in this fraction and the activity of some enzymes appears to be due to the current needs of microorganisms.

It seems that the main challenge in the context of the issues raised in this paper is to clearly determine which fractions of Mn, Fe and Co have a significant impact on the activity of the studied enzymes. Unfortunately, the number of factors that can influence enzymatic activity and the interactions between them makes it very difficult to confirm such a relationship. The most unpredictable factor in field research corresponds to climatic conditions, which affect a number of physicochemical properties of the soil and its microbiological activity. This factor also makes it difficult to compare the results of different studies.

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