

Assessment of extraction methods for studying the fractional composition of Cu and Zn in uncontaminated and contaminated soils

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Abstract

This study is aimed at elucidating the fractionation of Cu and Zn in Haplic Chernozem and its alteration under the contamination to evaluate the extraction selectivity of different extractants and thus the efficiency of three sequential extraction schemes (Tessier and McLaren five-step and modified BCR three-step methods). General trends in the distribution of Cu and Zn speciations revealed with these three fractionation schemes suggest that they can be applied for the soils and the obtained results can be compared. Low mobility of potentially toxic metals (PTM) in the studied soil is suggested not only by their high content in residual fraction, but also by low contents of exchangeable and carbonate bound fractions (not more than 4-5 % Cu and 5-7 % Zn in the first two fractions). The highest contribution to the absorption and retention of Cu delivered from anthropogenic sources is made by organic matter and sesquioxides (up to 29 %); for Zn, by the nonsilicate Fe and Mn compounds (up to 25 %). However, the pattern of PTM extraction from soils varies during the application of different fractionation methods. The Tessier method is distinguished by a higher extractability relative to organic matter and sesquioxides. Therefore, this method is more informative for the contaminated soils. The McLaren method makes it possible to track the weakly bound species of compounds without the risk of involving other soil components. The BCR method is marked by simplicity of application and, therefore, recommended only for the noncalic or low-calcic soils.

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Introduction

In recent decades, contamination of soils with the potentially toxic metals (PTM) has become a global ecological problem. In contrast to the majority of organic pollutants, which lose toxicity due to biodegradation, PTMs can provoke prolonged toxic effects serving as a source of contamination (Tashakor et al., 2014; Acosta et al., 2015). Urgency of this problem lies in the following fact: when PTMs fall into soil, they do not break down and can migrate to other compartments of ecosystem, creating severe problems for the human life and environment (Yaylali-Abanuz, 2011; Hu et al., 2013). Their contamination effect in soil

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depends on chemical associations. Therefore, determination of the chemical form of metal in soil has an important significance for evaluating its mobility and bioaccessibility (Naji et al., 2010; Gabarrón et al., 2017). Soil is a complex interface containing organic and inorganic compounds, as well as solution and gases, reacting under various physicochemical conditions. For example, it is known that ions of metals (Cu and Zn included) become more soluble, mobile and toxic for plants under conditions of high acidity (Anju and Banerjee, 2010). Metal speciation in the soil, i.e. determination of the phase (or phases) of chemical elements, is a scientific challenge (Doelsch et al., 2008).

The sequential extraction schemes (SES) methods are most widely used for determining the PTM speciation in soils. They yield additional information about the associations of metals with the geochemical phases of soil. Thus, they provide insight into processes, which regulate the mobilization of metals, and possible ecological risks (Silveira et al., 2006; Kennou et al., 2015). Although these methods do not give direct characteristics of metal speciation, they can imitate the chemical reactivity of metals during natural and anthropogenic alterations in the environment. Moreover, these methods comply with all requirements for the realization over large areas: simplicity, applicability to all soil and rock species, low cost, and understandable results (Rosado et al., 2016).

Metals can occur in soils as various physicochemical phases that govern different modes of their occurrence. The most commonly isolated phases in different SES methods are: exchangeable, carbonate bound, Fe-Mn oxide bound (reducible), organic (oxidizable) and residual (Rao et al., 2007; He et al., 2013).

Numerous different procedures of the sequential extraction have been proposed for the TM extraction from soils (Filgueiras et al., 2002; Hlavay et al., 2004). The most widely used SES methods are those proposed by McLaren and Crawford (1973) and Tessier et al. (1979). The McLaren method was initially designed for fractionating copper compounds. In practice, however, it is applied long ago for fractionating compounds of various PTMs. The classic sequential extraction method reported in Tessier method (Tessier et al., 1979), initially designed for bottom sediments, is widely known and applied often in Russia and Europe for evaluating the fractional PTM composition in soils (Rao et al., 2007; Orecchio, Polizzotto, 2013; Minkina et al., 2015; Rosado et al., 2016; Evans et al., 2019).

Diversity of the sequential extraction procedures makes it possible to use various reagents under different experimental conditions of the storage and preparation of samples, temperature, fraction size, reagent concentration, extragent application sequence, solid/liquid phase ratio, pH value control, and duration of stirring and filtration relative to centrifugation (Sutherland et al., 2000). Because of a lack of conformity in the applied procedures, however, it is rather difficult to compare the results obtained in different laboratories. The Community Bureau of Reference (BCR) of the European Commission attempted to standardize the three-step extraction (acid-soluble/exchangeable, reducible, and oxidizable fractions) scheme that was supplemented later with a new step providing determination of the residual fraction (Rauret et al., 1999; Ure et al., 1993; Pueyo et al., 2008). This method is used widely because of the presence of a certified reference material (BCR 701) as a control sample (Leermakers et al., 2019).

Although the sequential fractionation schemes are very popular, impeccability of these methods and univocacy (accuracy) of results are now being challenged with increasing frequency. The main problem in the sequential extraction is related to non-selectivity of extragents and redistribution of elements between phases in the course of extraction (Rapin et al., 1986; Raksataya et al., 1996; Sulkowski and Hirner, 2006). In addition, the preliminary sample treatment (freeze drying, air drying, oven drying, and others) also play a crucial role often leading to determination errors (van Hullebusch et al., 2005). Despite all these shortcomings described well in literature, the sequential extraction methods are widely used so far for evaluating the accessibility and mobility of metals in solid matrices (soils included) both in separate methods (Wisawapipat et al., 2017; Hasan et al., 2018; Minkina et al., 2018a; Al-Mur, 2020) and in combination with other instrumental identification methods (Scheinost et al., 2002; Hsu et al., 2015; Arenas-Lago et al., 2016; Mekapogu et al., 2018; Minkina et al., 2018b, 2019).

The present paper is aimed at a comparative evaluation of the results of Cu and Zn fractionation in Haplic Chernozem samples obtained by different sequential chemical extraction schemes to determine the most suitable version for specific features of soil without and with anthropogenic contamination.

Material and Methods

Soils

For the purpose of this study, two important locations were selected: (I) soil located away from any source of contamination (pure uncontaminated soil); (II) soil subjected to aerosol contamination by products of the fuel combustion. In both cases, soil is represented by Haplic Chernozem.

The pure (uncontaminated) soil was sampled from the upper layer of virgin Haplic Chernozem sector, where

the thick medium-humic, calcaric heavy loam overlies the loess-type sandy clay in the specially protected Persianovo steppe area (Rostov region).

Soil in this area, marked by a constant anthropogenic load related to aerosol emissions from the Novochoerkassk power station (NPS), was sampled at 1 km northwest (direction of the prevailing winds) from the emission source. It is believed that areas located at a distance of up to 4 km from power stations are subjected to the highest ecological disturbances (Minkina et al., 2013a). The power station, a year-round functioning, high electric and thermal output station mainly based on coal, is one of the largest producers of electricity in southern Russia and at the same time the major source of environment pollution (Linnik et al., 2019). Moreover, it is the sole hydroelectric station functioning on the local coal mining wastes and beneficiation products (anthracite fines).

All soils were sampled in the 0–20 cm layer. Only steel or plastic tools were used for sampling in order to avoid the PTM contamination of soils. After air-drying and declumping the aggregates, the soil was sieved through a 2-mm mesh sieve. In the collected soil samples, we analyzed the following main physicochemical parameters: pH value by the potentiometric method (soil:water ratio 1:2.5); C_{org} content by the titrimetric method using the biochromatic oxidation procedure according to Tyurin (Tyurin, 1965; Vorob'eva, 2006); cation exchange capacity (CEC) and exchangeable cations Ca^{2+} and Mg^{2+} according to the method proposed by Shaimukhametov (1993); carbonate content by the complexometric method according to S.A. Kudrin; Fe-Mn (hydr)oxides using the Meer–Jackson's reductive complex (Vorob'eva, 2006); and grain size composition of soil by the pipette method with the pyrophosphatic sample preparation (Vadyunina and Korchagina, 1986). Properties of the studied soil are presented in Table 1.

Table 1. Physical and chemical properties of Haplic Chernozem

Soil	Silt and clay (soil particle <0.01 mm), %	Silt (soil particle <0.001), %	C_{org} , %	pH _{H2O}	CaCO ₃ , %	Fe-Mn (hydro) oxides	Exchangeable cations, cmol kg ⁻¹		CEC, cmol kg ⁻¹
							Ca ²⁺	Mg ²⁺	
Uncontaminated soil	47.1 ± 3.8*	26.8 ± 1.8	3.7 ± 0.3	7.5 ± 0.1	0.03	3.8 ± 0.3	3.0 ± 0.1	0.6 ± 0.1	3.71 ± 0.3
Contaminated soil	55.3 ± 4.2	30.9 ± 2.0	2.6 ± 0.2	7.4 ± 0.1	0.07	3.9 ± 0.4	2.6 ± 0.1	0.5 ± 0.1	3.22 ± 0.3

* ±Standard deviation

Sequential extraction procedures

Sequential extraction was performed using two five-step (Tessier and McLaren) methods and the modified three-step BCR sequential extraction procedure (Table 2). After each extraction, the liquid and solid phases were separated by centrifuging. The TM content in extracts was determined by the atomic absorption spectrometry (AAS). The above-mentioned methods make it possible to extract the fractions of metals differing from each other in the strength of bond with soil components (Table 2).

Results and Discussion

Overview

The bulk content of Zn and Cu in soil of the virgin sector matching the background metal content in Haplic Chernozem (Mandzhieva et al., 2017) was almost two times lower than in the soil located under the influence of aerosol emissions (Tables 3, 4). The sum of fractions obtained by all sequential fractionation methods under consideration was very close and similar to the bulk content of Cu and Zn in soil, suggesting credibility of the data obtained by different methods (Tables 3, 4).

Exchangeable fraction

The exchangeable fraction includes the weakly adsorbed metals retained on the surface of soil particles by a relatively weak electrostatic interaction: (i) metals that can be released due to ion exchange processes; (ii) metals that can co-precipitate with carbonates occurring in many types of sediment. To determine the exchangeable forms of metals, CaCl₂ and MgCl₂ are used in the McLaren and Tessier methods, respectively. These extragents represent neutral salts that are used most commonly for extracting the above compounds (Rauret, 1998; Hall and Pelchat, 1999). In the BCR method, the exchangeable fraction is extracted not separately but together with the acid-soluble fraction.

Metals corresponding to the exchangeable fraction represent a minor portion of the total metal content in soil (Emmerson et al., 2000). In the uncontaminated Chernozem, the share of exchangeable Cu and Zn is low and makes up 1 % of the sum of fractions obtained by the McLaren and Tessier methods (Figure 1, 2).

Table 2. Different sequential extraction schemes of potential toxic metal extraction from soils

Authors of the method	Toxic metal fractions	Reagent and operating conditions
McLaren and Crawford (1973)	Exchangeable	0.05 M CaCl ₂ was shaken for 16 h. (the ratio of soil:solution 1:10)
	Acid soluble (bound to carbonates)	2.5% CH ₃ COOH was shaken for 1 h. (the ratio of soil:solution 1:10)
	Bound to organic matter	1 M K ₄ P ₂ O ₇ , pH 11 was shaken for 1 h. (the ratio of soil:solution 1:10)
	Bound to Fe-Mn oxides	0.14 M H ₂ C ₂ O ₄ + 0.2 M (NH ₄) ₂ C ₂ O ₄ (oxalate reagent) was shaken under ultraviolet irradiation for 1 h (the ratio of soil:solution 1:10)
	Residual	HF+HClO ₄ , then concentrated HNO ₃ after evaporation (the ratio of soil:solution 1:10)
Tessier et al (1979)	Exchangeable	1M MgCl ₂ (pH 7.0) with continuous agitation (1 h) (the ratio of soil:solution 1:8)
	Bound to Carbonates	1M NaCH ₃ COO, pH 5.0 (CH ₃ COOH) was leached at room temperature for 5 h. (the ratio of soil:solution 1:8)
	Bound to Fe-Mn oxides	0.04M NH ₂ OH·HCl in 25% CH ₃ COOH was heated at 96±3°C for 8 h. with continuous agitation (the ratio of soil:solution 1:20)
	Bound to Organic Matter	0.02M HNO ₃ and 5 ml of 30 % H ₂ O ₂ adjusted to pH 2 with HNO ₃ , and the mixture was heated to 85 ± 2 °C for 3 h with intermittent agitation. After cooling, 3.2 M NH ₄ CH ₃ COO in 20 % (v/v) HNO ₃ was added and agitated continuously for 30 min (the ratio of soil:solution 1:20)
	Residual	HF+HClO ₄ , then concentrated HNO ₃ after evaporation (the ratio of soil:solution 1:25)
Modified BCR three-step sequential extraction procedure (Pueyo et al., 2008)	Acid soluble (Exchangeable and weak acid soluble fraction, first step)	0.11 M CH ₃ COOH solution by shaking in a mechanical, end-over-end shaker for 16 h (the ratio of soil:solution 1:40)
	Reducible (bound to hydroxides and oxides of Fe and Mn, «ferrous», second step)	0.5 M NH ₂ OH·HCl was shaken for 16 h, pH 2, The acidification of this reagent is by the addition of a 2.5 % (v/v) HNO ₃ solution (the ratio of soil:solution 1:40)
	Oxidisable (bound to organic matter, «organic», third step)	8.8 M H ₂ O ₂ when heated (for 1 h at 85 ± 2 °C) to evaporate almost to dryness, then 50 ml 1 M NH ₄ CH ₃ COO, pH 2, The acidification of this reagent is by the addition of a 2.5 % (v/v) HNO ₃ solution (the ratio of soil:solution 1:70)
	Residual (Residue from the third step)	aqua regia (HCl (37 %) and HNO ₃ (70 %)) were added, after evaporation (the ratio of soil:solution 1:9)

Contents of Zn and Cu are increased appreciably (up to 4-6 %) in the exchangeable fraction due to pollution, resulting in their accumulation in plants (Figure 1, 2). The concentration of metals in the exchangeable fraction shows a high correlation with their content in plants (Gupta, 1993; Novozamsky, 1993; Aten, 1996).

Table 3. Fractional composition of Cu in Haplic Chernozem, mg kg⁻¹

Soil	Fractions						Total content
	Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	Sum	
The McLaren method							
Uncontaminated	0.3	1.5	3.9	6.5	26.7	38.9	44
Contaminated	5.7	11.5	21.1	38.5	85.6	162	180
The Tessier method							
Uncontaminated	0.5	1.9	5.1	8	27.2	42.7	44
Contaminated	7.4	10.6	30.6	49.1	73.8	172	180
Modified BCR method							
Uncontaminated	1.2		4.5	7.7	28.5	41.9	44
Contaminated	14.5		25.5	43.2	80.6	164	180

Table 4. Fractional composition of Zn in Haplic Chernozem, mg kg⁻¹

Soil	Fractions						Total content
	Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	Sum	
The McLaren method							
Uncontaminated	0.9	5	9	3.5	62.4	80.8	90
Contaminated	12.6	28.5	54.2	25.0	194.0	314.3	338
The Tessier method							
Uncontaminated	1	4.5	13.2	9.5	57.5	85.7	90
Contaminated	19.5	28.1	82.8	32.5	173.0	335.9	338
Modified BCR method							
Uncontaminated	6.0		11.3	6	60.7	84	90
Contaminated	38.6		67.6	23.3	192.8	322.3	338

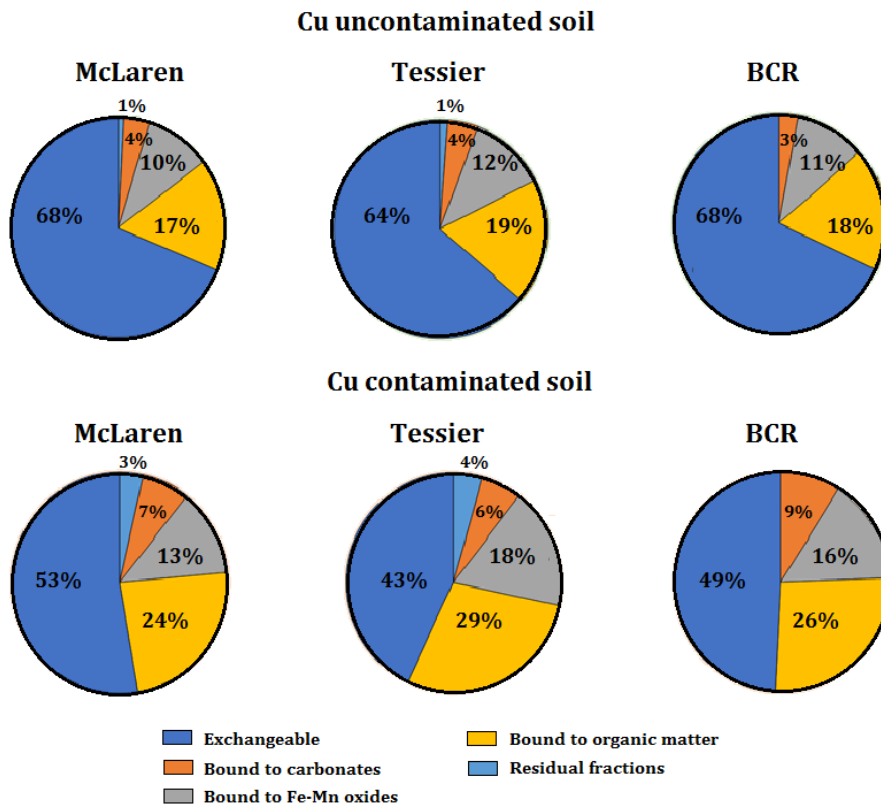


Figure 1. Cu distribution in uncontaminated and contaminated soils, % of the sum of fractions

Acid-soluble fraction

The acid-soluble fraction is represented by metals precipitating on carbonates (Clevenger, 1990). These compounds are sensitive to changes of pH values (Zorpas et al., 2000) and are commonly extracted with weak acids (Coetzee, 1993; Gauthreaux et al., 1998). To extract this fraction, researchers use the acetic acid (BCR and McLaren methods) or sodium acetate (Tessier method). In addition to the acetate buffer solution, these acids are the most commonly used extragents for the above-mentioned compounds (Diks and Allen, 1983; Lee and Kittrick, 1984; Gibson and Farmer, 1986; Ure et al., 1993).

The acid-soluble fraction is next to the exchangeable fraction in terms of the metal content. Similar results of metal content in this fraction are related to identical extraction conditions. The content of Cu and Zn in this fraction in the uncontaminated soil is 3-4 and 5-6 %, respectively.

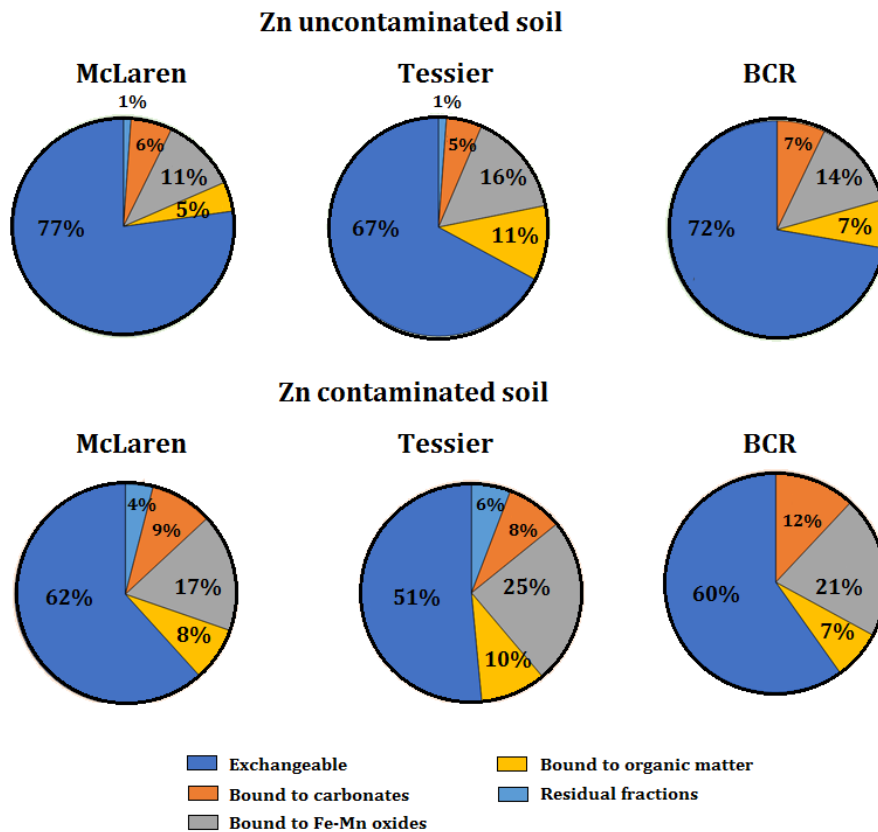


Figure 2. Zn distribution in uncontaminated and contaminated soils, % of the sum of fractions

Zn shows a higher affinity to carbonates than Cu (Stone and Marsalek, 1996; Li et al., 2001). Under conditions of anthropogenic load, this fraction accumulates up to 6-9 % Cu and 9-12 % Zn. Although the carbonate content in the studied soil samples is <1 % (Table 1), they demonstrate high activity in the retention of metals because of a highly dispersed mycelial form of carbonate occurrence in Haplic Chernozem, which is a specific feature of these soils (Minkina et al., 2017). The dispersed carbonates have a high specific surface area, which increases their interaction with PTM. Analysis of the K-edge XANES spectra of Zn adsorbed by the carbonate showed that Zn ions replace Ca ions in the octahedral positions and display 1s→4p electronic transition. Moreover, they are coordinated with carbonate ions as ligands making up adsorbed complexes on the surface of mineral calcite (Minkina et al., 2013a).

Usually, PTMs in the exchangeable and acid-soluble fractions are considered bioaccessible, whereas the fractions of metals bound to organic matter and Fe-Mn oxides are considered more stable under natural soil conditions (Wong et al., 2002).

Reducible fraction

In order to simplify the extraction of metals, the McLaren, Tessier and BCR methods, as well as the majority of recently proposed SES methods, take into consideration the reducible fraction that includes not only Mn oxides, but also the amorphous and crystalline Fe oxides (Lee and Kittrick, 1984; Gibson and Farmer, 1986; Ure et al., 1993).

During the analysis of this metal fraction, Fe and Mn (hydr)oxides are extracted jointly. They are good PTM absorbers (Baruah et al., 1996; Stone and Droppo, 1996). The absorption of metals by sesquioxides occurring in soil as a coating on the mineral surface or as separate fine particles can take place by one or combination of the following mechanisms: co-precipitation, adsorption, surface complexation, ion exchange and penetration into the mineral lattice (Hall and Pelchat, 1999).

In the case of low Cu ion concentration in the uncontaminated soil, a significant portion of this metal is associated with Fe-Mn oxides (10-12 %). Zn content in this fraction amount to 11-16 %. Under conditions of contamination, the content of Cu and Zn in the reducible fraction increases to 13-20 % and 17-25 %, respectively (Figure 2), because of a big role of hydroxides in the fixation of metals (Mellis et al., 2003). Metals are likely to fixed in the nonexchangeable form by chemisorption due to the replacement of two H⁺ ions incorporated in OH or OH₂ groups on the oxide surface by the bivalent metal cation (Pinskii et al., 2014). The highest increase of metals in this fraction was observed during the application of Tessier method. This method is marked by a higher extractability of PTMs bound to the ferruginous minerals because of the

application of hydroxylamine hydrochloride in acid—a strong reducer with high processing temperature (96°C) and high CH₃COOH concentration (25 %). The hydroxylamine hydrochloride can affect other soil components to a greater extent than Tamm's reagent used in the McLaren method. One of the problems arising in this case is as follows: the hydroxylamine hydrochloride releases a significant amount of metals bound to organic matter and, hence, reduction of metals in the Fe-Mn oxide fraction can be enhanced at the expense of the oxidizable fraction (Ahnstrom and Parker, 1999).

The lowest contents of Cu and Zn in soils are observed in the McLaren method. Moreover, discrepancies increase in the contaminated soil. The McLaren method uses ammonium oxalate, oxalic acid or oxalate buffer solution with pH=3. When evaluating the fraction of metals tightly bound to ferruginous minerals in the contaminated soils, this method, probably, yields not quite adequate estimate. Processing of soil samples is accompanied by the formation of low-soluble sediments of PTM oxalates, as suggested by low solubility products of salts. Influence of oxalate as PTM extragent decreases with the degree of soil contamination. In addition, the oxalate has a low selectivity (Vodyanitsky, 2010). Thus, the Tamm's reagent used in this method, probably, cannot decompose completely all ferruginous minerals even during the ultraviolet radiation (Ladonin and Karpukhin, 2003), which is particularly important for studying metals in the uncontaminated soils. It should also be noted that the extraction of organic fraction by the McLaren method precedes the extraction of Fe-Mn oxide (Shuman, 1983). At the same time, the ferruginous coating can partially be dissolved, resulting in a lower content of the fraction associated with ferruginous minerals.

Oxidizable fraction

Trace elements can be associated with the process of complexation or bioaccumulation with different forms of organic matter, such as living organisms, dead organic matter (detritus), or coating on mineral particles (Tokalioglu et al., 2000). It is presumed that metals bound to organic matter remain in soil in the tightly bound state for a long time (Kennedy et al., 1997). However, the decomposition of organic matter can foster the release of soluble metals associated with this component (Clevenger, 1990; Gauthreaux et al., 1998; Davidson et al., 1999). Since the metals bound to organic matter can easily be released in an oxidizing environment, the technique of oxidation is usually applied for their extraction. One of the most widespread oxidizers is represented by hydrogen peroxide in acid medium used in the BCR and Tessier methods due to requirement of more stringent extraction conditions. In the uncontaminated soil, the share of Cu and Zn bound to organic matter is 17-19 % and 5-11 %, respectively (Figure 1, 2). The highest metal content in this fraction was yielded by the Tessier method.

In the contaminated soil, the share of Cu bound to organic matter increases to 24-29 % (Figure 1). Physical analytical methods confirmed the organophilic nature of Cu substantiated by high values of the stability constant of this metal with organic ligands (McBride, 1989; Manceau, 2010; Nielsen, 2015). Analysis of the fine XANES spectra revealed that the interaction of Cu²⁺ ions with humic acids of soils can lead to the octahedral (intrasphere) coordination of chelate complexes (Xia, 1997; Strawn, 2008; Minkina et al., 2016).

The Zn content in the organic matter fraction does not practically change with the increase of contamination when the Tessier and BCR methods are applied (Figure 2). An appreciable increase of the metal (from 5 % to 8 %) in this fraction is only observed in the McLaren method, possibly, due to specific features of the latter method, which is based on the dissolution of humic acids in an alkaline medium and the retention of PTM ions in the solution owing to a strong complex-forming capacity of the pyrophosphate ion. However, though this reagent is likely selective for organic matter, efficiency of extraction remains low (Papp et al., 1991).

In the case of Tessier and BCR methods, the extraction takes place under rather aggressive conditions that affect the amorphous Fe-Mn oxides and needs a preliminary dissolution of the organic fraction (Elliot et al., 1990).

Residual fraction

Since PTM ions fixed in the crystal lattices of minerals are most firmly associated with the soil, they are difficultly extractable. Therefore, they are least accessible for plants and involvement in the biological cycle. The results obtained by the three fractionation methods revealed: in uncontaminated and contaminated soils the highest Cu content (45-68 % of the sum of Cu fractions) is recorded in the residual fraction associated with silicates (Figure 1). The distribution of Zn is similar to that of Cu: the Zn content in Haplic Chernozem is highest in the residual fraction (51-77 %). Moreover, the maximum Zn content is recorded in the uncontaminated soil (Figure 2), and Zn is more prone to interaction with soil mineral components (Mellis et al., 2003; Manceau, 2004; Ladonin and Karpukhin, 2011).

However, the share of this fraction of metals in the contaminated soil is lower than in the uncontaminated soil, probably, because of a very slow uptake of alien metal compounds into the crystal lattices of clay

minerals. Moreover, metals can penetrate mineral lattices and replace Al or make up intrasphere MeOH⁺-type complexes (Manceau, 2002; Minkina et al., 2013b, 2015; Bauer et al., 2018).

Fractional composition of PTM

Thus, the results of fractionation made it possible to unravel regularities in the fractional distribution of metals in the uncontaminated and contaminated soils and to define the role of soil components in the fixation of metals. Both uncontaminated and contaminated varieties of Haplic Chernozem are marked by the following Cu distribution pattern in fractions: residual > bound to organic matter > bound to Fe-Mn oxides > acid-soluble/exchangeable. However, when the BCR method is used, the Fe-Mn fraction prevails over the organic fraction, possibly, because of the sequential application of extragents.

The Zn distribution pattern in fractions of the uncontaminated and contaminated soils is similar: residual > bound to Fe-Mn oxides > bound to organic matter > acid-soluble/exchangeable.

Hence, irrespective of the level of Zn concentration, its distribution between different soils components is mainly governed by the interaction with soil mineral components (Fe-Mn silicates and oxides), as well as by the interaction with organic matter in the case of Cu contamination.

Conclusion

In general, the application of different sequential extraction methods revealed similar trends in the distribution of Cu and Zn in fractions. The obtained values are consistent and showed similar pattern for mobility of toxic metals. The results of Cu and Zn fractionation revealed that the Tessier method is more suitable for determining the total anthropogenic component in contaminated soils, but low-informative for the fractions nontightly bound to the soil. This is a significant shortcoming of this method, because precisely the most mobile PTM compounds are important for soil processes. The BCR method, which is broadly similar to and based on the Tessier method, has the following advantages: simplicity and accessibility. Besides, precisely this method is considered as standardized procedure in the global practice. This method can successfully be applied for the noncalcic or low-calcic soils. The McLaren method provides a better insight into metals in the nontightly soil-bound fractions owing to the application of “weaker” extragents, which is important in the study of their accessibility for plants. When evaluating the content of metal compounds tightly bound to the organic matter and ferruginous fraction, the McLaren method is likely less reliable.

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