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METALS IN THE ENVIRONMENT MOBILITY, BIOACCESSIBILITY, AND CONTAMINATION

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Abstract: This paper shall give a short overview about analytical methods to test environmental mobilities and bioaccessibilities of trace metals in soils, sediments and airborne particulate matter, focussing upon essentials and toxics, as well as methods to minimize possible hazards. Merits and limitations of interpretations, like grain size effects and matrix effects, are outlined. It is based primarily on the experiences of the author and his comprehensive review, but contains still unpublished graphics. Multi-element determination techniques should be used to characterize the release of main elements and nutrients from the solid also.

Keywords: soils-sediments-airborne particulates, trace metals, contamination, bioaccessibility, mobilizable fractions, column experiments, simulated digestive fluids

1. INTRODUCTION

Metals have been known and used since the bronze age, abova all gold, silver and copper, and also tin to harden the copper to make bronze weapons and ornaments. Gold and silver have been used by leaders and kings to show their power and wealth, and to make coins. Invention of cast iron to design knives, swords and hawks meant a huge advantage in fights. Lead has been used for water pipes till the 20th century.

Ore veins have been indicated by indicator plants, which can tolerate higher soil metal levels, or by odd colorations of the soils or rocks. Until the beginning of the industrial era, smelters operated with charcoal at a local scale. Intoxications occurred also at a local scale. Lead and antimony were used for paints and cosmetics, mercury against syphilis, and thallium for depiliation, which caused a lot of toxic side effects.

Metal ions can have structural, catalytic, and regulatory functions within an organism, they activate enzymes and activate metabolic reactions. They interact mainly with proteins to yield prostethic groups of enzymes, in order to promote e.g. redox reactions, whereas interactions with carbohydrates and fat are usually low. Binding to –SH, -OH, -NH groups of proteins changes their shape, structure and functionality. Cu, Mn, Fe, Zn, Co, and Mo are usually regarded as the essential elements. Too low levels means an interruption of a metabolic pathway, and too high levels often leads to malformations of proteins, paricularly enzymes. Complexation with DNA (like with Al) results in synthesis of wrong proteins. Further reasons of metal intoxications are formation of precipitates inside the cells, clogging of cell pores, and substitutions of an essential element by a chemically similar one, which cannot fulfill the tasks. Examples are Cd-Zn, Tl-K, Pb-Ca (in bones), Fe-Al. Sometimes, adaptation of the genom to use the „wrong“ element instead, might occur by genetical selection. like Rb for K (Baligar & Barber 1978; Wyttenbach et al., 1995, Drobner & Tyler 1998), Eu for Ca, V for Fe, and As for P (Froelich et al., 1985, Kuhn & Sigg 1993; Peryea 1998; Wang et al., 2002).

For some elements, a level of minimum need has not been established, because the ambient abundance is much higher. E.g. the essentiality of lead has been established for rats at about 45 µg/kg in the food (Reichlmayr-Leis & Kirchgessner 1993) but this remains still unknown in textbooks.

A compilation of older references can be found in the texts of Sager (1992), or Hlavay et al., (2004).

2. SOURCES AND SCALES OF CONTAMINATION

Contamination means enrichment of metals in the environment by human activities, thus the ambient levels of occurrence have to be considered as well. Metal emissions to the atmosphere are mainly smelters, combustion processes (coal, fuel), and abrasion of buildings. Cleaning of flue gases, substition of coal and ban of Pb-paintings and Cd-pigments have reduced emissions. The loads from used batteries and electronic devices, may however increase.

Metal emissions to surface waters from metal industries can be much more easily controlled then diffuse pollution from waste deposit sites, metal roofs, and corrosion of cars. Further sources of input to the watershed are sewage, manures, cosmetics and pharmaceuticals, but also corrorion of naturally occurring surficial ore bodies. Clean-up of sewage and waste water by co-precipitation of unwanted traces with iron hydroxide is state of the art (Dyck, and Lieser 1981).

For each element, the **Geo-accumulation index** (Müller 1979) can be defined as

$$I_{geo} = \log^2 \frac{[M]}{[M]_{bg} * 1,5}$$

If the actual (total) metal concentration (in mg/kg) and the concentration of the geological background are known. In case the geological background is unknown, the mean crust value will give a rough estimate. The resulting scale yields terms from unpolluted (Igeo = 0) to moderately polluted ($I_{geo} = 2$), heavily polluted ($I_{geo} = 4$) to extremely polluted ($I_{geo} > 5$) sites, irrespective of the environmental mobility and bioaccessibility.

The **Contamination Index** (CI) also refers to expectable background values (Lee et al., 2001), continental crust), but summarizes the multi-element-approach to one number, which smoothes individual outliers of a few contaminants. It is calculated as the average from the actual metal contents on site, over the respective crustal abundance, e.g.:

$$CI = \{(\frac{As}{1}) + (\frac{Cd}{0,1}) + (\frac{Cr}{85}) + (\frac{Cu}{75}) + (\frac{Ni}{105}) + (\frac{Pb}{8}) + (\frac{Zn}{80})\} / 7$$

The pollution index (PI) (Nishida et al., 1982) refers to the tolerable levels, which have been set by legal advices, and has to be calculated for each country separately. For Austria (Danneberg 1999) e.g.:

$$PI = \{(\frac{As}{20}) + (\frac{Cd}{1}) + (\frac{Co}{50}) + (\frac{Cr}{100}) + (\frac{Cu}{100}) + (\frac{Hg}{1}) + (\frac{Mo}{5}) + (\frac{Ni}{60}) + (\frac{Pb}{100}) + (\frac{Se}{5}) + (\frac{Zn}{300})\}/11$$

3. TOTAL ELEMENT CONTENTS VERSUS MOBILE FRACTIONS

The total element contents of a given sample has been clearly defined. All methods must yield the same results. Currently, X-ray fluorescence (XRF), activation analysis (by e.g. neutrons) or microprobe directly target on the solid. After dissolution, the digests are susceptible to spectroscopic methods (ICP-OES, ICP-MS, AAS), electrochemic methods or photometric methods. All methods must yield the same results, and for more common elements and matrices, reference materials available. Many legal thresholds refer to totals, but predictions of origin, mobilities, availabilities, risks are based on assumptions. Environmentalists assume that concentration levels above the mean crust levels are due to contamination, they neglect geogenic enrichments in ore veins and their weathering products. But from total amounts, predictions of origin, mobilities, bioaccessibilities, and risks are not feasible.

The most relevant question, how much is geogenic and how much is contamination, in order to find somebody as guilty to have caused the contamination, is not easy to be answered, but gets indicated from speciation.

Speciations in the solid are responsible, how much of the total amount will dissolve, transported or transferred to biota and thus enter metabolic pathways. Main minerals can be identified by X-ray-diffraction. However contaminant metals hardly occur in detectable (> 1%) mineralogical phases, but adsorbed to amorphous compounds, surface coatings and at exchangeable sites of clays. Thus, selective dissolution methods have been developed, which target to dissolve certain types of mineral phases, leaving the others untouched (details see below). Sequences can be defined, to dissolve more mobile phases at first, and to use the residue for the next selective dissolution. Because experimental errors and uncertainties propagate from one step to all subsequent ones, this is limited to about 6 steps in practical work. In spite, there are much more possibilities of speciation possible, the resulting picture yields much more information than the total contents, but it is 6-times more effort and costs. These element patterns are operationally defined, however, and very few reference materials are available. Precision, recovery and ruggedness (e.g. soli:liquid ratio) should be tested at the start of each project, in particular before interpretation of trends, hazards and the like (Sager 1999).

In practical work, it is sufficient to distinguish metal fractions due to their mobility, bio-acceibility or eco-toxicology by operationally defined fractionation, whereas real speciation remains an assumption.

4. WHICH MAIN PHASES MAY ACT AS A CARRIER FOR TRACE ELEMENTS?

In oxic soils, the humics, the pedogenic oxides (Fe-Mn-Al), the clay minerals and the carbonates are the main carriers for those traces, which do not form proper phases (Fig. 1). Because the sorptive phases also combine with each other, their action versus trace elements is not additive. When the redox potential gets anoxic (often in deeper layers of sediments), pyrite is formed from the pedogenic oxides, which coprecipitates a lot of metals, but phosphorus gets released.

Humics means a dead organic substance, which carries –OH, -NH and –COOH groups to enable formation of chelates. Among all trace metals, copper has the strongest affinity to humics, other trace metals can form stable complexes as well (Hirata 1981). The pedogenic oxides are primarily products from the weathering of silicates, and sorb or co-precipitate other metal ions from solution. The affinity increases with the tendency to form low soluble hydroxides. This has been used in water purification processes (Dyck & Lieser 1981). The clay minerals exchange their interlayer cations, and unlike others can also take K, Tl, as well as Li at intermediate positions. In presence of water, hardly soluble carbonates are formed, mainly by Ca, Mg, Sr, Ba, Mn and Pb; some other carbonate minerals have been found in deserts. They are usually crystallised and thus easily accessible by XRD. Their alkaline surface might sorbent for hydroxides. Boric acid as a neutral monomer at low concentrations, is the most mobile species apart from halogenides, though it has been proven to be adsorbed anywhere. (Meyer & Bloom 1997, Goldberg & Glaubig 1986, Goldberg et al. 1993). Addition of zeolitic resp. organo-zeolitic amendments significantly facilitated phytoremediation of Cu mine waste dumps because it lowered the transfer of Cu and other metal cations to green plant shoots (Damian et al., 2013, Damian et al., 2019).

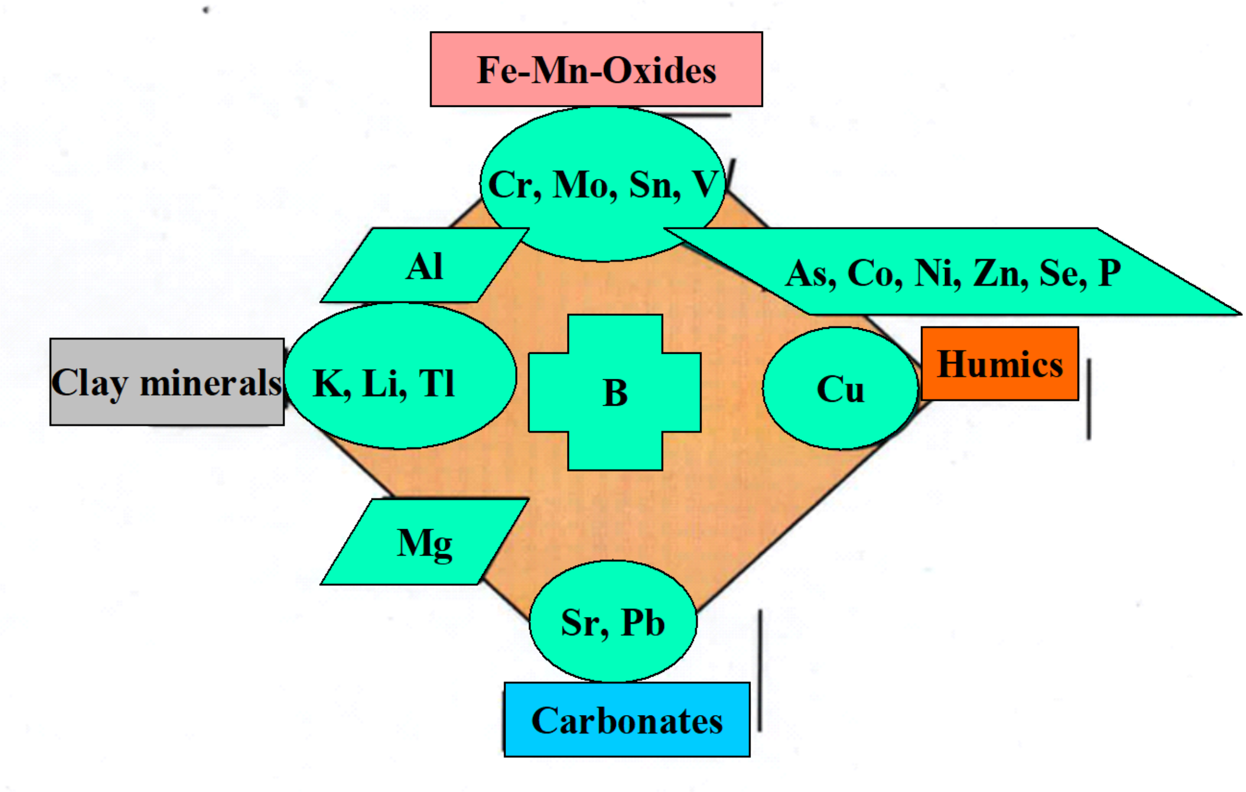


Figure 1. Affinity of trace elements to main solid phases in oxic soils (no sulphides)

5. EXPERIMENTS TO INVESTIGATE ENVIRONMENTAL MOBILITIES

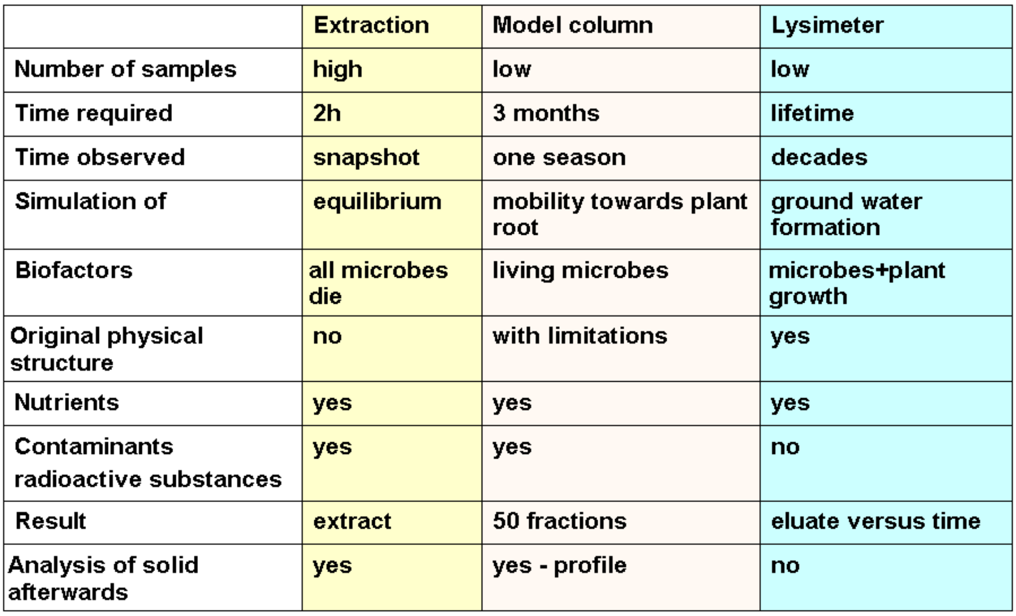
Metal hazards due not arise from metals bound to insoluble geogenic compounds, but from mobilized and solubilized fractions. Also, nutrients (like P) and essentials cannot be transferred into biota, if they occur in immobilized forms, except in case of inhalation of airborne particles. Thus, apart from total element contents, predictions of transport and runoff as well bioavailability should be considered.

Table 1 shows an overview about current methods to test environmental mobilities in soils and sediments. Usually, selective dissolution of mobile phases by extraction is done, because this is only limited by space and blanks, and the detection limit of the final determination method. ICP-OES (optical emission spectrometry) or ICP-MS (mass spectrometry) can measure every element which occurs above detectable concentrations within 2 ml in one run. Just spectral intereferences, or mass-interferences from chlorides and argon have to be considered. After centrifugation, the remaining solid can be used for a next reagent, which is termed sequential leaching. The result is a quasi-equilibrium of a snapshot of the moment of the start of extraction, because all microbes die, and amorphous phases have no further chance to recrystallize. Because one or a few grams of sample are used, the samples have to be homogenized, and the original physical structure gets destroyed (Sager 1992, Hlavay et al. 2004).

Within a model column, vertical mobilities of metals and nutrients during an entire season can be simulated. Cored columns contain the original soil structure, like stones and plant roots, which complicate hydraulic conditions. Columns filled with standard dried and sieved samples do not retain the original physical structure, but yield more defined hydraulic conditions to determine a breakthrough of applied substances. The test substances can either be applied as one cocktail, to continously, and washed out with water. The eluates are regularly collected and analyzed for a breakthrough peak or a breakthrough level of the applied substance. The area below the peak, resp. the turning point to the breakthrough plateau, can be used as significant figures to be combined with the properties of the solid. At the end, the column can be sliced, and investigated, to which depth and to which mobilizable fraction the added substance has gone. If the columns are kept in the dark, transfers to algae and green plants is prevented. Due to microbial activities and slow dissolution, the release in the runoff from soil columns during a 2 months period was much more than the quasi-equilibrium obtained after 2 hours extraction (Sager 2001, 2002, 2004).

Lysimeters are column in the open field, which can be seeded and harvested like their surrounding. Applications of toxic metals or radioactive substances is, however, limited to avoid the creation of hazardous waste. When they are established, the composition of the solid cannot be changed. A priori, a decision has to be made either to have cored or artificially filled lysimeters. During filling, stones and macropores are removed, and aeration changes microbial life. After some years, natural zonation gets established again. Percolation water is taken at the bottom, or in some cases at intermediate depths, but the solid inside is not directly accessible.

Table 1. Experiments to monitor environmental mobilities



	Extraction	Model column	Lysimeter
Number of samples	high	low	low
Time required	2h	3 months	lifetime
Time observed	snapshot	one season	decades
Simulation of	equilibrium	mobility towards plant root	ground water formation
Biofactors	all microbes die	living microbes	microbes+plant growth
Original physical structure	no	with limitations	yes
Nutrients	yes	yes	yes
Contaminants radioactive substances	yes	yes	no
Result	extract	50 fractions	eluate versus time
Analysis of solid afterwards	yes	yes - profile	no

A promising, but not often used technique is Electro-Ultrafiltration (EUF), which targets on the kinetics of dissolution (Fig. 2). A soil suspension is mixed in a cell with semipermeable wall, e.g. permeable for 20000 D, voltage (e.g. 200V, 400V) is applied by Pt-electrodes at constant current (e.g. 15 – 150 mA), to remove dissolved ions by an electric field into an anodic and a cathodic reservoir. Aqueous soil solutions have been used to extract nitrate, ammonium, K and B from arable soils, to predict fertilization needs for sugar turnips within a short time, which correlates with the $CaCl_2$-extract. For the dissolution of metals, a complexant (e.g. DTPA) has to be added, which is electrically inert, and which prevents precipitation of hydroxides at the cathode, where pH increases (Horn 2006). Because the back-reaction is prevented, 5-times sampling within minutes yields the dissolution kinetics, which is about linear (Jelecevic et al. 2019).

6. THE QUESTION OF BIOACCESSIBILITY

In agriculture, the main task is to find out, which part is available to green plants. Roots acquire their nutrients and trace elements via dissolution of their surroundings by root exsudates, or take it from the mycorrhiza. The latter are fungi, which penetrate soil and roots, and live on root exsudates. Fungi and soil bacteria gradually dissolve soil minerals.

Plant uptake thus depends on plant needs, the kind of plant, climate (water, temperature, sunshine), soil microbiology, and genetic adaptation to the respective site. Metal speciation, dissolution kinetics, and plant needs influence metal uptake and transport to leaves, shoots and fruits.

The main problem is to transfer a dissolution process of 2 hours till overnight to the uptake of a plant during the entire growing season, like to conclude from a snapshot to a film. Plant-available fractions have been established and standardized in agricultural labs, based on soil to plant transfer to cereals, maize and potatoes from arable soils (e.g. ÖNORM L-1089). These are all monocotyledones and annually seeded. They are growing for about 3 months, and take their metal load at about 15 days after germination. Plant physiology tells us that dicotyledones (e.g. fruit trees) or gymnospermae (e.g. fir trees) have a different transportation system. After loss of their leaves, the roots of perennial plants are still active to accumulate nutrients and metals, unless the soil gets frozen. Perennial greens like fir trees can assimilate and take nutrients and metals all the year round.

As the „plant available fractions“, various extracts have been defined in different countries, starting from the dry and > 2 mm sieved soil, which lead to 5 classes for interpretation: very low – low – sufficient – much – too much. The most simple extract is the water extract, which is surely correct for algal growth. It depends on the proportion solid/liquid, and the dissolution of colloids.

NH_4NO_3 extracts are easily compatible to subsequent ICP-MS measurements, they have been introduced to detect mobile Zn-Cd-Pb-Cu. They are not suitable for Ni and Cr(III) because of resorption of the hydroxides at the solids (Sager 1999, Traub & Scharf 2001). But it is applicable to extract Cr(VI) as such, unless the extract gets acid (pH>6) due to exchange of H^+ versus NH_4^+ (Sager 2005, Sager 2008).

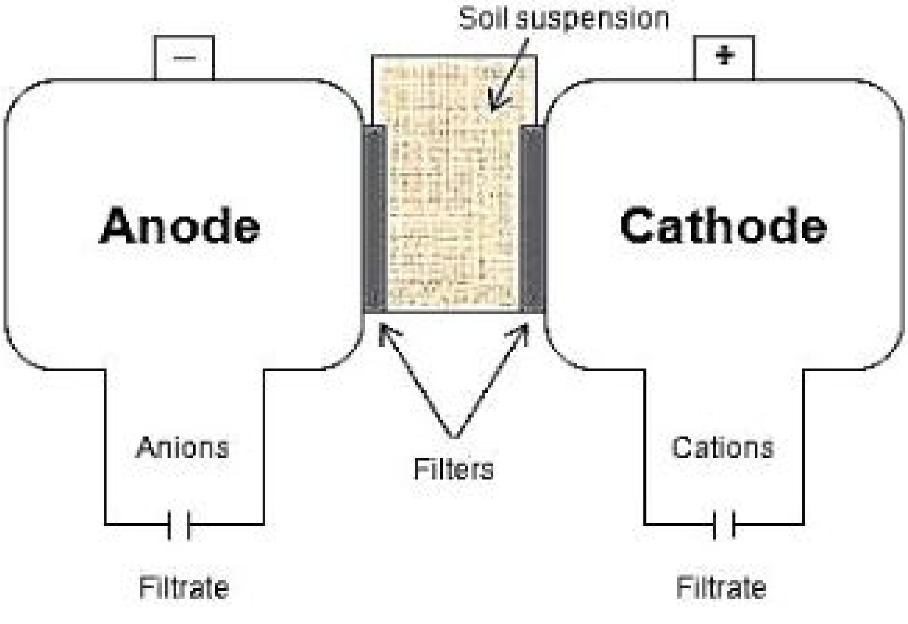


Figure 2. The EUF- method

Complexants take metals from any site, due to affinities and amounts of complexant used, which may be similar to plant roots, but not necessarily dissolve the same amounts. The Ca-acetate-lactate pH 4.0 extract has been primarily defined for the availability of phosphorus to wheat, maize and potatoes from soils in central Europe (Schüller 1969). Schüller was convinced that apatite is less available than other P-forms, and added Ca to prevent apatite dissolution.

Mehlich III is a complex mixture of chemicals, developed in the midwest of the US (0.2M CH_3COOH – 0.25M NH_4NO_3 – 0.015M NH_4F – 0.013M HNO_3 – 0.001M EDTA, pH 2.5), without giving any details of respective pot or field experiments (Mehlich 1984), but in subsequent papers of other authors. It is mainly used to determine available P, Zn, Cu, Mn and others.

EDTA extracts have been defined in 1989 to get plant-available fraction for Cu-Mn-Fe-Zn, which are termed as the „essentials“. Because Fe yields the most stable complexes, and is always more abundant than EDTA, the results may largely depend on reactive Fe-oxides, beneath ion exchange versus the double amount of Na (ÖNORM L 1089).

DTPA has been introduced as an extractant to monitor available metal fractions in 1978 (Lindsay & Norvell 1978). Compared with EDTA, it is the stronger complexant, but the weaker acid, thus it dissolves less at low pH, and more at high pH than EDTA.

Though too much Cu and Zn is also toxic, the more toxic metals Pb-Cd-Cr-Ni-Hg-As have been usually interpreted from data gained from the aqua regia (HCl/HNO_3) extract. Whereas Cd, Hg and As are totally recovered, Ni and Pb may be 10% lower, and recovery of Cr is highly variable, e.g. due to non aqua-regia soluble chromite $FeCr_2O_4$. In serpentine soils, Ni has been found to be dominantly bound to chlorite and serpentinite as a substitute for Mg, and this is not accessible without hydrofluoric acid treatment (Hseu et al. 2017). Dissolution kinetics of layers silicates in aqua regia necessitate strict standardization of conditions (liquid/solid, duration of reflux), if elements are determined, which are not completely soluble (like K, Al, Fe, Cr, Ni).

7. ASSIGNMENT TO MAIN PHASES

Clay minerals can be detected in X-ray diffraction. A sharp signal is gained from distances in the fixed layers, which coincides with feldspars. The distances between these layers, where exchangeable cations are situated, is slightly variable, and can be exactly determined after oriented sample preparation, to see, which clay minerals are present. Leaching with salt solutions are known to exchange their cations at the cationic interlayer sites of clay minerals. Apart from exchange reactions, the clays are very stable, and dissolve only in hydrofluoric acid. Salt solutions also kill all biota, and most of their contents gets released (Sager et al. 1990).

Carbonates usually give sharp signals in X-ray diffraction, and calcite, aragonite, dolomite and magnesite can be easily discriminated. In soil routine analysis, the sum of carbonates is determined by Scheibler´s method, that is to measure CO_2 evolved by addition of HCl within a closed system. Weak acids dissolve the carbonates, but also release everything which is exxchangeable versus H^+, like some mineral phosphates, or adsorbed hydroxides (Sager et al. 1989).

The **Fe-Mn(-Al) hydroxides** precipitate after weathering of the primary silicates, or after aeration of anoxic waters. They are formed amorphous in many cases, and thus cannot be seen by X-ray diffraction, but microlocally by microprobe (Latrille et al. 2001). Usually, a Mn-rich and an Fe-rich phase are formed. They are dissolved by reductants as well as by natural (e.g. citrate, tartrate) and artificial (e.g. EDTA) complexants (Stanjek 1998). Because reductants are assumed not to attack the humics, they seem to act more specifically. Na-dithionite is used at neutral pH; high contents of Na and S, and precipitation of elemental sulfur upon acidification are disadvantageous for ICP measurements. Hydroxylamine specifically dissolves the Mn-oxides, and NH_4-oxalate/ oxalic acid buffer pH 3 dissolves the Fe-oxides, without addition of alkali cations, and are thus favourable prior to ICP measurement of the leachates (Borggaard 1992, Del Campillo & Torrent 1992). The hydroxylamine can be acidified with 0,01M HNO_3, or with 25% acetic acid (Sposito et al. 1982).

In general, the proportion of poorly crystalline Fe-hydroxides over total iron increases with soil age, because of recrystallization of ferrihydrite to goethite (wet dry-cycles), which decreases the mobility of traces. Air-drying of soils, however, can also lead to an increased extractabilitry of Fe and Mn because of reduction of Mn-oxides by organic matter, alteration of soil functional groups, and further to release or recrystallisation of traces originally bound at these sites. Therefore, some authors have started leaching with freeze-dried soils. Grinding causes physical breakdown of soil micro-aggregates, may increase the extractability, but also re-adsorption because of formation of new surfaces (Hlavay et al. 2004).

Metal ions can be released from **organic substances** like humics by dissolution in alkali, oxidation or complexation. The soil or sediment organics are usually too hydrophilic to dissolve in organic solvents. Just covalently bound tin-organic compounds (Sager 1986), some Cu (Schlösser & Schwedt 1985) or chlorophyll are known to move into ethanol. Alkali add a lot of Na (or K) to the extract, and acidification, which is necessary to perform ICP or AAS determinations, may cause precipitation of the humics again. In addition, many metals reprecipitate as the hydroxides in alkali. Therefore wet oxidation is preferable, which is usually done by H_2O_2. HNO_3 combines oxidational and acid attack. $KClO_3$ is even stronger, but it needs some nitric acid, because it does not attack the organics itself, but re-oxidizes the nitrous acid (Sager & Hammerschmidt 2015).

Sulfides are hardly present in arable soils, but as residual minerals in orogenic zones, or as autochthonous precipitates under reducing conditions, particularly in sediments. Air-drying may oxidize some sulfides (e.g. CdS), thus, extractions in a nitrogen atmosphere, freeze-drying, or at least freeze-drying, are recommended (Förstner et al. 1990). Metals bound as sulfides are usually obtained after oxidation with H_2O_2, and can be hardly discriminated from humics; some are, however, quite resistant (Sager et al. 1990).

From this, the sequence given in table 2 has been designed by Tessier et al., (1979) and modified several times. It has been developed primarily for cations (Zn-Cd-Pb-Cu), but it is applicable for others also, but in this case there is no strict assignment to phases (Co, Ni, Cr; P, As, Mo, Sn, Si, La etc.). It has (to my knowledge) not been applied to Hg and Ag speciation.

Table 2. Sequence of extractions and assigned species (Sager et al., 1990)

I	ammonium acetate pH 7	exchangeables, living cells
II	ammonium acetate pH 5	carbonates, acid-exchangeables
III	0.1M $NH_2OH.HCl$ in 25% acetic acid	Mn-oxides
IV	oxalate buffer pH 3	Fe-oxides
V	oxidation with H_2O_2	humics, sulfides
VI	boiling HNO_3	residual

Feldspars, micas, clay minerals, spinells, rutil etc. need digestion with hydrofluoric acid. Exchangeables (fraction I) might be also extracted by $MgCl_2$/NaOH buffer pH 7. In case of much dolomite, pH in fraction II should be lowered to 4.5. The hydroxylamine (fraction III) has been also used in 0.01 M HNO_3, if the samples are not carbonaceous. The oxalate buffer has been introduced later (Hong & Förstner 1983), to discriminate a Mn-rich and an Fe-rich hydroxide phase, because these can be really sampled separately (Belzile et al., 1989b). Oxalate may release most of the metal loads, and releases less Fe in the dark (Borggaard 1992, Del Campillo & Torrent 1992).

Later, the sequence had been simplified (Quevauviller et al., 1997, Lopez-Sanchez et al., 1998), and a reference material certified for Cd/Cr/Cu/Ni/Pb/Zn (Table 3) and 0.5g in 20 ml extractant solution. Recently, this BCR-sequence has been modified (Table 4) and applied to apple orchard soils.

Table 3. Simplified sequence proposed by the BCR

I	0.11M acetic acid	1h/20°
II	0.1 M $NH_2OH.HCl$ in 0.01M HNO_3	16 h
III	+ 10 ml 30% H_2O_2 + 10 ml 30% H_2O_2 + 1M NH_4-acetate pH 5	1h boiling water bath 1h boiling water bath 16 h

Table 4. Simplified sequence, suitable also for speciation of B, S, and I (Sager 2016)

I	0.16M acetic acid	Exchangeables+carbonates
II	0.1 M oxalate buffer pH 3	Fe-Mn-oxides
III	Oxidation of the original sample by $KClO_3$-HNO_3-H_2O	All except most of silicates
IV	digestion of the original sample with $HClO_4$-HNO_3-HF	Total

The advantage of this modified sequence is to get additional information about sulfur, boron, silicon, iodine, titanium, and rare earths, if glass vessels are avoided. The $KClO_3$-HNO_3-H_2O digest replaces aqua regia, and non-volatile sulfate, iodate, and boric acid, and some silicic acid; the latter is limited by its solubility in acid (Sager 2016).

8. MERITS AND LIMITATIONS OF SEQUENTIAL LEACHING

Sequential leaching yield a pattern, which indicates mobilities under changing environmental conditions (acidification, oxidation, reduction). It does not give a „plant-available fraction“. Much more possibilities of speciations are possible than the number of fractions which can be obtained. Because the fractions operationally defined, change of the sequence may change the results, and the procedure has to be kept constant throughout a project. Ideally, main and trace elements dissolve from the same solid, which enables the assignment to carrier phases. Uncertainties are due to desorptions from non-soluble parts (e.g. clay minerals) and resorptions (e.g. hydroxides) at the remaining solid (Kheboian & Bauer 1987, Belzile et al. 1989a). Leaching patterns depend on the presence of minority phases, individual properties (solubilities of the hydroxide, affinities towards complexants), the occurrence of main sorbing phases, and the level of load of sorbing phases (Sager & Vogel 1993).

Multi-element determination devices (ICP-OES, ICP-MS) permit the simultaneous determination of main elements, trace elements and nutrients within one or two runs. For economical reasons, data about main elements, non-metals and nutrients should be included, because the fractionation procedure is at least half of the working time (Sager 1991, Sager 2016).

In sediments and soils, the abundance of rock particles from erosion is hardly dependent on grain size, and non surface dependent fractions can be regarded as geogenic. Fractions from surface coatings, however, have been precipitated from solution, their contents strongly increase with decreasing grain size, and they are probably largely of anthropogenic origin. Therefore, only samples wet-sieved to the same grain size should be compared. Fig. 3 shows the abundance of arsenic in Danube sediments at the reservoir at Altenwörth/Lower Austria (Sager et al. 1989).

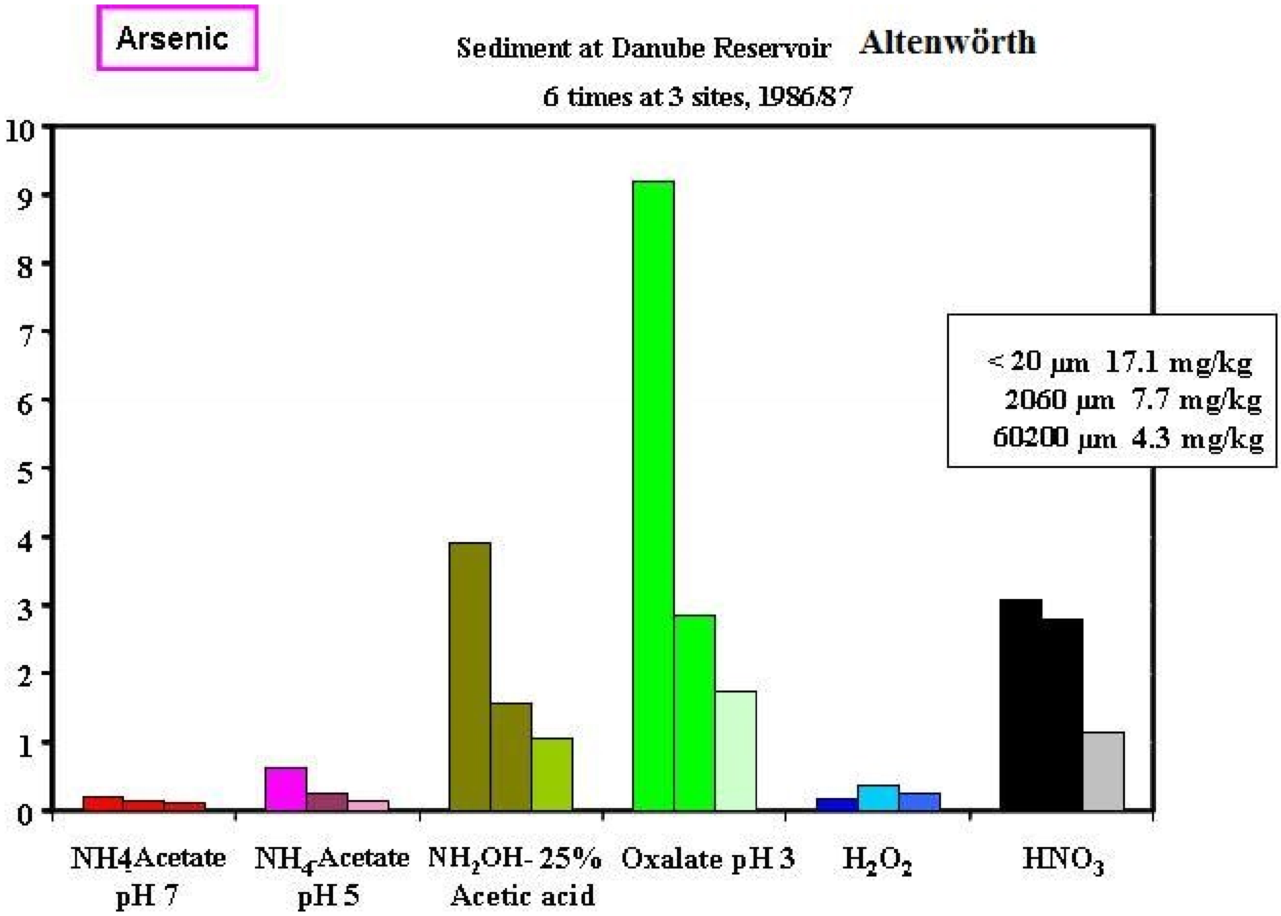


Figure 3. Grain size dependence in sediments (mg/kg); total amount is given in the box

In order to prove, which fractions increase by sorption from solution, a soluble salt (e.g. 1 mg of test ion) is shaken with 50 ml tap water (or better: river water) overnight. After centrifugation and filtering, the adsorbed amount can be determined (which is usually > 90%), and the loaded solid submitted to sequential leaching. The differences of concentrations from sequential leaching obtained before and after sorption depend on the substrate and the investigated ion. Fig 4 shows that short-term adsorbed Pb is recovered preferably already at pH 7, but from carbonaceous soils and iron oxides a pH 5 is needed. To the contrary, desorption of short-term adsorbed Cr(III) needs pH 5 and less, because it forms a hardly soluble hydroxide (Fig. 5).

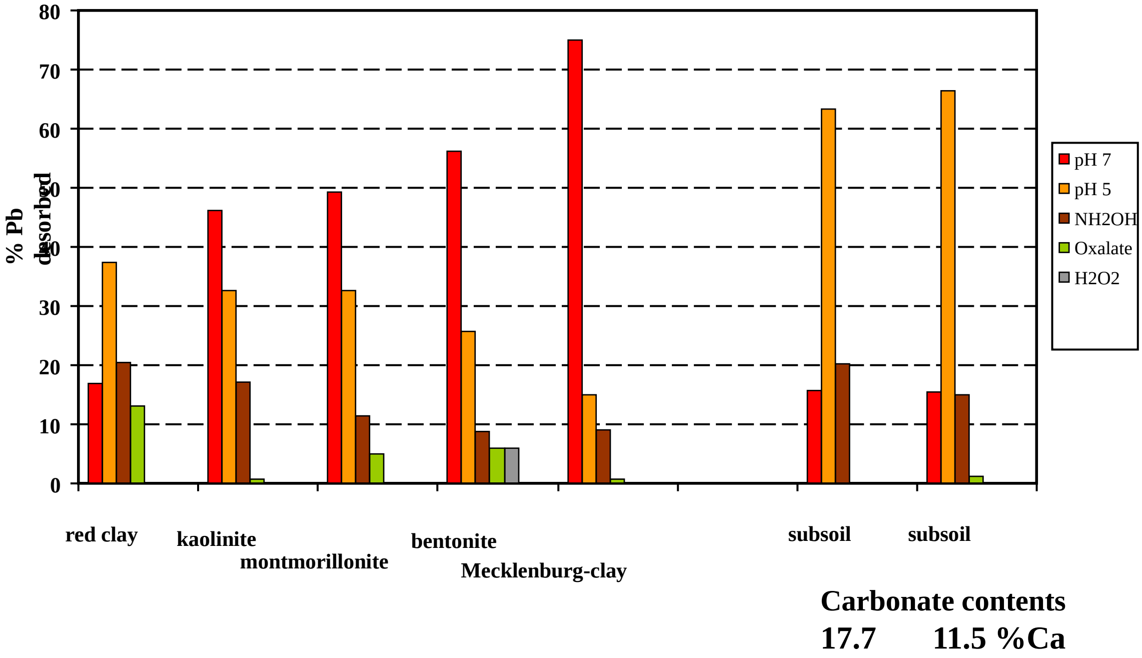


Figure 4. % Pb net desorption from clay minerals and soils

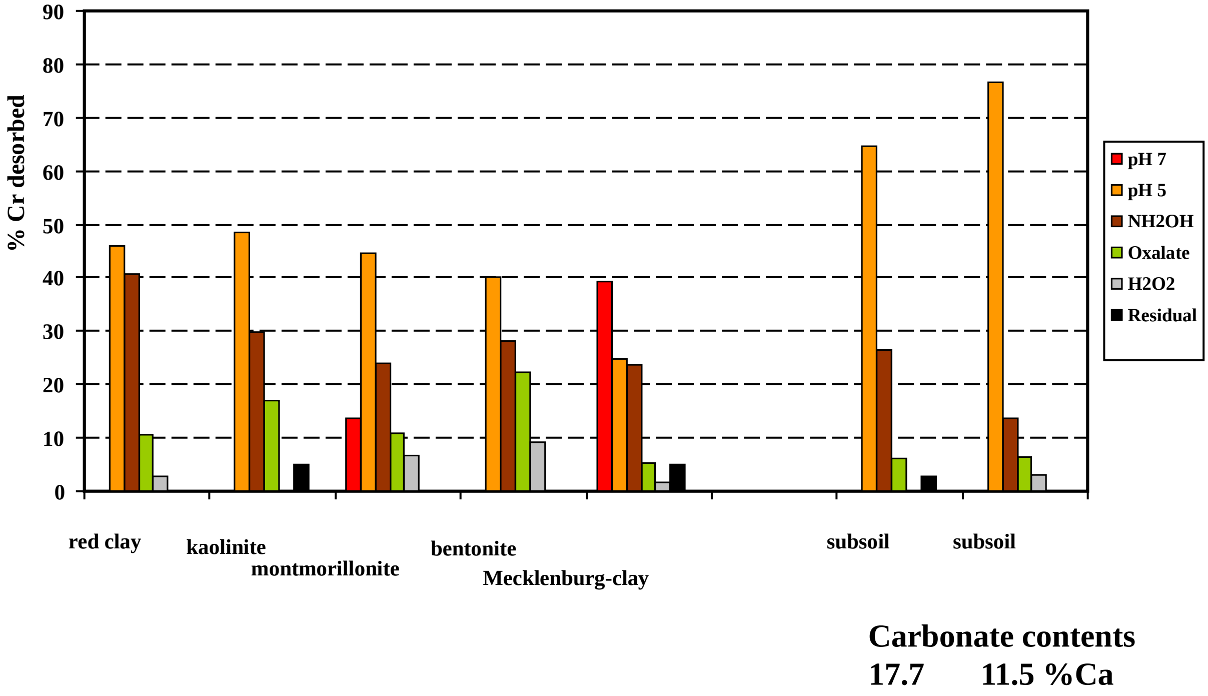


Figure 5. % Cr net desorption from clay minerals and soils

Figure 6 shows that different leaching patterns can be obtained due to different sources. On the left side there are three arable soils, in the middle is the desorption of previously adsorbed Cu, and at the right side the emission of a smelter (Brixlegg, The Tyrol).

At sites of high geochemical abundances, sequential leaching reveals different status of weathering in arable soils at historic mining sites, which can be demonstrated at an example from Northern Styria at Johnsbach (Fig. 7)(Compare Bodenschutzbericht 1999)

Common sequential leaching techniques, which have been developed for fractionation of sediments and soils, have also been applied to airborne particulate matter, reviewed by Mukhtar and Limbeck (2013). Tessier-like, or BCR-like sequences have been frequently used, but also simulated gastric juice or simulated lung fluid.

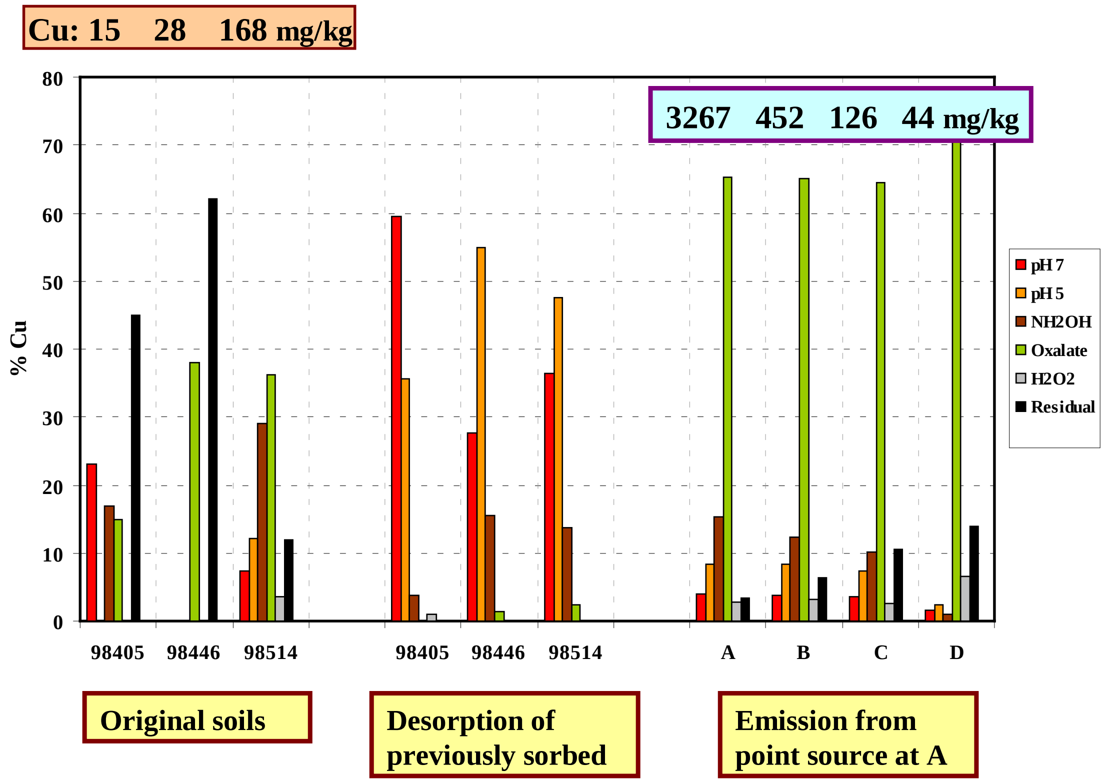


Figure 6. Different leaching patterns obtained from different sources

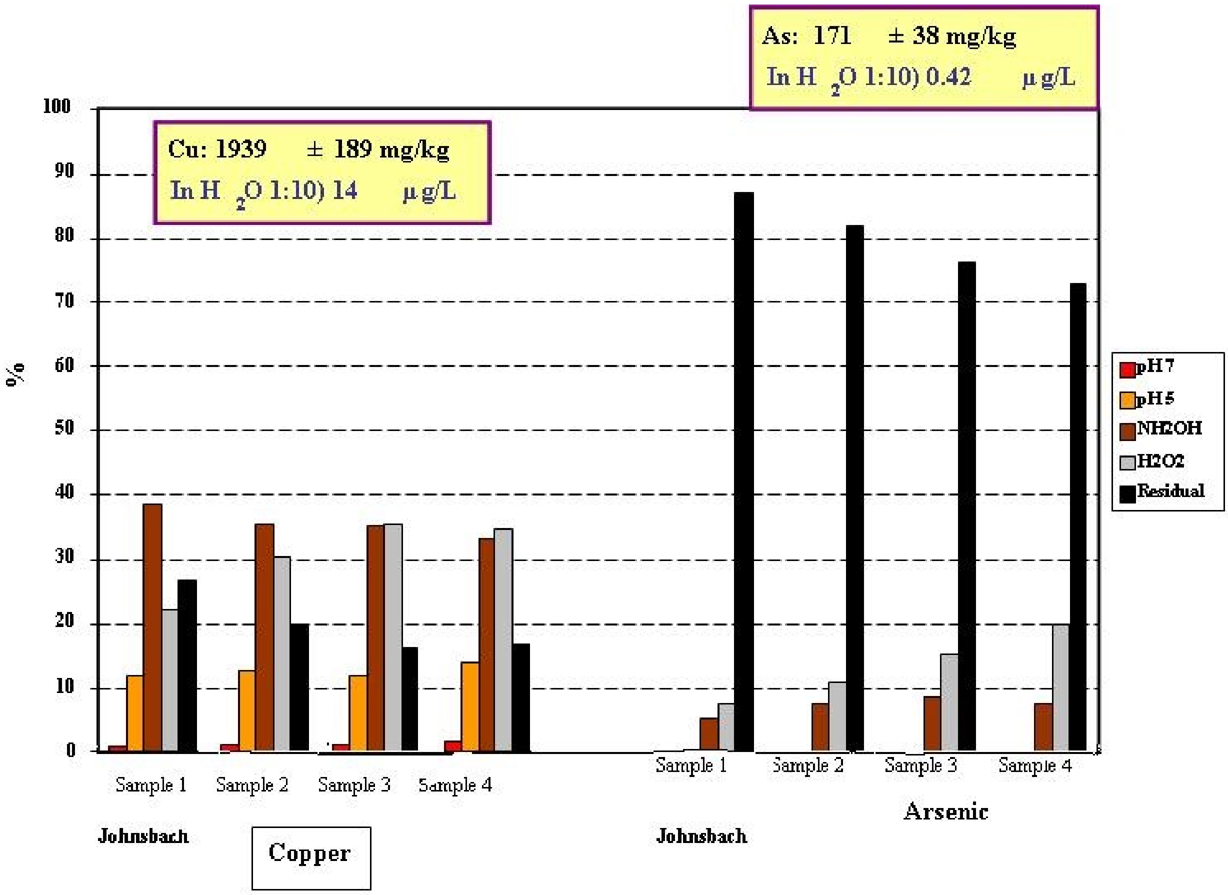


Figure 7. Different weathering status found within the same arable soil

The amount of solubles and aexchangeables is usually larger than in sediments and soils. Urban particulates may contain an oily organic fraction, which prevents wetting, and should be removed by ethanol as the first step. After collection by high-volume air samplers, urban atmospheric particulates in Syria were fractionated according to the Tessier-sequence, and major amounts of Pb have been found as exchangeable ($MgCl_2$) or soluble in acetate buffer pH 5, possibly as PbClBr or $PbSO_4.(NH_4)_2SO_4$, whereas Cu was mainly mobilized with $NH_2OH.HCl$ (Mn-OOH bound phase) (Al-Masri et al. 2006).

9. A TWO- STEP PROCEDURE FOR IN-VITRO IMITATION OF DIGESTION

Common risk assessment, which leads to recommended daily intakes or threshold levels, is based on total element contents and the assumption of usual speciation and bioavailability. Whereas bioavailability is the term for the amount actually taken across the cell membrane, bio-accessibility means the fraction dissolved in synthetic body fluids in vitro.

In vitro leaching methods are inexpensive alternatives to costly and lab-intensive animal models. Digestion of vertebrates can be approached in vitro by sequential leaching, imitating an acid and an alkaline step.

In vitro- models easy to repeat, use objective criteria, but it is not possible to imitate the complex mixture of proteins and enzymes released from the body, which govern the enzyme secretion from the actual needs of the organism. Of course, bioavailability is not only dependent on dissolution, but also on needs and duration of stay, apart from individual differences. If an animal is hungry, more digestive juices and enzymes and longer times are used. Besides dissolution and reprecipitation in the gut, bioaccessibility is also due to the time of stay governed by needs and successions of nutrient uptake, microbial activities, and acute intoxications.

The digestion within the stomach of monogastric mammals can be simply simulated by shaking in glycin/HCl buffer pH 1.5, containing pepsin, for 2h at 37°C (Chung et al., 2005). Other authors have used a gastric juice containing NaCl, pepsin, Na-citrate, maleic aic, acetic acid, lactic acid, adjusted to pH 2 with HCl (Mukhtar & Limbeck 2013). Subsequently, solubility in the gut can be simulated by phosphate/carbonate buffer pH 8 containing pancreatin (Chung et al., 2005). Green plants can be separated between the two steps by centrifugation, whereas in case of meat this is not impossible, and a one-pot method has been used. In brief, meat plus gastric juice (pH 3 ± 0.1) were incubated under shaking for 2.5 h at 37°C; then duodenal juice was added, pH was adjusted to 6.5 ±0.1 and digestion continued for 2 h at 37 °C. Finally, the slurry was centrifuged, the metal contents in the digestive fluid and the undigested solid residue were determined (Mateo et al., 2007).

Ruminants digest in part under reducing conditions, which might precipitate metal sulfides within their stomach, and may release other fractions of the ingested solids.

Metals, in particular contaminations, have been preferably released under acid conditions in the gastric juice from all plant leaves and rice grains investigated. The solubility in the simulated gut juice in presence of plant residues ranged at about 20% for As, Co, Cu, Mn, and Zn, and was below 10% for others. From contaminated soils in the Mežica region (Slovenia), simulated stomach fluid released 6.7-23% of total Pb (Finžgar et al., 2007).

The enzymes may also contain metals, and resorption of these blanks might yield even negative concentration values (Chung et al., 2005).

After deposition of airborne particles in the respiratory system, ultrafine particles interact with the lung fluid, whereas coarser ones are transported by the so-called mucociliary clearance adoral, are swallowed and reach the gastro-intestinal tract. The bio-accessibility of metals from airborne particulates from synthetic gastric juice, or synthetic lung fluid, which was simulated by a solution of NaCl, citric acid and albumin, is usually higher than from soils or sediments (Mukhtar & Limbeck 2013).

10. CONCLUSIONS

Whereas main minerals of soils and sediments are easily accessible by X-ray diffraction, speciation and mobilities of trace metals can be assessed much better by selective and sequential leaching techniques. Though they do not quite imitate natural conditions, because the long-term activities of micro-organisms is neglected, they show a picture of the current situation for many samples within reasonable time. Geogenic fractions have low solubility and grain-size dependency. The transfer to green plants, soil biota, or benthic organisms occurs preferably from easily soluble fractions.

Sequential leaching patterns depend on the source, the geochemical matrix (amount and proportion of adsorbent phases), and the chemical properties of the respective element. General assumptions a priori, which is the anthropogenic, the contaminating, the plant-available or the bio-accessible fraction, are not entirely true, but have a certain probability, which should be tested by additional experiments.

Sequential leaching can also be used to monitor speciation changes within soil profiles, like formation of a B-horizon, or speciation changes close to the root zone during plant growth, but this would beyond the scope of this paper.

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