Fractionation of Iron and Manganese in the Horizons of a Nutrient-Poor Forest Soil Profile Using the Sequential Extraction Method

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Abstract

Soil iron and manganese fractionation is evaluated in the soil profile sampled from a pine-covered area of Wielkopolski National Park (mid-western Poland), that for years has been exposed to acid rain. The soils studied are sands and loamy sands with a pH of 3.3-4.4. The content of iron and manganese was analyzed by sequential extraction in the following fractions: exchangeable, acid extractable, reducible, oxidizable, and residual. The soil profile displays a lithogenically dichotomous structure that is reflected in the iron and manganese content, especially in the Fe residual fraction and Mn oxidizable fraction. In terms of the lability of manganese, it can be stated that the amount of manganese in reducible fractions (Mn_{red}) is higher than in exchangeable and acid extractable ones (except in the surface layer), while the amounts of manganese in fractions in which it is poorly available (Mn_{ox}) and unavailable (Mn_{res}) are lower than that of Mn_{red}. For iron, this sequence looks as follows: $Fe_{ex}<Fe_{ac.ext}<Fe_{red}$, while the amounts of iron in fractions in which it is poorly available (Fe_{ox}) and unavailable (Fe_{res}) are similar and decidedly predominant. The availability of the most readily released iron and manganese lessens with depth, and of the remaining forms grows in accordance with the change in lithology.

Keywords: sequential extraction, iron, manganese, availability, fractionation

Introduction

Metal species identification is used by researchers studying soil fertility, water quality, soil genesis and geomorphology, environmental quality, soil ecology, and soil remediation. The ubiquity of metals combined with the complexity of soils makes the study of metals one of the most important disciplines of soil chemistry.

The total amount of iron in the soil is significant, up to several percent (5.8% of the Earth's crust), but the world of living organisms, including plants, uses this element in trace quantities. In the soil, iron forms a variety of minerals such as hematite, goethite, limonite, nontronite, and pyrite. Iron is also present in the form of grain-coatings in oxidized material, and in many secondary minerals. Iron hydroxides play a key role in the biogeochemical cycle, in the bioavailability of iron itself [1, 2], and in the bioavailability of other elements [3]. Iron is considered the main element in the redox activity of the rhizosphere responsible for crustal weathering in upland soils [4, 5], and is used by bacteria in geochemical transformation [6]. Iron is an indicator of oxidation-reduction conditions due to the change of valence. An analysis of the amount and profile distribution of iron forms is crucial in studies of the directions and intensity of

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soil formation processes [7-9]. Some role is also ascribed to iron oxides and hydroxides in buffering the impact of acid rain [10].

Iron oxides in soils are important sorbents as they bind heavy metals present in the soil solution as cations and anions [11-13]. As a result, heavy metals can assume occluded forms in hydrated oxides of manganese and iron [14]. The increased acidification of the soil due to acid deposition and/or fertilization offers a high potential for metal mobilization [15-20]. An increase in the concentration of the dissolved organic ligand causes mobilization of elements by complex formation and desorption [21, 22].

Manganese is a relatively abundant element, making up some 0.085% of the Earth's crust. It is broadly dispersed throughout soils, sediments, water, and biological materials. Manganese has three possible oxidation states in the soil: 2+, 3+, and 4+. Its most reduced form, Mn^{2+}, is the only stable form in the soil solution. Both Mn^{3+} and Mn^{4+} are stable only in the solid phase of soils, where they form insoluble oxide and hydroxide minerals of variable structure. Manganese solubility is controlled by the redox potential and pH of the soil. A low pH or low Eh favours the reduction of insoluble manganese oxides and increases the solubility of Mn^{2+}. The Mn^{2+} ion is released from solids by spontaneous dissolution or cation exchange, especially under acidic or reducing conditions [23]. Even small changes in the soil redox potential or pH can shift the Mn^{2+} – Mn oxide reaction. As a result, manganese solubility within any particular soil can fluctuate markedly over time, sometimes ranging from deficient to toxic levels [24-26]. The mobility of manganese defies classification because it is extremely sensitive to the soil condition (acidity, wetness, biological activity, etc.). Manganese is rated to have a fairy low intrinsic toxicity to plants, yet toxic effects are commonly seen because very high Mn^{2+} concentrations can develop in waterlogged soils [27, 28], and when manganese has accumulated in the soil, toxic effects are observed in some plants [29, 30].

The importance of microelements for plants has already been well documented in the literature. Both iron and manganese are essential to organisms and are cofactors in numerous enzymes. There is a close relationship between iron and other metals; of special interest is their influence on plant cell metabolism. Elevated amounts of metals, particularly manganese, limit iron uptake and suppress the production of chlorophyll in plant cells [15]. High manganese content hinders the uptake of calcium and magnesium. In turn, a deficiency of manganese is most often found in soils that are saline and alkaline, calcareous, or peaty [23].

In order to evaluate the state of the environment, it is important to determine not only the total concentration of metals, but also the anticipated impact of change (in the pH or Eh potential) on their mobility. The existing solid-state analytical methods intended to determine the form of a metal in the soil are often based on the application of single, parallel or sequential, selective chemical extractions [31, 32]. They allow an evaluation of the form in which the given element occurs, and thus an evaluation of its bioavailability and the possibility of its migration, accumulation, and biomagnification [33, 34]. After the first complete concept of the sequential extraction of metals had been worked out, several other procedures have been devised employing 3 to 5 steps. The European Community Bureau of Reference (BCR), now known as the Standards, Measurements and Testing Program, has attempted to unify the speciation procedure [35-38]. Generally, a few extractants are used in a sequence in which the earlier ones are the least aggressive and the most specific, and subsequent extractants are progressively more destructive and less specific. The technique described is not intended to identify all chemical individuals, but it only describes their types depending on the mode of binding and is defined by the type of chemical operations being carried out. In the literature there is an abundance of studies on the speciation of heavy metals in soils polluted by industrial and mining activities [39-42], and in the bottom deposits of lakes/rivers suffering from strong human impact [43]. Manganese speciation in all kinds of samples (seawater, salt marshes, clinical samples of cow and human milk) is described extensively in Pearson and Greenway [26], who recommended sequential extraction as a technique to employ when studying sediments and air-filter samples.

The aim of the present research was to determine the fractionation of manganese and iron in the soil in the conditions of long-lasting impact of acid rain and the variability of iron and manganese forms down the soil profile. Our study can contribute to a better understanding of the multi-directional transformation of those elements, and to an evaluation of the amount of bioavailable forms. The results described below complement those obtained earlier for the same soil in a study of changes in the composition of soil solutions [44] and observations of the dynamics of metal leaching from soil monoliths [18].

Materials and Methods

Site Description

The soils under study come from the horizons of a single profile in the woodland area of Wielkopolski National Park (west-central Poland) from terrain adjacent to the Jeziory Ecological Station of Adam Mickiewicz University. However, the Park is threatened by its location near (25 km) the city of Poznań (700,000 inhabitants) and the highly industrialized nearby town of Luboń (30,000 inhabitants). The pollution caused by industrial plants situated a few or a dozen or so kilometres from the forest complex and the airborne pollution carried from far away brings high amounts of sulfoxides, chlorides, and fluorides [45]. This pollution significantly influences the chemical composition of precipitation [46] and causes considerable acidification of the soils. During the past 18 years of observation, the mean annual pH of precipitation has varied slightly, never rising above 4.8, while minimum figures have ranged from 2.9 to 3.5. Studies have shown a higher deposition of acid-

Table 1. Sequential extraction procedure employed.

Fraction	Chemicals	Temperature	pH	Shaking time
Exchangeable	10 mL 1M CH_3COONH_4	room temp.	7.0	1 h
Acid-extractable	20 mL 1M CH_3COONa	room temp.	5.0	5 h
Reducible	20 mL 0.04 M $NH_2OH \cdot HCl$ in 25% CH_3COOH	96ºC		5 h
Oxidizable	5 mL 0.02M HNO_3 + 5 mL 30% H_2O_2	85ºC	2.0	2 h
	5 mL 30% H_2O_2	85ºC	2.0	3 h
	10 mL 3.2 M CH_3COONH_4 in 20% HNO_3	room temp.		0.5 h
Residual	2 mL $HClO_4$ + 10 mL HF evaporated to near-dryness, dissolved in 6M HCl			

forming compounds in the Park than in the European Monitoring Environmental Programme lowland monitoring stations [47]. A substantial part of the Park is covered with poor soils developed from loose or weakly loamy sand with a low water-holding capacity and poor sorption properties. The highly acidic nature of the soils inhibits several protective (buffer) mechanisms. An additional factor responsible for deteriorating conditions of plant growth has been a precipitation deficit: the mean rainfall figure is about 550 mm, but frequently it has been as low as 330 mm, e.g. in 2003.

Field Study

Samples were taken from under a pine stand about 80 years old. After digging an exposure 1.4 m deep, samples were collected from individual genetic horizons and placed in plastic bags. Only the so-called mineral horizons were sampled, i.e., down from the A horizon of humus accumulation. Overlying it, as in any forest soil, is an organic (O) top horizon of humus commonly called litter. In the profile under study, it was discontinuous and had a negligible thickness (about 1 cm). It was removed prior to sampling the mineral horizons.

Laboratory Study

The soil samples were dried to obtain an air-dry state, then crushed in a mortar and run through a 2-mm mesh sieve. The following physico-chemical determinations were carried out on the <2 mm fraction using the standard methods: granulometric analysis using the areometric method after Casagrande (supplemented with the sieve method to fractionate sand), pH in H_2O and 1M KCl, and the content of organic matter, as organic C, using an elementary method (Vario max).

Sequential Extraction

The sequential extraction technique employed to separate the various forms of iron and manganese was Tessier's procedure with some modification of exchangeable agent

[48] (Table 1). The successive stages of sequential extraction were carried out in centrifuge test tubes of 50 mL capacity. The samples were agitated in a horizontal shaker equipped with a water thermostat whose programmed temperature ranged from 20ºC to 100ºC with an accuracy of ±2ºC. After each extraction stage a sample was centrifuged for 30 minutes. The clear solution from above the precipitate was transferred to a calibrated polypropylene test tube with a cap. The solid remaining in the original test tube was washed with 5 mL of demineralized water, again centrifuged for 30 minutes, and combined with the first portion of the extract. Solutions from the first three extraction stages were acidified with four drops of concentrated nitric acid. The use of Tessier's procedure was insufficient to achieve complete digestion at the last stage – the residual fraction. To digest the precipitate completely, a mixture of the acids $HClO_4$ and HF was used.

The method employed for iron and manganese determination in all the extracts was atomic absorption spectrometry employing acetylene-air flame atomization with deuterium background correction. The spectrometer used was a Perkin Elmer A Analyst 300. Element standards were prepared by serial dilution of 1,000 $mg \cdot L^{-1}$ commercial standard solutions (Spectrosol, from Merck, Dorset, UK). To avoid the interference of the matrix, each of the standard solutions prepared was diluted with an extractant. Extracts with the content of the elements under analysis exceeding the range of determination were also diluted with extractants (e.g. the residual fraction).

To estimate the correctness of the results obtained, an analysis of the total content of manganese and iron was performed on the certified reference material RTH 907. The recovery level was 94% for manganese and 92% for iron.

The difference between the sum of the analytes in the five fractions and their total concentration was generally within 15%, well in line with the results reported in other studies [42]. All experiments were performed in triplicate and blanks were run simultaneously. Procedural blanks were below the detection limits of FAAS. The relative standard deviations of the results were typically below 12%.

Table 2. Basic properties and classification of the soils studied.

No.	Genetic horizon	Depth (cm)	Sand 2.0-0.05 mm (%)	Silt 0.05-0.002 mm (%)	Clay <0.002 mm (%)	Textural group	$C_{org.}$ (%)	pH_{KCl}	pH_{H_2O}	Classification
1	A1	0-3	80	19	1	loamy sand	5.68	3.2	3.8	very strongly acidic
2	A2	3-9	77	19	2	loamy sand	2.58	3.3	3.8	very strongly acidic
3	AB	9-18	79	16	2	loamy sand	1.50	3.5	3.9	very strongly acidic
4	Bv	18-42	82	15	3	loamy sand	0.35	4.0	4.1	strongly acidic
5	C1	42-65	83	13	4	loamy sand	0.12	4.1	4.2	strongly acidic
6	II C2	65-100	70	14	16	sandy loam	0.07	4.0	4.5	strongly acidic
7	II C3	100-140	67	16	17	sandy loam	0.07	4.4	5.2	strongly acidic

Results and Discussion

The particle-size distribution, classification into the particular genetic horizons, and other basic properties of the soil under study are shown in Table 2.

In terms of grain size, the soil displays a dichotomous structure called lithogenic discontinuity. The two parts differ substantially in their clay fractions. The soil profile is sandy in the upper part and loamy below 65 cm. Typologically, the soils are classified as rusty, developed on glaciofluvial sands underlain by till. According to the WRB classification, this soil belongs to the Ferralic Arenosols. The conducted pH determinations put them in the class of strongly acidic soils down to a depth of 18 cm and strongly acidic further down the profile. The lowest pH_{KCl} and the highest organic C content are characteristic of the top A horizon of humus accumulation. Further down, the pH_{KCl} rises, while the organic C content drops.

Both pedogenesis and the nature of the parent material influenced the levels of the metals examined in the soil horizons. The mineralogical composition of the soils had been studied earlier [49]; it showed a dominance of quartz. In each horizon there were feldspars, clay minerals, and accessory heavy minerals. The main source of iron and manganese could be amorphic and crystalline oxides and hydroxides, and in the case of iron, also clay minerals, among them illite/smectite and vermiculite. Vermiculite is here the main clay mineral which, in special conditions of strong acidification, can undergo a transformation and release iron ions [50, 51].

The above mineralogical and chemical properties of the soil samples under study decisively affect the dynamics of metal release and migration. These processes can be observed when analyzing the results of the successive stages of extraction presented below.

Sequential Extraction of Iron

The results obtained for iron with the method of sequential extraction are shown in Table 3.

There is a high variability in the amount of iron extracted. The content of exchangeable Fe divides the soil profile into two sections differing substantially in the concentrations of readily liberated iron. The layer representing the bedrock – sandy loam – displays the lowest values (0.6-1.1 mg·kg⁻¹, under 0.01%), while the surficial layers release ten times as much iron, which corresponds to 0.1%. The highest concentration of exchangeable iron (8.9 mg·kg⁻¹) was found to occur in the upper, A2, horizon of the soil profile (3-9 cm). This substantial quantity of the most readily extractable iron is due to the fact that soluble organic acids from the leaf leachate of soil organic matter may form aqueous complexes with iron. Moreover, mineral components in the soil are more weathered in the upper layer than in the deeper one because of rainfall and temperature variations and higher biological activity. Iron in soil solution, which is part of that fraction, mainly exists as a soluble organic complex rather than an inorganic ion, because free Fe^{2+} ions are scarce under aerobic conditions [20, 52].

A similar distribution pattern is shown by iron obtained as an acid-extractable fraction. According to Tessier et al. [32], an acid-extractable fraction yields the forms of the metal bound with carbonates. In the present case, however, because the pH of forest soils is low due to the prolonged exposure to both strong inorganic acids of anthropogenic derivation and natural organic acids, there can be no possibility of the presence of carbonates, as established in the mineralogical examination reported. The use of sodium acetate in acetic acid acidified to a pH of 5 can release iron bound more tightly than in the previous extraction; it releases non-specifically sorbed iron. The concentration of iron released as the acid-extractable fraction is higher than in the exchangeable fraction: it reaches more than 60 mg·kg⁻¹ in the A2 horizon, to decline from the Bv horizon to some 10 mg·kg⁻¹. Similar results were obtained by Wilcke et al. [53], who drew attention to the significant differences between core and surface concentrations resulting from the fact that metals of aggregate surfaces are less strongly bound than those aggregated in the deeper parts owing to preferential weathering. When calculating the contribution of this form

Table 3. The particular iron forms obtained at successive stages of sequential extraction (mg·kg⁻¹±SD), percentage of total content.

No.	Genetic horizon	Depth cm	Fe – forms										
			Exchangeable		Acid-extractable		Reducible		Oxidizable		Residual		Total
			mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹
1	A1	0-3	6.83±0.81	0.10	27.3±3.2	0.39	1,350±162	19	2,145±258	31	3,492±288	50	7,022±712
2	A2	3-9	8.90±1.10	0.13	61.9±4.9	0.87	1,696±135	24	2,064±168	29	3,281±249	46	7,112±557
3	AB	9-18	5.13±0.41	0.07	56.9±4.2	0.78	1,752±210	24	2,472±231	34	3,018±362	41	7,304±807
4	Bv	18-42	1.00±0.11	0.02	10.5±1.2	0.16	1,069±110	16	2,523±210	39	2,901±312	45	6,504±633
5	C1	42-65	1.06±0.08	0.02	9.1±0.7	0.14	1,280±141	20	2,399±263	37	2,839±227	43	6,528±632
6	II C2	65-100	0.63±0.07	<0.01	10.2±0.6	0.06	2,148±171	12	6,879±717	40	8,344±644	48	17,381±1,533
7	II C3	100-140	0.56±0.07	<0.01	9.7±1.0	0.06	2,373±121	14	6,610±698	40	7,559±631	46	165,520±1,451

to total iron content, similarly as for the exchangeable fraction, we can observe two parts of the soil profile differing substantially in the proportion of this fraction. And similarly, the differences are due to the release of weakly bound iron from organic complexes. This is also corroborated by the research conducted by Riise et al. [21] and Xue et al. [20], who suggested that the low molecular weight of organic acids contributes to the translocation of Fe in the podzolization process. It is well known that the solubility of iron compounds is inversely related to soil pH, hence a jump in the pH from 3.3 (A) to 4.0 (Bv) is an additional justification of the observed variability. Owing to their highly similar distribution patterns for a poor forest soil, those two fractions (exchangeable and acid extractable) are considered jointly, as they may indicate the form in which metals are most available for plant uptake. In the case of the soils under study, the sum of those two fractions is about 1% in the A2 and AB horizons, to fall below 0.2-0.1% in the lower part of the profile. The similarity of the iron distribution pattern between the exchangeable fraction and the acid-extractable fraction has been observed by some researchers (e.g. Maitz et al. [54]). The percentages of the exchangeable and acid-extractable forms of iron are extremely low because of its strong tendency to form oxides and hydroxides in such conditions [42].

The next potentially bioavailable fraction is the one bound to Fe-Mn oxides, also called reducible. The action of hydroxylamine hydrochloride in the environment of acetic acid at a prolonged high temperature releases much greater amounts of iron than at the previous stages. The effect of this extraction also shows a completely different distribution pattern (Table 3). The amount of the released iron varied between 1,350 mg·kg⁻¹ and 2,370 mg·kg⁻¹, locally exceeding 20%. The iron content in this fraction decreases from the AB to Bv horizons, and then rises as far down as C3. Because of the increasing total iron, this pattern looks a little different when seen in percentage terms: despite the increase in iron content in this fraction in the lower part of the profile, its proportion drops to 12-14% (Table 3).

The proportion of the metal released in the reducible fraction probably results from the dissolution of amorphous and, to a lesser extent, crystalline oxides [42]. A similar sequence of concentrations in the respective fractions and percentages of the reducible fraction can be found in the literature: 25% [55], 14-24% [42], and 12% [54]. This fraction is also important due to the heavy metals accompanying Fe and Mn oxides, often co-precipitated or strongly adsorbed on the surfaces of oxides and hydroxides [11, 56].

The next fraction of sequential extraction is termed oxidizable. The aim of this stage of extraction is the possible complete decomposition of organic matter attached to the various mineral components of the soil. The effects of hydroperoxide and nitric acid at an elevated temperature include not only the mineralization of organic compounds, but also the release of metals from interlayer spaces of clay minerals and from a partial decomposition of aluminosilicates [32, 57]. This is indicated by high iron concentrations obtained in samples from the topsoil down to 65 cm, while in the lower, loamy part of the profile they further increase more than twofold. Variations in the amount of this iron fraction clearly indicate two lithologically different parts of the profile.

The oxidizable and residual fractions represent maximum values of iron content – up to 6,880 mg·kg⁻¹ for the oxidizable fraction and 8,340 mg·kg⁻¹ for the residual one, or 40% and 48%, respectively (Table 3).

An analysis of the iron content in the individual fractions suggests that the dichotomous structure of this profile plays a fundamental role here. The substantial increase in the proportion of the clay fraction in the lower horizons (from a few to more than 10%) is correlated with a high level of iron, which almost doubles in the reducible fraction, almost triples in the oxidizable one, and grows 2-3 times in the residual one. The sum of all the iron fractions rises from about 7 g·kg⁻¹ in the upper part of the profile to about 17 g·kg⁻¹ in the lower part. In turn, what seems significant for the bioavailable forms is the relation of iron with the organic matter in the soil. Viewed in terms of the bioavailability and

Table 4. The particular manganese forms obtained at successive stages of sequential extraction (mg·kg⁻¹±SD), percentage of total content.

No.	Genetic horizon	Depth cm	Mn – forms										
			Exchangeable		Acid-extractable		Reducible		Oxidizable		Residual		Total
			mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg⁻¹	%	mg·kg-1	%	mg·kg⁻¹
1	A1	0-3	47.2±3.1	27	25.8±2.1	15	46.1±6.1	26	19.5±2.3	11	36.6±3.4	21	175.1±17.0
2	A2	3-9	16.7±1.3	15	10.2±0.9	9	27.4±2.9	24	13.2±1.6	12	46.2±3.9	41	113.8±10.6
3	AB	9-18	8.5±1.0	4	7.9±0.7	4	115.8±12.9	61	16.0±1.3	8	42.1±5.1	22	190.3±21.0
4	Bv	18-42	4.2±0.3	2	3.6±0.4	1	174.0±9.7	71	23.4±3.1	10	38.7±4.0	16	243.8±17.5
5	C1	42-65	2.5±0.2	1	1.9±0.1	1	154.8±16.1	73	17.4±1.4	8	36.0±4.0	17	212.6±21.8
6	II C2	65-100	8.0±0.6	3	4.7±0.5	2	149.9±12.2	61	61.5±8.2	25	22.3±1.8	12	245.5±23.3
7	II C3	100-140	2.5±0.2	1	3.9±0.1	1	164.9±12.4	59	61.4±7.3	22	32.3±1.9	12	281.2±21.9

lability of iron, the sequence reflecting the released amounts of the metal looks as follows: $Fe_{ex} < Fe_{ac.ext} < Fe_{red}$, while the amounts of iron in fractions in which it is poorly available (Fe_{ox}) and unavailable (Fe_{res}) are similar and decidedly predominant. The availability of the most readily released iron lessens with depth, and of the remaining forms grows in accordance with the change in lithology.

Sequential Extraction of Manganese

The results obtained for manganese with the method of sequential extraction are shown in Table 4.

The manganese content in the exchangeable fraction turned out to be decidedly higher than that of iron and ranged from 47 mg·kg⁻¹ in the A1 horizon to 2.5 mg·kg⁻¹ in the lower part of the profile. Interestingly, in the upper horizons exchangeable Mn made up as much as 27% (A1) and 15% (A2). The manganese content in the acid-extractable fraction varied between 25.8 mg·kg⁻¹ in the upper A1 horizon and 4 mg·kg⁻¹ in the C3 horizon. Its distribution pattern was similar to that of the exchangeable fraction: it decreased with depth and then showed a slight increase in the C2 horizon. The percentage of manganese varied from 15% in the upper horizon to 1% in the lower one. The considerable proportion of manganese in the upper horizons of the soil profile seems to indicate a connection with organic matter, i.e. a derivation from leaf litter [30]. The large quantities of manganese in those fractions are also due to its weak binding with organic matter [20]. As in the case of the exchangeable and acid-extractable fractions of iron, the similar variation patterns of the Mn fractions allow them to be treated jointly. Here the sum of the most bioavailable fractions is the highest in the surface horizons, at 73 mg·kg⁻¹, or 42% (A1), and 27 mg·kg⁻¹, or 24% (A2), while in the lower horizons this sum does not exceed 15 mg·kg⁻¹, or 5%.

The content of Mn that might affect plants is around 400-500 mg·kg⁻¹, whereas almost no toxic effect due to iron excess has been documented in the literature [29, 37]. Here total Mn in the soil sample is less than 300 mg·kg⁻¹.

In the next fraction, substantial amounts of manganese oxides were released due to powerful reducing conditions. This Fe-Mn oxide-bound fraction is the most important for manganese and could extract considerable amounts of Cd, Pb and Zn [53]. The results show that the upper horizons (A1 and A2) release less of this form of manganese, while starting with the AB horizon the manganese content exceeds 100 mg·kg⁻¹ and lower down even 150 mg·kg⁻¹, sometimes accounting for more than 70% of all manganese forms (Table 4). Similar results were obtained by Sanchez et al. [41] – 60% of manganese present in this fraction, Wang et al. [24] – 38%, Navas and Lindhorfer [37] – 52% and Fuikawa et al. [52] – 20-40%.

The next, oxidizable, fraction is indicative of the number of organic connections. In the profile under study, it contained manganese at concentrations ranging from more than 10 mg·kg⁻¹ in the sandy part to 61 mg·kg⁻¹ in sandy loam, which constituted, respectively, from 8% to more than 20% (Table 4). The jump in the content of this form occurred in the deepest section of the profile, 65-140 cm, in which the amount of clay (<0.002 mm) changed from 4% to 16%. It is also evidence of the release of other forms of manganese associated with secondary minerals. The remaining portion of manganese found in the so-called residual fraction showed fairly equal concentrations, 37 mg·kg⁻¹ on average. The percentages in the residue were lower than in the reducible fraction, and only in the A2 horizon did it exceed the reducible figure. The maximum value of this fraction was recorded in the A2 horizon (46 mg·kg⁻¹). The metals confined in the residual fraction are incorporated within the crystalline lattice and are considered the most stable and least, if at all, available to plants [24, 32]. Data from the literature confirm a similar percentage for the residual fraction: Navas and Linhorfer [37] – 23%, Wang et al. [24] – 26%, Abolino et al. [42] – 26%.

The sum of all the forms of manganese in the soil under study ranged from 114 mg·kg⁻¹ in the AB horizon to 265 mg·kg⁻¹ further down (exceeding 200 mg·kg⁻¹ already from the Bv horizon). These results agree with those presented in

the Geochemical Atlas [58], and are rather low in comparison with the mean for soils [26].

In terms of the bioavailability and lability of manganese, its highest concentrations were found in the reducible fractions (except in the surface layer), while its amounts in fractions in which it is poorly available, Mn_{ox}, and unavailable, Mn_{res}, are lower than that of Mn_{red}. The availability of the most readily released manganese lessens with depth, and of the less readily available forms increases in accordance with the change in the pH and lithology.

The mobility of metals in soil profiles may be assessed on the basis of the absolute or relative content of fractions weakly bound to soil components. The mobility factor could be calculated as the rate of the sum of the metal content in the exchangeable and acid-extractable fractions to the sum of all fractions (%) [59], or the sum of all fractions except the residual one [55]. When one looks at Tables 3 and 4 of iron and manganese content, it is readily apparent that the fundamental difference between those metals lies in their mobility, which is also significant from the point of view of bioavailability. When considering manganese, in Kabała's [59] approach, it is only in the upper horizons (0-18 cm) that 8-42% of the metal is mobile. In turn, in Sutherland's [55] approach, which includes in this pool the metal from the reducible and oxidizable fractions, the proportion of the labile fraction is substantial, even in excess of 88%. In the case of iron, the bioavailable fraction contributes less than 1%, while the labile one contributes a little over 50%.

It seems that despite some procedural difficulties of the sequential extraction method, it gives an insight into the quantities of the bioavailable and poorly available forms of manganese and iron in the soil, and indicates changes in the distribution pattern of each form.

Conclusions

- The concentrations of exchangeable and acid-extractable iron were low and decreased with depth, which may limit the availability of this important plant nutrient.
- Surface layers, richer in organic matter, contain greater amounts of exchangeable and acid-extractable iron and manganese than the deeper layers.
- Most of the readily available form of manganese was found at a depth where it could easily be reached by plants and where it accounted for as much as 68% of the entire pool of the metal.
- The quantity of readily available manganese did not exceed limit values because of its low total content in the soil under study.
- The highest concentration of iron was recorded in the residual fraction, unavailable to plants.
- The highest concentration of manganese was found in the reducible fraction (up to 154.8 mg·kg^{-1}) and showed an association with soil pH. It could be bioavailable to plants with a change in the redox or pH conditions.

- Acidic atmospheric deposition may affect transformation among speciations of iron and manganese through the formation of soluble organo-metal complexes or through a change in oxidation-reduction conditions.

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