



BIOAVAILABILITY OF TRACE METALS IN TERRESTRIAL ENVIRONMENT: METHODOLOGICAL ISSUES

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Beside anthropogenic sources, trace metals can be found in the parent material from which the soils develop. Whether these inputs will become toxic and to what extent mobility depends upon a number of factors: specific chemical and physical trace metal characteristics, soil type, land use, geomorphological characteristics within the soil type and exposure to emission sources. Processes that control the mobility, transformation and toxicity of metals in soil are of special importance in the soil root developing zone – the rhizosphere. For this reason, there is a considerable interest in understanding trace metals behaviour in soil, with special emphasis on the way they enter the soil and on processes by which plants take them up. Full understanding and prediction of chemical behaviour of an element in the environment is possible only by identification of all forms in which that element can be found under different environmental conditions. Various chemical methods, geochemical models and biotests are used for assessment of the bioavailable metal fraction in soil. However, these methods are not universally applicable for all elements and different soil characteristics. Chemical methods for assessment of metal bioavailability are commonly grouped within methods for identification of total metal content in soil, methods for assessment of currently available and potentially available fractions, methods for prediction of metal speciation in soil solution. This article offers a critical review of methodologies available for assessing metal speciation in solid and liquid phases in soils taking into consideration the array of parameters that might influence uptake and effects upon the plant.

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Introduction

Two thirds of all elements found in nature are metals. According to their chemical definition, metals are elements and as such cannot be synthesized or degraded by biological or chemical processes, though these processes can change chemical forms of metals. Metals are contained in the Earth's crust and in parent rocks. By weathering of rocks soils are formed, so their presence differs in different geographic regions. Terms like heavy metals, metalloids and microelements are the most commonly encountered in ecological studies. Among the 96 known metals, 17 are semimetals or metalloids (e.g., B, Si, Ge, As, Sn, Te, Po ...). The term heavy metal refers to a group of 53 metals with density higher than 5 g/cm³. From the geochemical point of view, trace elements are metals whose percentage in rock composition does not exceed 0.1% (e.g., Cu, Cr, F, Fe, Mo, Ni, Se, Zn, As, Cd, Hg, Pb). In very small amounts, some of these elements are essential for normal growth and development of living organisms and they are, from the physiological aspect, called micronutrients or microelements (e.g., Fe, Mn, Zn, Cu, Mo, Ni, Se), while others are toxic even in small concentrations¹. The issue of toxicity is usually simply a matter of quantity, with the range varying for each element. The concentration of metals in uncontaminated soil primarily depends upon the chemical composition of the parent material from which the soil was formed.

Metals are chemically very reactive in the environment, which results in their mobility and bioavailability to living organisms. Metals can be present in all environmental compartments as different species, with the parent element associating with different ligands, but never being irreversibly transformed or metabolized, and in those terms metals are different from organic compounds. People can be exposed to high levels of toxic metals by inhaling air, drinking water, or eating food products that contains them. As a consequence, metals get into the human body by different routes - by inhaling, over skin, and ingestion of contaminated food via food chain. The issue of toxicity is usually simply a matter of quantity, with the range varying for each element.

Trace metals occur naturally in rocks and soils, but increasingly higher quantities of them are being released into the environment by anthropogenic activities. There are environments (or areas) in which anthropogenic loading of trace metals puts ecosystems and their inhabitants at a health risk. Repeated use of metal-rich chemicals, fertilizers, and organic amendments such as sewage sludge and wastewater may cause contamination at a larger scale. So far, it is believed that most soils in Europe², have not been significantly rich in trace metals by anthropogenic activity. This is changing as livestock production expands, fertilizer application increases, and bio-solids and effluent applications to agricultural soils become more common. Increased concentration of Cu and Zn in soils under long-term production of grapevine, citrus and other fruit crops have been recorded in various studies^{3,4,5,6}. Phosphate and micronutrient fertilizers contain potentially harmful trace elements, such as arsenic (As), cadmium (Cd), and lead (Pb). Chen et al.⁷ observed significant correlations between Cd and Pb and soil phosphorus in California vegetable

croplands, indicating the application of P-fertilizers contributes significantly to the accumulation of Cd and Pb in soils. Romić et al.⁸ pointed out that the application of P-fertilizers also contributes significantly to the accumulation of Cd in horticultural soils of fluvial terraces in the Croatian coastal region.

The focus of this overview is methodologies available for assessing metal speciation in solid and liquid phases in soils to study the origin and fate of these metals in terrestrial environments.

Metal bioavailability approach

The concepts of «Bio-availability» and «Bio-accessibility» were introduced to express whether the actual concentration of a toxic element would have effects on organisms⁹. The main challenge that comes out from the assessment of loads of trace and toxic metals is the methodology of determination or prediction of the trace element content in a soil that results in toxicity.

Metal-soil interaction is such that when metals are introduced at the soil surface, their mobilisation does not occur to any great extent unless the metal retention capacity of the soil is overloaded, or metal interaction with the associated waste matrix enhances mobility¹⁰. The impact of trace metals on soil and the surrounding environment mostly cannot be predicted simply by measuring the total concentration. This is because only the soluble and mobile fractions have the potential to leach or to be taken up by plants and enter the food chain.

Metal bioavailability is a complex issue that depends on a series of properties pertaining to the soil matrix, plant properties and environmental conditions. Different definitions and uses of the term metal bioavailability can be found in scientific literature, but most of them refer to the proportion of total metals that are available for incorporation into biota. The issue of bioavailability and bio-accessibility often determines whether or not the concentration at which a chemical is present will have effects on organisms. Peijnenburg and Jager¹¹ define «bio-available fraction» as the fraction of the total amount of a chemical present in a specific environmental compartment that, within a given span of time, is either available or can be made available for uptake by micro-organisms or plants, from either the direct surrounding of the organisms or the plant, or by intake in food.

Distribution, mobility, bioavailability and toxicity of metals depend not only on metal species and concentration but also on the form in which metals exist in soil. Full understanding and prediction of chemical behaviour of an element in the terrestrial environment is possible only by identification of all forms in which that element can be found in soil under different environmental conditions.

Metal speciation is one of the most important properties that determine the behaviour and toxicity of metals in the environment. Chemical speciation of an element refers to its specific form characterized by a different isotopic composition, molecular structure, and electronic or oxidation state¹². Speciation is the process of identification and determination of different chemical and physical forms

of elements present in a sample¹³. Metals that occur in cationic forms have a higher ability of binding to negatively charged soil colloids, and are thus less bioavailable, but more easily accumulate in soil, unlike the anionic forms that are mainly present in soil solution and are more bioavailable, but are more readily leached from the soil.

Soil extraction methods

Soil properties, speciation of trace metals and transfer mechanisms between trophic levels (like human intake of Cd from plant seeds that is limited by nutritional factors) have to be characterized to assess the bioavailability of metals and to get the respective data gaps filled in. Major soil properties that affect changes in metal speciation, and thereby also their fractionation, are soil pH^{14,15}, redox-potential¹⁶, soil mineralogy^{17, 18} and existence of different organic and inorganic reactants – ligands^{19,20}.

Metals that occur in cationic forms have a higher ability of binding to negatively charged soil colloids, and are thus less bioavailable, but more easily accumulate in soil, unlike the anionic forms that are mainly present in soil solution and are more bioavailable, but are more readily leached from the soil. Various metals distribute them differently among fractions of soil solid and liquid phases. Several forms of trace metals in soils can be emphasised:

- *Soil solution forms* (ionic, molecular, chelated and colloidal forms), characterized by high metal mobility.
- *Ions at the exchange interface*, non-selectively sorbed, readily exchangeable ions in inorganic or organic fractions;
- *Ions specifically sorbed by inorganic colloids*, more firmly bound ions, medium mobility;
- *Ions complexed or chelated by organic colloids*, as well as elements present in decomposing organic materials and the soil biomass, medium to high mobility because of the decomposition of organic matter with time;
- *Ions occluded by, or structural components of, secondary minerals and other inorganic compounds*; strongly dependant on environmental conditions, medium metal mobility
- *Elements incorporated in precipitated sesquioxides and insoluble salts*, or fixed in crystal lattices of clay minerals, or present in the structure of primary minerals; low metal mobility, available after weathering or decomposition.

Single chemical extractions

All fractions of metals are in dynamic equilibrium, and only metals in aqueous soil solution are directly available to biota. Soil solution is in direct contact with the soil solid phase and transformations going on in it are a consequence of mineral equilibrium, exchange processes and sorption processes in the soil mineral phase and organic matter, as well as complex formation with organic matter in the solid phase and in solution²¹. The concentration of metals in the soil solution, at any given time, is governed by a number of interrelated processes, including inorganic and organic complex formation, oxidation-reduction, precipitation

/dissolution and adsorption / desorption reactions respectively. McLean and Bledsoe²² emphasize the importance of the accuracy with which the multiphase equilibria can be determined or calculated for the prediction of the concentration of a given metal in the soil solution.

Most assessments of metal availability have involved single chemical extractants (e.g. EDTA, DTPA, acetic acid, diluted inorganic acids) intended to remove the entire reservoir of reactive metal and have been used primarily in soil fertility assessment. Single chemical extractions provide inexpensive and rapid assessment methods, and they are generally used to assess available amounts of soil metals and usually aim to extract the water-soluble, easily exchangeable and some of organically bound metals. For example, dilute salt solutions of replacing cations, such as $MgCl_2$, $CaCl_2$, $NaNO_3$, are commonly used to extract the soluble and easily replacing cations.

Dilute HCl is one of the most widely used reagents, in techniques which employ acid solutions to isolate the non-residual phase, in a variety of solid environmental media, and it is assumed to extract metals on exchange sites, due to its acidic properties²³. Several studies suggest that HCl extraction might lead to overestimation of soil available metals²⁴ or that its correlation with metal plant uptake is generally low²⁵.

Single chemical extractability of trace metals by using 0.43 M HNO_3 appeared strongly related to the estimated anthropogenic enrichment and therefore used to assess the hazard of human-induced enrichment of Cd, Cu, Pb and Zn²⁶. The procedure recommended for estimation of “mobile fraction” indicating the “potential availability” has been standardized within the framework of harmonization of leaching procedures for risk assessment of trace metals in soils²⁷.

Diethylenetriaminepentaacetic acid (DTPA) is a potent synthetic chelating agent, and the method of extraction with DTPA was developed for the purpose of determining zinc, iron, manganese or copper deficiency in neutral and carbonate soils²⁸. Haq and Miller²⁹ reported negative results of the DTPA test, which they explained by their failure to determine important significant relations between concentrations of metals (copper and manganese) extracted from soil and those found in the tested plants. O'Connor³⁰ gave a number of comments on the DTPA test, based also on non-significant correlation between DTPA-extractable metals in soil and their concentrations in plants. Regardless of the above considerations, DTPA is the most widely used agent for extraction of “available” cadmium, copper, nickel and zinc, and thereby also the most standardized one^{31,32,33}. Starting from the fact that the data on total copper content reveals very little about its bio-availability, such strong correlation between copper extracted with *aqua regia* and DTPA actually indicates that neither the latter extraction method is suitable for assessing copper availability to plants.

Romic et al.³⁴ applied multiple linear regression analysis to establish the relation between copper fractions after particular single extractions (*aqua regia*, DTPA and $CaCl_2$) and soil properties that may affect their behavior in soil and availability to plants. Most of the colloidal particles in soil strongly adsorb copper, which forms stronger organic

complexes than other bivalent transition metals and therefore soils rich in organic matter can retain it more, without causing plant toxicity. As shown in Table 1, DTPA-extractable copper was largely explained by the total copper contents, but it was also found that the DTPA-extractable copper decreased with increasing cation exchange capacity of soil.

Table 1. Linear regression of DTPA-extractable (Cu_{DTPA}) as a function of $CaCl_2$ -extractable (Cu_{CaCl_2}) and soil organic matter (Org.C)³⁵.

Source of variation	Degree of freedom	Sum of squares	F	Pr > F
Cu_{CaCl_2}	1	685.7	240.0	0.0000
Org.C	1	44.54	15.59	0.0002
Total	63	1224		

In the same study, two parameters were included into the regression model of $CaCl_2$ -extractible copper: organic matter content (Org-C) and pH, and the model explains 62% of total variance (Table 2). Concentrations of $CaCl_2$ -extractable copper mainly depend on pH, which relation was also confirmed by this investigation. However, since these are predominantly alkaline soils, this relation is not as strong as in the case of soils with a more varying pH³⁶.

Soil extraction 0.01 M $CaCl_2$ is the method that was increasingly used in the last decades for soil testing to determine soil fertility and the behaviour of nutrients and contaminants in it^{37,38}. Due to the availability of advance instrumental techniques it has become possible to determine very low concentrations of nutrients and pollutants in soil extracts. The advantage of this method for determining metal concentrations in soil is that the concentration of electrolytes stays practically constant and metal concentrations reflect the difference in binding strength or solubility between soils. The extractant is an un-buffered solution and therefore the measured metals reflect their availability at the pH of the soil. The best criterion of the efficiency of the method for determining the soil bio-available fraction is the high correlation between the Cu content observed in plants grown in situ, at least for neutral to acid soils³⁹.

Peijnenburg et al.⁴⁰ gave the comprehensive overview of empirical methods for extraction of metals from soils and noted that, depending on the purpose of the study, information on general soil properties is needed to properly interpret the results of any extraction.

Sequential extraction procedures

Changes in soil conditions may cause trace metal mobilization and favour the contamination of surrounding environmental compartments. Major soil properties that affect changes in metal speciation, and thereby also their fractionation, are soil pH, redox-potential, and existence of different organic and inorganic reactants – ligands. Therefore, identification of the main binding sites and phase associations of soil trace metals is needed to evaluate their remobilisation potential and the risks induced⁴¹.

The sequences of different chemical extractions, usually starting with the weakest, least aggressive and ending with

the strongest and most aggressive, are commonly used to quantify the different fractions of metal retention in soils. Despite the fact that the procedures have been widely used since early 1970s⁴², many questions regarding the uniformity in procedures, problems of poor selectivity, redistribution during extraction, and the dependency of results on operating conditions have been still frequently raised^{43,44,45}. The principal advantage claimed for sequential extraction over the use of single extractants is that phase specificity is improved. One of the most widely-applied extraction procedures proposed by Tessier et al.⁴⁶ distributes metals into five operationally – defined chemical fractions:

Exchangeable fraction

Metals extracted are weakly-sorbed species, retained on the soils surface by relatively weak electrostatic interactions or those released by ion-exchange processes, commonly extracted using dilute salt solutions of replacing cations (MgCl₂, CaCl₂, NH₄NO₃, NH₄-acetate).

Acid-soluble fraction

Metals bound to carbonates, susceptible to changes of pH, a buffered acetic acid/sodium acetate solution generally used.

Fraction bound to hydrous oxides of Fe, Mn and Al

Iron and manganese oxides exist in soil as nodules, concretions, cement between particles or coatings on them; these oxides bond trace metals and their dissolution rate is controlled by Eh and pH of reagents used; reducing agents such as ammonium oxalate, hydroxylamine and sodium dithionite have been mostly used to release metals bound in oxides.

Fraction bound to organic matter

Soil trace metals are bound to various form of soil organic matter, and organically bound fraction may be released using oxidising agents such as hydrogen peroxide, pyrophosphate or sodium hypochlorite.

Residual fraction

Residual fraction basically contain primary and secondary minerals holding metals within their crystal structure; strong, concentrated or boiling nitric acid, with or without hydrofluoric acid or perchloric acid, have been used to assess occluded metals in soil.

Selective sequential extraction was applied to investigate the potential of mobilizing trace metals in agricultural soils of Northwestern Croatia⁴⁷. In alluvial soils developed on Quaternary (Upper Pliocene to Holocene) deposits, extraction with 1M Mg(NO₃)₂ (pH 7)⁴⁸ indicated possible remobilization of elements from the solid phase into soil solution, particularly in the case of copper. There are several possible mechanisms that increase solubility of metals in the surface layer: 1) the soil mineral component is more susceptible to weathering in shallower than in deeper soil layers owing to faster infiltration of precipitation, higher biological activity and greater changes in temperature; 2) shallower soil horizons are richer in organic matter, which can stimulate metal desorption by formation of soluble organic complexes; and 3) exchangeable complex of shallower soil layers contains more basic cations (Na⁺, K⁺, Ca²⁺, Mg²⁺), which can also reduce sorption of metals by increasing the competition for exchange sites. All these processes are even more pronounced in the anthropogenic horizon of arable soils.

Table 2. The partitioning of metals among different extraction reagents in selective extraction of soil samples and comparison with *aqua regia* extraction

Metal	Soil depth, cm	Exchangeable Metal concentration, (EXC), mg kg ⁻¹	Carbonates, (CARB), %	Fe/Mn oxides, (OXD), %	Organic matter, (ORG), %	Residue, (RES), %	Total extract by aqua regia, %	Total extract, by SSE, %	RE ¹
Cr	0-25	n.d.	0,2	0,51	2,05	35,06	33,79	37,82	10,7
	25-40	n.d.	0,2	0,51	1,02	37,1	34,82	38,83	10,3
	40-100	n.d.	0,21	1,03	1,03	42,63	39,22	44,9	12,7
Cu	0-25	2,05	2,05	1,02	4,61	11,3	18,43	20,99	12,2
	25-40	2,25	2,25	1,02	1,54	13,3	17,41	20,37	14,5
	40-100	1,86	2,89	1,03	0,52	17,5	21,67	23,84	9,1
Ni	0-25	0,41	0,2	1,54	2,56	25,6	28,67	30,31	5,4
	25-40	0,2	n.d.	1,02	2,56	29,7	31,74	33,48	5,2
	40-100	0,62	0,21	1,55	2,58	36,1	39,22	41,08	4,5
Pb	0-25	0,82	3,28	4,61	5,12	20,48	35,84	34,31	-4,5
	25-40	0,82	1,64	2,05	0,51	18,43	26,62	23,45	-13,5
	40-100	0,62	1,86	3,61	1,55	19,61	28,9	27,25	-6,1
Zn	0-25	4,3	5,94	5,63	4,1	64,5	78,85	84,48	6,7
	25-40	4,3	2,66	2,05	3,07	58,4	69,63	70,45	1,2
	40-100	3,51	2,68	2,58	3,1	56,8	71,21	68,63	-3,8

¹RE (%) = (Total extracted by SSE – Total extracted by *aqua regia*) * 100 / Total extracted by *aqua regia*

Table 2 shows the distribution of metals among the defined geochemical fractions of the studied soil. Only a small portion of metals (less than 6% for zinc and less than 4% for copper) was in the exchangeable fraction. A significant portion of copper and lead was associated with the organic fraction, in agreement with the known affinity of Cu for organic matter. Metal fractions of Pb and Zn associated with the oxides reflect the dynamic of eluviation processes within the soil profile. Metals were mostly associated with the residual fraction.

Table 2. The partitioning of metals among different extraction reagents in selective extraction of soil samples and comparison with *aqua regia* extraction

The authors concluded from this study that the relative mobility of the metals is as followed: Cu>Zn>Pb>Ni>Cr.

Conclusions

The most common observation in most of the articles that study sequential or partial extraction of soil trace metals is that they are not completely specific to metals or chemical phases. The complexity of bioavailability phenomenon comes out from an array of matrix-related⁴⁹, species-related⁵⁰ and metal-related⁵¹ issues.

Generally, most of the laboratory methods for evaluating bioavailability of metals in soil overestimate actual available fraction. Therefore, further studies using biota have to be performed to understand the contribution of biological factors that control bioaccumulation and toxicity of trace metals.

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