Simultaneous extractions as a tool for estimation of bioavailable manganese fraction in soils

Tatiana GABLOVIČOVÁ[*], Gabriela NÁDASKÁ, Juraj LESNÝ

Abstract

The paper intends to contribute to the objectification of bioavailable manganese quantification in some typical soils via application of specific analytical methods including a series of simultaneous extraction fractionations. The investigated soil samples were obtained by own sampling and represent two Slovakian soil types, namely Calcic Phaenozem, Siltic, Jaslovské Bohunice and Haplic Cambisol, Dystric, Sihla. Extraction procedures based on $\mathrm{CaCl_2}$, $\mathrm{MgCl_2}$, EDTA as well as Mehlich 2 and Mehlich 3 extraction procedures were used. The total manganese contents were determined by X-ray fluorescence analysis and the fractional analyses were accomplished applying galvanostatic stripping chronopotentiometry. Comparing six investigated soil samples (3 horizons of Calcic Phaenozem and 3 horizons of Haplic Cambisol), the highest total manganese contents were determined in horizon A ($800\ \mathrm{mg\cdot kg^{-1}}$ in Calcic Phaenozem) and in horizons A and B respectively (both $400\ \mathrm{mg\cdot kg^{-1}}$ in Haplic Cambisol). The highest extractable fractions of total manganese content were found in horizon A of Calcic Phaenozem (46,9 % utilizing EDTA) and in horizon A and B of Haplic Cambisol (12.5 and 13.9 % using Mehlich 2 and Mehlich 3 respectively). The lowest total Mn content in Calcic Phaenozem was found in horizon C ($600\ \mathrm{mg\cdot kg^{-1}}$) while in the relevant soil sample the highest extractable Mn-fraction was determined using the Mehlich 3 (12.5 %). The lowest total Mn content in Haplic Cambisol was determined in horizon C as well ($300\ \mathrm{mg\cdot kg^{-1}}$) and in the relevant sample, the highest extractable Mn-fraction was determined using Mehlich 3 (9.2 %). In all accomplished experiments, the lowest extractable manganese fractions - not exceeding $0.025\ \mathrm{mg\cdot kg^{-1}}$ - were observed using $\mathrm{MgCl_2}$.

Keywords: *manganese, simultaneous extractions, bioavailable fraction*

1 Introduction

According to the well known general definition soil represents an extremely complex natural body composed of mineral and organic constituents in solid, aqueous and gaseous states. Moreover, soil consists of soil horizons of variable thickness, which considerably differ from the parent materials in their physical, chemical and biological characteristics. The study of physical-chemical behaviour of trace elements in soils and sediments brings daily increasing amount of valuable information concerning their occurrence, sources, mobility and toxic effects on biota. In spite of the fact that the knowledge of total content of particular elements in the environment (in investigated systems) is of essential importance, the more detailed study of issues related to the behaviour of trace elements in the environment requires a series of advanced and often very complex analytical methods. The recent development in analytical procedures enables - in particular cases - a significant progress in detailed knowledge of sorption mechanisms of a a large group of trace elements, reaction kinetics of their chemical species including their concentration and accumulation in the food chain. Nevertheless, from the point of view of plant production, the determination of the relevant trace element's bioavailability for plants is of crucial significance. In the last decades a large number of sophisticated analytical procedures have been developed. However, reliable determination of the relevant data is, as a rule, very complex and in most cases even impossible. The reason is straightforward: bioavailable quantities of trace elements depend on a large spectrum of parameters, among them the physical and chemical composition of the soil, the chemical speciation of the elements in question, the different properties of investigated plants and the ruling climate play the most influencing roles. For dependable mobility assessment of the studied elements in soils and sediments, as well as their bioavailability for plants, a number of simultaneous and sequential extraction procedures have been suggested and tested.

Most of sequential extraction procedures, especially for metals, are designed for separation of investigated elements in soils or sediments, into five operationally-defined geochemical fractions. The particular ones are defined

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as follows: exchangeable fraction, acido-soluble fraction, fraction bound to hydrous oxides of iron, manganese and aluminium, oxidising fraction and residual fraction. The bioavailable fractions, accessible for plants, are considered most commonly to be identical with both the exchangeable and the acido-soluble fractions. Portions of metals which are attracted to the surface mostly by weak electrostatic interactions include weakly sorbed chemical forms of the relevant metal. These can be freed by ion-exchange processes. The choice of applicable leaching reagents depends on a series of phenomena influencing the character of interactions in question. The extensive list of extractants includes aqueous solutions of electrolytes, first of all salts of strong bases and acids as well as weak bases and acids, mostly at concentration of $1\ \mathrm{mol} \cdot \mathrm{L}^{-1}$ and at pH 7. The most frequently used electrolytes are as follows: $\mathrm{MgCl_2}$ [16], $\mathrm{CaCl_2}$ [9], $\mathrm{KNO_3}$ [5], $\mathrm{Ca(NO_3)_2}$ [5], $\mathrm{Mg(NO_3)_2}$ [14, 6], $\mathrm{CH_3COONH_4}$ [1, 4, 18], $\mathrm{NH_4Cl}$ [13], $\mathrm{NH_4NO_3}$ [19].

In contrast to the exchangeable fraction, the acido-soluble one is sharply pH sensitive. The dissolution of the investigated solids is carried out in most of the proposed procedures at $\mathrm{pH} \leq 5$. Most frequently $\mathrm{CH_3COOH}$ [8] or acidified sodium acetate $\mathrm{CH_3COONa/CH_3COOH}$ are utilized [6, 16]. However, for a number of metals ethylenediaminetetraacetic acid is oftentimes used as well [9, 15]. Considering the well known complexing ability of the last mentioned extractant, its utilization for separation of the acido-soluble fraction is less specific owing to its affinity to metals bound to organic matter. The paper represents a continuation of our further efforts [11] and intends contribute to further soil manganese data acquirement as well as to the critical assessment of the gained values.

2 Experimental

Soil samples and their basic attributes: A, B and C horizons of two typical Slovakian soil types were chosen for this study. Some important physical and chemical properties of the six relevant samples are given in Table 1. The investigated samples were obtained by own sampling. They represent Calcic Phaenozem (Siltic) (Jaslovské Bohunice) and Haplic Cambisol (Dystric) (Sihla). The related sampling sites are shown in Fig. 1.

Figure 1 Sampling sites of investigated soils

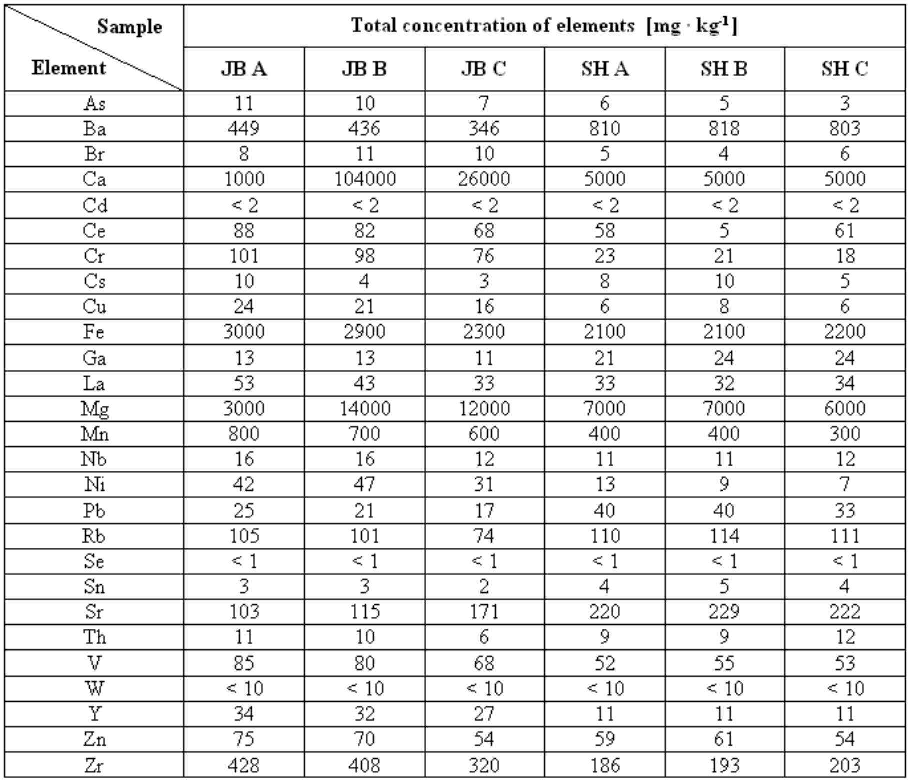
Table 1 Important properties of studied soil samples

Sampling site	Co-ordinates	Sign in text	Classification	Horizon	pH		CEC meq/100g	$\mathrm{CaCO_3}$ [%]
					$\mathrm{H_2O}$	KCl		
Jaslovslé Bohunice	E Long:17°64'20" N Lat:48°47'02"	JB A	Calcic Phaenozem	horizon A (0-20 cm)	8,30	7,53	18,05	0,7
Jaslovské Bohunice	E Long:17°64'20" N Lat: 48°47'02"	JB B	Calcic Phaenozem	horizon B (35-45 cm)	8,58	7,80	16,61	2,75
Jaslovské Bohunice	E Long:17°64'20" N Lat:48°47'02"	JB C	Calcic Phaenozem	horizon C (≥ 50 cm)	8,92	8,34	14,45	14,8
Sihla	E Long:19°65'28" N Lat:48°65'41"	SH A	Haplic Cambisol	horizon A (0-15cm)	4,6	3,8	10,99	0,5
Sihla	E Long:19°65'28" N Lat:48°65'41"	SH B	Haplic Cambisol	horizon B (15-25 cm)	4,7	4,0	6,67	0,5
Sihla	E Long:19°65'28" N Lat:48°65'41"	SH C	Haplic Cambisol	horizon C (≥ 50 cm)	4,7	4,2	8,11	0,5

The preparation of samples: Before the subsequent analytical procedures the soil samples were air-dried (at room temperature) and after removal of plant residues, homogenization and sieving (2 mm) they were stored at dark and dry place.

Determination of elemental concentrations: The total Mn concentrations as well as total concentrations of chosen elements were determined by röntgenfluorescence analysis using the high performance X-ray fluorescence spectrometer X-LAB 2000, SPECTRO, Germany (Table 2).

Table 2 Concentrations of chosen soil constituents in studied samples

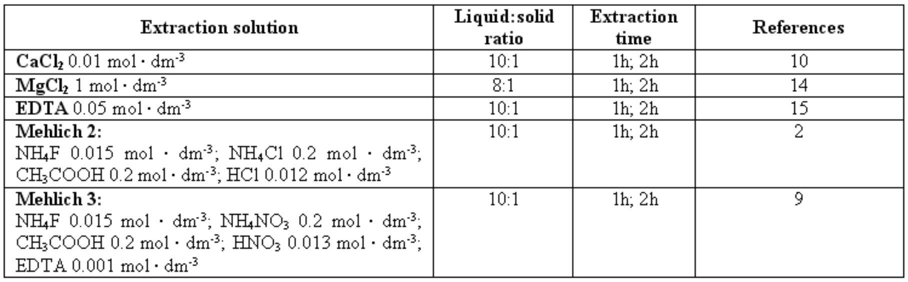


Sample	Total concentration of elements $[\mathrm{mg \cdot kg^{-1}}]$					
Element	JB A	JB B	JB C	SH A	SH B	SH C
As	11	10	7	6	5	3
Ba	449	436	346	810	818	803
Br	8	11	10	5	4	6
Ca	1000	104000	26000	5000	5000	5000
Cd	< 2	< 2	< 2	< 2	< 2	< 2
Ce	88	82	68	58	5	61
Cr	101	98	76	23	21	18
Cs	10	4	3	8	10	5
Cu	24	21	16	6	8	6
Fe	3000	2900	2300	2100	2100	2200
Ga	13	13	11	21	24	24
La	53	43	33	33	32	34
Mg	3000	14000	12000	7000	7000	6000
Mn	800	700	600	400	400	300
Nb	16	16	12	11	11	12
Ni	42	47	31	13	9	7
Pb	25	21	17	40	40	33
Rb	105	101	74	110	114	111
Se	< 1	< 1	< 1	< 1	< 1	< 1
Sn	3	3	2	4	5	4
Sr	103	115	171	220	229	222
Th	11	10	6	9	9	12
V	85	80	68	52	55	53
W	< 10	< 10	< 10	< 10	< 10	< 10
Y	34	32	27	11	11	11
Zn	75	70	54	59	61	54
Zr	428	408	320	186	193	203

Determinations of manganese fractions: After simultaneous extractions with $\mathrm{MgCl_2}$, $\mathrm{CaCl_2}$, Mehlich 2, Mehlich 3 and EDTA (detailed data shown in Table 3) the particular Mn-fractions were determined by galvanostatic stripping chronopotentiometry using EcaFlow model GLP 150, ISTRAN Ltd., Slovakia. For the fractions gained by separation processes using Mehlich 2, Mehlich 3, $\mathrm{CaCl_2}$ and $\mathrm{MgCl_2}$ the determinations of Mn was accomplished directly after the extractions, while the determination of Mn in the EDTA extract were carried out after microwave mineralization using the Multiwave 3000, ANTON PAAR GmbH, Austria.

Agitation, extraction solutions and extraction times: The agitation of soil samples with particular extraction media was accomplished at frequency $150\ \mathrm{min}^{-1}$. The simultaneous extractions were carried out at room temperature utilizing the experimental conditions outlined in Table 3.

Table 3 Experimental conditions applied for single extractions of manganese from soil

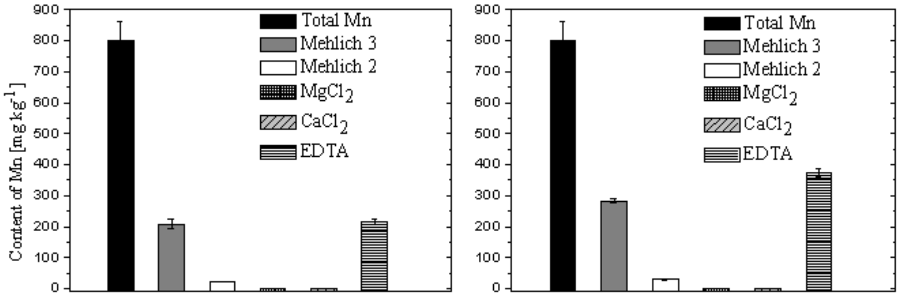


Extraction solution	Liquid:solid ratio	Extraction time	References
$\mathrm{CaCl_2}$ $0.01\ \mathrm{mol \cdot dm^{-3}}$	10:1	1h; 2h	10
$\mathrm{MgCl_2}$ $1\ \mathrm{mol \cdot dm^{-3}}$	8:1	1h; 2h	14
EDTA $0.05\ \mathrm{mol \cdot dm^{-3}}$	10:1	1h; 2h	15
Mehlich 2: $\mathrm{NH_4F}$ $0.015\ \mathrm{mol \cdot dm^{-3}}$; $\mathrm{NH_4Cl}$ $0.2\ \mathrm{mol \cdot dm^{-3}}$; $\mathrm{CH_3COOH}$ $0.2\ \mathrm{mol \cdot dm^{-3}}$; HCl $0.012\ \mathrm{mol \cdot dm^{-3}}$	10:1	1h; 2h	2
Mehlich 3: $\mathrm{NH_4F}$ $0.015\ \mathrm{mol \cdot dm^{-3}}$; $\mathrm{NH_4NO_3}$ $0.2\ \mathrm{mol \cdot dm^{-3}}$; $\mathrm{CH_3COOH}$ $0.2\ \mathrm{mol \cdot dm^{-3}}$; $\mathrm{HNO_3}$ $0.013\ \mathrm{mol \cdot dm^{-3}}$; EDTA $0.001\ \mathrm{mol \cdot dm^{-3}}$	10:1	1h; 2h	9

3 Results and discussion

In Figures 2 - 8 the relevant total manganese contents and the fractions of Mn in soils obtained by $\mathrm{MgCl_2}$, $\mathrm{CaCl_2}$ and EDTA, as well as by Mehlich 3 and Mehlich 2 extraction agents are shown. $\mathrm{MgCl_2}$ is the most frequently used extraction agent for determination of ion-exchangeable amounts of different metals including Mn. The effect of this reagent combines two important properties, namely the high ion-exchange capacity of Mg(II) and the almost negligible complex-formation ability of chloride ions. In addition, $\mathrm{MgCl_2}$ do not attack organic matter, nor silicates and metallic sulphides. Application of $\mathrm{MgCl_2}$ may actually lead to dissolution of carbonates in some extent, but as it is in general assumed, this problem can be easily handled by reduction of contact times [3, 6]. Other salts of different anions and cations are applicable for separation of exchangeable metals as well. Beside magnesium and chlorides the most frequently used ionic extractants are calcium, sodium, potassium and nitrates. As it is generally known, the total occurrence of magnesium in the body of Earth is almost 30-times higher as the total occurrence of calcium. However, the comparison of concentrations of these highly important alkaline earth metals in the earth's crust shows an inverted relation, namely the magnesium content in the terrestrial crust is observable lower (1.88 atomic percentage) in comparison with that of calcium (1.78 atomic percentage). Subsequently - partially as a result of the above mentioned fact - Ca(II) is the main cationic component of the soil sorption complex, it is considered to be the most effective cation exchanging ion for soils and consequently $\mathrm{CaCl_2}$ is the most effective cationic soil extractant. Mehlich 2 represents a specific multicomponent extraction solution, which, as a rule, is utilized as separation agent for bioavailable (plants available) soil P, K and Mg estimation, as well as for mobile Ca fraction estimation. Its components - first of all ammonium ions, present in relatively high concentration - enable in the same time the extraction of exchangeable cations, including the manganese ones. Mehlich 3 contains - in comparison with the Mehlich 2 - ammonium nitrate instead of ammonium chloride and nitric acid instead of hydrochloric acid. The last mentioned components may lead to higher solubility of cationic soil constituents. The presence of EDTA - even at rather low concentration ($0.001\ \mathrm{mol \cdot dm^{-3}}$) - may increase the generally low complex-forming properties of the discussed extractant and consequently extends the efficiency of soil manganese solubilisation. In correspondence with its above mentioned properties, EDTA in higher concentrations ($0.05\ \mathrm{mol \cdot dm^{-3}}$) acts as a powerful complexing reagent for a wide scale of metallic elements, including manganese bound to humic matter. Its ability of stronger metal extraction (not excluding manganese) from upper soil horizons is generally expected.

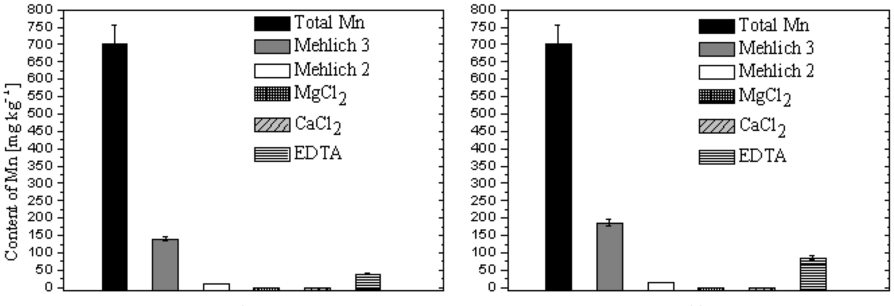
Soil sample Jaslovské Bohunice horizon A: After 1 hour agitation time, the highest relative manganese fraction (the portion of manganese comparing to the total manganese content) was determined using EDTA (27.1 %), while the Mehlich 3 extract showed a similar value (26.1 %). In the same time $\mathrm{MgCl_2}$-, $\mathrm{CaCl_2}$- and Mehlich 2 extracts showed negligible manganese contents. Applying 2 hour agitation, both EDTA and Mehlich 3 extract contained significantly higher manganese contents (46.9 % and 35.3 % respectively), while the manganese concentrations in relevant $\mathrm{MgCl_2}$-, $\mathrm{CaCl_2}$- and Mehlich 2 extracts remained practically unchanged. It is straightforward that the manganese content in the upper horizon of the investigated soil sample is bond mainly to the humic matter, while the presence of easily exchangeable manganese content is of negligible value.



a) b)

Fifure 2 Concentration of manganese in soil sample Jaslovské Bohunice horizon A and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h

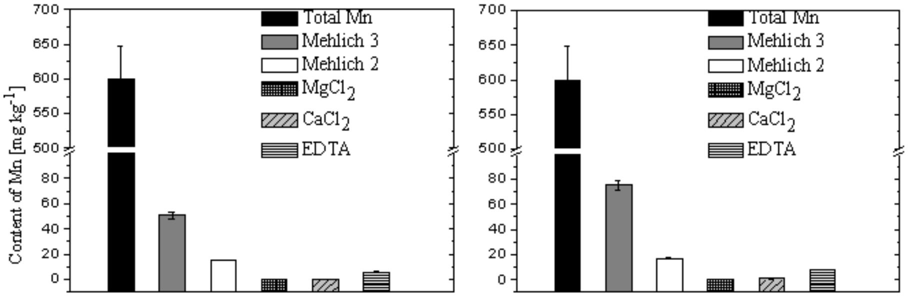
Soil sample Jaslovské Bohunice horizon B: After 1 hour agitation time, the highest relative manganese fraction was determined applying Mehlich 3 (20.1 %). In the same time in EDTA extract we found 5.5 % of the total manganese content. The $\mathrm{MgCl_2}$-, $\mathrm{CaCl_2}$- and Mehlich 2 extracts showed negligible manganese contents. Applying 2 hour agitation, the manganese content in the Mehlich 3 showed appreciable increase (26.7 %), while in the EDTA extract the manganese content increased significantly (12.3 %). The manganese contents in $\mathrm{MgCl_2}$-, $\mathrm{CaCl_2}$- and Mehlich 2 extracts showing negligible manganese concentrations remained unchanged. As Table 2 shows, the total manganese concentration ($700\ \mathrm{mg \cdot kg^{-1}}$) of the investigated soil sample - horizon B of Haplic Phaenozem - was in comparison with the manganese concentration ($800\ \mathrm{mg \cdot kg^{-1}}$) of relevant horizon A by 12.5 % lower. In the same time, both Mehlich 3 as well as EDTA extracts contained by roughly comparable relative proportions lower amounts of manganese. These facts meaningfully support the expectations related to the ability of EDTA to extract manganese from humic matter as well to the significant agitation time dependence of extractable manganese from the investigated soil samples.



a) b)

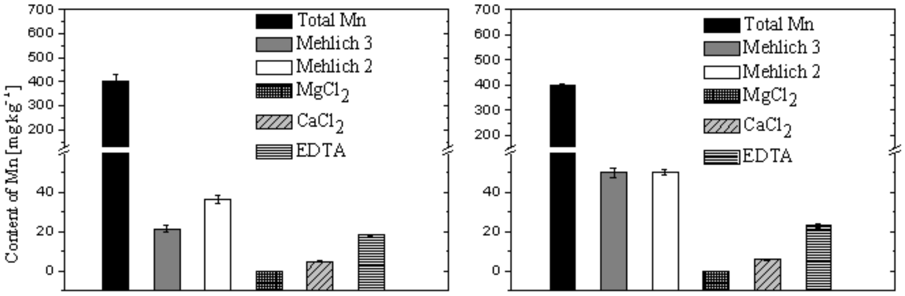
Fifure 3 Concentration of manganese in soil sample Jaslovské Bohunice horizon B and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h

Soil sample Jaslovské Bohunice horizon C: The total manganese concentration ($600 \ \mathrm{mg \cdot kg^{-1}}$) in the horizon C of Haplic Phaenozem - in comparison with the upper horizons - showed further decrease. In the same time the relative manganese portion in Mehlich 3 extract after 1 hour agitation significantly decreased as well (8.5 %), while the one in EDTA extract (0.2 %) became of negligible value. The $MgCl_2$- and $CaCl_2$ extracts practically did not contain manganese. The elongation of agitation time to 2 hours led to measurable increase of manganese contents in Mehlich 3 extract (12.5 %), while in the EDTA extract (1.3 %) the relevant increase is of negligible value. Similarly to the upper horizons the $MgCl_2$- and $CaCl_2$ extracts did not contain manganese, but an easily measurable manganese amount was found in Mehlich 2 extracts (2.7 %) which was practically independent on contact time. As it is shown, the decrease of humic mater concentration in horizon C led to two consequences. The first is the further decrease of relative manganese content in Mehlich 3 extracts and the second - the measurable increase of Mn in the Mehlich 2 extracts. This knowledge is in good agreement with the corresponding gradual humic mater concentration decrease.



a) b)

Figure 4 Concentration of manganese in soil sample Jaslovské Bohunice horizon C and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h

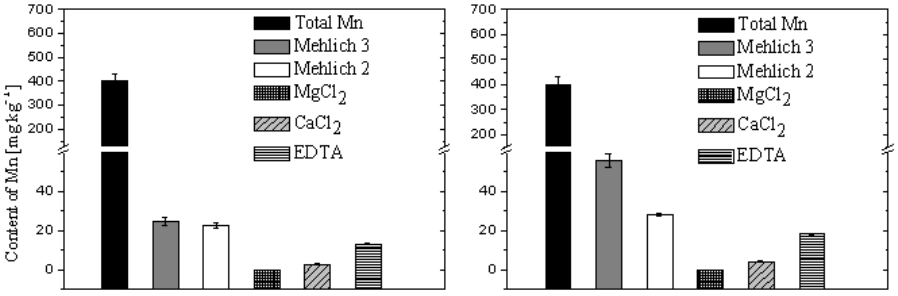


a) b)

Figure 5 Concentration of manganese in soil sample Sihla horizon A and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h

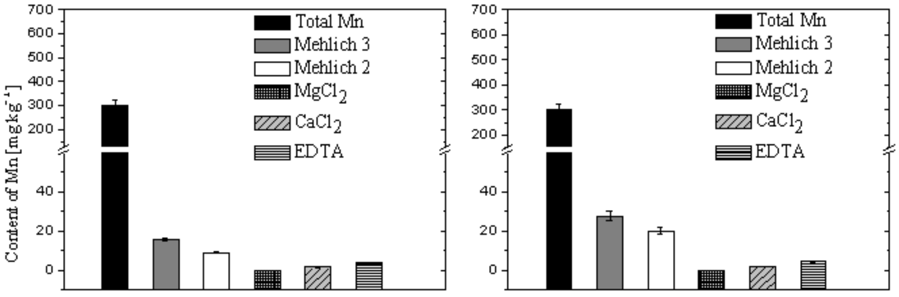
Soil sample Sihla horizon A: The studied samples of Haplic Cambisol show in comparison with the above depicted Calcic Phaenozem lower values of total manganese content in general. Haplic Cambisol represents a silicic and acidic soil type possessing a comparatively low cation exchange capacity, while the former described samples of Calcic Phaenozem belong to calcareous soil types showing basic reaction and relatively high cation exchange capacity. Horizon A of the investigated Haplic Cambisol contained $400\ \mathrm{mg}\cdot\mathrm{kg}^{-1}$ of total manganese. Taking into account, that the humic matter concentration of the sample is much lower in comparison with the horizon A of Calcic Phaenozem, it may be expected, that the EDTA and Mehlich 3 extracts will show lower manganese concentrations as the relevant Mehlich 2 extract. Our results confirm the mentioned anticipation. After one hour agitation the EDTA and Mehlich 3 extracts contained 4.6 and 5.4 % of manganese respectively, while the Mehlich 2 extract showed 9.1 % of the total manganese concentration. In the same time the $\mathrm{CaCl_2}$ extract showed - unlike the one of horizon A Calcic Phaenozem - low, but measurable value (1.2 %). The $\mathrm{MgCl_2}$ extract did not contain manganese at all. Elongation of agitation to two hours brought two measurable changes, namely the increase the Mehlich 3 and Mehlich 2 fractions (both 12.5 %). The presumable reason of the last mentioned result is the progressive solubilization of manganese containing constituents of sample due to the presence of nitrates in the extraction solution.

Soil sample Sihla horizon B: As the comparison of Fig. 5 and Fig. 6 implies, the total manganese concentrations in both A and B horizons of the investigated Haplic Cambisol (depth up to 25 cm) are identical ($400\ \mathrm{mg}\cdot\mathrm{kg}^{-1}$) and moreover, the one hour lasting extractions gain very similar manganese concentrations. The only notable difference appears in Mehlich 2 extracts, where in the horizon B we found out a decrease of manganese concentration (5.7 % instead of 9.1 %). Comparing the results of two hour lasting agitations both the horizon A and B, the only substantial change is the decrease of manganese concentration in Mehlich 2 extract (7.0 % instead of 12.5 %). Regardless of both horizon and agitation time, we found similar manganese concentrations in all other extracts, namely zero manganese concentration in $\mathrm{MgCl_2}$ extracts, < 1.5 % in $\mathrm{CaCl_2}$ extracts and approx. 4.5 % in EDTA ones. We may conclude that the soil composition differences in the upper soil horizons including the manganese speciation differences are not substantial and the bioavailable manganese concentrations in the investigated soil samples of low value in general.



a) b)

Figure 6 Concentration of manganese in soil sample Sihla horizon B and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h



a) b)

Figure 7 Concentration of manganese in soil sample Sihla horizon C and concentration of various extracts presents weight of manganese in one kilogram of soil
a) time of extraction: 1 h; b) time of extraction: 2 h

Soil sample Sihla horizon C: The total Mn concentration in horizon C decreases to $300\ \mathrm{mg}\cdot\mathrm{kg}^{-1}$ and this decrease brings about an observable decrease of manganese concentration in all investigated extraction media both in case of one hour agitation time as well as applying the elongation of extraction time. The most significant decrease is to seen by EDTA extracts, where, in comparison with the horizon B, the manganese concentration sank after one hour extraction from 3.3 % to 1.2 %, and after two hours extraction from 4.5 % to 1.5 %. These correspondent decreases may be caused not only by the general decrease of manganese in soil samples, but simultaneously by progressive decrease of humic substances concentrations. The manganese exchangeability of both $\mathrm{MgCl_2}$ and $\mathrm{CaCl_2}$ remained negligible, while the last mentioned reagent is of generally observable higher efficiency.

4 Conclusions

The investigated six soil samples representing horizons A, B and C of two typical Slovakian soil types, namely Calcic Phaenozem and Haplic Cambisol, sharply differ in their total manganese content. From the point of view of plant cultivation horizons A are evidently the most important soil layers and therefore the determination of bioavailable fractions concerning the relevant ones is, as a rule, the most necessary requirement. The highest total Mn concentration was determined in the horizon A of Calcic Phaenozem ($800\ \mathrm{mg \cdot kg^{-1}}$), while the lowest one in the horizon C of Haplic Cambisol ($300\ \mathrm{mg \cdot kg^{-1}}$). The total Mn concentration showed in both soil types an unambiguous decrease with depth. The essentially basic Calcic Phaenozem contained higher total manganese concentration in general compared to the essentially acidic Haplic Cambisol (horizon A: $400\ \mathrm{mg \cdot kg^{-1}}$). In the same time Calcic Phaenozem showed a significantly enhancing carbonate content and a slight pH increase with soil depth (horizon A: $\mathrm{pH(H_2O)}$ = 8.30 and pH(KCl) = 7.53), while Haplic Cambisol displayed with growing depth an unchanged carbonate concentration and a rather stable pH value (horizon A: $\mathrm{pH(H_2O)}$ = 4.6 and pH(KCl) =3.8). As it has been mentioned, among all of the studied soil samples the highest total manganese concentration was found out in horizon A of Calcic Phaenozem and the highest manganese portion was determined in the relevant EDTA extract (46.9 %). Even the EDTA extract of soils, as a rule, is considered to be the representing bioavailable fraction of manganese content of soils and sediments, its complex forming nature extends the extractable manganese fraction beyond the exchangeable Mn content, namely regarding the manganese fraction bound to broad spectra of soil organic matter. In the same time the generally accepted $\mathrm{CaCl_2}$ extract like the often used $\mathrm{MgCl_2}$ one showed negligible and not detectable manganese concentrations respectively. The above presented high value of manganese content of EDTA extract is undoubtedly due to both the high total manganese content of the relevant soil sample and the high humic matter content of the sample. Concerning Haplic Cambisol after one hour of agitation the highest manganese fraction was found out in Mehlich 2 extract (9.1 %), while the second highest value was achieved in Mehlich 3 extract (5.4 %). The elongation of the extraction time to 2 hours resulted in the slight increase of Mehlich 2 and a significant increase of Mehlich 3 manganese fractions (both 12.5 %). The one hour lasting extraction with EDTA resulted in 4.6 % Mn fraction while the elongation of extraction time to 2 hours brought only insignificant increase (5.7 %). The mentioned outcome apparently coincides with the low humic matter content as well as with the acidic character of the investigated sample. In the same time, the $\mathrm{CaCl_2}$ extracts (applying both agitation times) showed a tiny manganese fraction (1.2 and 1.5 % respectively) while the $\mathrm{MgCl_2}$ extracts did not contain manganese at all. The gained results accentuate the necessity of further efforts in the development of more efficient methods of soil fractionation/speciation analysis aimed to acquirement of more reliable data concerning in particular the determination of plant-available portion of manganese in soils. The above declared necessity concerns a number of soil constituents while manganese is only one of typical ones. Among viable approaches the application of radioindicators (more recently stable isotopic indicators) seems to be promising, including the enhancement of E-value and L-value determination procedures, as well as procedures aimed at proper extraction process objectification [7].

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