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A comparison of sequential extraction procedures for fractionation of arsenic, cadmium, lead, and zinc in soil

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Abstract: Twelve soil samples differing in physicochemical properties and total element contents were extracted by three sequential extraction procedures to determine As, Cd, Pb, and Zn bound to individual soil fractions and are defined by individual operational procedures. In the case of arsenic, two additional sequential extraction schemes were designed entirely for fractionation of soil containing arsenic were tested. The results confirmed that determination of element proportions bound to individual soil fractions is strongly dependent on the extracting agent and/or procedure applied within individual extracting schemes. As expected, absolute values of the elements released among the individual extracting procedures are weakly comparable. More reliable results were determined for the more mobile soil elements i.e. cadmium and zinc, in the fractions characterizing the most mobile proportions of investigated elements where significant correlations with basic soil characteristics were observed. In contrast, ambiguous results were observed for As and Pb, for both the individual extraction procedures and the effect of the soil characteristics. Regardless of the studied element, the poorest results were determined for reducible and oxidizable soil fractions. The application of at least two independent procedures or modification of the extraction scheme according to element investigated and/or particular soil characteristics can also be helpful in definition of element pattern in soils in further research.

Keywords: Arsenic, cadmium, lead, soil, sequential extraction, zinc

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1 Introduction

The methods of sequential extraction of soils were developed in order to more precisely define the single fractions of elements in a soil. Sequential extraction usually necessitates from three to seven steps. They are time consuming and require skilled personnel and adequate analytical instrumentation techniques. Nevertheless they give the most accurate information about fractionation and transformation of elements in the soil, especially in relation to different soil physicochemical properties, such as soil pollution and long-term effect of soil amendments, for example, liming, application of sewage sludge, coal ash etc. [1-6]. In this context, such parameters as the redistribution index and reduced partitioning parameter were evaluated for the quantification of the redistribution processes of heavy metals in both contaminated [7] and uncontaminated [8] soils. Moreover, the sequential extraction methods are suitable for an evaluation of element distribution into individual soil fractions after experimental soil amendment by potential risk elements [9] as well as for evaluation of remediation potential for these elements [10].

A large number of different methodological approaches were developed over the last two decades defining the individual fractions of given elements. The first sequential extraction procedure was described by McLaren and Crawford [11] in 1973 but an extended method was developed by Tessier et al. [12], who performed fractionation of metals in samples of sediments into 5 parts: (1) exchangeable fraction representing the most easily available metals, (2) carbonate fraction, (3) Fe, Mn and Al oxides fraction, (4) organic matter fraction and (5) residual fraction, tightly bound on silicate matrix of samples. Filgueiras et al. [13] reviewed a few hundred of these applications of various sequential extraction procedures applied to the fractionation of elements in environmental samples such as sediment, soil, sewage sludge, coal fly ash, solid waste incineration bottom ash, airborne dust, etc. A large diversity of sequential extraction schemes concerning the extraction reagents, operating conditions and number of stages involved is evident. The authors emphasized that small changes in the experimental conditions (e.g. pH, temperature, contact time, solid to extractant volume ratio, particle size, sample pretreatment) can lead to large variations in the fractionation, makeing it troublesome for comparisons between results. These findings were confirmed by many soil scientists e.g. [14-16] for both single and sequential extraction procedures. As well as conventional extraction methods, accelerated sequential extraction procedures such as continuous – flow procedure [17], microwave [18, 19], and ultrasound assisted [20] procedures were also published. Recently, continuous leach inductively coupled plasma mass spectrometry (CL-ICP-MS) was described [21, 22]. This method provides information on the specific geochemical sites and mineral phases from which elements are being released using real-time data generated by continually analysing progressively reactive solutions from water through to 30 % nitric acid as they are pumped directly into a high resolution ICP mass spectrometer. Mineral breakdown reactions can be monitored from the major elements released, thereby eliminating uncertainties related to host phase/trace element associations. By comparing major and minor element release patterns, trace elements can be reliably assigned to host phases. Results from single mineral phases, mixtures of mineral phases, and natural ore samples indicate that the release of elements from specific minerals is not obscured in more complex samples and that reprecipitation and back reactions are not a concern with this method. Scanning electron micrograph (SEM) examination of the reaction products has been used to verify and support the CL-ICP-MS data interpretation. Results for natural soil samples indicate that 'false' mobile element anomalies can be identified using CL-ICP-MS and underscore the importance of understanding where trace elements reside in samples used for environmental studies or mineral exploration [22]. However, the instrumentation necessary for these experiments is not available in most routine agrochemical laboratories.

In the European Union (EU), unification of methods for a simple and sequential extraction and also the preparation of certified reference soil materials is one of the aims of the Standards, Measurement and Testing (SM&T) department of the EU (now the Institute for Reference Materials and Measurements) [23]. The proposed SM&T sequential extraction scheme separates the extractable elements into three fractions: (1) acid soluble fraction, (2) reducible fraction, and (3) oxidizable fraction and was developed for harmonization of different extraction schemes. Validation and harmonization of sequential analytical schemes was fulfilled for two sediment samples (CRMs, BCR, 601, and 701) while validation of extractable metal contents in additional solid materials such as sewage sludge, coal fly ash or airborne dust require further research. Concerning the standardized sequential extraction procedure developed by the Standards, Measurement and Testing Programme, the most important source of variability appeared to be the pH of the $NH_2OH.HCl$ reagent in step 2 and to a lesser extent alteration of mass of soil sample [24, 25]. Moreover, the extractants are not completely specific and efficient. Shan and Chen [26] documented the readsorption of elements onto other solid geochemical phases during sequential extraction. Ayoub et al. [27] assessed the labile pools of Cd and Zn in soils using isotopic exchange techniques and concluded the lack of specificity of chemical extractants (EDTA and CH_3COOH) to release quantitatively the bioavailable metal pool.

However, the interlaboratory trial of sediment samples showed unacceptable spread of results in some cases resulting in indicative values instead of certified ones. Evidently, the operationally defined character of the sequential analytical scheme means that the conditions established must be strictly followed if a good agreement among fractionation results obtained in different laboratories are to be obtained [13]. Mossop and Davidson [28] presented the results of sequential extraction procedure for a set of soils and sediments. The samples were extracted into two batches in separate weeks and the reproducibility of the extraction procedure was evaluated. The between-batch variability of the results differed according to sample type and element determined, for example, in the case of Fe content in freshwater sediment resulted as follows: 4.3 % (fraction 1) and 83.4 % (fraction 3) higher in the first batch and 33.6 % lower in fraction 2. These results documented the relatively poor reproducibility and repeatability of the sequential extraction scheme even in the same laboratory under the same operational conditions.

Although various excellent reviews summarize the existing sequential analytical procedures, their applications, validation of the methods, analytical aspects, and interpretations of the data, were recently published [13, 29-31], they evaluated predominantly results from different laboratories representing a wide spectrum of different environmental and agricultural materials. The investigations comparing the data obtained by at least two extraction procedures applied to the same set of samples as presented for example by Mester et al. [32] and Alvarez et al. [33] are relatively limited. However, such comparisons can still be helpful for better knowledge of applicability of the extraction methods and more reasonable interpretation of the data. This study compares the results of fractionation of As, Cd, Pb, and Zn in twelve different soils using the SM&T extraction scheme [34] with two frequently applied sequential extraction procedures [35,36] which have been developed for determination of wide spectrum of elements, especially metals. Because of different chemical properties of arsenic, two single-purpose sequential extraction schemes reflecting the variable behavior of this element in soil have been included into the investigation and tested in the frame of this experiment [37,38].

2 Experimental

Twelve soil samples differing in their physical-chemical properties and total element contents (Table 1 and 2) were extracted by three sequential extraction procedures (Table 3) involving SM&T extraction scheme - method A [34], classical Tessier's scheme slightly modified by Li et al. – method B [35], and the scheme published by Zeien – method C [36]. Additional sequential extraction procedures according to Wenzel et al. – method D [37] and Azcue et al. – method E [38] were applied for fractionation of soil containing arsenic. Before extraction the soil samples were air-dried at 20 °C, ground in a mortar and passed through a 2-mm plastic sieve. Each extraction was provided in five replications, all the chemicals used were of electronic grade purity and were purchased from Analytika and Lach-Ner Ltd., Czech Republic. For the centrifugation of the extracts, the Hettich Universal 30 RF (Germany) device was used. The reaction mixture was centrifuged at 3000 rpm (i.e. 460xg) for 10 minutes at the end of each step and supernatants were kept at 6 °C before measurement. Blank extracts representing 5 % of total number of extracts were prepared using the same batch of reagents and the same apparatus and analyzed in the same time and in the same way as soil extracts. The residual fraction was calculated as the difference of total element content and sum of released fractions. Validation of the SM&T extraction procedure in our laboratory conditions was presented separately [39].

The total element concentrations in the soil samples were determined separately in digests obtained by a two-step decomposition as follows: half of the sample was decomposed by dry ashing in a mixture of oxidizing gases (O_2+O_3+NO_x) in an Apion Dry Mode Mineralizer (Tessek, CZ) at 400 °C for 10 h; the ash was then decomposed in a mixture of HNO_3 + HF, evaporated to dryness at 160 °C and dissolved in diluted a*qua regia* [4]. Certified reference material RM 7001 Light Sandy Soil (Analytika, CZ) containing 12.3 ± 1.1 mg As kg^{-1}, 0.32 ± 0.05 mg Cd kg^{-1}, 43.8 ± 3.7 mg Pb kg^{-1}, and 120 ± 7 mg Zn $kg^{-1}$was used for quality assurance of the analytical data of total element determination.

In this material it was determined that the elemental content was as follows 14.7 ± 2.4 mg As kg^{-1}, 0.29 ± 0.00 mg Cd kg^{-1}, 35.9 ± 1.3 mg Pb kg^{-1}, and 112 ± 4 mg Zn kg^{-1}.

The element concentrations in soil extracts were determined by atomic absorption spectrometry as follows: Arsenic was determined by a continuous hydride generation technique using the Varian SpectrAA-300 (Australia) atomic absorption spectrometer equipped by hydride generator VGA-76. A mixture of potassium iodide and ascorbic acid was used for pre - reduction of the sample and HCl acidified the extract before measurement. For cadmium and lead determination a Varian SpectrAA-400 (Australia) flameless atomic absorption spectrometer with GTA-96 graphite furnace atomizer was applied. The pyrolytically coated tubes with L'vov platform and matrix modifier based on $NH_4H_2PO_4$ solution were used for all the measurements. For the determination of zinc, the flame atomization (air-acetylene flame) was applied (Varian SpectrAA-300 atomic absorption spectrometer). Analytical results for the elements are based on standard addition measurement mode in all the cases. All reagents used were of electronic grade purity (Analytika, Ltd., Czech Republic).

Wilcoxon's non-parametric test ($\alpha = 0.05$) and linear regression model were applied for the evaluation of the analytical data using Statgraphics 5plus statistical software.

3 Results and discussion

Complete results of the elemental contents in individual fractions within the investigated sequential extraction schemes are summarized in Tables 4 and 5. Compared to SM&T extraction, methods B and C for the first fraction were divided into two parts so as to describe in more detail, the most mobile portion of the elements. Method C was concentrated on detailed characterization of elements binding onto clay minerals resulting in resolution of element portions in three soil oxide fractions. For the comparison of analytical data with the SM&T method, the sum of mobile fractions (fraction 1+2) was calculated for methods B and C and the sum of oxide fractions (fractions 3+5+6) for method C. Similarly, the same approach was applied for the methods D and E, where the sum of mobile fractions (method D) and sum of oxide fractions (methods D and E) were calculated, as well. Figure 1 illustrates relative distribution of determined elements into individual groups of soil fractions.

3.1 Arsenic

The arsenic proportion released as the sum of mobile fractions among methods A, B, and C varied in range from 0.85 % (method B) to 4.2 % (method A) of total arsenic content in soil, and the differences among the methods were significant at $\alpha = 0.05$. A different pattern was observed for methods D and E designed especially for arsenic fractionation. Arsenic portions released as sum of mobile fractions reached 7.1 % for method D, and 7.8 % for method E, respectively. The methods D and E did not differ significantly and correlated tightly with r = 0.92 while most of the correlations with some of the methods A, B, and C were negligible. The results were not affected by any of the soil characteristics (pH, soil organic matter, CEC) except that for method D, where the correlation coefficients between extractable arsenic for soil pH (0.46) and CEC (0.57) indicated the relationships among these parameters. According to the methods A, B and C, the highest mobility of arsenic was observed in the soil sample No. 8, light sandy Fluvisol, whilst the methods D and E showed the highest mobility in the sample of neutral Chernozems No. 1. For a sum of oxide fractions, medians of released arsenic represented between 2.9 % (method B) and 25.6 % (method D) of the total arsenic content. In this case, the extractable arsenic contents were also not related to the measured soil characteristics. However, regardless of the different values determined by individual procedures the highest extractability was reported for Cambisols No. 3 and 4, and sandy-loam Fluvisol No. 12. These soils are characterized by relatively low cation exchange capacity but differed by organic matter content (Table 1) as well as by the total arsenic (Table 2). In the case of determination of organically bound arsenic, arsenic proportions varied between 2.2 % (method C) and 7.4 % (method B) differing significantly at $\alpha = 0.05$. The data obtained by using procedure E were not significantly related to any of the remaining extraction methods. The high proportion of arsenic in this fraction was reported for soils with a high content of oxidizable carbon (Cambisol No. 4, and Fluvisol No. 12) as well as in the samples with low C_{ox} level (Fluvisol No. 8, and Cambisol No. 3). This trend was confirmed by all the investigated extraction procedures. Concerning residual fraction representing the most stabile and non-extractable element fraction the results confirmed low mobility of soil arsenic and ranged between 71 % (method C) and 87 % (method B). The comparison of results obtained by five different sequential extraction procedures is very difficult, especially in the case where arsenic is most easily extractable. The data did not indicate any relationships among the extraction procedures as well as among the basic soil characteristics. In this case, verification of the selectivity of the individual extraction steps will be necessary, including readsorption and redistribution of the arsenic during fractionation. [40] Using a higher number of samples within the experimental sets will be necessary for further research. The results showed also that an important source of inconsistency of the results obtained by individual extracting procedures is caused by different approaches to dissolution of the arsenic bound onto Fe/Mn oxides in soil. Oxalate buffer extracted arsenic bound to crystalline Fe oxides, while hydroxylamine hydrochloride solutions are characterized as the agents releasing predominantly Mn oxides – associated elements. Moreover, re-adsorption of arsenic on goethite surfaces was observed if acidified hydroxylamine hydrochloride (0.25 mol L^{-1}) was applied as the extractant while an excess of oxalate present during extraction minimized the re-adsorption [37]. For the evaluation of the effect of soil characteristics on the arsenic distribution into soil fractions defined by individual extraction procedures more detailed description of soil organic matter composition as well as mineralogical composition of the soils should be substantial according to the recommendation published by Hudson-Edwards et al. [31].

3.2 Cadmium

The sum of the mobile fractions released by the individual extraction procedures differed significantly in most of the cases according to the extraction procedure applied. The results confirmed high mobility of cadmium compared to arsenic, lead and zinc and the sum of mobile fractions ranged between 33.3 % for method C and 39.7 % for method B. For method C, strong relationships with individual soil characteristics were determined for NH_4NO_3 extracts (r = -0.61 for C_{ox}, r = -0.53 for CEC, and r = -0.75 for pH levels, respectively). In contrast, the most mobile cadmium portions determined with $MgCl_2$ extracting agent within the method B did not significantly correlate with any of the tested soil characteristics. Method A where the strongest extracting agent (acetic acid) indicated also the effect of soil CEC (r = -0.64), and pH (r = -0.42). Evidently, the individual extractants characterizing the most mobile soil cadmium fractions are able to attack different types of weak element bounds in individual soils. Moreover, a wide spectrum of physicochemical parameters of the tested set of soils introduced too many factors for statistical evaluations of the results. According to Gray et al. [41] the weak extractants such as 0.01 mol L^{-1} $CaCl_2$ and 1 mol L^{-1} NH_4NO_3 are sensitive to soil pH changes because of their low buffering capacity and are considered as representative of plant-available metal conditions. For oxide fractions of cadmium ranged between 26.3 % for method C and 48 % for method A. The methods A and B showed fairly good positive correlations between cadmium fractions bound on Fe/Mn soil oxides, and soil CEC and pH (r = 0.69, and 0.87 for method A, and r = 0.64, and r = 0.87 for method B, respectively). For method C dividing the oxide fraction into three separate fractions no significant influence of soil characteristics on cadmium extractability was found. In this case, correlations of individual cadmium portions with the contents of individual Fe/Mn oxides in soil will be more reasonable for such evaluations. Ambiguous results were determined for the organically bound fraction (from 1.4 % for method A to 27.1 % for method C) where positive correlations were determined for method C (significant in the case of soil CEC; r = 0.49) whereas the organically bound fractions determined by the methods A and B were not correlated except for the C_{ox} soil in the case of method B (r = -0.52). Surprisingly, the highest organically bound fraction was determined in the Cambisol No. 3 (70 % by method B, 50 % by method C, not confirmed by method A – only 21 %) characterized by the lowest content of oxidizable carbon. Probably, composition of organic matter will play an important role in this case. The low total content of cadmium in this soil result in cadmium contents extracted within individual fractions close to detection limit of analytical method and subsequently in lower precision of the results and possible overestimation of the data in this case.

Gray et al. [42] adapted the sequential extraction procedure from Shuman [43] for fractionation of cadmium in a set of New Zealand soils resulting in mean proportions of Cd present in the individual fractions in the order: residual (38 %) > organic (35 %) > > amorphous oxide (13 %) > crystalline oxide (12 %) > exchangeable (3 %). The relatively high Cd portion in organic fraction, and low portion of this element in the two oxide fractions were explained by high content of organic carbon connected with low content of iron in the soils studied. The application of original Tessier's scheme [11] for fractionation of cadmium in highly contaminated soils showed 37 % of this element in exchangeable fraction and 15 % in residual fraction [44]. In our case, similar results were confirmed for Cambisols while low portions of residual cadmium was determined in the case of Fluvisols and Chernozems where the values varied between 0.2 % (Chernozem No. 1), and 5.2 % (Fluvisol No. 8) of total cadmium content. An exception was represented by acid Cambisol No. 3 (0.1 %) where the dominant portion of cadmium was represented by an organically bound fraction as mentioned above. The role of soil type and soil physicochemical parameters in cadmium, fractionation in soils is evident in the case of cadmium to a higher extent compared to other elements.

3.3 Lead

A high portion of this element in the sum of oxide fractions (from 31.0 % for method B to 32.7 % for method A) and organically bound fraction (from 28.2 % for method C to 42.1 % for method B was typical. However, the relationships of these lead portions with soil characteristics were ambiguous. Method A suggested a slightly lower proportion of lead was bound to Fe/Mn oxides in Cambisols No. 3, 4, 9, and 10, characterized by comparable soil sorption capacity, while method B showed the lower extractability of this fraction in Chernozems No. 1 and 7. In the case of method C, the total sorption capacity of the soil without detailed determination of the contents of Fe/Mn oxides in soil is insufficient for these relations. For the organically bound fraction, the dominant lead fraction in the investigated soils, no significant relations to the soil characteristics were calculated whereas Fernández et al. [8] showed positive correlations between soil pH and oxidizable fraction of Pb by using method A. Bacon et al. [45] emphasized that the organic nature of the soils must be taken into account in this case. The sum of mobile fractions varied in the range between 0.8 % for method A and 3.7 % for method B, where the carbonate bounded Pb was the most important. In comparison to cadmium, among the extractants releasing the most mobile element fractions only NH_4NO_3 extractable lead portions within method C were related to the investigated soil parameters (r = 0.62 for soil C_{ox}, r = 0.63 for CEC, and 0.41 for pH). The high affinity of lead to organic matter in soil as well as a low proportion of mobile Pb in soil supporting low plant-availability of this element was confirmed also by other authors [46].

Parat et al. [47] compared three sequential extraction procedures to determine element fractions in acid sandy soil differing in number and/or order of extracting agents in sequence. Lead distribution varied according to the extractant used to release organic fraction of the elements where sodium hypochlorite (pH 8.5) was able to release lower portion of this element compared to hydrogen peroxide (pH 2) probably due to higher pH of the reagent. In our experiment, neutral or acidic extractants were applied and this effect was not the source of inter-procedural differences. Most probably, more detailed description of the origin and composition of soil organic matter and Fe/Mn oxides in soil should be included into these evaluations.

3.4 Zinc

Zinc belongs to the relatively mobile elements as reflected in the sum of mobile fractions ranging between 3.5 % for method A and 7.6 % for method B. Among the extractants applied to release the most mobile portions of zinc was $MgCl_2$ (method B) which showed the closest correlations with the investigated soil characteristics (r = -0.62 for C_{ox}, r = -0.70 for CEC, and r = -0.64 for soil pH, respectively). In contrast, the following evaluated fractions varied broadly where the sum of oxide fractions was between 7.3 % for method A and 22.6 % for method C and the organically bound fraction between 7.4 % for method C and 22.3 % for method A. While the method A did not show any relationship between the organically bound zinc fraction and total oxidizable carbon in the soil, the organically bound fractions released by methods B and C had significant positive correlations (r = 0.69, and 0.58, respectively). Generally, the extractable zinc portions within individual extraction procedures were not significantly affected by soil type and soil texture occurring in the investigated set of soil samples. The residual fraction of zinc ranged between 70.2 – 82.8 %. Comparable to cadmium, the residual fractions evaluated where the closest relationships among the extraction methods suggested a stability of the sum for the potentially mobilizable portions of zinc but the sum of oxide fractions and organically bound fraction considerably differed in the individual extraction procedures. However, poor reliability of the SM&T extraction scheme (method A) for Zn contents in individual fractions of uncontaminated soils was documented by Fernández et al. [8]. In our case except for soils No. 4 and 12 the soil samples can also be considered as representatives of low zinc contamination.

The application of Shuman's sequential extraction procedure [43] for the set of New Zealand soils showed substantial proportions of zinc associated with amorphous (18.5 %) and crystalline (24.2 %) iron oxides and average 40 % of total zinc remained in residual fraction [48]. Thus, predominant effect of soil physicochemical parameters on distribution of this element into main soil fractions is evident in this case as for other elements, and confirmed by other authors [48]. Parat et al. [47] concluded that the use of sodium acetate as the agent extracting active calcium carbonate affected significantly the distribution of zinc within the reducible fractions while the results of procedures II and III, where this extraction step was omitted, were comparable. Our results showed complementary effect of the reducible fractions and the organically bound fraction where sum of these fractions was comparable regardless of the extraction procedure.

4 Conclusion

The results confirmed that determination of element portions bound in individual soil fractions is strongly dependent on extracting agent and/or procedure applied within individual extracting schemes. Even small modification of sequential extracting procedure can result in significant variability of results among laboratories. Procedural variables of individual extraction procedures can lead in some cases to the contradictions in practical interpretation of analytical data [44]. Additionally, different approaches to the statistical evaluation of the data should also affect the interpretation of the data. As expected, absolute values of elements released among individual extracting procedures are weakly comparable. Many authors compared the existing sequential extraction schemes, most frequently SM&T extraction scheme [34] and classical Tessier's scheme [11] where most of the studies were targeted on sediment and sewage sludge samples [13]. Concerning soils, higher extraction capacity of lead in the case of soil oxide fraction was reported [49] for Tessier's scheme compared to SM&T extraction scheme and confirmed by our results. In the model systems, the manganese oxide and humic acid phases were found to be responsible for most of this redistribution in both schemes. In contrast to our results Tessier's scheme showed higher overall extractability for elements from montmorillonitic soils [50] but our set of experimental soils was characterized mostly by lower sorption capacity (Table 1).

Exceptional position of arsenic among the other potentially toxic elements in soil chemistry led to an individual approach in development of sequential extraction schemes. The sequential release of loosely and strongly adsorbed arsenic, arsenic coprecipitated with metal oxides or amorphous monosulfides, As co-precipitated with crystalline iron oxyhydroxides, As oxides, As co-precipitated with pyrite, and As sulfides was described by Keon et al. [51]. Mihaljevič et al. [52] compared four sequential extraction procedures including the classical Tessier's scheme for arsenic fractionation from a geochemical point of view. They considered only the procedure published by Hall et al. [53] suitable for arsenic fractionation since, in this case, good agreement between observed and expected extractions was found on simple mineral mixtures. The method characterizing i) exchangeable fraction by 1 mol L^{-1} CH_3COONa, ii) fraction bound to amorphous Fe oxyhydroxides by 1 mol L^{-1} $NH_2OH.HCl$ in 0.25 mol L^{-1} HCl, iii) fraction bound to crystalline Fe oxyhydroxides by 0.25 mol L^{-1} $NH_2OH.HCl$ in 25 % CH_3COOH, iv) fraction bound to sulfides and organic matter by 8.8 mol L^{-1} H_2O_2 + 3.2 mol L^{-1} CH_3COONH_4, and v) residual fraction by HF + $HClO_4$, was evaluated as insufficient for accurate arsenic fractionation, but applicable for approximate identification of distribution into labile, medium labile, and residual forms in polluted soils. As summarized by Hudson-Edwards et al. [31], a detailed description of the soils to be analyzed is necessary before choosing the extraction procedure where the application of more than one procedure is recommended as well as the application of complementary techniques for verification of the results.

A complete summary of existing sequential extraction procedures including detailed comparison of these methods and/or extracting agents applied within individual extraction schemes was recently covered by Filgueiras et al. [13]. Instead recommendations for further research, the necessity to prepare new reference materials with certified extractable contents of elements, harmonization of sequential extraction schemes, development of extraction schemes specifically optimized for the characteristics of the target samples, and the use of chemometrics for assessment of sequential extraction protocols and for finding relationships between soil fractions and plant uptake of elements have to be emphasized in this context. These conclusions are fully acceptable for our experiment as well. The differences in the extractability of individual elements where both extracted element and the extracted soil sample influenced the distribution of elements into individual fractions as documented by previous comparative studies [32, 45]. More reliable results, better related to the soil characteristics, were determined for more mobile soil elements i.e. cadmium and zinc in the fractions characterizing the most mobile portions of investigated elements. In contrast, ambiguous results were observed for As and Pb for both the individual extraction procedures and the effect of the soil characteristics. Regardless of the studied element, less reasonable results were determined for reducible and oxidizable soil fractions. Moreover, analytical problems given by low level of elements in uncontaminated soils represented substantial problems especially if the element content is to be divided to higher number of fractions (method C). From this point of view, a more simple procedure such as method A would be able to give more informative results. For more detailed evaluations of elements bound to individual soil fractions and information concerning the nature and composition of experimental soils will be substantiated in future research. The application of at least two independent procedures or modification of the extraction scheme according to element investigated and/or particular soil characteristics can also be helpful in definition of element pattern in soils.

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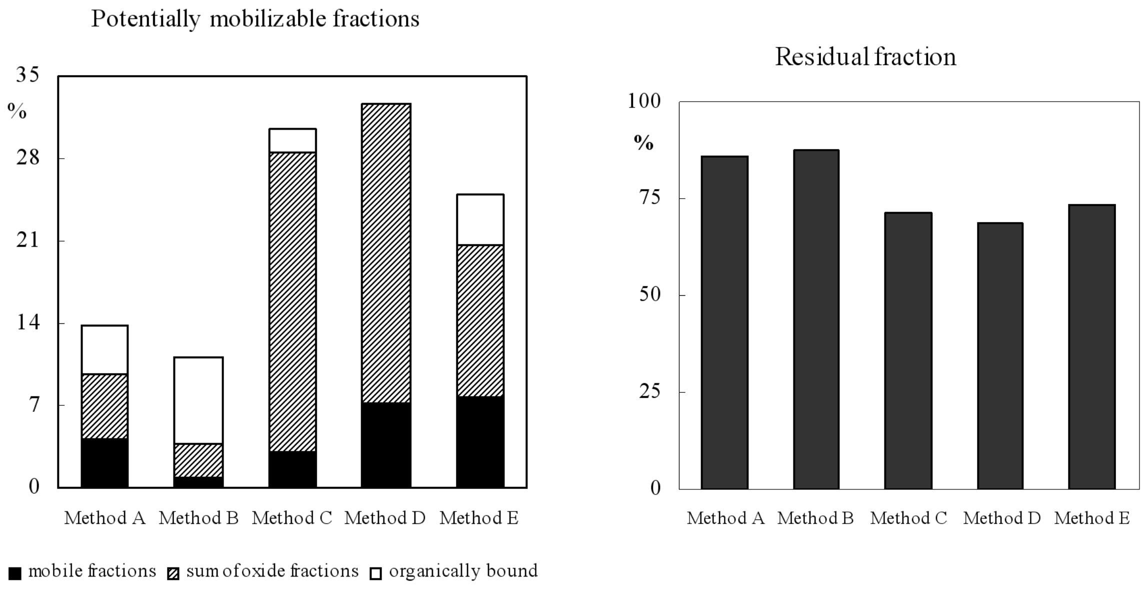
Arsenic



Cadmium

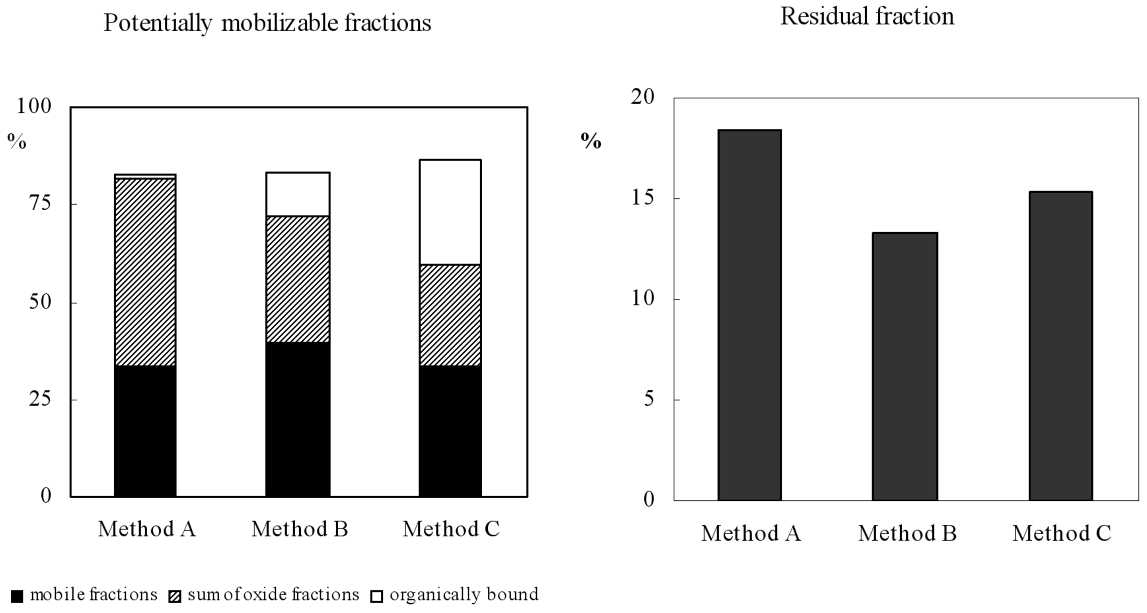
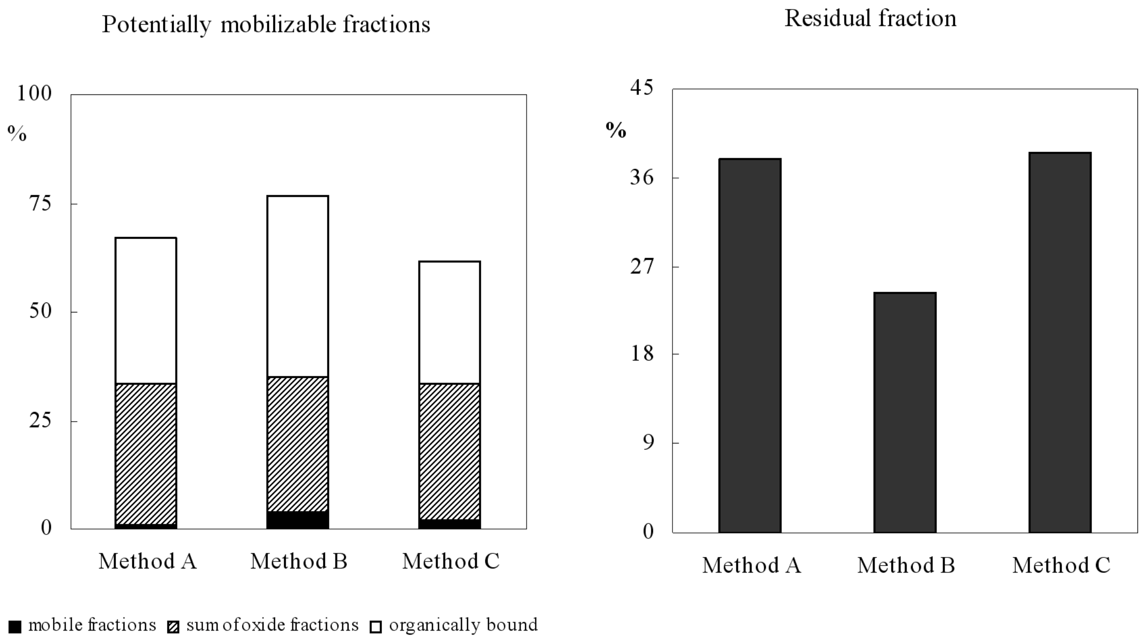


Fig. 1 Relative distribution of elements into different soil fractions according to individual sequential extraction procedures (sum of mobile fractions (fraction 1+2) was calculated for methods B, C, and D and sum of oxide fractions (fractions 3+4+5) for methods C, D, and E).

Lead



Zinc

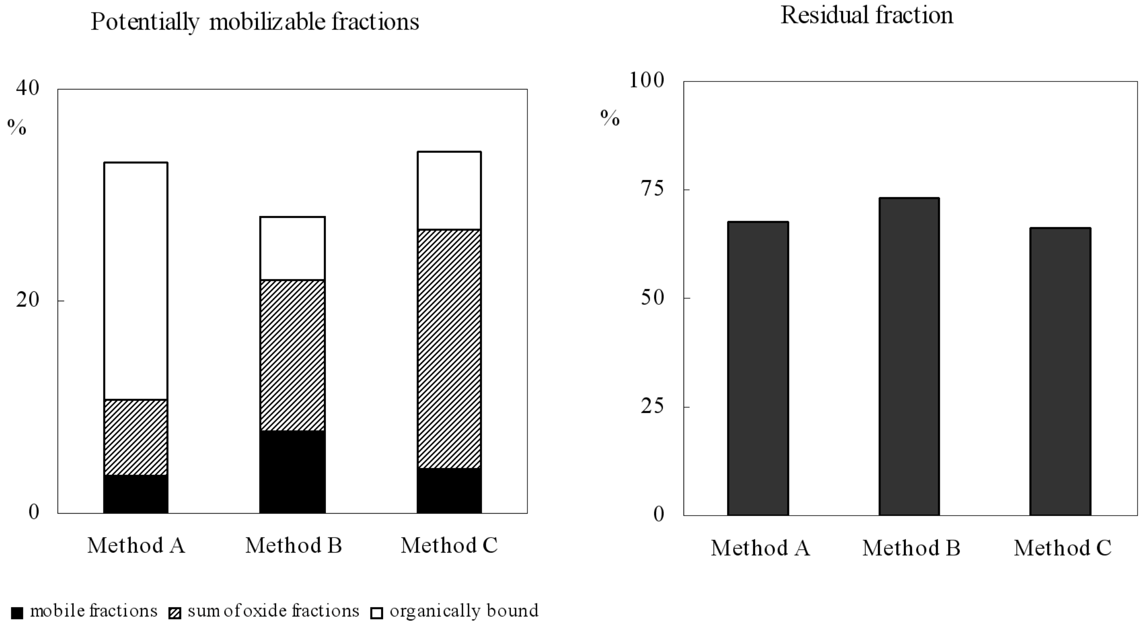


Fig. 1 (continued) Relative distribution of elements into different soil fractions according to individual sequential extraction procedures (sum of mobile fractions (fraction 1+2) was calculated for methods B, C, and D and sum of oxide fractions (fractions 3+4+5) for methods C, D, and E).

Soil No.	C_{ox} %	CEC mmol kg^{-1}	pH	soil type	texture
1	1.61	252	7.1	Chernozem	clay-loam
2	1.71	162	6.8	Luvisol	clay-loam
3	0.41	110	5.0	Cambisol	sandy
4	2.40	171	5.8	Cambisol	loam
5	1.13	135	5.7	Luvisol	loam
6	1.14	118	6.4	Luvisol	loam
7	1.39	232	7.2	Chernozem	clay-loam
8	0.68	85	5.7	Fluvisol	sandy
9	1.64	153	5.1	Cambisol	loam
10	1.06	129	5.1	Cambisol	loam
11	1.25	134	5.1	Cambisol	loam
12	2.24	212	6.4	Fluvisol	sandy loam

C_{ox} – total content of oxidizable carbon,
CEC – cation exchange capacity

Table 1 Basic characteristics of the set of experimental soils.

Soil No.	As		Cd		Pb		Zn	
	AVG	SD	AVG	SD	AVG	SD	AVG	SD
1	15.0	2.8	0.239	0.003	30	1.1	108	6
2	13.1	0.8	0.219	0.013	17.8	0.7	85.5	4.8
3	254	38	0.025	0.001	28.9	8.4	69.6	3.1
4	38.0	3.0	9.89	1.27	1777	33	323	21
5	11.8	0.4	0.234	0.055	16.6	0.7	70.2	3
6	11.7	0.8	0.194	0.004	15.8	0.5	63.2	1.7
7	18.7	3.1	0.256	0.019	46.8	18.4	112	5
8	5.04	0.5	0.156	0.001	9.29	2.6	51.5	5.4
9	15.6	1.6	0.392	0.064	46.0	9.3	141	2
10	13.8	2.5	0.167	0.004	30.5	1.8	119	8
11	15.0	0.6	0.277	0.009	21.6	2.4	106	3
12	22.2	3.6	2.76	0.31	99.1	2.3	413	4

AVG – average
SD – standard deviation

Table 2 Total contents of investigated elements in the experimental soils (mg kg^{-1}), n = 3.

Method A (SM&T), Quevauviller [32]

	Fraction	
1	exchangeable	0.11 mol L^{-1} acetic acid,1:40 w/v for 5 h
2	Fe-Mn oxides	0.1 mol L^{-1} $NH_2OH.HCl$,1:40 w/v for 16 h
3	organically bound	8.8 mol L^{-1} H_2O_2 + 1 mol L^{-1} CH_3COONH_4, 1:100 w/v for 16 h

Method B, Li et al. [33]

	Fraction	
1	exchangeable	0.5 mol L^{-1} $MgCl_2$,1:9 w/v for 1 h
2	carbonate	1 mol $L^{-1}$$CH_3COONa$, 1:8 w/v for 5 h
3	Fe-Mn oxides	0.04 mol L^{-1} $NH_2OH.HCl$, 1:20 w/v for 6 h
4	organically bound	8.8 mol L^{-1} H_2O_2 + 3.2 mol L^{-1} CH_3COONH_4, 1:5 w/v for 30 min

Method C, Zeien [34]

	Fraction	
1	mobile	1 mol L^{-1} NH_4NO_3, 1:25 w/v for 24 h
2	weakly bound	1 mol L^{-1} CH_3COONH_4, 1:25 w/v for 24 h
3	Mn oxides	0.1 mol L^{-1} $NH_2OH.HCl$, 1:25 w/v for 30 min
4	organically bound	0.025 mol L^{-1} NH_4EDTA, 1:25 w/v for 90 min
5	Fe-Al amorphous oxides	0.2 mol L^{-1} $(COONH_4)_2$, 1:25 w/v for 4 h
6	Fe-Al crystalline oxides	0.1 mol L^{-1} ascorbic acid + 0.2 mol L^{-1} $(COONH_4)_2$, 1:25 w/v for 30 min at 96 ± 3 °C

Method D, Wenzel et al. [35]

	Fraction	
1	exchangeable	0.05 mol L^{-1} $(NH_4)_2SO_4$, 1:25 w/v for 4 h
2	specifically sorbed	0.05 mol $L^{-1}$$(NH_4)H_2PO_4$, 1:25 w/v for 16 h
3	Fe-Al amorphous oxides	0.2 mol L^{-1} $(COONH_4)_2$, 1:25 w/v for 4 h
4	Fe-Al crystalline oxides	0.1 mol L^{-1} ascorbic acid + 0.2 mol L^{-1} $(COONH_4)_2$, 1:25 w/v for 30 min at 96 ± 3 °C

Method E, Azcue et al. [36]

	Fraction	
1	exchangeable	1 mol L^{-1} CH_3COONa, 1: 20 w/v for 6+6 h
2	Fe -Mn amorphous oxides	0.25 mol L^{-1} $NH_2OH.HCl$ in 25% HCl, 1:20 w/v for 2+0.5 h
3	Fe crystalline oxides	1 mol L^{-1} $NH_2OH.HCl$ in 25% HCl, 1:20 w/v for 3+1.5 h
4	organically bound	2 mol L^{-1} HNO_3, 1:10 w/v for 6 h

Table 3 Sequential analytical procedures compared in the experiment Selective sequential extraction schemes reflecting different behavior of arsenic in soil.

Fraction		As mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}
Method A (SM&T), Quevauviller [32]					
F 1	exchangeable	0.047 - 29.2 (4.2)	0.011 - 4.11 (35)	0.086 - 111 (0.75)	0.751 - 89.2 (3.5)
F 2	Fe-Mn oxides	0.206 - 106 (5.5)	0.023 - 4.12 (43)	0.591 - 896 (33)	2.51 - 151 (7.2)
F 3	organically bound	0.179 - 16.4 (4.2)	<0.001 - 0.053 (1.4)	0.879 - 443 (34)	9.09 - 100 (22)
Method B, Li et al. [33]					
F 1	exchangeable	<0.004 - 0.667 (0.16)	<0.001 - 4.14 (21)	<0.01 - 9.29 (<0.1)	<0.053 - 8.22 (2.2)
F 2	carbonate	0.015 - 0.893 (0.69)	0.004 - 1.19 (17.6)	0.255 - 186 (3.7)	3.03 - 51.6 (5.4)
F 3	Fe-Mn oxides	0.151 - 54.5 (2.9)	0.006 - 2.47 (32)	2.04 - 566 (31)	4.08 - 202 (14.4)
F 4	organically bound	0.385 - 35.5 (7.3)	0.007 - 0.684 (11.1)	0.574 - 554 (42)	1.14 - 64.7 (6.0)
Method C, Zeien [34]					
F 1	mobile	<0.03 - 2.45 (0.57)	0.011 - 0.866 (8.9)	0.065 - 13.8 (0.60)	<0.022 - 12.4 (1.2)
F 2	weakly bound	0.036 - 6.77 (2.4)	0.007 - 2.55 (24)	<0.037 - 17.9 (1.3)	1.16 - 54.6 (2.9)
F 3	Mn oxides	<0.02 - 1.20 (0.45)	<0.004 - 0.498 (4.5)	<0.048 - 84.5 (0.27)	0.325 - 20.5 (1.1)
F4	organically bound	0.049 - 14.1 (2.1)	0.017 - 4.49 (27)	0.827 - 990 (28)	1.84 - 136 (7.4)
F 5	Fe-Al amorph. ox.	0.952 - 1 21 (14.9)	<0.017 - 0.896 (10.2)	1.24 - 209 (22)	5.03 - 104 (11.6)
F 6	Fe-Al crystall. ox.	0.510 - 48.7 (9.9)	<0.017 - 0.438 (8.7)	0.923 - 83.1 (8.8)	4.91 - 83.6 (10.1)

Table 4 The range of element contents in individual soil fractions according to investigated sequential extraction procedures (mg·kg^{-1}), in parentheses median of ratio of the extraction (%), n = 5.

Method D		
F1	exchangeable	<0.015 – 3.62 (0.58)
F2	specifically sorbed	0.275 – 56.7 (6.5)
F3	Fe-Al amorphous oxides	1.13 – 32.3 (13.1)
F4	Fe-Al crystalline oxides	0.398 – 35.6 (12.5)

Method E		
F1	exchangeable	0.073 – 43.5 (7.8)
F2	Fe -Mn amorphous oxides	0.257 – 67.0 (5.1)
F3	Fe crystalline oxides	0.348 – 137 (7.7)
F4	organically bound	0.027 – 11.6 (4.3)

Table 5 The ranges of arsenic contents in individual fractions according to Method D, Wenzel et al. [35] and Method E, Azcue et al. [36] ($mg \cdot kg^{-1}$), in parentheses median of ratio of the extraction (%), n = 5.