CHAPTER 3

BIOAVAILABILITY OF TRACE ELEMENTS AND RISK ASSESSMENT

In the field of medical geology, bioavailability and risk assessments are two important aspects that need serious consideration. The mere presence of a toxic trace element or a species in a specific locality for example, does not necessarily imply a serious health hazard. The nature of the species concerned and the bioavailability of the species are the main factors that influence the impact of an environmental chemical species on human and animal health. The bioaccumulation of the species concerned is an important step in the food chain and the processes controlling it needs to be well understood.

BIOACCUMULATION

In a very general sense, bioaccumulation could be defined as "the process by which organisms absorb chemicals or elements directly from the environment". It should however be noted that the term "bioaccumulation" has to be specified by quantitative data, comparing concentrations of a compartment in relation to another (e.g. plant vs. soil parts, adsorbed amount vs. dissolved amount) (Streit, 1992). The selective concentration of elements is inherent in any life process and the process of bioaccumulation therefore leads to indications of pollution of the environment. The chemistry of the elements and the biochemical structure of the biological species are the main factors that influence the bioaccumulation process.

The passage of metal ions into the plants through the soil solution and root cells depends on a number of factors such as redox potential, pH, interaction with ligands and properties of soil matrix such as cation exchange capacity. The metal speciation in soil solution and on soil surfaces and metal uptake by the root is illustrated in Figure 3.1. The root cell membranes are highly selective to trace elements. The phospholipid based membranes are highly impermeable to ions or (large) polar molecules, whereas non-polar

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molecules (such as O_2) pass rapidly. The electric potentials between membrane separated compartments, known to correlate with pH gradients play an important role in soil solution-plant pathways (Streit and Stumm, 1993). The mechanism of element passage through the cell membranes is complex and very little is still known about the actual selective process.

BIOAVAILABILITY

The term bioavailability has been defined in a number of ways. In a very general way, one could define bioavailability as "the extent to which a substance can be absorbed by a living organism and can cause an adverse physiological or toxicological response" (NEFESC, 2000). For environmental risk assessments which involve soil and sediment, the above definition implicitly includes the medium in which it occurs to become available for absorption.

Other definitions of bioavailability depend on the scientific discipline which requires such a definition. For example:

(i) Environmental bioavailability: Physiologically driven uptake process (Peijnenburg et al., 1997)

(ii) Toxicological bioavailability: The fraction of the total available dose absorbed by an organism which is distributed by the systematic circulation and ultimately presented to the receptor or sites of toxic action (Landrum and Hayton, 1992)

(iii) Bioremediation bioavailability: The extent to which a contaminant is available for biological conversion (Juhasz et al., 2003).

Other terms such as pharmacological bioavailability, phytobioavailability and bioaccessibility have also been used (Landrum and Hayton, 1992).

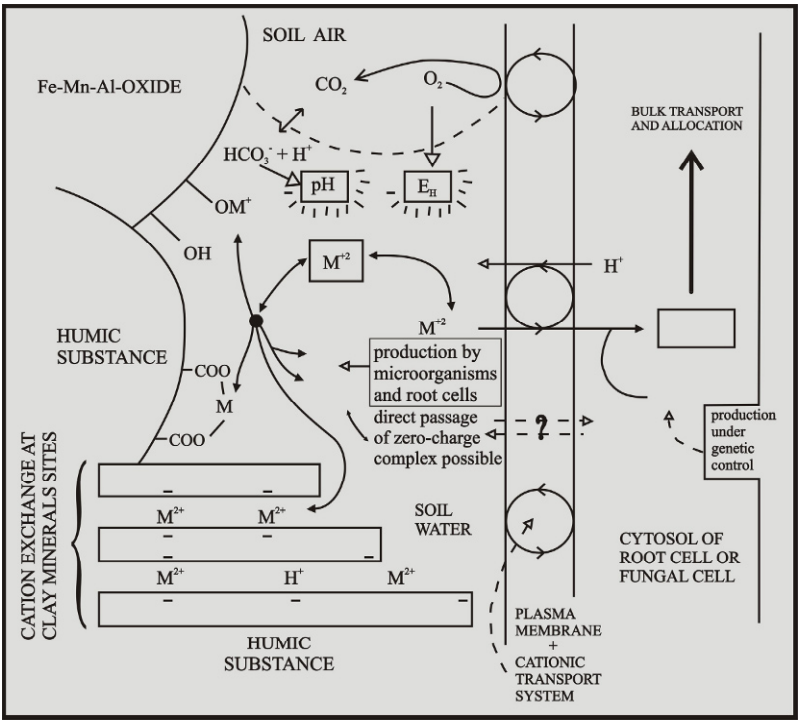

Fig. 3.1. Highly schematic diagram to show metal speciation in soil solution and on soil surfaces and root metal ion uptake, assuming a divalent metal cation (M^{2+}) and a monovalent soluble external ligand. The uptake rate into the cell is determined (in a given individual) by the number of metal ions bound to receptor sites on membrane transport molecules, e.g. metal ion ATPases. At equilibrium the amount of metal bound to these sites and therefore the transport rate, would be directly related to the soil metal ion concentration (Streit and Stumm, 1993)

The three main factors that control bioavailability are:

(a) there has to be an opportunity for the receptor (or organism) to be in contact with the matrix in which the contaminant is found.

(b) the contaminant must be potentially available at least in part.

(c) the receptor (or organism) must be able to absorb or assimilate the potentially available fraction.

If these three requirements are not fulfilled, a contaminant is not bioavailable (Juhasz et al., 2003). In the case of human health risk assessment, two types of bioavailability, namely absolute bioavailability and relative

bioavailability are measured. Absolute bioavailability is the fraction or percentage of a compound which is ingested, or applied on the skin surface that is actually absorbed and reaches the systemic circulation, and is defined as:

$$\text{Absolute Bioavailability} = \frac{\text{Absorbed dose}}{\text{Administered}} \times 100$$

Since toxicity parameters are based on an administered dose rather than an absorbed dose, absolute bioavailability in often not determined in the case of human health risk assessments.

Relative bioavailability on the other hand, is a measure of the extent of absorption among two or more forms of the same chemical, different media (e.g. food, soil, water) or different doses. Since matrix effects can substantially decrease the bioavailability of a soil or sediment bound metal compared to the form of metal or dosing medium, relative bioavailability is more important in environmental studies. It is defined as:

$$\text{Relative Bioavailability} = \frac{\text{Absorbed fraction from soil}}{\text{Adsorbed fraction from dosing medium used in toxicity study}}$$

The relative bioavailability thus expressed is termed the relative absorption fraction (RAF). In the case of trace elements, bioavailability varies considerably depending on factors such as food source, oral intake, chemical form or species, nutritional state (deficiency vs. excess), age, gender, physiological state, pathological conditions and interaction with other substances.

The determination of bioavailability is carried out in a number of ways. Among these are:

(a) microbial tests
(b) soil invertebrate tests
(c) amphibian tests
(d) plant tests
(e) tests using higher organisms

These tests use the measurement of a toxic or a mutagenic response, inhibition of a metabolic function, changes in microbial population structure, mortality or malformations, accumulation of chemical species in organs or

the blood streams, and dissolution of contaminants after extraction of particular mineral phases (Juhasz et al., 2003).

In the soil environment several processes affect the mobility of metals. These are: (a) dissolution and precipitation (b) sorption (c) ion exchange and (d) oxidation-reduction reactions. In such an environment, the mobility and hence the bioavailability of metals is reduced by conditions that promote precipitation or sorption. The most bioavailable metals are those that form weak outer complexes with inorganic materials such as clay, iron and manganese oxides or organic soil matter or those that form complexes with ligands while in solution. These are therefore not sorbed. On the other-hand, metals that tend to form inner sphere complexes do not desorb easily and hence less bioavailable. Table 3.1 shows the relative mobility of some metals in soils. The bioavailability of these metals can be inferred from these mobilities.

In the case of sediments, metals may be to a large extent incorporated into the structure of the mineral itself and hence may not be easily bioavailable. Only the remaining metals which are adsorbed or complexed may become bioavailable (Table 3.2).

RISK ASSESSMENT

Risk assessment is a method which assesses the actual or potential adverse effects of contaminants to plants and animals and which focuses on the damage that has been or will be done by contaminants. The information obtained from risk assessment helps one to identify populations or areas that are likely to be adversely affected by soil, water or air contamination.

The technique of risk assessment (WHO, 2002) is based on a causal stress-response model where a contaminant is transported from its source to the receptor (humans, animals, plants) through a definite pathway. The main components of a risk assessment are:

a. Identification of the problem
b. Characterization of the receptor
c. Assessment of the exposure
d. Assessment of the toxicity
e. Characterization of the risk

Table 3.1. Relative mobility of selected metals in soil (Hayes and Traina, 1998)

Metal	Most common Oxidation states in soil[(a)]	Predominant Forms and Distribution in soil systems	Mobility
Arsenic	III	Oxyanion: sorbs more weakly than As(V) to metal oxides and only at higher pH	Moderate
Arsenic	V	Oxyanion: sorbs strongly to metal oxides; forms relatively insoluble precipitates with iron	Low
Cadmium	II	Cation: sorbs moderately to metal oxides and clays; forms insoluble carbonate and sulphide precipitates	Low to moderate
Chromium	III	Cation: sorbs strongly to metal oxides and clays; forms insoluble metal oxide precipitates	Low
Chromium	VI	Oxyanion: sorbs moderately to metal oxides at low pH, weaker sorption at high pH	Moderate to High
Lead	II (IV)	Cation: sorbs strongly to humus, metal oxides, and clays; forms insoluble metal oxides and sulphides; forms soluble complexes at high pH	Low
Mercury	II (O-I)	Cation: sorbs moderately to metal oxides and clays at high pH; relatively high hydroxide solubility; forms volatile organic compounds	Low
Nickel	II (III)	Cation: sorbs strongly to humus, metal oxides, and clays; forms insoluble metal oxides and sulphides; forms soluble complexes at high pH	Low

[(a)] Possible, but less common, oxidation states in soil systems are shown in parentheses.

Risk assessment involves ecological and human risk assessment. The former, in view of the large number of receptors involved is far more complicated than the latter. Human risk assessment however, involves serious ethical considerations and in vivo studies using many animal models are therefore more often used.

Table 3.2. Dominant absorbed or complexed phases of metals in oxic and anoxic sediments (from Brown and Neff, 1993) [-CO_3 = carbonates; FeO = iron oxyhydroxides; Fe/MnO = iron and manganese oxyhydroxides; OM = organic matter; S = sulfides (dominant species given); TBL-Cl-OH-CO_3 and S = tributyltin chloride, hydroxide, carbonate, and sulfide]

Metal	Association in Oxic Sediments	Association in Anoxic Sediments
Arsenic	AsO_4^{3-} - Fe/MnO	$As_2(SO_3)_2$, As_2S_3, FeAsS
Cadmium	Fe/MnO, OM/S, -CO_3	CdS
Chromium	OM, FeO	OM, $Cr(OH)_3$
Copper	OM, Fe/MnO	Cu_2S, CuS, FeCuS
Lead	Fe/MnO	PbS
Mercury	OM	HgS, OM
Nickel	Fe/MnO	OM/NiS, organic thiols
Tin[(a)]	TBL, –Cl, –OH, -CO_3	TBL-S, OH, -CO_3
Zinc	Fe/MnO, OM	ZnOM/S

(a) Only butyl tins are considered

The ecological risk assessment involves the uptake of metals by plant and animals from soils, sediments and water. The processes that occur within the food chain are clearly complex and ecological risk assessment needs to consider several aspects of metal uptake. Among these are the bioavailability of metals by plants and animals from soils, sediments and water by contact with external surfaces, ingestion of contaminated soil, sediment or water and inhalation of vapour phase metals or airborne particles. Food is another source for the bioavailability of metals in animals. Based on these factors, bioavailability is evaluated by estimating:

(a) available fraction of metals present in the environmental media (e.g. sediment or soil)
(b) bioaccumulation directly from environmental media
(c) uptake from ingestion of food

It has now become clear that bioavailability must necessarily become an integral part of the method of risk assessment. Risk-based approaches for the assessment of contaminated sites using total soil metal concentrations rather than the bioavailable metal concentrations, for example, may lead to erroneous conclusions. A good understanding of the bioavailability of the contaminant concerned is therefore a definite pre-requisite for all risk assessment procedures. Of special importance is the need to understand the factors that affect bioavailability, human health implications, plant uptake

and ecosystem health of metals. The field of medical geology clearly recognizes the impact of studies of bioavailability on epidemiology.

ASPECTS OF EPIDEMIOLOGY IN MEDICAL GEOLOGY

Epidemiology is that branch of medicine that investigates the frequency and geographic distribution of diseases in a defined human population for the purpose of establishing programmes to prevent and control their development and spread. Geochemistry plays a major role in the field of epidemiology, particularly in diseases of tropical environments in developing countries, where large human and animal populations live in intimate contact with soil, water and plants of their habitat. The link between geology and epidemiology becomes direct in such cases. The term prevalence, in relation to a disease, indicates the proportion of the population that has a particular disease, at a specific time. Incidence measures the frequency of new cases of the disease.

Epidemiology involves the investigations of the origins of a specific disease, also termed aetiology, and to develop and test the hypotheses. It also attempts to discover the likelihood of a population being exposed to the disease concerned and identifies risks in terms of probability statements and studies trends over time to make projections for the future. New risk factors are discovered and studied in detail. Epidemiology deals extensively with mortality or death rates and morbidity and the rate of incidence of the disease concerned.

One of the terms used frequently in epidemiological investigations is “risk factor”. The risk factor could be associated with the natural soil, water, plant, air and biological environment or an anthropogenically originated medium. The risk factor is any characteristic or condition that may occur with greater frequency in people with a disease than it does in people known to be free from that disease. Based on the information on the risk factor, the chance of increased spread of the disease can be studied and remedial measures recommended. The general term “risk” is often classified in chemical epidemiology as relative and absolute risk. The former is used to denote the ratio of incidence or prevalence in the exposed group to that of the unexposed groups. The absolute risk is the chance of a person to develop a disease.

The epidemiologists are often confronted with 'competing risks' which in fact are other sets of risk factors than can cause the condition of interest and which coexist with the set of factors of interest. These are known as "red herring" cases in out break investigations.

CAUSATION AND CORRELATION

These two terms are very frequently used in both medical geology and epidemiology. The term 'association' is also used in conjunction with correlation if two variables appear to be related by a mathematical relationship, indicating that a change of one appears to be related to the change in the other. Causation is used in epidemiology when the following conditions are satisfied;

(a) A dose-response relationship exists between the condition and the disease.
(b) The prevalence or the incidence of the disease is reduced when the condition under study is removed.
(c) The condition precedes the disease.
(d) A cause and effect relationship is physiologically plausible.

It is however, most important to note that a correlation does not always imply a causal relationship even though a correlation is necessary for a causal relationship. A correlation can be of two types, namely a negative correlation and a positive correlation. In the former, the magnitude of one variable moves in the opposite direction to the other associated variable. Here the correlation coefficient is negative. If the relationship is definitely causal, then the higher levels of the risk factor are protective against the outcome. In the case of a positive correlation, the two variables change in the same direction and the correlation coefficient is positive. Here, the higher the levels of the risk factor, the higher are the outcome. Correlation however measures only linear association. The fact that a statistical correlation alone does not prove causation may be due to the presence of other contributing or confounding factors. This provides misleading evidence and lead researchers to find an association for the wrong reason.

HOMEOSTASIS IN MEDICAL GEOLOGY

The geochemical cycles of the elements trace their pathways in the lithosphere, atmosphere, hydrosphere and biosphere. When these elements (both essential and toxic) enter the food chain and the human body, a process termed 'homeostasis' operates maintaining equilibrium within the constituent cells.

Homeostasis is the maintenance of equilibrium, or constant conditions, in a biological system by means of automatic mechanisms that counteract influences tending towards disequilibrium. This concept was developed by the French physiologist Claude Bernard in the middle of the nineteenth century and is presently considered as one of the most fundamental concepts in modern biology. The term homeostasis was introduced by Cannon in 1932 and was defined as "a condition which may vary, but remains relatively constant" (Clancy and McVicar, 1995).

In the human body all the systems are involved in homeostasis with greater involvement of endocrine, nervous, respiratory and renal systems. In the case of an imbalance, these regulating systems work towards restoring the optimum conditions. This is done by a process termed 'negative feedback' in which a deviation from the normal level is detected and the restoration process initiated.

The process 'contact inhibition' is an example of homeostasis where cell division in a population of cells ceases when they are too numerous or touch each other. A chemical 'messenger' is thought of as passing from cell to cell thereby causing the inhibition. Cancer cells on the other hand are propagated without any inhibition and hence they have lost the mechanism of homeostasis.

In the case of trace elements in humans, the principle of homeostasis can be applied in order to trace the pathways of the elements concerned within the human body. Even though the study of the elemental pathways within the human body lies in the domain of human physiology, the elemental pathway is part of the natural cycle of the element and hence it overlaps with the domain of geochemistry.

The World Health Organization (WHO, 2002) in their report on "Principles and Methods for the Assessment of risk from Essential Trace Elements (ETE)" classified the ETEs as comprising of three groups. These are

(a) cations (Zn, Fe, Cu, Mn, and Cr), (b) anions (those of Mo, I, Se) and (c) those forming bioinorganic complexes (e.g. Co). For each category the body has evolved specific mechanisms for the acquisition and retention, storage and excretion of the various elements.

Cationic ETEs: via gastrointestinal tract and liver. Homeostasis regulates uptake and transfer of the metal by the gut (e.g. Fe, Zn, and Cu). Copper for instance shows a decrease in absorption from 75% at 0.4 mg/day to 12% at 7.5 mg/day even though the total amount absorbed increases.

Anionic ETEs: These are more water soluble and less reactive with N, S, P, O, and OH groups than are cationic ETEs. They are absorbed very efficiently (>70%). Homeostasis is managed by manipulation of oxidation and methylation states. Total body burden is regulated by renal excretion.

ETEs forming bioinorganic complexes: Since cobalt can compete with many other cationic ETEs effectively, it is possible that cobalamin forms to avoid such problems (WHO, 2002).

The WHO has applied the homeostatic model in human health risk assessment for ETEs as follows:

> Homeostatic mechanisms should be identified for the selected ETE.
> Variation of the population's homeostatic adaptation must be considered.
> There is a "zone of safe and adequate exposure for each defined age and gender groups "for all ETEs – a zone compatible with good health. This is the acceptable range of oral intake (AROI).
> All appropriate scientific disciplines must be involved in developing an AROI.
> Data on toxicity and deficiency should receive equal critical evaluation.
> Bioavailability should be considered in assessing the effects of deficiency and toxicity.
> Nutrient interactions should be considered when known.
> Chemical species and the route and duration of exposure should be fully described.
> Biological end-points used to define the lower recommended dietary allowances (RDA) and upper (toxic) boundaries of the AROI should ideally similar degrees of functional significance. This is particularly relevant where there is a potentially narrow AROI as a

result of one end-point being of negligible clinical significance. All appropriate data should be used to determine the dose-response curve for establishing the boundaries of the AROI.

These are several steps involved in the application of the principles for the assessment of risk from essential trace elements. These are:

Step 1: Data selection. This develops a data base for analyses. When human data to evaluate a functional effect is lacking, animal data is often used.

Step 2: Hazard identification (deficiency and excess end points). From among the factors needed to establish these end points are homeostatic mechanisms, bioavailability, nature of exposure, population variability and age-sex variations.

Step 3: Quantitative evaluation of critical affects. This involves the evaluation of the various dose-response curves for each end-point from deficient and excess exposure.

Step 4: Balanced quantitative assessment to determine acceptable range of oral intake (AROI). The effects of both deficient and excess exposure in healthy populations are considered in the derivation of AROI.

Step 5: Exposure assessment. This identifies and quantifies exposure sources (e.g. water, food, supplements, soil and dust), bioavailability and exposure patterns of the populations.

Step 6: Risk characterization. Integration of the AROI and the exposure information.