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Fractions of Ni, Pb, Cr, and Their Impact on Enzyme Activities of Arable Land Cultivated by the Simplified Method

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Abstract: Total metal content is not representing its availability and thus does not provide the details about potential environmental hazards, including the impact on soil enzyme activities. To understand metal availability, chemical fractions must be considered. The goal of this study was to evaluate the influence of Ni, Cr, and Pb fractions on the enzymatic activity of soils cultivated by the simplified method, which is rare not only in Poland. The percentage of studied metals in fractions was determined according to the Community Bureau of Reference (BCR) method. Four fractions were extracted: acid soluble and exchangeable (F1), reducible (F2), oxidizable (F3), and residual (F4). The highest Ni and Cr percentages were noted in fraction F4, and for Pb, they were noted in fraction F2. The smallest Ni and Pb percentages were observed in fraction F1 (most mobile) and for Cr, they were observed in fraction F2. In soil samples collected in spring, the significant relationship was stated between F1/Ni/dehydrogenase, F2/Pb/dehydrogenase, and F2/Pb/urease. Such dependence occurred between F1/Ni/phosphatase and F4/Ni/urease during summer as well as between F1/Ni/phosphatase and F4/Ni/dehydrogenase in autumn. F1/Pb caused a drop in phosphatase activity, whereas F4/Cr influenced its increase. The study results indicated that metal fractions influenced phosphatase activity the most, while protease activity in the soil was not affected.

Keywords: metal fraction; enzyme activity; heavy metal; arable soil; BCR method

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1. Introduction

Soil is a very important part of the ecosystem. Heavy metals in soil represent destructive and potent environmental hazards [1]. They are a source of concern because of their potential reactivity, toxicity, and mobility [2,3]. High concentrations of metals in soil can cause the limitation of vegetation [4]. According to Xia et al. [5], nickel toxicity toward microorganisms in soil appears at concentrations higher than 100 mg kg^{-1}, causing the decrease of microbial biomass carbon and soil basal respiration. As to lead, the toxic level is 150 mg kg^{-1} for microbial biomass carbon and 100 mg kg^{-1} for microbial biomass nitrogen [6]. Chromium belongs to the metals that are extremely harmful to microbes. In the environment, only trivalent and hexavalent forms of chromium are prevalent. They are differentiated in terms of biological reactivity and physicochemical properties. Hexavalent ions are more toxic due to the high mobility in biological systems [7]. As stated by ul Hassan et al. [8], the 50% reduction in overall microbial soil activity is observed at a total chromium concentration of 263 g kg^{-1}. The toxicity of heavy metals to plants is dependent on soil composition, plant species, metal concentration, soil pH, and the chemical form of the metal [9]. Critical concentrations in soil are within the range of 10–100, 30–300, and 5–30 mg·kg^{-1} for Ni, Pb, and Cr, respectively [10]. The persistence of enzymes in the soil is influenced by parameters such as soil temperature, depth, organic matter, acidity, and

granulometric composition. It ranges from a few days to several years [11]. Studies investigating enzyme activities are the source of details about the soil biochemical processes [12]. In this study, we investigated the activities of four enzymes, namely dehydrogenase, phosphatase, urease, and protease, because they are commonly used as indicators of the soil overall microbial activity. Dehydrogenase is one of the most important enzymes in this context, as it exists in active form only inside the living cells [13]. It is involved in C-cycling and microbial oxidative activity. Other studied enzymes take part in the transformation of phosphorus compounds (phosphatase) and N-cycling (urease and protease). Research on the decreased enzyme activities in the presence of heavy metals is a useful tool for the assessment of soil degradation [1]. The high level of heavy metals that act as micronutrients and low concentration of nonessential metals has a negative influence on bacteria in the soil [14]. A decrease in the number and activity of soil microorganisms imposes an impact on soil enzymes. The influence of heavy metals on enzymatic reactions is mainly related to binding to the enzyme active sites, complexation of substrate, and reacting with the enzyme–substrate complex.

Study results show that activities of dehydrogenase, urease, catalase, acid and neutral phosphatase, and sucrose can be reduced by heavy metals [15]. Donderski and Swiontek Brzezinska [16] observed that Cu, Zn, Ni, Pb, Cd, and Cr could inhibit the activity of alkaline phosphatase. The above-mentioned research, and many more [17–20], have referred to the total form of metal. However, the impact of metal fractions on the soil enzymatic activity was investigated to a small degree. It is an important issue, since even the low level of metal that occurs in unpolluted soil can affect the soil microbial activity and the soil enzyme activities. We must know which part of the total metal content can interact with the soil microorganisms and enzymes. Fractional metal composition gives us this knowledge.

The aim of the study was estimation of the fractional composition of nickel, lead, and chromium and its influence on enzymatic activity (protease, dehydrogenase, urease, alkaline phosphatase) of arable soils cultivated by the simplified method.

2. Materials and Methods

2.1. Collection of Soil Samples

Nine samples of agricultural soils (*Albic Luvisols*) were taken from the arable layer (0–25 cm) in the area located in northeastern Poland, around Gawliki Wielkie (P1) and Radzie (P2) (Figure 1). Each sample (about 6 kg) was obtained from a different field in six replicates and mixed the same day (see Figure S1 in Supplementary Materials). They were collected three times in 2015 (April, July, October) during the growing season. Soils in this region have mostly boulder clay origin. In the study area, for the last five years, no-till farming was used. Winter wheat was cultivated in site P1, while broad bean was cultivated in area P2. The following N/P/S/CaO fertilization rates were used: 287.5, 75, 30, 120/45, 75, 8, 120 kg·ha^{-1} in areas P1/P2, respectively.

Monthly rainfall and temperatures in the study area, provided by the Institute of Meteorology and Water Management-National Research Institute [21], are shown in Table 1. Rainfall data come from the weather station located in Siedliska and temperatures come from Olecko.

2.2. Physicochemical Analysis

Soil samples were dried at room temperature, sieved through a 2 mm sieve, and stored at 4 °C for analysis. The following determinations were performed: organic carbon content by oxidation with potassium dichromate (VI) in the presence of sulfuric (VI) acid according to the PN-ISO 14235:2003 [22]; pH in 1 mol/dm^3 KCl by the potentiometric method based on PN-ISO 10390:1997 [23] and Kjeldahl nitrogen by the steam distillation with titration for quantification the amount of ammonia, using an Omnilab FoodAlyt D5000 apparatus, after previous sample digestion in concentrated sulfuric (VI) acid according to the PN-ISO 11261:2002 [24]. The soil granulometric composition was determined by

the sieve method in accordance with the Polish regulations: PN-R-04032:1998 [25] and PN-R 04033:1998 [26]. The total content of Ni, Pb, and Cr was determined by the flame atomic absorption spectrometry (FAAS) method, after previous digestion with aqua regia according to the PN-ISO 11466:2002 [27] using the Ethos Easy microwave digestion system (Milestone, Sorisole, Italy). The content of metals in fractions was assayed by means of graphite furnace atomic absorption spectrometry (GFAAS). In both cases, iCE 3500 apparatus (Thermo Scientific, Waltham, MA, USA) was used. The percentage of each fraction in the total metal content was calculated.

Figure 1. Sampling site location. P1—Gawliki Wielkie, P2—Radzie (sample numbers in parentheses).

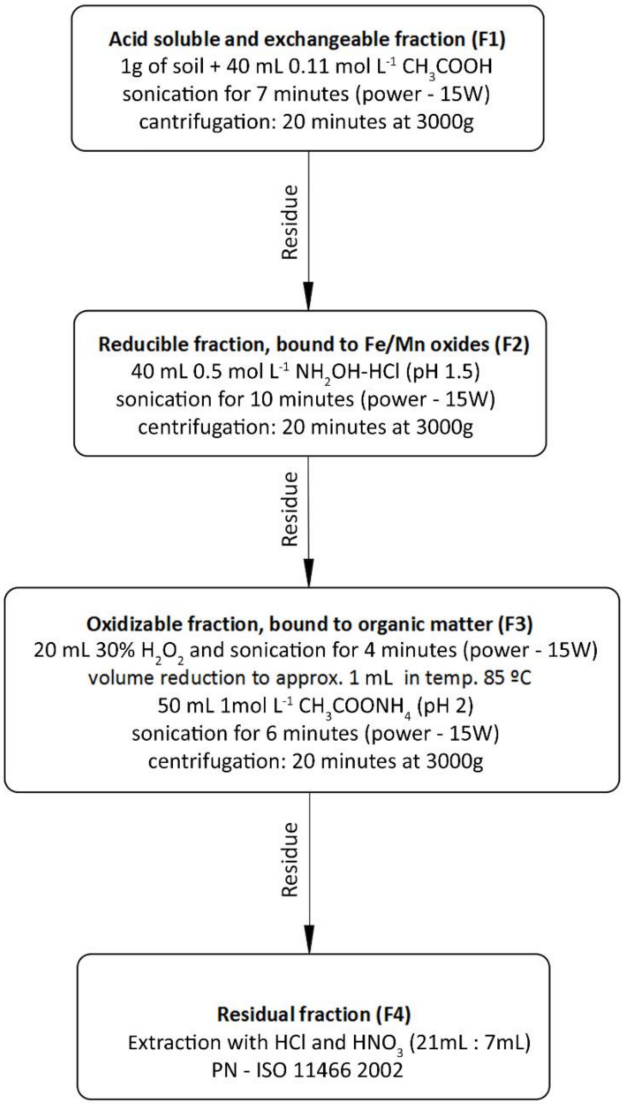
Table 1. Monthly rainfall and temperatures in the year 2015, in the study area.

	Month											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Rainfall Summary, mm	64.2	18.9	52.0	36.1	49.3	32.6	70.1	12.9	84.1	14.9	118.4	54.5
Average Temperature, °C	− 0.7	− 1.0	4.2	6.8	11.3	15.4	17.6	20.3	14.0	6.1	4.2	2.6

1—January, 2—February, 3—March, 4—April, 5—May, 6—June, 7—July, 8—August, 9—September, 10—October, 11—November, 12—December.

Detection limits for Ni, Pb, and Cr during FAAS analysis were 7.5, 12.7, and 8.4 $\mu g \cdot L^{-1}$, respectively. The certified reference material CRM BCR-701 (sediment, LGC Standards, Poland) was used for a quality control of the BCR method. In addition, the recovery rates for metals in the soil samples were evaluated using the following formula: ((F1 + F2 + F3 + F4)/total content) × 100. For Ni, recovery ranged from 84% to 139%; for Pb, it ranged from 88% to 123%, and for Cr, it ranged from 112% to 147%.

The fractional composition of Ni, Pb, and Cr in soil samples was determined with the ultrasound accelerated Community Bureau of Reference (BCR) method, which consisted of four stages [28] (Scheme 1).



Scheme 1. The ultrasound accelerated Community Bureau of Reference (BCR) sequential extraction scheme for metal partitioning.

2.3. Enzyme Activities

Dehydrogenase activity was evaluated by spectrophotometry at a wavelength of 485 nm [29] after 24 h incubation at 30 °C using as a substrate 1% triphenylotetrazole chloride (TTC) and expressed in μg of triphenylformazan (TPF) g^{-1} DM $24h^{-1}$. Alkaline phosphatase was analyzed according to the procedure by Tabatabai and Bremner [30]. Protease activity was assayed using azo-casein according to the method of Macura and Vágnerová [31], and urease activity was assayed according to the method of Hoffmann and Teicher [32]. A more comprehensive description of the determination of enzyme activities was given in our previous paper [33].

2.4. Statistical Analysis

Analysis of variance (ANOVA) performed by using Statistica 13.1 software (TIBCO Software, Palo Alto, CA, USA) was calculated based on all the results (except soil granulometric composition). To evaluate the significant differences among the means of Ni, Pb, and Cr fractions, soil enzyme activities, and soil physicochemical properties, we used the least significant difference test. Statistica 12.5 software (TIBCO Software) was used to calculate the correlations between studied parameters (Pearson's correlation factor for

$P \leq 0.05$). Two versions of the same software were used due to the expiration of Statistica 12.5 licence.

3. Results

3.1. Physicochemical Properties of Studied Soils

The studied soils represented three textural classes: loam, clay loam, and sandy loam (Table 2). They were slightly acidic and acidic according to pH values. Most of them had high organic carbon and nitrogen content. The level of enzyme activities was differentiated, and for dehydrogenase, it amounted to 0.02 to 0.60 μg·TPF·g^{-1}·DM·24·h^{-1}; for alkaline phosphatase, it was 0.32–2.88 mM·pNP g^{-1}·h^{-1}; for protease, it was 18.29–34.15 mg azo-casein·g^{-1}·h^{-1}; and for urease, it was 4.12–8.90 μgN·g^{-1}·DM h^{-1}. The average enzyme activities are shown in Table 2.

Table 2. Characteristics of studied soils (mean ± standard deviation). Values refer to the average from all sampling [33]; sand (2–0.05 mm), silt (0.05–0.002 mm), clay (<0.002 mm).

	Sampling Site								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Protease mg azo-casein g^{-1}·h^{-1}	24.26 ± 2.07	22.27 ± 1.91	21.04 ± 2.05	24.27 ± 0.72	22.40 ± 1.64	21.08 ± 1.91	26.68 ± 5.56	22.43 ± 1.55	24.39 ± 1.78
Phosphatase mM pNP g^{-1}·h^{-1}	0.89 ± 0.24	1.66 ± 0.17	0.96 ± 0.29	0.93 ± 0.21	1.66 ± 0.88	0.72 ± 0.27	1.41 ± 0.80	1.07 ± 0.61	0.38 ± 0.05
Dehydrogenase μg TPF g^{-1} DM $24h^{-1}$	0.11 ± 0.05	0.11 ± 0.01	0.22 ± 0.12	0.34 ± 0.18	0.23 ± 0.14	0.18 ± 0.11	0.19 ± 0.15	0.16 ± 0.15	0.04 ± 0.02
Urease μgN g^{-1} DM 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
pH	6.5 ± 0.3	5.7 ± 0.7	4.9 ± 0.7	6.5 ± 0.9	5.7 ± 0.7	5.2 ± 0.9	5.7 ± 0.1	5.9 ± 1.2	5.1 ± 1.8
Organic C, %	1.87 ± 1.68	2.05 ± 0.35	2.02 ± 0.21	2.37 ± 1.52	2.25 ± 0.30	1.19 ± 0.33	3.36 ± 2.68	1.48 ± 0.35	1.12 ± 0.27
N, %	0.31 ± 0.02	0.19 ± 0.01	0.27 ± 0.03	0.32 ± 0.13	0.31 ± 0.15	0.22 ± 0.05	0.23 ± 0.04	0.28 ± 0.07	0.21 ± 0.02
Sand, %	20.9 ± 14.5	21.9 ± 1.1	22.4 ± 6.6	27.2 ± 9.1	23.4 ± 0.7	39.0 ± 11.6	22.1 ± 1.8	36.2 ± 24.2	56.1 ± 8.3
Silt, %	25.0 ± 9.0	15.6 ± 4.9	24.6 ± 4.7	29.9 ± 1.9	22.7 ± 5.2	32.0 ± 4.6	30.1 ± 3.9	31.5 ± 2.4	16.5 ± 1.3
Clay, %	27.6 ± 4.1	27.4 ± 2.0	30.7 ± 5.2	7.6 ± 0.9	31.4 ± 3.5	8.2 ± 1.5	29.6 ± 3.8	7.3 ± 2.1	8.3 ± 1.4
Ni, mg kg^{-1}	13.0 ± 6.2	21.9 ± 2.2	17.1 ± 4.0	9.5 ± 2.6	13.0 ± 7.7	10.2 ± 6.0	10.5 ± 3.7	8.1 ± 3.1	10.3 ± 9.4
Pb, mg kg^{-1}	11.7 ± 4.7	16.6 ± 2.0	13.8 ± 2.5	12.2 ± 5.2	15.8 ± 9.2	10.1 ± 1.8	13.8 ± 6.0	11.0 ± 0.4	7.1 ± 1.9
Cr, mg kg^{-1}	28.8 ± 13.7	45.6 ± 3.8	35.3 ± 10.0	22.0 ± 5.7	27.8 ± 13.1	24.9 ± 14.4	19.1 ± 3.5	21.5 ± 2.9	12.6 ± 3.1

Only the activities of protease and dehydrogenase were seasonally differentiated. The activity of protease in April was the highest and significantly higher than that in July (Figure 2). Similarly, the activities of all other enzymes were also the highest during the spring. Dehydrogenase activity was clearly differentiated between April and October.

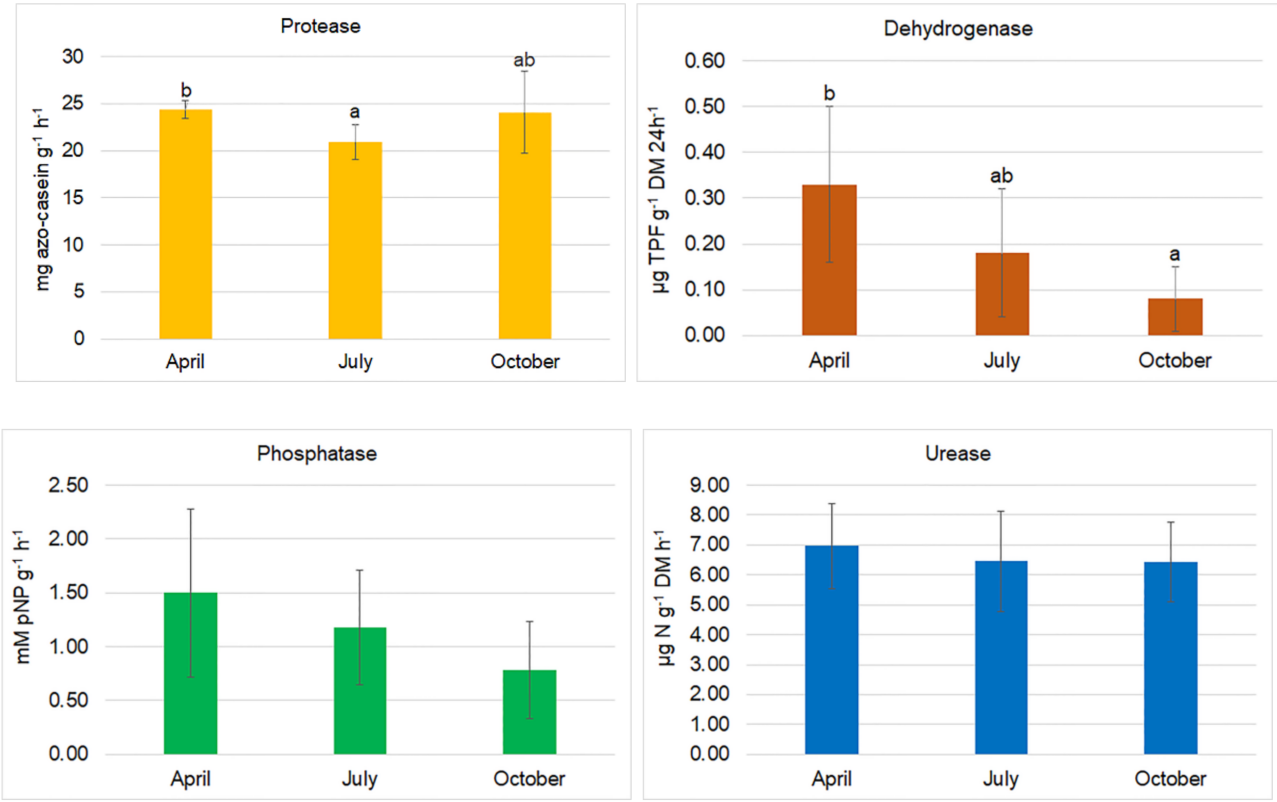


Figure 2. Seasonal changes of enzyme activities. Different letters above bars (mean ± standard deviation) indicate significant difference ($P < 0.05$).

We have not found strong correlations between the physicochemical properties of the studied soils—such as pH, organic C, N, and granulometric composition—and enzyme activities (Table 3).

Table 3. Correlations between enzyme activities and characteristics of studied soils [33]; sand (2–0.05 mm), silt (0.05–0.002 mm), clay (<0.002 mm); $n = 27$.

	pH	Organic C	N	Sand	Silt	Clay
Protease	0.253	0.139	0.001	−0.124	0.047	−0.030
Phosphatase	−0.204	0.035	−0.044	−0.306	−0.149	0.200
Dehydrogenase	−0.240	−0.299	0.290	−0.061	0.038	−0.154
Urease	0.070	0.022	−0.283	−0.341	−0.168	0.266

In the case of Ni, the positive correlation between fraction F1 and sand (r = 0.667) as well as negative correlation between this fraction and clay (−0.497) was observed (Table 4). A significant relationship between F1/Pb and sand (0.628) was also stated. The same fraction correlated negatively with clay (−0.487). F4/Pb negatively influenced silt content (−0.482). Most of the dependences were noted between fractions of Cr and the characteristics of the studied soils. Sand content influenced positively F1/Cr (0.398) and organic carbon F3/Cr (0.524). Negative correlation between F4/Cr and sand (−0.499) as well as silt (−0.390) was also noted.

Table 4. Correlation coefficients between characteristics of studied soils and fractions of Ni, Pb, Cr; sand (2–0.05 mm), silt (0.05–0.002 mm), clay (<0.002 mm); n = 27.

	Ni				Pb				Cr			
	F1	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
pH	0.080	0.037	−0.070	−0.022	0.326	−0.135	0.002	−0.142	−0.115	−0.266	0.120	−0.149
Organic C	0.299	0.099	0.054	−0.277	0.293	−0.264	0.117	−0.328	−0.009	0.197	0.524 *	−0.301
Sand	0.667 *	−0.050	0.082	−0.209	0.628 *	−0.312	−0.184	−0.254	0.398 *	0.244	0.224	−0.499 *
Silt	0.271	0.312	0.331	−0.079	0.184	0.249	−0.121	−0.482 *	0.100	0.276	0.129	−0.390 *
Clay	−0.497 *	−0.282	−0.124	0.209	−0.487 *	0.228	−0.098	0.044	−0.352	−0.272	−0.177	0.298

* significant for P < 0.05.

3.2. Total Metal Content and Fractions

Total metal content was characteristic for noncontaminated arable soils and ranged 8.1–21.9, 7.1–16.6, 12.6–45.6 mg kg^{-1} on average for Ni, Pb, and Cr, respectively. The highest amount of Ni was observed in fraction F4 (66.2% of total content on average) and the lowest was observed in fraction F1 (15.0%). Fractions F2 and F3 constituted 15.3% and 16.0% of total Ni, respectively. In the case of Pb, the highest percentage was stated in fraction F2 (44.0%) and the lowest was stated in fraction F1 (10.8%). Fractions F3 and F4 had 31.0% and 26.4% total Pb, respectively. Fraction F2 gathered the lowest amount of Cr (5.3%). Its highest content (67.3%) was observed in fraction F4. Fractions F1 and F3 constituted 11.3% and 42.6% of total Cr, respectively. Fraction F1 gathered the most of Ni in the autumn (Figure 3). It was significantly more than in the summer. In the case of Cr/F2, we have noted the same dependence. The percentage of Pb/F4 clearly decreased in October in comparison with July.

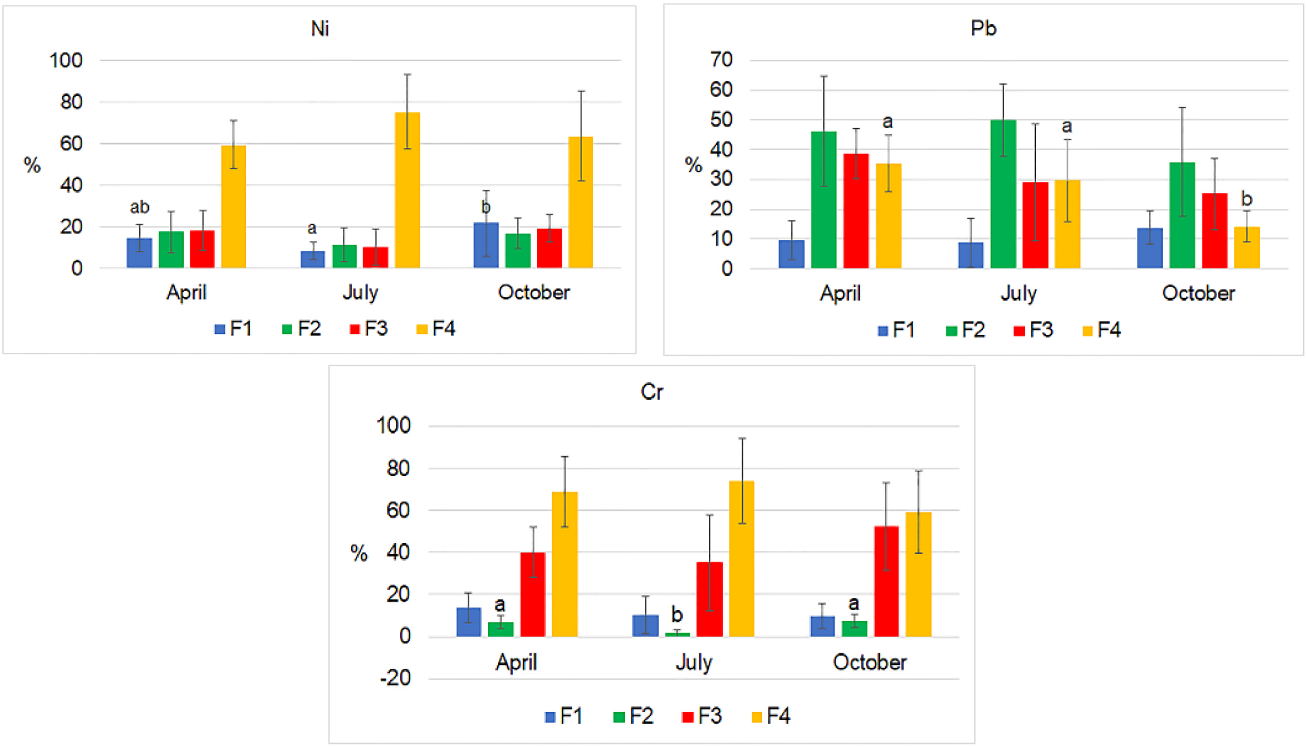


Figure 3. Seasonal changes of fractional composition of metals. Different letters above bars (mean ± standard deviation) indicate significant difference (P < 0.05).

3.3. Impact of Metal Fractions on Enzyme Activities

The influence of metal fractions on enzyme activities was varied in sampling dates (Table 5). Phosphatase activity was moderately influenced by F1/Ni during the summer (−0.688) and strongly correlated with the same fraction in the autumn (−0.731). In October, we have also stated significant correlations between phosphatase and F1/Pb (−0.702) as well as F4/Cr (0.708). Dehydrogenase was affected in April by F1/Ni (r = 0.806) and F2/Pb (0.742). Its activity correlated negatively with F4/Ni (−0.772) during the autumn. Urease activity was correlated with F2/Pb in April (−0.843) and F4/Ni (0.707) in July. We did not notice any impact of metal fractions on protease activity during the study.

Table 5. Correlation coefficients between pH, organic carbon, enzyme activities, and fractions of Ni, Pb, and Cr in seasons (n = 27).

	Ni				**Pb**				**Cr**			
	Soil Sampling in April											
	F1	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
pH	−0.420	−0.289	−0.201	−0.096	−0.291	−0.394	0.124	−0.100	−0.237	−0.261	−0.278	0.037
Organic C	−0.177	−0.001	0.297	−0.806 *	−0.091	−0.043	0.832 *	−0.640	0.330	0.292	0.783 *	−0.261
Protease	−0.291	−0.263	0.103	0.058	−0.325	−0.101	−0.011	−0.171	−0.107	−0.137	−0.093	0.002
Phosphatase	−0.033	0.087	−0.050	−0.650	−0.047	0.047	0.491	−0.505	0.354	0.181	0.508	−0.379
Dehydrogenase	0.806 *	0.441	0.527	0.130	0.570	0.742 *	−0.416	−0.008	0.464	0.595	0.166	−0.473
Urease	−0.641	−0.165	−0.057	0.124	−0.235	−0.843 *	0.419	0.234	−0.271	−0.356	−0.134	0.438
	Soil Sampling in July											
pH	0.611	0.515	0.177	−0.305	0.600	0.206	0.453	0.275	0.521	0.214	0.448	−0.139
Organic C	−0.498	−0.330	−0.478	−0.331	0.220	−0.501	0.558	−0.191	0.628	−0.263	0.405	−0.003
Protease	0.637	0.653	0.263	−0.360	0.490	0.260	0.210	0.266	0.218	0.121	0.182	−0.024
Phosphatase	−0.688 *	−0.378	−0.602	−0.284	0.355	−0.387	0.316	−0.310	0.422	−0.435	0.258	−0.201
Dehydrogenase	0.564	0.532	0.346	−0.094	0.040	0.146	−0.007	0.080	0.195	0.057	−0.083	0.015
Urease	−0.202	−0.427	0.290	0.707 *	−0.262	0.324	−0.374	−0.453	−0.462	−0.053	−0.093	−0.574
	Soil Sampling in October											
pH	0.047	0.113	−0.107	−0.022	0.433	−0.056	−0.087	0.118	−0.502	−0.543	−0.116	−0.195
Organic C	0.342	0.247	−0.101	−0.191	0.517	−0.238	0.038	−0.098	−0.324	0.142	0.545	−0.348
Protease	0.003	0.022	−0.152	0.366	0.417	−0.038	−0.120	0.567	−0.183	0.036	0.261	0.146
Phosphatase	−0.731 *	0.058	−0.423	0.242	−0.702 *	0.207	−0.233	0.134	−0.464	−0.632	−0.473	0.708 *
Dehydrogenase	−0.356	−0.248	0.392	−0.772 *	−0.266	0.544	0.518	−0.241	−0.279	−0.291	0.053	−0.382
Urease	−0.437	−0.118	−0.360	−0.471	−0.089	0.522	0.029	0.282	−0.497	−0.622	−0.282	0.103

* significant for $P < 0.05$.

4. Discussion

4.1. Distribution of Heavy Metals in Chemical Fractions

The total metal content provides incomplete information about its bioavailability and possible environmental threats [34], including the effect on soil enzyme activities. More valuable results can be achieved studying metal fractions in the soil. We used the BCR method to evaluate the fractional composition of the studied elements and thus their mobility and bioavailability, which are the essential factors affecting the impact of metal on the enzymatic activity of soil [35]. This extraction method, among others, is well standardized, which is its great advantage [36]. Metal fractions obtained with this method are considered as operationally defined [37]. It means that the extraction scheme reflects the natural processes that lead to heavy metals fractionation in the environment, which can cause their mobilization [38].

We have stated 66.2% and 15.0% Ni on average in fractions F4 and F1, respectively. The same dependence was noted by Zeiner et al. [39]. In the orchard soils, they have found in fraction F4 the most Ni (40.6%) and the least in fraction F1 (6.36%). Cheng et al. [40] also reported that fraction F1 comprised the lowest amount of Ni (about 7% of total content) in garden soils. This fraction corresponds to the part of metal that is bound to the soil matrix with weak bonds and thus is highly mobile [41]. Pb in the studied soils was present mainly in fraction F2 (44.0%), which is in accordance with the results of Boughattas et al. [36]. The authors stated in fraction F2 of control soil approximately 95% of the discussed element. Most of Cr (67.3%) was in fraction F4. Gattullo et al. [42] observed in unpolluted soil,

in fraction F4, even more Cr (90%). Fraction F4 is considered as stable under natural conditions, which means low mobility and accessibility to soil microorganisms. Metals in this fraction are embedded in the crystalline structure of the minerals [43].

The first three fractions obtained according to the BCR method are considered as mobile. They describe the extractability of metals [41]. Our results show that the mobile pool of Pb amounted to 85.7%, which means that Pb was characterized by the highest extractability, while Ni was characterized by the lowest (46.3%).

4.2. Correlations between Soil Characteristics and Heavy Metal Fractions

Generally, the distribution of studied metals in fractions was not influenced by the physicochemical properties of the soil. Most of the correlations we have noted are between fraction F1 and granulometric composition. The relationship between that fraction and clay content, which in the case of Ni and Pb is statistically significant, reflects the high affinity of metal to the smallest soil particles, which makes the extraction more difficult. Zong et al. [44], studying urban soils, found most of the heavy metals exactly in the fraction of <2 µm. According to the authors, it is related to the clay properties, since this soil fraction is characterized by a negative charge, large surface area, and high content of organic matter, which has the ability to absorb the metals very easily.

4.3. Effect of Heavy Metal Fractions on Soil Enzyme Activities

The activity of soil enzymes can be considered as indicators, since it depends on the content of pollutants, including heavy metals [45]. Among soil enzymes, dehydrogenase seems to be the best indicator, which is characterized by the highest sensitivity [46]. Wyszkowska et al. [47] observed that enzymes may be arranged according to the metal sensitivity in the following order: dehydrogenase > urease > alkaline phosphatase > acid phosphatase. The negative correlation between total metal contents in the soil and enzymatic activities was reported many times [46], but the bibliography on the relationship between soil enzymatic activity and fractional metal composition is very poor.

Surprisingly, we observed a high correlation of some enzyme activities with fraction F4, which is considered as almost unavailable, as mentioned before. A strong positive correlation (0.86) between urease activity and F4/Ni was reported by Miśkowiec and Olech [48]. According to the authors, part of this enzyme can be strongly adsorbed on the clay minerals in a functioning and stable form. They also emphasized that Ni could be an indicator of urease activity and the quality of unpolluted soils, independently of land management practices. The negative correlation between F4/Ni and dehydrogenase activity observed in our research seems to prove the fact that it is an essential trace element for dehydrogenase [49], since the increase of F4 fraction leads to the decrease of its overall availability. It is unclear why all the significant correlations between enzyme activities and residual metals have not occurred in all three sampling dates. It may be related to the differentiated climatic conditions between the seasons. We can assume that rainfall and temperature changes (Table 1) influenced to some degree the enzyme activities and fractionation of the studied metals. Apart from fraction F4, fraction F1 affected the activity of enzymes the most. We have noted a negative and significant influence of Ni and Pb accumulated in this fraction on the activity of phosphatase. The discussed fraction accumulated most of the Pb in October, which was the cause of such dependence in this month. In the case of Ni, spring was the only time that fraction F1 did not correlate strongly with phosphatase activity. It could be the result of the rainfall and simultaneously the low temperature in April. It is confirmed by the largest phosphatase activity in this season as compared to the summer and autumn, since its activity increases with increasing soil moisture content [50]. We have also stated strong correlations between F2/Pb and the activity of two enzymes, but only in spring. It could be ascribed, once again, to the high soil water content. Firstly, it contributed to the highest dehydrogenase activity in this season, thus neutralizing the adverse impact of F2/Pb on its activity. Secondly, it enhanced the

negative effect of Pb contained in fraction F2 on urease activity, since extremely high soil moisture may lead to an inhibition of urease activity [51].

4.4. Impact of Sampling Date on Enzyme Activities and Fractional Composition of Heavy Metals

The activities of protease and dehydrogenase were seasonally significantly differentiated, which was probably due to the weather conditions. The climate influences the soil enzymatic activities, which is a well-known fact [52–54]. Substantial differences between sampling dates were also obtained for some metal fractions. In the case of Ni and Cr, it was related to the redistribution. For Ni, it was shifting from fraction F4 to F1, and for Cr, it was shifting from F4 to F2 and F3. Heavy metal release from fraction F4 can be observed due to the changes of soil reaction [34] or microbial activity [55]. In this case, it could be the second reason, since significant pH changes were not stated during the study. The activity of microorganisms probably caused also the significant change of Pb percentage in the fraction F4.

5. Conclusions

According to our knowledge, this is the first study regarding the impact of Ni, Pb, and Cr fractions on the enzyme activities of arable soils cultivated by the simplified method, depending on season. We have observed that nickel fractions (F1 and F4) had the greatest impact on enzyme activities. It was most evident in the case of phosphatase, where the inhibitory effect of F1/Ni was confirmed by correlation coefficients, which were significant in the summer and autumn. It is essential, since this fraction comprised the smallest part of the total Ni content, and nevertheless, it influenced very clearly the activity of phosphatase. As a result, the availability of phosphorus for plants was in some degree lowered, because phosphatase is controlling the decomposition of organic matter, which is its main source. This could be a limiting factor for the plant growth. Phosphatase was the most sensitive to the metal fractions, since Pb and Cr also influenced its activity.

It is important to say that the significant impact of residual Ni on the urease and dehydrogenase activities can be related to the presence of abiontic enzymes, which are enzymes that are immobilized in the soil matrix but remain active. We can say the same about F4/Cr and phosphatase activity. Currently, it is not possible to determine conclusively the degree of participation of abiontic enzymes (not connected with viable cells) and viable cells in the soil enzymatic activity. This issue requires further research.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/min11060584/s1, Figure S1: Sampling protocol. Six subsumples (about 1 kg each) from every field were collected using Egner's stick.

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