

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION\*

## DETERMINATION OF TRACE ELEMENTS BOUND TO SOILS AND SEDIMENT FRACTIONS

(IUPAC Technical Report)

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# Determination of trace elements bound to soils and sediment fractions

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*Abstract:* This paper presents an overview of methods for chemical speciation analysis of elements in samples of sediments and soils. The sequential leaching procedure is thoroughly discussed, and examples of different applications are shown. Despite some drawbacks, the sequential extraction method can provide a valuable tool to distinguish among trace element fractions of different solubility related to mineralogical phases. The understanding of the speciation of trace elements in solid samples is still rather unsatisfactory because the appropriate techniques are only operationally defined. The essential importance of proper sampling protocols is highlighted, since the sampling error cannot be estimated and corrected by standards. The Community Bureau of Reference (BCR) protocols for sediment and soil give a good basis for most of the solid samples, and the results can be compared among different laboratories.

## INTRODUCTION

In environmental sciences, the development of monitoring systems is of main importance. Increasingly strict environmental regulations require the development of new methods for analysis and ask for simple and meaningful tools to obtain information on metal fractions of different mobility and bioavailability in the solid phases. The objectives of monitoring are to assess pollution effects on humans and the environment, to identify possible sources, and to establish relationships between pollutant concentrations and health effects or environmental changes [1–7]. Thus, it is necessary to investigate and understand the transport mechanisms of trace elements and their complexes to understand their chemical cycles in nature. Concerning natural systems, the mobility, transport, and partitioning of trace elements are dependent on the chemical form of the elements. The process is controlled by the physicochemical and biological characteristics of that system. Major variations of these characteristics are found in time and space due to the dissipation and flux of energy and materials involved in the biogeochemical processes that determine the speciation of the elements. Solid components govern the dissolved levels of these elements via sorption–desorption and dissolution–precipitation reactions. For the assessment of the environmental impacts of a pollutant, some questions regarding the solid-phase water system must be answered [8]:

- What is the reactivity of the metals introduced with solid materials from anthropogenic activities (hazardous waste, sewage sludge, atmospheric deposits, etc.) by comparison with the natural components?
- Are the interactions of crucial metals between solution and solid phases comparable for natural and contaminated system?
- What are the rules of solid–solution interactions on the weaker bonding of certain metal species, and are the processes of remobilization effective in contaminated as compared with the natural system?

Nowadays, it is evident that element speciation has become a major aspect in analytical and bioinorganic chemistry. In an IUPAC guideline for terms related to speciation of trace elements: “Definitions, structural aspects and analytical methods”, definitions of terms related to speciation and fractionation are [9]:

- Speciation (in chemistry) of an individual element refers to its occurrence in or distribution among different species (chemical speciation).
- Speciation analysis is the analytical activity of identifying and quantitating one or more chemical species of an element present in a sample.
- Species (in chemistry) denotes an element in a specific and unique molecular, electronic, or nuclear structure (chemical species).

Chemical extraction is employed to assess operationally defined metal fractions, which can be related to chemical species, as well as to potentially mobile, bioavailable, or ecotoxic phases of a sample. According to Verloo et al. [10], the mobile fraction is defined as the sum of the amount dissolved in the liquid phase and an amount, which can be transferred into the liquid phase. It has been generally accepted that the ecological effects of metals (e.g., their bioavailability, ecotoxicology, and risk of ground-water contamination) are related to such mobile fractions rather than the total concentration [11–12]. Short-term effects have been related to metal concentrations, frequently referred to as the intensity factor [13], while medium- to long-term effects are mainly governed by the kinetics of desorption and dissolution of metals from solid-phase species, representing a capacity factor of metal solubility [12]. The use of selective extraction methods to distinguish analytes, which are immobilized in different phases of soils and sediments, is also of particular interest in exploration geochemistry for location of deeply buried mineral deposits. Fractionation is usually performed by a sequence of selective chemical extraction techniques, including the successive removal, or dissolution of these phases and their associated metals.

The concept of chemical leaching is based on the idea that a particular chemical solvent is either selective for a particular phase or selective in its action. Although a differentiated analysis is advantageous over investigations of bulk chemistry of soil and sediments, verification studies indicate that there are many problems associated with operational fractionation by partial dissolution techniques. Selectivity for a specific phase or binding form cannot be expected for most of these procedures. There is no general agreement on the solutions preferred for the various components in sediment or soils to be extracted, due mostly to the matrix effect involved in the heterogeneous chemical processes [14]. All factors have to be critically considered when an extractant for a specific investigation is chosen. Important factors are the aim of the study, the type of solid materials, and the elements of interest. Partial dissolution techniques should include reagents that are sensitive to only one of the various components significant in trace metal binding. Whatever extraction procedure is selected, the validity of selective extraction results primarily depends on the sample collection and preservation prior to analysis.

In this work, trace element determination in sediment and soil samples is described in more details with respect to sampling, sample preparation, and the sequential extraction procedure. Moreover, a brief description of the analytical techniques will also be given.

## SAMPLING

A sampling plan has to be established prior to sampling. The purpose and expectation of a sampling program must be realistic and can never surpass the measurement and sample limitations. Moreover, costs and benefits must be considered in the design of every measurement program.

The total variance of an analysis ( $s^2_{\text{total}}$ ) is expressed as:

$$s^2_{\text{total}} = s^2_{\text{measurement}} + s^2_{\text{sampling}} \quad (1)$$

where  $s^2_{\text{measurement}}$  and  $s^2_{\text{sampling}}$  are the variances due to the measurement and sampling, respectively [15]. The measurement and sampling plans and operations must be designed and accomplished so that the individual components may be evaluated. Sampling uncertainty may contain systematic and random components arising from the sampling procedure. In environmental sampling, the act of sample removal from its natural environment can disturb stable or meta-stable equilibria. If the test portion is not rep-

representative of the original material, it will not be possible to relate the analytical result to the original material, no matter how good the analytical method is nor how carefully the analysis is performed. Further, sampling errors cannot be controlled by the use of standards or reference materials.

### Sampling of sediments

Because of the heterogeneity and complex nature of sediments, care should be taken during sampling and analysis to minimize changes in speciation due to changes in the environmental conditions of the system. Sampling for pollution mapping has to consider the heterogeneity of the deposit by methods such as particle size analysis and geochemical normalization. Sediment sampling must avoid alteration of natural biogeochemical processes, which would affect results by the unrepresentativeness of the original equilibrium. Consequently, sampling variance and artifacts introduced during processing of samples can be more than an order of magnitude greater than analytical measurement variances in trace element speciation [8].

Schoer [16] has studied the effect of particle size of sediments on the adsorption capacity. Variations in the behavior of different elements with particle size is attributed largely to differences in their relative potential for sorption on clay minerals, hydrous oxides, and organic matter surfaces, all of which tend to be concentrated in smaller grain sizes. The maximum concentration of organic carbon in the sediment samples was found in a size range of 2–6.3  $\mu\text{m}$ , whereas smaller fractions showed only traces of organic carbon. On the other hand, easily reducible manganese reached its highest concentration in the fraction of  $<2 \mu\text{m}$ . Appropriate comparability among oxide sediment samples collected at different times and places from a given aquatic system and between different systems can be obtained most easily by analyzing the fine-grained fraction of sediment.

Some investigations have also pointed to a relation between specific surface, grain size fraction, and the speciation of trace elements in sediments. Amorphous Fe-oxide precipitates appear to be most significant in affecting both surface area and sediment trace metal levels. It was found that external surface area, determined by Brunauer–Emmett–Teller (BET) method, is a function of both grain size and of composition of geochemical phase [17]. Suspended particulate matter sampling is mainly carried out by filtration. Such samples are of limited utility for studies of the speciation of elements in solids. In recent years, suspended sediment recovery by continuous-flow centrifugation has commonly been used to obtain sufficient sample for speciation, up to a few grams to carry out all the analysis: particle size distribution, mineralogy, total and sequential extractions content. Etcheber et al. [18] provided a comparative study of suspended particle matter separation by filtration, continuous-flow centrifugation, and shallow water sediment traps. Although particles were separated by density, rather than size, the continuous-flow centrifugation technique was preferred due to its speed and high recovery rate. The continuous-flow separation technique is simpler to use especially on the open sea, where suspended sediment concentrations are low. Trace elements in suspended particulate matter from open North Sea have been measured for particle size distribution, specific surface, bulk concentration, and partitioning between five sequential extraction fractions [19].

### Sampling of soil

Spatial [20,21] and seasonal variability [22–24] are known to influence significantly the results of sequential extraction schemes in soils. Wenzel et al. [25] showed that no general trend exists that would predict mobile metal fractions to have more pronounced partial variability than less mobile ones. Despite limitations in comparability of data, this may be explained by the influence of variation in total metal concentrations. The opposite effects of the spatial variation are in factors governing metal solubility (e.g., pH and organic matter contents). Accordingly, the spatial variability of mobile metal fractions may either be increased or decreased by these factors. The coefficients of variation for metals extractable by neutral salt solutions or complexing agents are usually high, often exceeding 50 %, limiting

the potential use of these extractants for monitoring temporal changes of metal mobility for environmental or soil management purposes. For total Pb, this problem was addressed by Schweikle [26]. Given coefficients of variation (CV) of extractable metals are up to 340 %. This problem has to be faced when soil tests for bioavailability or ecotoxic relevant metal fractions are designed, i.e., for legislative purposes. Soil management practices (fertilizing, liming, sludge application) may cause significant seasonal changes in mobile fractions, but also natural seasonal variation of extractable metals in extensively used forest and range soils or undisturbed ecosystems may occur as well [27–30]. Seasonal variation of extractable metals is an inherent process that is at least as significant as spatial variability [31–33]. Due to the variation in weather conditions, seasonal patterns of extractable fractions are not necessarily predictable from a few years observation and may differ from site to site. Accordingly, there is a clear possibility of obtaining biased results when sampling only once. Distinction should also be made between sampling of (1) natural, agricultural, grassland, forest, or moorland soil where to some extent element distribution and speciation can be regarded as homogeneous and (2) industrially contaminated soils will usually have an element distribution and speciation that is heterogeneous not only over the surface area but also with depth. In the first case, representative samples of the area topsoils may be required. In the second case, statistical sampling may be desirable but will often be uneconomic, and the so-called judgmental sampling using selected pit sampling of soil profiles may be required.

Soil properties may vary considerably on a micro scale of about 1 to 100 mm. Thus, metal solubility and extractability may be affected either directly by micro inhomogeneity of the total metal contents or by simultaneous variation in soil properties (pH, CEC, organic matter, mineral composition, and soil texture). Differences in the fraction of outer- and inner-sphere aggregates may be caused either by natural processes of soil formation or by anthropogenic inputs. It was found that moderately acidic soils with high silt and clay contents had significantly higher CEC and exchangeable Mg (0.1 mol/l BaCl<sub>2</sub>), but lower amounts of exchangeable Ca and K in the outer sphere aggregates [30,34]. As indicated by higher levels of exchangeable Al and lower amounts of basic cations, aggregate surfaces are frequently more acidified than homogenized bulk soil, particularly in well-aggregated soils low in basic cations [35]. This is also reflected by higher concentrations of Al<sup>3+</sup>, Fe<sup>2+</sup>, and H<sup>+</sup> ions in the saturation phase of acidic soils [35–39]. Wilcke et al. [40] revealed that the sorption capacity of the outer-sphere aggregates in acidic soils is lower than that of the inner sphere. Total and mobile Pb fractions were usually enriched on aggregate surfaces, probably due to widespread Pb deposition [40].

It has been concluded that the mobility of metals may frequently be underestimated when assessed by chemical extraction of disturbed, homogenized, and sieved soil samples of well-aggregated, acidic soils, particularly when anthropogenically polluted, and probably overestimated in soils with organic fillings and linings in macropores. These chemical effects are obviously confused with transport nonequilibria in aggregated soils [41–43]. That should commonly lead to lower metal concentrations in the real soil solution than predicted by structure-destroying equilibrium methods, i.e., the saturation phase.

### Storage and preparation of sediment samples

Sample preparation is one of the most important steps prior to analysis, and not many experiments, so far, have been addressed to avoiding extraction procedures using continuous percolation with different extractants. Knowledge of the biogeochemical diagenesis history of sediments is essential to understand the contamination mobility in marine and freshwater environments. The oxidized sediment layer controls the exchange of trace elements between sediment and overlying water in many aquatic environments. The underlying anoxic layer provides an efficient natural immobilization process for metals. Significant secondary release of particulate metal pollutants can be obtained from the accumulated metals as a result of processes such as:

- desorption from clay minerals and other substrates due to formation of soluble organic and inorganic complexes,
- post-depositional redistribution by oxidation and decomposition of organic materials,
- alteration of the solid–solution partitioning by early diagenetic effects such as changing the surface chemistry of oxyhydroxide mineral, and
- dissolution of metal precipitates with reduced forms, (metal sulfides) generally more insoluble than the oxidized form (surface complexes).

The mechanism of sorption of trace metals on hydrous Fe/Mn-oxides and calcite has been recently revealed by speciation analysis with X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS) [44]. Transformations of metal forms during early diagenesis have also been successfully studied by sequential chemical leaching. However, many of these studies did not consider that sample preservation techniques in trace element speciation studies of oxic sediments and sludges are different from those that should be used for anoxic samples [45]. Air- and oven-drying caused major changes in overall sediment and soil equilibrium by converting fractions relevant to trace element binding into highly unstable and reactive forms [46]. Increased organic matter and manganese solubility and exchangeability were observed as effects of soil-drying. Drying of sediments was also reported to reduce the quantity of Fe extracted by techniques which remove amorphous iron oxides ( $\text{CH}_3\text{COOH}$ , pyrophosphate, hydroxylamine), suggesting an increase in the oxide crystallinity [47]. Extractability of copper by oxalate acid, pyrophosphate, and DTPA was found to be enhanced by a factor of 2 compared to that of the control by sediment-drying, reflecting the predominant binding of this metal by organic matter [47].

In practice, it is usually impossible to retroactively correct data obtained from dried sediments and soils to those that existed originally in field. Such data may even be of limited value for comparison of bioavailable concentrations of trace metals in samples collected within the same environment. Bartlett et al. [46] found that manganese extractability changed as a function of storage time. Sieving and mixing in order to obtain a representative sample for bioavailability analysis may lead to precise but inaccurate results. These effects make the preparation of stable sediment and soil reference materials for comparative speciation studies extremely difficult.

Wet storage of oxidized sediments and soils is inadequate because of microbially induced shift from oxidizing to reducing conditions in the stored sediments. Extractability of the metal with the most insoluble sulfide (Cu) was reported to decline rapidly during wet storage [47]. Freezing is usually a suitable method to minimize microbial activity. Freezing was found to enhance water solubility of metals in the order of Mn (8–17 %) > Cu (7–15 %) > Zn (6–12 %) > Fe (3–7 %). Storage subsequent to freezing significantly affected extractability of these metals by weak reagents (ammonium acetate, DTPA) [47]. To prevent exposure to atmospheric oxygen is of importance since several significant changes in trace metal concentrations have been observed in all but the residual fractions of the five- to six-step sequential extraction procedures used.

Another problem is the solubility of a variety of metal sulfides in acidified extractants ( $\text{pH} < 5$ ). Among the various trace metals, only Cu and Cd sulfides are stable enough to survive the initial extraction steps before they are oxidized by  $\text{H}_2\text{O}_2$  [48]. It was observed that the high concentration of dissolved organic substances found in the first extraction steps of fresh anoxic sediments suppressed the amount of Cd and other metals found. This effect was not experienced with dried samples. Storage of anoxic sediments in a freezer was found to cause change in the fractionation pattern of various metals studied. It has been found that a double wall sealing concept (i.e., an inner plastic vial with the frozen sediment contained under argon in an outer glass vial) proved to be suitable. However, it seems to be impossible to totally avoid changes in the in situ chemical speciation of trace elements found in nature, unless the sediment and soil samples are extracted immediately upon collection [8].

### Storage and preparation of soil samples

Sample preparation generally involves the following steps: (1) drying or rewetting, (2) homogenizing and sieving, (3) storage, and, occasionally, (4) grinding. Usually, soil samples are air-dried prior to extraction. Although changes in the extractability of some elements (i.e., of Mn) have been reported earlier [49], this problem only recently received more attention [50–55]. Air-drying prior to extraction is a standard procedure, but leads to an increased extractability of Fe and Mn, whereas other metals are more or less unaffected [50–55]. As the effect of air-drying depends on soil properties and the initial moisture conditions, no general regression equations are available for prediction of metal levels in the field moist soils from analysis of air-dried samples. Since extraction of field moist samples cannot be recommended for routine analysis, individual relations on a local or regional scale should be obtained to avoid errors in the determination of mobile pools of Mn and other metals in soil. Several authors identified possible mechanisms of these changes in metal extractability upon air-drying. The observed decrease in easily reducible (oxidic) Mn-fraction was related to (i) dehydration of Mn-oxides [49], (ii) reduction of Mn-oxides by organic matter [56], and (iii) alterations of soil functional groups that were forming unavailable Mn complexes [57]. In summary, drying of samples prior to the determination of mobile metal fractions usually results in unrealistically large amounts of extractable Mn, Fe, Cu, and Zn, and underestimation of Ca, Mg, K, and probably Co, Ni, and V. The changes in extractability upon air-drying are related to soil properties (i.e., pH and organic matter content) and to the initial soil moisture conditions. Prediction of changes in metal extractability upon air-drying seems to be possible for most metals when individually based on selected soils of a data set.

Although homogenizing and sieving are essential steps in performing representative and repeatable soil analysis, these procedures suffer from some serious drawbacks. Firstly, the effects of structure disturbing soil sampling are obviously reinforced, thus creating new surfaces for reactions with metals in the solute phase, giving raise to adverse readsorption or desorption processes during metal extraction [58]. Secondly, homogenization of soil material from different horizons may result in erroneous changes in pH and carbonate content of the fine earth. In soils with high variability on a microscale, sieving and homogenization may cause erroneous results (i.e., by the destruction of weathered rock fragments or carbonate nodules).

Navo et al. [59] reported frequent nitrification during storage of air-dried samples to nonmicrobial changes in the physical structure (i.e., to an increase in the surface area of the organic fraction). Based on these results, Wenzel et al. [30] concluded that Mn was continually mobilized through the reduction of Mn-oxides by electron transfer from newly created organic surfaces. Accordingly, air-drying may reduce microbial activity in soils effectively, but physical changes of the organic fraction may affect the extractability of Mn and probably of other metals sensitive to changes in the redox potential.

As a conclusion, sample storage seems to be generally less critical to the analysis of extractable metal fractions than air-drying, but it is likely to enhance the effects of air-drying in the case of redox-sensitive elements. Occasionally, soil samples are ground prior to extraction. This procedure causes physical breakdown of soil microaggregates, thus potentially altering the extractability of metals from soil samples [50]. The exposure of fresh surfaces may, depending on soil properties, increase the extractability of some metals, but potentially may also cause readsorption of metals during the batch process [50].

### SEQUENTIAL EXTRACTION TECHNIQUES

Sequential extractions have been applied using extractants with progressively increasing extraction capacity, and several schemes have been developed to determine species of the soil solid phase. Although initially thought to distinguish some well-defined chemical forms of trace metals [60,61], they rather address operationally defined fractions [58,62]. The selectivity of many extractants is weak or not sufficiently understood, and it is questionable as to whether specific trace metal compounds actually exist

and can be selectively removed from multicomponent systems [12]. Due to varying extraction conditions, similar procedures may extract a significantly different amount of metals. Concentration, operational pH, solution/solid ratio, and duration of the extraction affect considerably the selectivity of extractants. The conventional approach of equilibration during a single extraction step is the shaking or stirring of the solid-phase/extractant mixture. Recently, an accelerated extraction has been presented using an ultrasonic probe [63]. The resolution sought in the chemical fractionation depends on the purpose of the study, as does the choice of the single extractant in each step in a sequential scheme. The selectivity of the procedure can be considerably improved by incorporation of the various nonselective single extraction steps into a carefully designed sequential extraction scheme.

There is no general agreement on the solutions preferred for the extraction of various components in sediment or soils, due mostly to the matrix effects involved in heterogeneous chemical processes [14]. The aim of the study, the type of the solid materials and the elements of interest determine the most appropriate extractants. Partial dissolution techniques should include reagents that were sensitive to only one of the various components significant in trace metal binding. In sequential multiple extraction techniques, chemical extractants of various types are applied successively to the sample, each follow-up treatment being more drastic in chemical action or different in nature from the previous one. Selectivity for a specific phase or binding form cannot be expected for most of these procedures. In practice, some major factors may influence the success in selective leaching of components, such as

- the chemical properties of the extractant chosen,
- experimental parameter,
- the sequence of the individual steps,
- specific matrix effects such as cross-contamination and readsorption, and
- heterogeneity, as well as physical associations (e.g., coatings) of the various solid fractions.

All these factors have to be critically considered when an extractant for specific investigation is chosen. Fractions of sequential extraction schemes include the following:

- Exchangeable fractions: Most of the recommended protocols seek to first displace the exchangeable portion of metals as a separate entity using  $\text{MgCl}_2$  or  $\text{NH}_4\text{Ac}$  (pH = 7) treatments.
- Bound to carbonates: Removal of carbonates using  $\text{HAc}$ , with or without buffering by  $\text{NaAc}$  (pH 5).
- Easily reducible fractions:  $\text{NH}_2\text{OH}\cdot\text{HCl}$  at pH 2 is generally used, but procedures differ in minor operational details such as solid/solution ratios, treatment time, and interstep washing procedure.
- Oxidizable oxides and sulfides fractions:  $\text{H}_2\text{O}_2/\text{NH}_4\text{Ac}$  is used most frequently.
- Residual minerals: Strong acid mixtures are applied ( $\text{HF}/\text{HClO}_4/\text{HNO}_3$ ) to leach all remaining metals.

The fractions of a sequential extraction procedure can be divided into the following steps:

- **MOBILE FRACTION:** this fraction includes the water-soluble and easily exchangeable (non-specifically adsorbed) metals and easily soluble metallo-organic complexes. Chemicals used for this fraction fall commonly in one of the following groups [58,64]:
  1. Water or highly diluted salt solutions (ionic strength  $<0.01$  mol/l),
  2. Neutral salt solutions without pH buffer capacity (e.g.,  $\text{CaCl}_2$ ,  $\text{NaNO}_3$ ),
  3. Salt solutions with pronounced pH buffer capacity (e.g.,  $\text{NH}_4\text{Ac}$ ),
  4. Organic complexing agents (e.g., DTPA, EDTA-compounds).
- **EASILY MOBILIZABLE FRACTION:** This fraction contains the specifically bound, surface occluded species (sometimes also  $\text{CaCO}_3$  bound species and metallo-organic complexes with low bonding forces).



- **CARBONATE-BOUND FRACTION:** To dissolve trace elements bound on carbonates, commonly buffer solutions (e.g., HAc/NaAc; pH = 4.75) are used. Zeien et al. [65] proposed to dissolve carbonates by adding equivalent amounts of diluted HCl to 1 mol/l NH<sub>4</sub>Ac/HAc-buffer, addressing specifically adsorbed and surface-occluded trace element fractions of soil with 5 % m/m carbonates.
- **ORGANICALLY BOUND FRACTION:** Various approaches for the dissolution of organic bound elements are known: (i) release by oxidation, (ii) release by dissolution, and (iii) addition of competing ligands. Different methods extract the organically bound fraction before the oxide fraction, before the carbonate-bound fraction or directly after the carbonate-bound fraction or after the oxide-bound fraction. The organically bound fraction itself can again be divided into up to three separate fractions [62].
- **Mn-OXIDE BOUND FRACTION:** This fraction is sensitive to drying procedures prior to extraction. They are most susceptible to changes in pE and pH. Trace metals bound to Mn-oxide may be readily mobilized upon changed environmental conditions. This fraction is to be separated prior to Fe- or Al-oxides.
- **Fe- and Al-OXIDE BOUND FRACTION:** In this fraction, the Fe-bound fraction can also be distinguished in AMORPHOUS Fe-BOUND FRACTION and CRYSTALLINE Fe-BOUND FRACTION.
- **RESIDUAL FRACTION:** This fraction mainly contains crystalline-bound trace metals and is most commonly dissolved with high concentrated acids and special digestion procedures.

### Main parameters for a sequential extraction schemes

A wide range of extraction procedures is readily available for different metals and variations of the extraction conditions are utilized due to varying sediment and soil composition. The following points have to be considered when designing an adequate extraction procedure:

- *Extractants:* Chemical and physical interferences both in extraction and analysis steps, respectively.
- *Extraction steps:* Selectivity, readsorption processes, and redistribution processes. If the single extractants for the different steps are chosen with respect to their ion-exchange capacity or reduction/oxidation capacity, each step has to be designed individually following special considerations [30].
- *Concentration of the chemicals:* The efficiency of an extractant to dissolve or desorb trace metals from sediment and soils will usually be increased with increasing concentration or ionic strength. Thermodynamic laws predict the efficiency of an extractant to dissolve or desorb trace metals from solid samples [66–70].
- *Extraction pH:* Extractants with a large buffering capacity or extractants without buffer capacity can be used [66,70–74].
- *Solution/solid ratio and extraction capacity:* The relative amount of extractant added to the sediment and soil has various implications on the results. Essentially, Wenzel et al. [30] distinguished four cases, e.g., (1) pure dissolution of metal compounds according to the solubility product, (2) pure ion exchange by 0.1–1 mol/l neutral salt solutions, or (3) by water or highly diluted neutral salt solutions ( $\ll 0.1$  mol/l), and (4) combinations of (1) with either (2) or (3). If, over a sufficiently wide solution/solid ratio, the capacity of the extractant to dissolve a metal fraction exceeds its total amount present in the solid sample, then the metal concentration in the extract (mg/l extract) will decrease with an increase in solution/solid ratio. However, the total amount (mg/kg) extracted will be constant with increasing solution/solid ratio. Nevertheless, as sediment and soils are multiphase/multicomponent systems, dissolution of other compounds due to the nonselectivity of the extractant may confuse this behavior [66,67,75–79]. Wenzel et al. [30] concluded that

the efficiency of mild reagents for extraction of abundant metal cations (e.g., Ca, Al, Mg, K) usually increased by increasing the solution/soil ratio, although often the concentrations in the extract concurrently decreased. With stronger reagents, this should also be valid for the more abundant metal cations as long the capacity of the extractant to dissolve a particular compound exceeded the amount present in the soil.

- *Extraction time and batch processes:* The effect of extraction time is related to the kinetics of the reactions between solid sample and extractant. Extractions may be predominantly based either on desorption or dissolution reactions. For desorption of metal cations from heterogeneous soil systems, Sparks [80] identified four rate-determining steps, e.g., (i) diffusion of the cations in the (free) bulk solution, (ii) film diffusion, (iii) particle diffusion, and (iv) the desorption reaction. Accordingly, the rates of most ion-exchange reactions are film- and/or particle diffusion-controlled. Vigorous mixing, stirring, or shaking significantly influences these processes. Film diffusion usually predominates with small particles, while particle diffusion is usually rate-limiting for large particles. Dilute solutions usually favor film-diffusion-controlled processes. The time to reach equilibrium for ion exchange on soils varies between a few seconds and days and is affected by soil properties [81]. For mineral dissolution, essentially three rate-controlling steps have been identified, e.g., (i) transport of solute away from the dissolved crystal (transport-controlled kinetics), (ii) surface reaction-controlled kinetics where ions are detached from the surface of crystals, and (iii) a combination of both [81]. Batch processes (e.g., stirring or shaking) increase the rate of transport-controlled reactions, while they do not affect surface-controlled reactions. Shaking and other batch processes may enhance the dissolution of readily soluble salts effectively, but are unlikely to affect the dissolution rate of less soluble minerals. Experiments reported by several authors generally revealed an increase of the extractable amounts of metals with time of extraction as expected from the theory of reaction kinetics [66,68,70,82–85].
- *Extraction temperature:* Within the normal range of extraction temperatures (20–25 °C or room temperature), the effect of temperature on metal extractability is usually small, but has to be considered for interpretation of small differences [70,83]. Finally, the whole procedure has to be optimized with regard to selectivity, simplicity, and reproducibility.

### **Standardization and standardized sequential extraction procedure as proposed by BCR**

Sequential extraction schemes have been developed during the past 20 years for the determination of binding forms of trace metals in sediment. The lack of uniformity of these schemes, however, did not allow the results so far to be compared worldwide or the procedures to be validated. Indeed, the results obtained by sequential extraction are operationally defined (i.e., the “forms” of metals are defined by the procedure used for their determination). Therefore, the significance of the analytical results is related to the extraction scheme used. Another problem, which hampered a good comparability of data, was the lack of suitable reference materials that precluded control of the quality of the measurements. Thus, standardization of leaching and extraction schemes is required, which goes hand in hand with the preparation of sediment and soil reference materials that are certified for their contents of extractable trace element, following standardized single and sequential extraction procedures [86]. Owing to this lack of comparability and quality control, the Community Bureau of Reference (BCR, now Standards, Measurements and Testing Program) has launched a program of which one of the aims was to harmonize sequential extraction schemes for the determination of extractable trace metals in sediment [87]. This program involved the comparison of existing procedures tested in two interlaboratory exercises, and it developed into a certification campaign of extractable trace element contents in a sediment reference material, following a three-step sequential extraction procedure duly tested and adopted by a group of 18 EU laboratories.

The significance of the analytical results depends on the “operationally defined characters” of the used extraction schemes, which requires the use of standardized protocols. Moreover, those schemes have to be validated and require the preparation of certified reference materials with certified contents of leachable elements if analyzed following standardized single and sequential extraction procedures [86]. BCR has proposed a standardized 3-stage extraction procedure (BCR EUR 14763 EN), which was originally developed for the analysis of heavy metals in sediments [88]. This procedure is currently used and evaluated also as extraction method for soils [89,90]. So far, the BCR procedure has been successfully applied to a variety of sediment [91–95], sludge [95], and soil samples [89,96].

The BCR scheme was recently used to certify the extractable trace element contents of a certified reference material (CRM 601, IRMM). Although this procedure offers a tool for obtaining comparable data, poor reproducibility and problems with lack of selectivity were still reported [89,97–100]. Various research groups used this technique and found partially discrepancies when applying the scheme. The same extraction scheme was used for the determination of extractable elements in soils, as well [90–101]. Sahuquillo et al. [102] investigated potential sources of irreproducibility when applying the BCR three-stage procedure to the lake sediment CRM 601. Factors such as the type of acid used for pH adjustment, temperature, and duration of extraction did not affect the precision. The most critical factor was the pH of step 2 ( $\text{NH}_2\text{OH}\cdot\text{HCl}$  extraction). Improved precision could be obtained when the  $\text{NH}_2\text{OH}\cdot\text{HCl}$  concentration was increased from 0.1 to 0.5 mol/l and the centrifugation speed was doubled [97]. The use of filtration did not affect the reproducibility, but it was not recommended since it promoted the dissolution of nontargeted phases. Neither ammonium hydrogen oxalate nor oxalic acid proved suitable alternatives in step 2 owing to precipitation of insoluble lead salts, particularly in the presence of calcium. A modified BCR procedure incorporating these changes has been applied to a sludge-amended soil (CRM 483) and provides indicative values for Cd, Cr, Cu, Ni, Pb, and Zn. It also recommends the use of an aqua regia digestion of the residue after the three steps of the extraction procedure for comparison with an aqua regia digestion of the original material. This approach is often referred to as “pseudototal digestion”. This is a vital quality control procedure. There is an increasing tendency to establish the “mass balance” of a sequential extraction, namely to compare the sum of the steps with the results of a separate total or pseudototal digestion. A comparison with the Tessier procedure showed identical correlation between the metals extracted in the corresponding steps of the BCR and the Tessier procedure.

### **Validation of sequential extraction procedures [103], NIST sequential extraction scheme**

#### *Sequential extraction schemes applied to sediment samples*

Sediments are basic components of lakes, as they provide foodstuffs for living organisms and serve as sinks for deleterious chemical species. The main mineralogical components of sediments, which are important for controlling their metal concentrations, are hydrous oxides of iron and manganese, organic matter, and clay. The degree of interaction between sediment samples and extractant solutions can be altered by changes in experimental parameters such as reagent concentration, final suspension pH, solid/solution ratios, temperature and contact time, and intensity of mixing. The absence of standardized conditions makes it difficult to compare experimental data derived from studies in which such parameters are significantly different or even not listed [104]. Recently, researchers have tended to use similar extraction protocols, mostly by adapting or modifying Tessier’s scheme [61].

Salomons et al. [105] used sequential extraction techniques to determine the chemical associations of heavy metals with specific sedimentary phases, whereby the potential availability of toxic compounds for biological uptake could eventually be estimated. They found that the Cd concentrations in the Rhine sediment increased more than 100-fold in 80 years. Five major mechanisms could be distinguished for metal accumulation on sedimentary particles: (1) adsorptive bonding to fine grained substances, (2) precipitation of discrete metal compounds, (3) coprecipitation of metals with hydrous Fe-

and Mn-oxides and carbonates, (4) association with organic compounds, and (5) incorporation in crystalline material. It was pointed out that the standard extraction method should be relatively simple, in order to make routine analysis of large numbers of sediments possible. At the same time, it should provide sufficient information for a tentative assessment of the environmental impact of particulate metals.

Tessier et al. [106] collected sediment samples from streambeds in an undisturbed watershed in eastern Quebec (Gaspé Peninsula). Two sampling sites were located on a stream draining an area of known mineralization (Cu, Pb, Zn) and two on a control stream. The sediment samples were separated into 8 distinct particle size classes in the 850  $\mu\text{m}$  to  $<1 \mu\text{m}$  size range by wet sieving, gravity sedimentation, or centrifugation. Each sediment subsample was then subjected to a sequential extraction procedure designed to partition the particulate heavy metals into five fractions: (1) exchangeable, (2) specifically adsorbed or bound to carbonates, (3) bound to Fe/Mn-oxides, (4) bound to organic matter, and (5) residual. Comparison of samples from the mineralized area with control samples revealed the expected increase in total concentrations for Cu, Pb, and Zn. Non-detrital metals were mainly associated with Fe-oxides (specifically adsorbed, occluded) and with organic matter or resistant sulfides. For a given sample, variation of trace metal levels in fractions 2 and 3 with grain size reflected the changes in the available quantities of the inorganic scavenging phase ( $\text{FeO}_x/\text{MnO}_x$ ); normalization with respect to Fe and Mn content in fraction 3 greatly reduced the apparent dependency on grain size. The results of this study suggested that a single reducing extraction ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ) could be used advantageously to detect anomalies in routine geochemical surveys. A second leaching step with acidified  $\text{H}_2\text{O}_2$  could also be included, as the trace metal concentrations in fraction 4, normalized with respect to organic carbon content, also showed high irregularity/background ratios.

The bonding stability of selected metals (Al, Fe, Pb, Zn, Cd) within the sediment core collected in the Wildsee (Black Forest, Germany) has been evaluated by applying sequential chemical extraction, which differentiates between residual, labile, and intermediate compounds. Increases in total concentrations of Al, Pb, and Zn, as well as losses in bonding strength of these metals, appear to be caused by acidification; Cd appears to be derived principally from direct deposition on the lake and its catchment rather than from acidification-mediated soil release [107].

The chemical speciation of several metals (base cations: Mg, Ca, Al; heavy metals: Fe, Mn, Cu, Pb, Cr, Zn, and Cd) was evaluated applying sequential chemical extraction in sediment core of the Huzenbachersee (northern Black Forest, Germany) [108]. Two distinct periods (2<sup>nd</sup> half of both 19<sup>th</sup> and 20<sup>th</sup> centuries) of increased amounts of Pb, Zn, Cd, and Fe were found, indicating phases of industrialization. Local glass factories caused elevated contents, particularly of Cr, in lower sediment layers. In the uppermost sediment layers, the bonding strength of several metals showed decreasing tendency (increasing for exchangeable and easily reducible fractions). As a result, secondary contamination of the water column could occur through sediment release especially of Zn and Cd [108].

A six-step sequential chemical extraction procedure was used to determine the association of Cu, Zn, Pb, and Cd with the major sedimentary phases (exchangeable cations, adsorbed and bound to the carbonate component, readily reducible and moderately reducible compounds, on sulfides/organic phases and residual components) in the stream sediment of the central part of the Labe River [109]. It was found that most of the Cd and part of the Zn was adsorbed and bound in readily reducible compounds together with Pb, and on an oxidizable organic phase together with Cu. A large part of Pb was immobile and could be brought into solution only through the use of hot concentrated  $\text{HNO}_3$  [109].

A simultaneous (SIM) sediment extraction procedure for low carbonate sediments, which partitions sediment-bound trace metals (Fe, Mn, Zn, Cu, and Cd) into easily reducible (associated with Mn-oxides), reducible (associated Fe-oxides), and alkaline-extracted (bound to organic) metal was investigated. The SIM method was compared to the sequential (SEQ) extraction procedure of Tessier [61]. Both methods showed good agreement for the partitioning of Zn and Cd among the easily reducible, reducible, and organic components of the sediment. Both methods also showed the same general distribution of Mn, Fe, and Cu among the three sediment components. However, concentrations of metals recovered by the two methods differed; less Mn and Fe and more Cu were recovered from sed-

iments by SEQ vs. SIM procedure. Less recovery of Mn was, in part, attributed to the loss of this metal in the “in between” reagent rinses required in the SEQ procedure. Greater recovery of Cu by SEQ vs. SIM method might be due to the pretreatment of the sediment with strong reducing agents prior to the step used for liberating organically bound metals. Advantages of SIM over SEQ included rapid sample processing time (i.e., the treatment of 40 samples/day vs. 40 samples/in 3 days) and minimal sample manipulation. Hence, for partitioning metals into easily reducible and organic sediment components in sediments low in carbonate, the use of a SIM extraction over that of a SEQ procedure was recommended [110].

Cr, Mn, Ni, V, and U have been determined in inter-tidal sediments collected from locations along the Cumbrian coast [111]. Elevated levels of Cr ( $39.5 \pm 0.9 \mu\text{g/g}$ ), V ( $33.0 \pm 0.6 \mu\text{g/g}$ ) and U ( $39.0 \pm 1.2 \text{ Bq/kg}$ ) were observed at Whitehaven, whereas concentrations of Mn were highest in samples from more northerly locations. The U enhancement was due to the extraction of phosphates from ore naturally rich in radionuclides at the nearby chemical manufacturing plant. The Cr contamination might also arise from chemical manufacturing, whereas the V was thought to originate from oil spillage. Interferences associated with the use of the BCR sequential extraction protocol were investigated, and the operationally defined speciation of Cr, Mn, Ni, and V was then determined. Cr, Ni, and V were found mainly in association with the residual sediment phase. A large proportion of the Mn at all sites was present as exchangeable species (i.e., soluble in 0.11 mol/l  $\text{CH}_3\text{COOH}$ ), and this was not affected by sample drying (at  $60^\circ\text{C}$ ) nor by storage (for 6 months) prior to extraction [111].

The labile metal content of sediments can be evaluated by equilibrating sediment suspensions with ion-exchange resins. By use of a sequence of strong-acid and weak-acid cation-exchangers ( $\text{H}^+$ - and  $\text{Na}^+$ -form) and chelating resins, extraction can be performed at pH values ranging from 2 to 10. The results allowed the total metal content to be subdivided into seven categories designated as (i) low-pH labile, (ii) weak-acid labile, (iii) exchangeable and readily desorbed at sediment-suspension pH, (iv) weak-base labile, (v) high-pH labile, (vi) nonlabile soluble forms, and (vii) detrital metal content. The sediment suspensions were mixed overnight with the different types of exchanger (held in porous containers), and the cations transferred from the sediment were subsequently back-extracted from the resin into 0.05 mol/l EDTA (pH 7.5). Analysis of the aqueous phase left in contact with the sediment residue gave the amount of nonlabile species released. Eighteen sediments, containing various levels of metal contamination, and effluent dam sludge have been examined by this technique. All the exchangers released Ca and Mg from the sediments, and the  $\text{H}^+$ -form exchangers also released Fe and Al. Some of the Fe, Al, and, to a lesser extent, Zn released by the sediment/exchanger interactions was present as nonlabile “soluble” species [112].

A three-stage sequential extraction procedure, following a protocol proposed by a European working group coordinated and supported by the BCR, has been applied to two river sediments from an industrial region of East Catalonia, Spain. The extractant solutions were as follows: step 1,  $\text{CH}_3\text{COOH}$  (0.11 mol/l); step 2, hydroxyl ammonium chloride (0.1 mol/l, pH 2); step 3, hydrogen-peroxide (8.8 mol/l) oxidation followed by extraction with ammonium acetate (1 mol/l, pH 2). No significant matrix interferences were found except for Cr in the  $\text{CH}_3\text{COOH}$  and hydroxyl ammonium chloride extracts [113].

In unpolluted soils and sediments, the trace metals exist mainly as relatively immobile species in silicate and primary minerals. As a result of weathering, a fraction of the trace element content is gradually transferred to forms accessible to plants. In polluted soils, the metal pollution input in nearly all cases is in nonsilicate bound forms and contributes to the pool of potentially available metals. The situation in sediments is in principle very similar. The metal species arising from these transfers or pollution processes can exist in several different soil or sediment phases [114]:

- in solution, ionic or colloidal;
- in organic or inorganic exchange complexes as readily exchangeable ions;
- in complexes in which they are strongly bound;

- in insoluble mineral/organic phases;
- in precipitated major metal (Fe, Mn, Al) oxides or insoluble salts; or
- in resistant secondary minerals.

The use of ammonium acetate (1 mol/l at pH 7) for extraction of soils and sediments for the speciation analysis of metal ions was investigated [114]. Because the sensitivity of flame atomic absorption spectrometry (FAAS) was insufficiently sensitive for the determination of many of the heavy metals in ammonium acetate extracts of unpolluted, and even in some polluted soils, the use of electrothermal atomic absorption spectrometry (ETAAS) was studied. A general procedure, using graphite furnace atomization and the “universal” matrix modifier, palladium, was developed, that was sufficiently sensitive for the determination of Cd, Cr, Cu, Ni, Pb, and Zn even in unpolluted soils. The concentration of Zn, however, would almost always be high enough for determination by FAAS, and this method was preferred to ETAAS for this element. While for Cr, Cu, Ni, and Pb, direct calibration with external standard solutions was applied, it was necessary to use standard addition calibration for Cd to avoid matrix interference effects. This is particularly important for interlaboratory comparisons or for certification analyses in the preparation of reference materials.

Sediments are the ultimate sinks for pollutants. Before these sediments become part of the sedimentary record (deeply buried), they are able to influence the composition of surface waters. The sediments can be divided in two sections: oxic surface layer and anoxic sediment. In anoxic systems when sulfide is present, Zn, Cd, and Cu are likely to be present as sulfides. Remobilization of the deposited sediments is possible when the overlying surface water changes (pH and complexing agent). In addition, changes in the surface water composition may enhance or prevent the removal of dissolved trace metals by particulates and subsequent removal by sedimentation. Remobilization also occurs when sediments are brought from anoxic to an oxic environment as takes place during dredging and disposal on land. Salomons et al. [115] reviewed the processes affecting trace metals in deposited sediments. The sediment-water system could be divided in three parts: the oxic layer, the anoxic layer and the oxic–anoxic interface. Available data showed that trace metals like Cu, Zn, and Cd occurred as sulfides in marine and estuarine anoxic sediments. Calculations showed that organic complexation was unlikely, and the dominant species were sulfide and bisulfide complexes. Cr and As were probably present as adsorbed species on the sediments. Their concentrations in the pore waters therefore depended on the concentrations in the sediments. The oxic–anoxic interface played a major role in the potential flux of trace metals from the sediments, however, this interface has not been well studied. Changes from an anoxic to an oxic environment as occurs during dredging and land disposal of contaminated sediments might cause a mobilization of some trace metals.

The chemical forms of many elements in the sediments of St. Gilla Lagoon (Sardinia, Italy) were evaluated [116]. Five fractions, consisting of an exchangeable metal fraction, metals bound to carbonates, metals bound to iron and manganese hydroxides, metals bound to organic matter, and a fraction of residual metals, were separated from sediments by sequential chemical extraction. The metals in each fraction were determined by the total-reflection X-ray fluorescence (TXRF) technique. Both principal and trace element distributions in the sequential phases were discussed in terms of pollution sources, metal transport, and deposition/redeposition in air-dried sediments. The use of a sequential extraction procedure could be an effective method for comparative studies between natural and contaminated areas, as well as between areas subjected to different chemical stresses. The results showed that in the examined area the lithogeneous fraction was the most relevant for total metal content. However, under oxidizing conditions among the “mobile” fractions, the reducible fraction proved to be the most important sink for Zn and Pb, the oxidizable fraction was only relevant for Cu at almost natural level.

Trace metals were leached from sediments and suspended particulates by using phthalate buffers at pH values of 2.2–6 [117]. Cd, Cu, Fe, Mn, Pb, and Zn were determined in the leachates by flame or flameless AAS. The fraction of total metal removed varied with sample composition, final pH, and element determined. The effects of equilibration time, aqueous/solid ratio, solution matrix, wet/dry sam-

ple, and final pH on the technique were evaluated. Depending on the element and sample type, metal removal increased linearly or exponentially with decreasing pH. Metal release rates were rapid with 35–85 % of the leachable metal removed within 0.5 h of the 48 h experiment. Results can be used for studying biological availability and uptake/release processes for metals in sediment and soil as a function of pH.

Various criticisms have been formulated against sequential methods, including that of inaccuracy in releasing metal from specific geochemical phases. Whalley [98] examined the selectivity of a leaching technique by analyzing individual mineral phases previously equilibrated with metal-spiked artificial seawater. Substrates were then sequentially extracted according to the three-step BCR procedure. The distribution of recovered metal between extracts was compared to that expected if reagents were acting on a specific phase.  $\text{CH}_3\text{COOH}$  released most of the metal associated with calcium carbonate, kaolinite, potassium-feldspar, and ferrihydrite. Hydroxylamine hydrochloride extracts contained most of the recovered metal from montmorillonite and  $\text{MnO}_2$ , as well as nickel from humic acid. Iron oxides are expected to be attacked by this reducing agent, but the majority of the metal had already been removed by the first extract ( $\text{CH}_3\text{COOH}$ ). This may reflect the high adsorption capacity of ferrihydrite. Zinc on humic acid was split between the first two reagents. The third extraction,  $\text{H}_2\text{O}_2/\text{NH}_4^+$ -acetate, which might be expected to release metal from organic or sulphidic material, only significantly recovered the added copper from humic acid. Total recoveries of the added metal were high, except from humic acid, feldspar, and montmorillonite, suggesting strong metal binding by these substrates. Variability between carefully controlled experiments raised some questions about the reproducibility of the extraction procedure, which may be made worse when applying the method to real sediments.

The BCR sequential extraction procedure provides a method for releasing metal from sediment under different chemical regimes. The geochemical phase has been shown to be of varying quality, as determined upon single substrates. Calcium carbonate and  $\text{MnO}_2$  released most bound metal into the expected reagents ( $\text{CH}_3\text{COOH}$  and  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , respectively), while humic acid generally released metal earlier in the procedure than might be expected. An amorphous iron-oxide, ferrihydrate, released most bound metal into  $\text{CH}_3\text{COOH}$  rather than into the second extract, but this may be the result of a large number of adsorptive sites on this phase. Extractions carried out upon feldspar and kaolinite removed most metal in the first step, while in the second step metal release from montmorillonite proved to be important. Stepped recoveries for humic acid and montmorillonite, where metal was found in more than one extract, suggested that metal release was more depending upon ion-exchange equilibria than on chemical alteration of binding sites. It is recommended that extracts be acidified after separation from sediment to ensure that released metal stays in solution. Significant differences in metal concentration were found in extracts from calcium carbonate, potassium-feldspar, ferrihydrite, and humic acid between samples analyzed before and after acidification.

A five-step sequential technique was used to determine the chemical association of heavy metals (e.g. Zn, Cd, Pb, Cu) with major sedimentary phases (exchangeable cations, easily and moderately reducible compounds, organic/sulphidic phases, residual components) in samples from polluted rivers in Central Europe (Middle Rhine River, Lower Rhine/Rotterdam Harbor, Weser Estuary, Neckar River) [118]. Data suggested that the surplus of metal contaminants introduced into the aquatic system from anthropogenic sources was usually found in relatively unstable chemical forms. Extractions with acidified hydroxylamine solution seemed to yield the metal fractions, which might predominantly participate in short-term geochemical and biochemical processes. Rates of mobilization were significantly higher for Zn and Cd than for Cu and Pb. The uptake of heavy metals by organisms occurred chiefly from the dissolved phase.

The availability of heavy metals depends greatly on the properties of particle's surface, on the kind of strength of the bond, and on external conditions such as pH, Eh, salinity, and concentration of organic and inorganic complexation agents. Most particle surfaces have an electrical charge, in many cases, a negative one. In solutions, an equivalent number of ions of opposite charge will gather around the particle, whereby an electric double layer is created. The surface charge is strongly affected by pH

and the composition of surface. Especially hydrous oxides of Fe, Al, Si, and Mn and organic surfaces (e.g., functional amino and carboxyl groups) participate in the  $H^+$  transfer. Lattice defects of clay minerals and the adsorption of ions also contribute to surface charges. The sorption process can be physical or chemical adsorption as well as sorption by ion exchange. Physical adsorption on the external surface of a particle is based on van der Waals forces or relatively weak ion dipole or dipole-dipole interactions (ca. 1 kJ/mol). Additional reactions could occur with physical sorption on the inner surfaces and in pores; capillary condensation within the pores or inclusion of molecules or ions that fit easily into the pore system. Solids include Fe-oxides, Al-hydroxides, clay minerals, and molecular sieves like zeolites.

Two sequential extraction schemes (a modified Tessier procedure with five steps and the three-step protocol designed by BCR) were applied to four sediment samples with different heavy metal contents [119]. The results obtained for Cd, Cr, Cu, Ni, Pb, and Zn partitioning showed that the metal distribution with the two procedures was significantly different. With the three-step protocol significant amounts of all the heavy metals were extracted with the oxidizing reagent (third fraction), whereas with the modified Tessier procedure the nonresidual metals were distributed among the second, third, and fourth fractions ( $CH_3COOH$ -acetate buffer, pH = 5; reducing and oxidizing reagents, respectively). The residual fraction obtained applying the three-step procedure was in general larger than that obtained using the five-step procedure, except for cadmium.

Contaminated dredged sediments were thermally treated at temperatures ranging from 120 to 450 °C [120]. The leaching behavior of selected metals (Cd, Cu, Pb, Zn, Fe, and Mn) was studied using  $NH_4Ac$ -EDTA extraction. In addition, solid-phase fractionation was carried out by means of a sequential extraction procedure. The  $NH_4Ac$ -EDTA extraction showed a sharp increase in metal leachability from sediments treated at intermediate temperatures (120–350 °C). The metal fractionation of the solid phases treated at the different temperatures did not show relevant shifts except for Cu. The decrease of the oxidizable Cu fraction was highly correlated ( $P < 0.001$ ) with the disappearance of organic matter at higher treatment temperatures. It is suggested that binding energy changes within the fractions rather than shifts between chemical forms account for the observed leaching behavior. Assessing the environmental quality of sediments based solely on total chemical analysis does not allow a differentiated approach toward a cost-effective and environmentally acceptable treatment strategy for contaminated dredged sediments.

Eight heavy metals from the clay fraction of sediments from major rivers within Germany were determined by atomic absorption spectroscopy (AAS) [121]. Heavy metals especially known for their high toxicity were enriched most (Hg, Pb, Zn by a factor of 10, Cd by 50), as compared to the natural background of these elements. A mobilization of heavy metals from the suspended load and from the sediments, as expected in rivers approaching the marine environment, could endanger marine organisms, thus negatively influencing the aquatic food chain.

Arsenic was partially extracted with 4.0 mol/l HCl from samples collected at 25-cm intervals in a 350-cm column of sediment at Milltown Reservoir, Montana, and from a 60-cm core of sediment collected at the Cheyenne River Embayment of Lake Oahe, South Dakota [122]. The sediment in both reservoirs was highly contaminated with arsenic. The extracted arsenic was separated into As(III) and As(V) on acetate form Dowex 1-X8 ion-exchange resin with 0.12 mol/l HCl eluent. Residual arsenic was sequentially extracted with  $KClO_3$  and HCl. Oxidized and reduced zones in the sediment columns were defined based on the results.

Applying a sequential extraction procedure, Coetzee carried out speciation analysis of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn in Hartheespoort Dam sediment [123]. Environmental risks associated with the potential remobilization probability of these metals were evaluated. The results showed that with regard to total metal content, the sediments of the dam would be comparable with moderately to heavily polluted fluvial systems in Europe and North America. The observed metal distribution patterns in the different sediment fractions, however, indicated that major proportions of most metals seemed to be associated with the inert fraction and could therefore be classified as to be of geochemical origin.



The extraordinary metal-rich rock types of the Transvaal complex in the area surrounding the dam support this result.

The three-step sequential extraction protocol designed by the BCR was evaluated with regard to total recovery, reproducibility, selectivity of extractants, and extent of phase exchanges or redistribution of metals during the extraction [124]. Model sediments of known composition were prepared comprising humic acid and natural minerals like kaolin, quartzite, and ochre. Synthetic compounds like calcium carbonate (calcite) and iron oxide (goethite), which could be used to simulate typical components of sediments, were used for comparison. Results indicated that the procedure gave excellent recoveries for all six metals studied (Cu, Cr, Cd, Zn, Ni, and Pb). Reproducibility was 3 %. Redistribution of Pb and Cu in the presence of humic acid was demonstrated. The selectivity of the reagents was insufficient to warrant interpretation of results in terms of a specific origin of a metal in a particular phase. It was shown that the chemistry of a metal could be a more important parameter than its actual phase location in the sediment in determining its response to the extractants.

The reproducibility of Tessier's extractions and the total content of Cd, Cr, Cu, Fe, Mn, Pb, Zn, and Ca in river sediment have been evaluated [125]. The accuracy of the dissolution procedures was evaluated using a reference material, BCR 145. None of the methods applied proved optimal for all the metals determined. The concentrations of metals extracted by the various reagents were characterized by good reproducibility on species bonded to the carbonates, to Fe/Mn-oxides, and in the residual fraction, precision was lower in the other cases. The sequential extractions also showed a satisfactory mass balance.

A modified sequential extraction procedure was applied for determination of the distribution of seven elements (Cd, Cu, As, Pb, Cr, Ni, and Zn) in sediment samples collected at Lake Balaton [126]. The fractions were (1) exchangeable and bound to carbonate, (2) bound to Fe/Mn-oxides, (3) bound to organic matter and sulfide, and (4) acid-soluble. Samples were taken in three seasons, and the average concentration of the elements was calculated. Based on the results determined at 15 sampling points along the lake, it seems that Lake Balaton has not yet been polluted. Concentrations of fractions were below the Hungarian standard levels set for soils, and most of the elements were found in the acid-soluble fraction, indicating stable compounds in sediments. There were no significant changes in the individual seasonal concentrations of elements during this short monitoring period.

The conventional Tessier and BCR sequential extraction methods were applied to metal partitioning in sewage sludge samples collected from a wastewater treatment plant. The results obtained by sequential extraction methods for Cu, Cr, Ni, Pb, and Zn were compared with those estimated from single extractions using identical operating conditions applied in each individual Tessier and BCR fractions. In all fractions of the BCR method, and in the three first fractions of the Tessier method, the results obtained from single extractions were in good agreement with those obtained using the sequential procedures. The oxidizable fraction of the Tessier method should be leached from the solid residue of the reducible extraction, since very low extraction efficiency was obtained for this fraction when it was directly extracted. The total extractable metal contents obtained by both single and sequential extractions were similar for all metals in the BCR method and for Cr, Ni, and Pb in the Tessier method. The recoveries obtained ranged from 93.5 to 105.8 % in the two samples. For Cu and Zn, the overall extraction efficiency of the proposed method was slightly lower than that obtained with the sequential procedure (recoveries around 90 %). The precision of the proposed Tessier and BCR single extraction methods was better than 8 % (RSD) for all metals.

## SEQUENTIAL EXTRACTION SCHEMES APPLIED TO SOIL SAMPLES

To assess the metal mobility of trace elements in soils on different time scales, a wide range of extraction schemes have been employed [55,58,82]. These methods vary with respect to the extraction conditions: chemical nature and concentration of extractants [58], solution/soil ratio, operational pH, and extraction time. If more than one extractant is used, differences occur due to variation in the extraction

sequence. The most critical steps are soil sampling, sample preparation, and the selectivity and accuracy of the extraction procedure [55]. As for total metal concentrations, spatial heterogeneity [127], as well as seasonal variation of extractable metal fractions [55] may bias the results. The use of correlation coefficients for choosing extractants for assessment of plant availability of elements needs consideration [128]. For extraction of the exchangeable fraction, almost all possible combinations of major cations with either  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , or acetate has been used, with concentrations ranging between 0.05 and 1 mol/l, and pH in the neutral range. The solution/soil ratios vary from 4:1 to 100:1, the extraction times between 30 min and 24 h. In ideal systems, the relative exchangeability of trace metals is determined by the affinity of the exchanging cation for the soil solid phase. This affinity increases with increasing valency and decreasing radius of the hydrated cation [82]. Although heterogeneous soil systems may deviate from this ideal behavior, the selectivity of soils for cations was frequently observed to increase according to  $\text{Na} < \text{K} < \text{Mg} < \text{Ca}$ . Consequently, under comparable conditions (e.g., concentration, extraction time, soil/solution ratio, the efficiency of cations to exchange trace metals usually increases according to  $\text{Li} < \text{Na} < \text{K} < \text{Mg} < \text{Ca} < \text{Ba} < \text{La}$ ) [81]. Because Mg is a harder Lewis acid than Ca, it was reported to displace also specifically adsorbed trace metals [129]. Therefore, Ca- and Ba-salts are regarded as most effective and selective agents in extracting exchangeable trace metals. Unfortunately, both cations may cause serious background problems (interferences) during determination of Pb and other trace metals. Usually, this can be resolved only by dilution of the extracts by  $>1:10$  prior to measurement decreasing the detection limits by the same ratio [130]. For that reason, the use of easily volatilizable salts (i.e.,  $\text{MgNO}_3$  or  $\text{NH}_4\text{NO}_3$ ) has been proposed [61,65,131–133]. Compared to 0.1 mol/l solutions of  $\text{CaCl}_2$  or  $\text{BaCl}_2$ , 1 mol/l  $\text{NH}_4\text{NO}_3$  in 2.5:1 ratio was found to extract about equal amounts of Al, Fe, Mn, Ni, Pb, V, and Zn, while Cu at concentrations of  $>0.1$  mol/l, and Cd were less efficiently extracted by 1 mol/l  $\text{NH}_4\text{NO}_3$  [64]. This can be explained by a more effective extraction with  $\text{CaCl}_2$  through the formation of chlorocomplexes with Cd and Cu. Other anions frequently used are either acetate or nitrate. At equal concentrations, the complexing ability increases in the order nitrate  $<$  chloride  $<$  acetate. The selectivity for extraction of the unspecifically sorbed (exchangeable) fraction should be decreased in the same order.

The specifically sorbed fraction is explicitly addressed only by few methods. In addition to other differences, the wide range of cations used suggests that most methods do not address any “specifically sorbed” fraction, and, likely even do not extract the same operationally defined solubility class. To extract specifically sorbed trace metals,  $\text{Pb}(\text{NO}_3)_2$  seems to be most adequate, due to its low  $pK$  (7.7) and large atomic radius, and it is being effective in displacing other trace metals, i.e., Cd ( $pK = 10.1$ ), Ni ( $pK = 9.9$ ), Co ( $pK = 9.7$ ), Zn ( $pK = 9.0$ ) and Cu ( $pK = 7.7$ ), with smaller atomic radius than Pb [134].  $\text{Pb}(\text{NO}_3)_2$  was found to extract less metal than HAc, probably indicating that the later was more specific [135]. For similar reasons,  $\text{Cu}(\text{Ac})_2$  was chosen by Mandal et al. [136]. Unfortunately, those trace metals being constituents of the extractants cannot be determined. Therefore, Zeien et al. [65] proposed 1 mol/l  $\text{NH}_4\text{Ac}$  and 1 mol/l  $\text{NH}_4\text{NO}_3$  in sequence to extract an operationally defined fraction under optimized analytical conditions by using only one cation ( $\text{NH}_4^+$ ) and decreasing the pH throughout the extraction sequence.

Among the extractants most frequently used to dissolve trace metals bound to carbonates are acids such as HCl and HAc ( $\text{pH} = 3\text{--}3.5$ ) ( $3 < \text{pH} < 3.5$  would be better), buffer solutions of HAc/NaAc ( $\text{pH} = 5$ ), and the buffering complexing agent  $\text{Na}_2\text{EDTA}$ , at  $\text{pH} = 4.6$ . Any of these extractants seems to have some potential to extract carbonates from soils, but is probably neither effective for quantitative dissolution (i.e.,  $\text{CH}_3\text{COOH}$  buffers), nor selective (cold diluted acids, i.e., HCl), or both, i.e.,  $\text{Na}_2\text{EDTA}$  [82]. Zeien et al. [65] proposed a procedure to dissolve carbonates by adding equivalent amounts of diluted HCl to a 1 mol/l  $\text{NH}_4\text{Ac}$ /HAc buffer, addressing the specifically sorbed and surface occluded trace element fraction of soils with  $<5\%$  carbonates.

For extraction of organically bound trace metals, various approaches were used, e.g., their release by oxidation or dissolution of the organic matter, or through addition of competing, e.g., complexing or

chelating ligands. Among the oxidizing extractants,  $\text{H}_2\text{O}_2$ , either purely or combined with  $\text{HNO}_3$  or  $\text{NH}_4\text{Ac}$ , extracts more trace metals from soils than  $\text{NaOCl}$  [82], i.e., from Fe/Mn-oxides [137].  $\text{K}_4\text{P}_2\text{O}_7$  and  $\text{Na}_4\text{P}_2\text{O}_7$  were reported to dissolve organic matter by dispersion and to efficiently complex the released metals [138,139]. Again, there is evidence from Mössbauer spectrometry [140] and other investigations that, depending on the extraction conditions [141], these extractants dissolve trace metals also from amorphous Fe-oxides [138,142], or from organo-mineral associations [140,143]. Accordingly, the variation in extraction parameters with concentrations between 0.1 mol/l and 1 mol/l, solution/soil ratios between 10:1 and 100:1, and extraction times from 1 h to 24 h indicate that results obtained by different procedures are hardly comparable and are likely to extract non-organically bound trace metals to a varying extent.  $\text{K}_4\text{P}_2\text{O}_7$  was found to extract more metals when used before Mn-oxide extraction with  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , while the latter extractant has little effect on the organically bound fraction [135]. Appropriately, pyrophosphate should be used first. Nevertheless, some methods followed the reverse extraction sequence [144,145].

As an alternative to pyrophosphate salts, some procedures employ  $\text{NaOH}$  [60] or  $\text{NaOH/EDTA}$  mixture [146] to extract organically bound trace metals by dissolution of organic matter. The selectivity of these methods is considered low, and the extracted metals may precipitate as hydroxides [82]. Alternative to destruction of the organic ligands, organically bound trace metals may be extracted by competing synthetic chelates (e.g., EDTA or DTPA) [89]. In sequential extractions, EDTA [57] or its ammonium salt [65] was less frequently used than the advantages would suggest. As  $\text{NH}_4\text{EDTA}$ , adjusted with  $\text{NH}_4\text{OH}$  to pH 4.6, was reported to dissolve considerable amounts of amorphous sesquioxides, it may be less selective than some pyrophosphate methods. Nevertheless, it should be considered as an alternative to extractants with alkaline pH (e.g.,  $\text{Na}_4\text{P}_2\text{O}_7$ ,  $\text{K}_4\text{P}_2\text{O}_7$ ,  $\text{NaOH}$  or  $\text{NaOCl}$ ). Thus,  $\text{NH}_4\text{EDTA}$  (pH 4.6) can be fitted in a sequence of extractants with decreasing pH that is thought to increase the selectivity by minimizing adverse effects on each subsequent extraction step (i.e., readsorption or precipitation of trace metal compounds) [65]. Moreover, the procedure is nondestructive to organic matter and organo-mineral associations, thus creating no new surfaces that may cause adsorption of trace metals during subsequent extraction steps as discussed by Beckett [58]. The dissolution of amorphous sesquioxides is probably limited by choosing a reasonable extraction sequence, extracting organically bound trace metals after removal of the most labile oxide fraction (e.g., the Mn-oxides) [84,135], and by a comparably short extraction time of 90 min, as proposed by Zeien et al. [65]. Accordingly, good correlation were found between organic carbon and  $\text{NH}_4\text{EDTA}$ -extractable metal fractions [147], although there was evidence that EDTA extractants may dissolve trace metals from (amorphous) sesquioxides. Among sesquioxides, the Mn-oxides are most susceptible to changes in pE and pH. Therefore, trace metals bound to Mn-oxides (i.e., Pb) may be readily mobilized upon changed environmental conditions (e.g., flooding) [144,148]. For that reason, this environmentally significant fraction is separated prior to Fe- and Al-oxides by most sequential extraction procedures. Essentially, Mn-oxides were extracted by reducing agents, e.g.,  $\text{NH}_2\text{OH}\cdot\text{HCl}$  or hydroquinone, either pure or mixed with  $\text{NH}_4\text{Ac}$ ,  $\text{HAc}$ , or diluted  $\text{HNO}_3$ . Some procedures extract Mn-oxides by 0.1 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 0.01 mol/l  $\text{HNO}_3$  (pH = 2) as initially proposed by Chao [84], or modified, i.e., 0.01 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 0.1 mol/l  $\text{HNO}_3$  (also reported at pH = 2). Results indicate that the modified method is probably less selective to Mn-oxides [84]. Chao's method dissolved about 50 % of total Mn and <1 % of total Fe from highly weathered soils when applied without previous extraction of exchangeable and specifically sorbed trace metals [84], indicating a good selectivity for Mn-oxides. Conversely, with higher concentrations (i.e., 0.25 mol/l) and higher temperatures during extraction (i.e., 50–100 °C),  $\text{NH}_2\text{OH}\cdot\text{HCl}$  extracted considerable amounts of trace metals from sesquioxides with a wide range of crystallinities [58]. As intended by Tessier et al. [61], 0.04 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 25 %  $\text{HAc}$  (pH = 2 at 85 °C for 5 h) actually should extract most of the sesquioxides, including the crystalline fractions.

Zeien et al. [65] proposed 0.1 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 1 mol/l  $\text{NH}_4\text{Ac}$  (pH 6, 30 min at 20 °C), although Chao [84] selected pH 2 to avoid hydrolysis and subsequent readsorption or precipitation of trace metals. Nevertheless, the procedure of Zeien et al. [65] seems to be comparably selective, hence

it dissolves on an average 37 % (0.12–73.9 %) of total Mn, but only 0.02–2.9 % of total Fe from a variety of soils. A negative correlation among Fe and Mn extracted by 0.1 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$ /1 mol/l  $\text{NH}_4\text{Ac}$  (pH = 6) indicated that only for low levels of Mn-oxides present in the soil, this reagent dissolved some Fe-oxides up to 2.9 % of total Fe. Since  $\text{NH}_2\text{OH}\cdot\text{HCl}$  has little effect on the organically bound metal fraction, it should be applied prior to extractants like  $\text{K}_4\text{P}_2\text{O}_7$ ,  $\text{Na}_4\text{P}_2\text{O}_7$ ,  $\text{NH}_4\text{EDTA}$  [135]. Instead of  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , some authors [84,149] proposed a mildly reducing mixture of 0.2 % hydroquinone and 1 mol/l  $\text{NH}_4\text{Ac}$ , dissolving considerably less Mn than  $\text{NH}_2\text{OH}\cdot\text{HCl}$  [58]. Applied repeatedly, hydroquinone may be used to partitioning of “easily reducible” and more “resistant” Mn-oxides [57]. In this context, it should be remembered that, along with sorbed fractions, the Mn-oxides are most sensitive to drying procedures prior to extraction, usually causing underestimation of the Mn-oxide fraction upon air-drying. The release of Mn and associated trace metals may be partly reversible, given sufficient time for equilibration after rewetting [51]. Since the required period of equilibration was frequently found to be about one week, the selectivity should be considerably improved by rewetting the soils and allowing them to equilibrate prior to sequential extraction.

Trace metals bound to Fe- and Al-oxides were extracted either by one step or were partitioned in two fractions, referred to as amorphous and crystalline Fe-oxides. Essentially, trace metals bound to amorphous Fe-oxides were removed by various modifications of Tamm's [150] acid oxalate solution in the dark [58]. In addition to X-ray amorphous Fe-oxides [151], acidic ammonium oxalate in the dark has been claimed to extract fulvic Fe complexes [138,143] and magnetite [151], but only little crystalline goethite or hematite [152]. This indicates that the selectivity of the method may be satisfactory as long as the extraction time is between 3–48 h [151,154]. The effects of variation in other extraction parameters, e.g., pH (3–3.5), concentrations of reagents (0.113–0.2 mol/l for  $(\text{NH}_4)_2\text{C}_4\text{O}_4$ , 0.087–0.2 mol/l for  $\text{H}_2\text{C}_2\text{O}_4$ ) and solution/soil ratio (5:1–100:1) on the extractability of trace metals can hardly be evaluated. Particularly, the great variation in solution/soil ratios leaves doubts on the comparability of these procedures. Moreover, instead of acidic ammonium oxalate solutions, 0.25 mol/l  $\text{NH}_2\text{OH}\cdot\text{HCl}$  + 0.25 mol/l HCl was also used to extract trace metals from amorphous Fe-oxides [155].

To extract either the total amount of Fe-oxides, or the crystalline fraction subsequent to removal of the amorphous Fe-oxides, acid oxalate solutions were frequently employed either under diffuse illumination or UV radiation at 20–100 °C and solution/soil ratios between 10:1 and 50:1 for 0.5–3 h. The concentrations of the  $(\text{NH}_4)_2\text{C}_4\text{O}_4\cdot\text{H}_2\text{O}/\text{H}_2\text{C}_2\text{O}_4$  reagents were either 0.175 mol/l/0.1 mol/l or 0.2 mol/l/0.2 mol/l, occasionally used along with 0.1 mol/l ascorbic acid. This variety of conditions and the pronounced effects of varied illumination and temperature on Fe extractability would suggest that hardly two procedures extract equal amounts of trace metals from soils [58,151]. Despite variation in extraction parameters, most procedures may fairly selectively remove the crystalline Fe-oxides when employed subsequent to extractions of Mn-oxides, amorphous Fe-oxides, and organic and carbonate fractions [58]. Uncertainties remain as to whether different extraction conditions may result in dissolution of varied amounts of trace elements from clay minerals [156,157]. Instead of acidic ammonium oxalate, some sequential extraction procedures [155,158] employ dithionite-citrate-bicarbonate (DCB) extractions according to Mehra et al. [159]. This method was widely used for individual extraction of “free” Fe-oxides, extracting similar amounts of Fe as acidic ammonium-oxalate under illumination, but it was not available as a pure reagent for trace metal analysis [58]. Also, at pH < 4.5, DCB precipitates sulfur and Fe-sulfides [160]. Accordingly, other trace metal sulfides may precipitate as well, so trace extractants were usually employed at pH = 7.8 as proposed by Mehra et al. [159]. This implies that DCB extractants do not fit into a sequence of extractants with decreasing pH. Summarized, acidic ammonium oxalate (3 < pH < 3.5) sequentially employed in the dark and under illumination is obviously adequate for dissolution of trace metals from amorphous and crystalline Fe-oxides.

A list of the most commonly used extraction procedures and the addressed fractions is given in Table 1. The procedures contain in general the extraction steps as described previously (in changed order or more compressed). Slight or significant modifications of these most commonly used proce-

dures are widely reported in the literature. Most extraction procedures address a wide range of heavy metals, but some extraction schemes were developed for specific elements or groups of elements.

**Table 1** Overview of sequential extraction schemes [30].

Reference	I	II	III	IV	V	VI	VII	VIII	IX	X
Tessier et al. [61]	–	1	–	2	4	3	3	3	–	5
Kersten et al. [173]	–	1	–	2	4	3	3	–	–	5
Hirner [62]	1	2	–	5	3,4,7	5	5	5	5	6
Sposito et al. [60]	–	1	2	4	3	–	–	–	5	–
Shuman [133]	–	1	–	–	2	3	4	5	–	6
Zeien et al. [65]	–	1	2	–	4	3	5	6	–	7
Kotuby-Amacher et al. [155]	– <sup>a</sup>	1	2	–	–	3	4	5	–	6
Sims et al. [144]	1	2	–	–	3	4	5	–	6	7
Saha et al. [174]	1	1	–	–	2	3	4	5	–	–
Kuo et al. [158]	–	1	–	–	4	–	2	3	–	(4)
Liang et al. [175]	1	1	2	–	3,5	3,4	6	–	–	7
Jarvis [176]	–	1	–	–	4	2,3	5	5	–	(6)
Goldberg et al. [57]	1	1	–	–	2	3	4	4	–	5
Miller et al. [135]	1	2	3	–	4,6	4,5	7	8	–	9
Mandal et al. [136,177]	1	1	–	–	2	–	3	4	–	–
Murthy [178]	–	1	–	–	1	–	2	3	–	(4)
McLaren et al. [83]	–	–	–	–	1	2	3	3	–	(4)
Soon et al. [179]	–	1	2(?)	–	2(?)	–	–	–	–	3(?)
Shuman [180]	–	–	–	–	2	–	3	3	–	4
Rauret et al. [181]	–	1	–	2	4	3	3	3	4	5
McLaren et al. [182] <sup>b</sup>	1a	1a	2a	–	1b	2b	2b	2b	–	3b

*Chemical species tentatively being extracted:*

I: water-soluble

II: unspecifically adsorbed (exchangeable)

III: specifically adsorbed (sorbed components)

IV: bound to carbonates

V: organically bound

VI: Mn-oxides

VII: amorphous Fe-oxides

VIII: crystalline Fe-oxides

IX: sulfides

X: residuals (silicate bound)

*Sequence: indicated by arabic numbers*

<sup>a</sup>Water-soluble fraction determined on separate subsamples by extraction with 0.005 M Ca(NO<sub>3</sub>)<sub>2</sub>, soil/solution ratio = 1:10 for 16 h.

<sup>b</sup>McLaren and Crawford employed two individual extraction schemes (referred to as a and b).

A sequential dissolution/precipitation procedure has been applied for the study of Hg speciation in soil, which produced 26 fractions. The final determinations of Hg were performed by the cold vapor technique [161]. The Hg was determined with a recovery of 96–98 % by an extraction scheme using 1 mol/l ammonium acetate, 1 mol/l NH<sub>2</sub>OH·HCl in 25 % CH<sub>3</sub>COOH, 0.1 mol/l HCl, 0.5 mol/l NaOH, and 8 mol/l HNO<sub>3</sub> [162].

A chemometric mixture resolution procedure suitable for determining the number and composition of physicochemical components in data derived from soil leachates is described [163]. The procedure is used to determine the number of components in sequential leachate data obtained for a NIST-certified soil (SRM 2710) using a widely applied leaching scheme. The resulting data show that the sequential leaching media are not specific for their designated target fractions and that erroneous

identification of fractions occurs. A study in which a new non-specific extraction method was tested was described. There was an evidence that the Tessier method extracted both Fe- and Mn-oxides simultaneously, whereas the nonspecific method has resolved the Fe- and Mn-oxides as separate entities.

Rare earth elements (REEs) have recently been extracted using a modified Zeien and Brümmer scheme [164]. Results showed that REEs have been fractionated during weathering. Moreover, organic matter seems to be important for the particulate transport of REE. A 3-step extraction was applied for the determination of the REE availability and their uptake by plants. Radionuclides and their distribution in soil phases and the physiochemical association in soil near the subsurface are of increased interest. Pu in surface soils seems to be primarily associated with the hydrous oxide coatings of the soil, organic matter, and carbonates [165]. The NIST standard sequential extraction protocol is used for identifying the fractions of radioactive elements in soils and sediments in six operationally defined fractions [166]. Cesium was extracted sequentially by a modified Tessier procedure [167]. A sequential chemical extraction method for the determination of the geochemical fractionation of Am, Pu, and U was evaluated by Schultz [166]. Pu and Cs mobility was examined by sequential extraction (modified Tessier procedure) and indicated that radiocesium is presently more mobile in the deeper soil layers.

## **ANALYTICAL METHODS FOR THE ELEMENT DETERMINATION IN SINGLE FRACTIONS**

The following methods are generally used for the determination of trace elements in the single fractions: electrochemical techniques, atomic absorption spectrometry (GF-AAS, F-AAS), inductively coupled plasma atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectrometry (ICP-MS), neutron activation analysis (NAA), spark source mass spectrometry (SSMS), ultraviolet spectrometry (UV-Spec), X-ray fluorescence spectrometry (XRF), and atomic fluorescence spectrometry (coupled with hydride generation).

More direct methods for the determination of trace elements on soil and sediment fractions are used to allow a direct assessment of the different phases in soils and the determination of trace elements bound to these particular phases. Investigation of the relevance of the results with respect to bioavailability is still under discussion. The direct instrumental speciation approach has been successfully developed recently. Trace metal sulfides in anoxic sediments have been identified by microbe techniques [168–169]. The mechanism of sorption of trace metals on hydrous Fe/Mn-oxides and calcite have been recently revealed by speciation analysis using XANES and EXAFS [44], XANES [170]. Laser ionization breakdown spectrometry is still a rather “exotic” method having a certain potential to analyze soils directly in the field. In this technique, a laser is used for the direct determination of Cd, Cr, Fe, Hg, Mn, Pb, Ti, and Zn in subsurface soils [171,172]. Alpha proton X-ray spectrometry (APXS) on board the Mars Pathfinder mission measured the composition of six soil samples and five rock samples at the Ares Vallis landing site on Mars.

## **CONCLUSIONS**

Despite all limitations, sequential extraction schemes can provide a valuable tool to distinguish among trace metal fractions of different solubility. These fractions are empirically related to mobility classes in different solid samples. The speciation of metals governs their availability to plants and their potential to contaminate the environment. Available forms of metal cations are not necessarily associated with one particular chemical species or a specific sediment and/or soil component. Speciation of trace elements may vary with time, depending on the solid-phase components that are present, pH, and the number and accessibility of adsorption sites. Soluble and exchangeable forms of metal ions will decrease with time if there are other solid components present that can adsorb the metal more strongly and have free sites that are accessible (e.g., hydrous oxide, organic matter).

The present state of knowledge on solid matter speciation of trace elements is still somewhat unsatisfactory because the appropriate techniques are only operational with associated conceptual and practical problems. With respect to estimating bioavailable element concentrations, one such conceptual problem is the effect of competition between binding sites on the solid substrate and selective mechanisms of metal translocation by the different organisms involved. This situation cannot yet be improved by more sophisticated analytical approaches to speciation. On the other hand, the usefulness of a differentiated approach, even if only operationally defined, to the interactive processes between water-biota and solid phases has been clearly proven. The possible environmental implications (e.g., during dredging operations) of land disposal of waste material, of acid precipitation, of redox changes in sub-soil, and of ingestion of polluted urban dust, can be qualitatively estimated, particularly when the physicochemical conditions of the interacting compartments of the environment are taken into consideration. The method of sequential chemical extraction is the least sophisticated and most convenient technique available for a speciation assessment. However, we must be certain that we fully understand what is happening during extraction to minimize the risk of producing artifacts and choose standard procedures to ensure that results are comparable. The primary importance of proper sampling protocols has been emphasized, since the sampling error can cause erroneous results even using highly sophisticated analytical methods and instruments. The number of fractionation steps required depends on the purposes of the study. The BCR protocols give a simple guide for most of the solid samples and the results can be compared among different laboratories.

Geoscientists and environmental engineers extensively use results of chemical speciation analysis and our responsibility is to show the pitfalls and limitations of sequential extraction procedures developed. Declaration of the uncertainty of results is a must and greatly improves the quality of these activities.

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