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16. Bioavailability in soils

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Abstract

In this chapter we review and discuss the commonly used phrase or concept "bioavailability". This concept is key to Risk Assessment as it assesses what proportion of a contaminant present at a contaminated site is available for uptake by organisms and is thus potentially able to cause harm. Whilst this is a relatively straightforward concept the reader will discover that in reality life is not that simple. We start by reviewing the different definitions of bioavailability currently in use. We go on to discuss how soil properties impact on the bioavailability of both metal, metalloid and organic contaminants. Next we review the different methods people currently use to determine bioavailability, concentrating on chemical extractions, but also covering modelling approaches. We conclude that a precise definition of bioavailability equally applicable to all different contaminated sites, contaminants and organisms is unlikely to be achieved. Similarly, a single chemical extraction is unlikely to give a universal measure of bioavailability. However, the message is not all doom and gloom. On a contaminant by contaminant or species by species level chemical extractions and other measurement techniques can accurately predict bioavailability. Modelling techniques are constantly improving and offer hope for the future in terms of predicting bioavailability. At present however, the best method of determining the amount of contaminant available for uptake by an organism is to measure the concentration of the contaminant in the organism. Even this method, however, is open to question as organisms can and have evolved methods of regulating metal uptake.

16.1 Introduction

The initial measurements of contaminants during site investigation are usually total concentrations of contaminants. However, it is usually the "bioavailable" fraction that is relevant to whether the contaminants present in a soil pose a hazard or not. A search on the Internet based search engine Web of Knowledge for peer-reviewed journal articles concerned with "Bioavailability" and "Soil" returns 7703 entries with a near perfect linear increase in journal articles for over a decade ($y = 55x - 109969$, $p \leq 0.001$ as of August 2008!). Add to this the vast number of government and agency reports that exist on the subject and the reader will appreciate that this chapter can not be fully exhaustive on its subject! Given the large amount of research effort and funding that has been expended on the subject it is perhaps surprising that there is neither a universally accepted definition of bioavailability nor a standardized means of measuring it. This deficiency reflects in part the complexity and diversity of the soil system and in part the legislative requirements of different jurisdictions. This chapter aims to summarise for organic and inorganic substances: 1) current thoughts on what bioavailability is, 2) how soil properties impact bioavailability and, 3) methods for measuring and predicting bioavailability. Covered elsewhere in this book, but of relevance to bioavailability, are chapters on oral bioavailability to humans (Wragg et al. 2009; Chapter 7 of this book), uptake of metals from soil into vegetables (McLaughlin et al. 2009; Chapter 8) and uptake of organic contaminants from soil into vegetables (Trapp and Legind 2009; Chapter 9 of this book). Besides, bioavailability is relevant for site specific ecological Risk Assessment (Rutgers and Jensen 2009; Chapter 15 of this book).

From the perspective of contaminated sites and their remediation the important question is not "How bioavailable is a particular element?" but, "Will this contaminant have a toxic effect at this soil concentration on this organism or other receptor?". Clearly bioavailability is an important part of this question but for the assessment of contaminated sites bioavailability needs to be tied to a consideration of toxic effects or legislatively permissible concentrations of contaminants in selected target organisms.

16.2 What is bioavailability?

In the last five years a variety of international working parties and governmental organisations have produced reports on bioavailability. There has also been a healthy discussion in the scientific literature regarding the term. The emerging consensus appears to be that, given the variability present in the environment, in terms of soil properties and soil organisms a definition of bioavailability that is precise, universal and useful is unlikely to be attained. However, there is an emerging consensus on what the term bioavailability encompasses, how it should be used and what information should be provided on a case by case basis to make the term meaningful and useful.

In 2002 the United States of America National Research Council published a report on bioavailability on soils (National Research Council 2002) which was usefully summarised by Ehlers and Luthy (2003). The report notes that the term bioavailability has been defined by various disciplines and goes on to state that this has led to some confusion over the term and that therefore further definitions will be avoided. Instead the report focuses on *bioavailability processes,* which are the biological, chemical and physical processes that result in an organism being exposed to a contaminant present in the soil. These processes are: release of the contaminant from the solid phase, transport of the contaminant to and across a biological membrane and, incorporation into a living organism. The National Research Council report is Chapter goes on to document methods for measuring these processes. Partially in response to the National Research Council report, but also noting the legal and regulatory implications of the concept of bioavailability within the Risk Assessment process, Semple et al. (2004) proposed the introduction of the term “bioaccessibility” to complement bioavailability. According to Semple et al. bioavailable material is “freely available to cross an organism’s cellular membrane from the medium the organisms inhabits at a given time”, whilst bioaccessible material is “that which is available to cross an organism’s cellular membrane from the environment, if the organisms has access to the contaminant”. The key advance offered by Semple et al’s definitions is the inclusion of a time element. Within their paper Semple et al. note that many classical chemical extraction methods for measuring “bioavailability” (see Section 16.4.1) actually measure or predict bioaccessibility and that, for Risk Assessment and remediation it is often bioaccessibility rather than bioavailability that is of concern. Semple et al. also make the points that bioavailability and bioaccessibility will vary between organisms and that the “membrane” in question can vary with the organism, for example being just the cellular membrane for a bacterium, but including both the epidermis and gastrointestinal tract of an earthworm. Following Semple et al. (2004) the term “bioaccessibility” is gaining usage in the literature. Reichenberg and Mayer (2006) made a further contribution to the debate over what bioavailability is when they proposed that bioavailability comprised both “accessibility” and “chemical activity”. Their “accessibility” term is essentially the same as the Semple et al. (2004) bioaccessibility term. Chemical activity is a classically defined term related to fugacity and freely dissolved concentrations, which quantifies the partial molar free energy of a substance (Faure 1992). A variety of *bioavailability processes* are driven by chemical activity, for example, sorption, diffusion and partitioning occur from high to low activities. The use of chemical activity as a component of bioavailability had already been incorporated into such models as the *biotic ligand model* (e.g. Di Toro 2001).

The International Standards Organisation (ISO) has established a working group on bioavailability (ISO/TC190 – Soil Quality). Harmsen (2007) summarises their activities. The definition of bioavailability used by this organisation is “Bioavailability is the degree to which contaminants present in the soil may be absorbed or metabolised by human or ecological receptors or are available for interaction with biological systems” (ISO 2005) which is similar to the definition of bioaccessibility given by Semple et al. However, Harmsen (2007) argues that bioavailability is best presented as a concept that is related to specific situations or measurements on a case by case basis.

To conclude, a variety of definitions for bioavailability have been proposed in the literature. There is a growing consensus that in broad terms it is a measure of uptake of and consequent cellular interaction with a contaminant by an organism. There is also a growing acknowledgement that bioavailability will: 1) vary between organisms, 2) be a function of time of contact between the organism and contaminated media and, 3) that further, more precise definitions will only be applicable on a case by case basis. The rest of this chapter will review the impacts that soil properties have on bioavailability and the various methods (bioassays, chemical extractions and models) that exist to measure or predict bioavailability.

16.3 Impact of soil properties on bioavailability

To be bioavailable molecules must cross a biological membrane. In effect this means that the molecules have to interact with the aqueous phase, either the soil's pore water or in soil passing through the gastrointestinal tract of invertebrates. Therefore, soil properties which control partitioning between the solid phase in soil and the pore water, such as pH, organic matter content, Eh, cation exchange capacity (CEC), and the concentration of clay minerals and oxyhydroxides, have a significant impact on bioavailability. Generally, molecules held on exchange sites can readily become bioavailable by desorption from these sites. Indeed many studies treat molecules held on exchange sites as part of the bioavailable fraction despite the contradiction that this generates with the above definitions of the term. As a consequence, an increase in the number of exchange sites aids the retention of molecules in a bioavailable form. Molecules sorbed more strongly to surfaces or in solid form are generally not available, whilst molecules in the pore water are available, but are also prone to being removed from the soil system as solution leaches through the soil.

From here on, inorganic and organic contaminants will be discussed separately, because their chemical and ecotoxicological properties are rather different. This results in different fate and consequently different interactions with living cells. The main characteristics of each group are summarized in Tab. 16.1 (taken from Campbell et al. 2006).

Tab. 16.1: Summary of the general characteristics that determine the kinetics of uptake and depuration of organic contaminants, metals, and inorganic metal contaminants in biota (taken from Campbell et al. 2006).

ORGANICS	METALS AND METALLOIDS
Tissue uptake is most commonly a blood flow limited process, with linear partitioning into tissues	Metals and their complexes are often ionized, with *tissue uptake* (membrane transport), having greater potential to be diffusion-limited or to use specialized transport processes
Metabolism is generally extensive and often species specific	*Metabolism* is usually limited to oxidation state transitions and alkylation / de-alkylation reactions
Persistence in body fat is common, because of lipid solubility (not capacity-limited)	Often *sequestered*, bound to specific plasma or tissue proteins (intrinsically capacity-limited), or deposited in inert forms like waste nodules, mineral concretions and granules
May be *eliminated* by excretion in urine after biotransformation from lipophilic forms to hydrophilic forms, due to complex metabolism	Predominantly *eliminated* by excretion because metals contaminants are generally small molecules and are hydrophilic. Thereupon excretion of inert species
Generally substance-specific *homeostatic mechanisms*[1] are not available	Essential metals have *homeostatic mechanisms* that maintain optimum tissue concentrations over a range of exposure rates
Interactions with other structurally similar contaminants may occur, especially during	*Interactions* among metals and between metals and organics are numerous and occur

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[1] Homeostatic mechanisms are physiological mechanisms by which organisms maintain concentrations of essential elements inside their body within specific fixed levels. These fixes levels are often termed the "window of essentiality".

metabolism	commonly during the processes of absorption, excretion, and sequestration

16.3.1 Metals and metalloids

For inorganic contaminants present as cations the bioavailability tends to decrease from acid to neutral through alkaline conditions. Typically, metals are more soluble under acidic conditions. However, over time, a highly acid soil is likely to loose solution cations through leaching. As a consequence, in the longer term the concentration of bioavailable contaminants can be reduced by acidic conditions, unless there is a significant source of contaminants to replenish those lost, for example the gradual dissolution of metal-bearing materials. Loss of cations through leaching can be offset by exchange processes. Cations held on exchange sites are normally considered to readily become bioavailable. As pH increases from acid to alkali, metal ions are more likely to displace protons from exchange sites and be held in the soil in a form that allows rapid replenishment of the reservoir of bioavailable contaminants as they are taken up by organisms. Lindsay (1979) presents a series of empirical equilibrium constants and phase diagrams for the reaction:

$$\text{Soil-metal} + x\ \text{H}^{+} \Leftrightarrow \text{soil-H} + \text{metal}^{x+} \qquad \text{(Eq. 16.1)}$$

Soil-metal and soil-H indicate metal ions and protons sorbed to exchange sites
H^{+} and metal^{x+} represent protons and metal ions in solution

Besides, Lindsay (1979) gives partition co-efficients and solubility data for a range of metals, that allow predictions of conditions under which specific metal ions are likely to reside in the pore water or on exchange sites in soils.

In contrast to cationic contaminants, contaminants present as anions such as arsenate and chromate tend to be more significantly sorbed at lower pH, thus becoming more bioavailable (but also more mobile in the soil) towards more neutral pH conditions.

pH influences bioavailability via the generation of exchange sites as well as exerting controls via solubility and competition for exchange sites between protons and other cations. The charge of mineral surfaces may be permanent or pH-dependent. The former, i.e. the permanent charge of minerals, results from structural imperfections in mineral structures. The latter, i.e. the pH-dependent charge of minerals, results from dissociation (leading to negative surface charge) or protonation (leading to positive surface charge) of hydroxyls associated with the surface of clay minerals, oxyhydroxides or organic matter. The relative importance of permanent and pH-dependent charge of minerals varies between metals. Generally, permanent charge is most important for so-called *2:1 clays* such as vermiculite, which comprise a basic repeating structure of Al-octahedral layers with a Si-tetrahedral layer sandwiched in between. pH dependent charge is more important for *1:1 clays* such as kaolinite (i.e. a clay with alternating layers of Al-octahedral and Si-tetrahedral sheets) and oxyhydroxides. The pH at which the surface charge switches from positive to negative (the point of zero charge, PZC) varies with sorbent, being typically 7 – 9 for Fe-oxyhydroxides, 8 – 9.2 for Al-oxyhydroxides, 1.5 – 4.6 for Mn-oxyhydroxides and 5 – 6 for clay minerals. These PZC values mean that in "typical" soils with a slightly acidic pH, Fe- and Al-oxyhydroxides are important for retaining bioavailable anions in the soil and Mn oxyhydroxides for retaining cations.

Surfaces of organic matter can have a negative charge through the dissociation of carboxylic acid and phenolic acid groups and thus can provide exchange sites for cationic metals. However, metals and organic matter also interact to form chelate complexes in which the metal is sorbed to the organic matter through more than one bond so that a ring structure is formed. As an illustration of the formation of chelate complexes, cadmium is complexed with one deprotonated carboxylate group (Fig. 16.1a), and copper is complexed with one carboxylate and one neighbouring phenolate group (Fig. 16.1b):

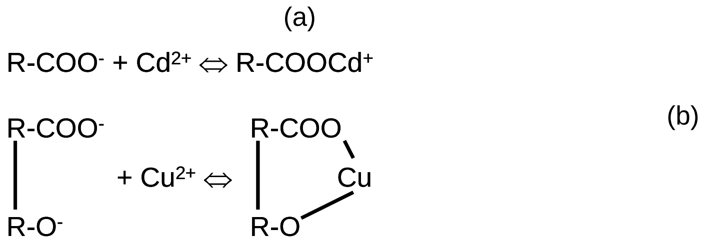

Fig. 16.1 Cadmium complexed to one deprotonated carboxylate group (a), and copper complexed to one carboxylate and one neighbouring phenolate group, as an illustration of the formation of chelate complexes

The resulting ring structures are very stable. There is an element of covalency in the bonds and thus metals held in this way are not readily bioavailable. Thus, increasing organic matter often leads to a decrease in the bioavailability of metals. Different metal cations show different tendencies to form complexes with organic matter (McBride 1994), with copper in particular able to form a strong bound. Overall, total metal-binding concentrations to organic matter decrease in the order copper > nickel > lead > cobalt > cadmium > calcium > zinc > manganese > magnesium (McBride 1986). Unfortunately, though there is currently no systematic explanation for this. The difficulty in determining the reason for this variation is probably due to the wide variety of functional groups present on organic matter. In the “real” world, the strength of metal binding is to a considerable extent dependent on competition for binding sites with other commonly occurring cations, such as H^+ and macro-ions like Ca^{2+}, Mg^{2+}, Na^+, etcetera.

The redox conditions in soil will also impact bioavailability of metals. In general terms, for elements that can exist in more than one oxidation state the lower oxidation state ions are more soluble. So under more reducing conditions the concentration in the pore water often increases. If soils are water-logged and become anaerobic, oxyhydroxides of Fe and Mn become unstable and dissolve. Any sorbed ions are released and there is an initial increase in bioavailability. Over time, bioavailability can decrease as pore water is leached from the upper soils. In waterlogged soils, however, leaching rates are relatively slow. Release of ions into the pore water initiated by waterlogging is partially offset by the precipitation of sulphides, which can reduce metal availability. Indeed, some in situ remediation techniques rely on the generating reducing conditions to render metals unavailable.

Finally, there is increasing evidence that speciation of metals governs their bioavailability. Indeed, models such as the *Biotic ligand model* (Section 16.4.2.1) rely on this phenomenon (Di Toro et al. 2001, Allen et al. 2008, Lock et al. 2007, Thakali et al. 2006a, 2006b; Van Gestel and Koolhaas 2004; Steenbergen et al. 2005; Arnold et al. 2007). Generally, metals present as free ions or as simple, relatively small inorganic complexes are viewed as being available, whilst larger, often organic complexes are not. In general, the higher the ionic strength of the pore water, the less free ions are present, and therefore the lower the bioavailability of that metal. .

16.3.2 Organic contaminants

The factors affecting the bioavailability of organic contaminants are fundamentally the same as those for metals, but the magnitude of effects varies. To assess the bioavailability of organic contaminants it is also necessary to determine the physical properties of the contaminants and to understand their biogeochemical fate, requiring the need to investigate transport properties, sorption, volatilization, and biodegradation of organic contaminants. The more of the contaminant present in the pore water the more bioavailable it is. A popular aphorism used for predicting solubility is "*like dissolves like*" (Kenneth and Williamson 1994). This indicates that a contaminant will dissolve best in a solvent of similar polarity. This is a rather simplistic view, since it ignores many solvent-solute interactions, but it is a useful rule-of-thumb. The behaviour of organic contaminants is often characterized by octanol-water partitioning, expressed as the octanol-water partitioning coefficient (K_{ow}). The larger the K_{ow}, the stronger the contaminants are bound to the soil solid phase and the less bioavailable they are. In general, the hydrophobicity and hence the K_{ow} increase with increasing number of C-atoms.

The solubility of organic contaminants nearly always increases with temperature. The solubility equilibrium is relatively straightforward for non-ionic and non-polar substances such as benzene. When dissolved in water, the benzene molecules remain intact and are generally surrounded by water molecules. This behavior differs from that of ionic contaminants or metal salts. When an ionic contaminant such as sodium chloride (NaCl) dissolves in water, the sodium chloride lattice dissociates into individual ions that are solvated or surrounded by water molecules, thus increasing the ionic strength of the solution. In turn, increases of ionic strength affect the surrounding by water of neutral organic contaminants. Effectively, at high ionic strength conditions, the solubility of organic contaminants and hence their bioavailability is reduced.

Apart from temperature and ionic strength, sorption to (dissolved) organic matter is the main actor affecting bioavailable concentrations of organic contaminants. Sorption increases with increasing organic matter content of the soil, whereas concentrations of organics in the pore water increase with increasing dissolved organic carbon (DOC) concentrations. In contrast to a large number of aquatic organisms, for which only the truly dissolved contaminant has been shown to be bioavailable, it can, however, not be ruled out that contaminants sorbed onto dissolved organic carbon can interact with biota. Amongst others, this is due to differences in the rates of release of organics from dissolved organic carbon versus release kinetics from soil solid organic material. Karickhoff *et al.* (1979) were one of the first authors to show the equilibrium concept of partitioning of organic contaminants by reporting that the contaminant-specific organic-carbon normalized partition coefficient (K_{oc}) is proportional to K_{ow}:

$$(\mathrm{Log})\ K_{oc} \approx a * (\mathrm{Log})\ K_{ow} + b \qquad \text{(Eq. 16.4)}$$

where a and b are contaminant specific constants.

Subsequently, the K_{oc} may be used to predict the degree of contaminant partitioning of hydrophobic organics between soil organic carbon and pore water:

$K_{oc} = K_d / f_{oc}$, with $K_d = C_w / C_{solid\ phase}$ (Eq. 16.5)

where f_{oc} is the fractional organic matter content of the soil and C_w and $C_{solid\ phase}$ are the concentrations of the contaminant in the pore water and soil solid phase, respectively.

Based on additional work of Sabljić et al. (1995), 19 quantitative structure-activity relationships (QSARs) for a variety of contaminant classes are listed in the EU Technical Guidance Document (TGD – European Commission 2003) for Risk Assessment of chemicals in the form of Eq. 16.4 (Tab. 16.2). Low polarity organic contaminants, however, may, on top of equilibrium partitioning, also bind to solid phases through adsorption mechanisms which result in greater (non-linear) binding coefficients. Thermally or diagenetically altered forms of carbonaceous materials such as coal, kerogen from shales, soot, and charcoal, have a particularly high binding affinity and nonlinear adsorption behaviour, with carbon-normalized Freundlich sorption coefficients that are as much as 50 to 250 times higher than typically reported K_{oc} values (Binger 1999; Bucheli and Gustaffson 2000; Grathwohl 1990).

Tab. 16.2: Overview of quantitative structure-activity relationships (QSARs) (Koc/Kow relationships) for a variety of contaminant classes, included in the TGD (European Commission 2003).

Contaminant class	**Equation**
Predominantly hydrophobics	$Koc = \frac{1.26 \cdot Kow^{0.81}}{1000}$
Nonhydrophobics	$Koc = \frac{10.47 \cdot Kow^{0.52}}{1000}$
Phenols, anilines, benzonitriles, nitrobenzenes	$Koc = \frac{7.94 \cdot Kow^{0.63}}{1000}$
Acetanilides, carbamates, esters, phenylureas, phosphates, triazines, triazoles, uracils	$Koc = \frac{12.30 \cdot Kow^{0.47}}{1000}$

Alcohols, organic acids	$Koc = \frac{3.16 \cdot Kow^{0.47}}{1000}$
Acetanilides	$Koc = \frac{13.18 \cdot Kow^{0.40}}{1000}$
Alcohols	$Koc = \frac{3.16 \cdot Kow^{0.39}}{1000}$
Amides	$Koc = \frac{17.78 \cdot Kow^{0.33}}{1000}$
Anilines	$Koc = \frac{7.08 \cdot Kow^{0.62}}{1000}$
Carbamates	$Koc = \frac{13.80 \cdot Kow^{0.37}}{1000}$
Dinitroanilines	$Koc = \frac{83.18 \cdot Kow^{0.38}}{1000}$
Esters	$Koc = \frac{11.22 \cdot Kow^{0.49}}{1000}$
Nitrobenzenes	$Koc = \frac{3.55 \cdot Kow^{0.77}}{1000}$
Organic acids	$Koc = \frac{2.09 \cdot Kow^{0.60}}{1000}$
Phenols, benzonitriles	$Koc = \frac{12.02 \cdot Kow^{0.57}}{1000}$
Phenylureas	$Koc = \frac{11.22 \cdot Kow^{0.49}}{1000}$
Phosphates	$Koc = \frac{14.79 \cdot Kow^{0.49}}{1000}$
Triazines	$Koc = \frac{31.62 \cdot Kow^{0.30}}{1000}$
Triazoles	$Koc = \frac{25.70 \cdot Kow^{0.47}}{1000}$

Hydrophobic partitioning is less important for polar and ionisable contaminants. These contaminants are involved in more diverse binding mechanisms that contribute to contaminant retention, including ion bonding or ligand exchange, covalent binding to the soil molecular structure, ion-dipole and dipole-dipole interactions, charge transfer, hydrogen bonding and hydrophobic bonding (Van der Waals forces) (Von Oepen et al. 1991). The chemically most active component of the soil is the colloidal fraction which consists of organic matter and inorganic clay minerals (Stevenson 1994). Both components display a negative electrical charge at the surface, resulting in weak binding forces and typically reversible sorption, as sorption often is restricted to a limited number of binding sites at the surface layer. The effect of the negative electrical charge at the surface can be measured by the cationic exchange capacity, which on average is 50 meq/100g for clays and 290 meq/100g for humic acids (Krogh 2000). Electrical forces involving charge transfer (~40 kJ/mol) are stronger than hydrophobic bonding (~4 kJ/mol) (Von Oepen et al. 1991) so that they dominate when present. Thus, a different degree of sorption of anions, cations and neutral molecules can be expected, with cations showing the highest potential for sorption, due to electrical attraction. Although lipophilic interactions are weaker than the other interactions mentioned, they are the most important for the majority of organic contaminants.

A typical example of this general sorption behaviour is provided by Vasudevan et al. (2002). These authors studied the sorption of two ionic contaminants (2,4-D and quinmerac) and one neutral chemical (norflurazon) onto iron oxide-rich, variable charged soils. It was found that sorption was strongly influenced by mineralogy, particularly soil iron and aluminium oxides, whereas sorption of the neutral norflurazon was only related to total soil carbon. An appreciable fraction of the mass sorbed in stirred-flow studies was easily desorbed by deionised water, and desorption of ionic contaminants was initially more rapid than sorption. This sorption-desorption behaviour, although contrary to desorption hysteresis commonly observed in batch studies, suggests that the reversibly sorbed fraction is weakly bound to the soil surface. 2,4-D sorption to iron oxide-rich soils and pure-phase metal oxides appears to be driven by non-specific electrostatic attraction, with specific electrostatic attraction and van der Waals interactions being secondary. Both the carboxylate and the heterocyclic nitrogen groups may participate in sorption of quinmerac, facilitated by specific and non-specific electrostatic attraction with charged soil mineral surfaces, and surface complexation. The heterocyclic nitrogen, amine, and carbonyl groups of norflurazon do not appear to interact with soil minerals.

A phenomenon that is observed when studying sorption of lipophilic, polar and ionic contaminants is related to the displacement of contaminants initially sorbed to the solid phase. This is for instance reported for sorption of pharmaceuticals to sewage sludge by Jones et al. (2006). This phenomenon may be ascribed to initially fast binding of the contaminant to a large number of low affinity binding sites (like weak binding to the surface layer), followed by displacement of the contaminants by for instance more hydrophobic constituents of the DOC or by (newly formed) DOC constituents capable of interacting more strongly with the sorption sites. Thereupon, these constituents may out-compete micro contaminants as they may be formed in relative high concentrations due to (microbial) degradation of the organic matter present in either the solid phase or in the pore water.

Bintein and Devillers (1994) suggested an empirical regression using K_{ow} and pK_a as molecule descriptors and pH and f_{OC} to describe the sorbent properties for the estimation of the K_d of both ionised and non-ionised contaminants:

$$\log K_d = 0.93 \cdot \log K_{OW} + 1.09 \log f_{OC} + 0.32 \cdot CF_a - 0.55 \cdot CF_b + 0.25 \qquad \text{(Eq. 16.6)}$$

where the correction factors CF_a and CF_b quantify the fraction of dissociated acids and bases in the system:

$$CF_a = \log \frac{1}{1+10^{pH-pKa}}$$

$$\text{(Eq. 16.7)}$$

$$CF_b = \log \frac{1}{1+10^{pKa-(pH-2)}}$$

According to these authors, the soil pH should be entered for acids into the equation for the correction factor CF_a. The pH required for the correction factor CF_b is the pH at the surface of the soil colloids and is lowered 2 pH units below the pH of the bulk soil solution. Even though the coefficient of determination of this equation was very high ($R^2 = 0.93$), a short-coming of the regression, regarding charged organic contaminants, is their insufficient representation in the test set (nine acids and three bases out of 87 contaminants). Besides, it may also surprise that the fraction of bases, CF_b, was negatively correlated to the sorption coefficient K_d, even though it might be expected that electrical attraction of the positively charged cations by the negative potential of soil colloids would increase sorption of bases.

In summary, although the predictability of newly developed empirical formulae is large, there is no rigorously tested method available to predict the Kd or the Koc of the majority of contaminants. Separate expressions are needed for the neutral and the ionic molecule fraction in order to account for dissociation of organic acids, bases and amphoters.

16.4 Measurement of bioavailability

The simplest and most straightforward method of determining the bioavailable fraction of a contaminant in a soil is to expose the organism of interest, be it an earthworm or a geranium, and measure the uptake of the contaminant into that organism. In the case organic contaminants the bioavailable fraction could be assessed by measuring the rate of biodegradation, i.e. perform a bioassay. This gives a direct measure of what is bioavailable to a specific organism over a specific period of time. Clearly this is not practical as there are too many contaminated sites, too many potential receptors and too many contaminants for direct bioassays to be performed as a matter of standard practise. For this reason, a variety of methods have been proposed to determine bioavailability. These can be divided into models that quantify scenarios on bioavailability and chemical extractions that mimic bioavailability and subsequent uptake. Because of the differences in their behaviour (Table 16.1), measurement procedures vary for inorganic and organic contaminants. Measurement procedures which are the same for the two groups of contaminants are the physical sampling of pore water (see MacDonald et al. 2008 for a review of methods) allied to the assumption that contaminants in the pore water are bioavailable. Methods for sampling pore water include simple centrifugation of the soil (e.g., Nahmani et al. 2007a), vacuum sampling (e.g., Tipping et al. 2005) and passive sampling systems based on a zero-tension principle (e.g. Haines et al. 1982). The most common vacuum sampler is the suction cell, where an imposed vacuum sucks pore water into an in-ground porous cup. In simple systems, the water is stored in the suction cell and is subsequently sucked or blown into a sample flask placed on the soil surface. When employing these methods in the laboratory it is common to standardize the soil in advance. The most common standardization treatment is fixing the water content in the soil and allowing the system to equilibrate for a specific period of time (e.g. Tipping et al. 2005). Water concentrations are commonly expressed in terms of percent of the maximum water holding capacity.

16.4.1 Extractions for determining bioavailability

Chemical extractions represent perhaps the most straightforward method for determining bioavailability of a contaminant. These methods typically involve shaking contaminated soil material with a solution for a period of time and then analysing the concentration of contaminants in the solution. Contaminants that are more readily extracted are more bioavailable. For standard extractions the ratio of solid to solution, period of shaking, nature of shaking, temperature, etcetera, are stipulated in guidelines. Any chemical extraction of this nature should have been validated against field based measurements, showing good correlations between extractable chemicals and tissue concentrations measured in an organism of interest. A problem is that the extractions are often applied outside the limits of their validation, e.g., different soil texture, pH, contaminant concentration, organic matter concentration.

The majority of chemical extractions have been developed for plants. There are some attempts in the literature to apply these to soil biota, e.g., earthworms (Langdon et al. 2001, Conder et al. 2001), but these are rather limited and are currently used far less frequently than plant correlated extractions.

Chemical extractions as a proxy for bioavailability are popular due to their rapidity and cost effectiveness. Provided that the limitations of such methods are borne in mind, and that results are presented as extractable total concentrations rather than bioavailable concentrations, such extractions are useful tools in Risk Assessment. However, there will always be a problem with extractions. Due to the infinite variability between organisms, soils and different elements, it will always be hard to have a high degree of confidence that a particular extractable concentration is a direct measure of bioavailability for a specific organism, especially under changing conditions. For this reason, calculating procedures perhaps offers a better solution to measure the bioavailability in the long term.

16.4.1.1 Metals and metalloids

Chemical extractants used to assess the bioavailability of inorganic contaminants involve solutions of either simple ionic solids, e.g., $CaCl_2$, or more complex organic reagents such as ethylenediaminetetraacetic (EDTA). The simple ionic solutions are used as a proxy for pore water. They are used in order to 1) dissolve readily soluble contaminants and 2) displace contaminants from exchange sites into solution. The more complex organic reagents are supposed to mimic the organic exudates produced by plants, which are able to more aggressively remove contaminants from exchange sites into solution. There are too many different extractions to review them all in this chapter. Different extractions are well documented in, amongst others, Dean (2007), McLaughlin et al. (2000), Houba et al. (1996) and Ross (1994). Perhaps the most commonly used chemical extractants to assess the bioavailability of metals are 0.01 M $CaCl_2$, diethylenetriamenepentaacetate (DTPA) and EDTA. They are described briefly below.

The first suggestion that 0.01 M $CaCl_2$ extractions can be used as a general assay for bioavailability that the authors are aware of was made by Houba et al. (1996). They suggest that < 2 mm air-dry soil is extracted at a ratio of 10 mL of 0.01 M $CaCl_2$ to 1 g of soil by shaking at 20 °C for 2 hours, after which the pH is measured, the mixture centrifuged and the centrifugate analysed for the elements of interest. Advantages of the 0.01 M $CaCl_2$ extraction procedure are that the concentration of the solution is in the range of that of “average” soils, the Ca^{2+} causes clays to coagulate as the dominant adsorbed cation on soil exchange sites Ca is better able to displace metals from exchange sites than other extractants. Thereupon, minimal chemicals are used reducing extractant disposal issues, and a single extractant can be used for a multitude of metals. Houba et al. (1996) are careful to avoid attributing all $CaCl_2$ extractable metals to an adsorbed fraction, recommending instead, expressing extractable metal concentrations in terms of solute concentrations in the extractant pore water concentrations. A variety of studies were was cited by Houba et al. (1996) in which 0.01 M $CaCl_2$ extractable metals have been correlated with metal concentrations in plants and many studies have been carried out since (e.g., Mench et al. 1997, Brun et al. 2001, Perez-de-Mora et al. 2006, Fang et al. 2007). A slightly different approach is to use $CaCl_2$ as the extractant but, in an effort to mimic pore water conditions, to tailor the concentration of solution used on a soil by soil basis. Thus, to simulate the metal concentrations in the pore water of a soil, the first step would be to determine the ionic strength of the pore water. For example, for clayish soils ionic strengths are typically around 2.5 mM (Schröder et al. 2005), implying that 0.01 M $CaCl_2$ will overpredict the pore water concentrations. As indicated by Schröder et al., 2.5 mM $CaCl_2$ as extractant would be a better choice in this example to mimic pore water.

DTPA extractions are perhaps the most used and abused procedures to measure bioavailability. Lindsay and Norvell (1978) proposed a method (shake 10 g < 2 mm air dry soil at a rate of 120 cycles min^{-1} on a horizontal shaker with a stroke of 8 cm in a solution comprising 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA buffered to pH 7.30 using HCl, then filter through Whatman No. 42 filter paper and analyse) for determining deficiencies of iron, manganese, zinc and copper in soils. The method was adopted as a mean to assess plant bioavailable metals at contaminated sites. O'Connor (1988) documents the main problems with using this method for soils containing high concentrations of metals, the most significant being that the chelating capacity of the solution (10 mmol kg^{-1} of soil) can be exceeded quite readily. However, Norvell (1984) proposed using a modified method with a 5:1 extractant to soil ratio for acid and metal–contaminated soils.

EDTA based extractions have been used for longer than the DTPA based extractions, with references dating back to the 1950s (Cheng and Bray 1953, Viro 1955a, b). Unlike the DTPA extraction of Lindsay and Norvell (1978) there is no single usual extraction with standardized concentration (e.g., in the range 0.1 – 0.01 M) and standardized pH value. There are many documented cases of EDTA extractions correlating well with plant metal concentrations (e.g., Cajuste and Laird 2000; Michaud et al. 2007; Hooda et al. 2007).

A rather different approach worthy of mention is *diffusive gradient in thin films* (DGT). The DGT methodology was originally developed for assessing the water chemistry (Davison and Zhang 1994), but has more recently been applied to measure bioavailability in soils, e.g., Zhang et al. (2001). The technique involves applying an arrangement of a filter, layer of diffusive gel and layer of resin to a moist soil surface. Over time, contaminants diffuse through the gel and are adsorbed by the resin. This approach samples both contaminants in solution and those held loosely on exchange sites which come into solution to replace contaminants that are sorbed by the resin. Zhang et al. (2001) report good correlations between copper uptake by *Lepidium heterophyllum* and the concentration of Cu measured using DGT. Good correlations between plant and DGT concentrations have also been reported for copper in other studies (e.g. Song et al. 2004) and zinc (e.g. Cornu and Denaix 2006, Sonmez and Pierzynski 2005). Cornu and Denaiz (2006), however, found a weak correlation between plant and DGT concentrations, for cadmium. The use of DGT for organisms is debated, for instance Koster et al. (2005), suggest that the DGT is often poorly representative for the metal concentrations to which the organism is exposed. In general, DGT will deplete the pore water from metals and replenishment of metals towards the solution phase is a slow process. However, organisms move around and are likely exposed to concentrations that are controlled by equilibrium between pore water and the solid phase, while metal concentrations are not depleted.

16.4.1.2 Organic contaminants

Organic contaminants have been recognised as contaminants in soils for a shorter period of time than inorganic contaminants. This is due to the historic importance of metals, the relative ease of analysis of metals compared to organic contaminants, and the mere fact that the spectrum of chemical structures is more diverse for organic contaminants than for metals and metalloids. Although the list of potential approaches to simulate bioavailability of organic contaminants is long, consequently far fewer chemical extraction procedures have been proposed as proxies for the availability of organic contaminants compared to metals. Paradoxically, this makes extraction procedures for organic contaminants harder to summarise and review, because there has been less time for the establishment of one or several standardized procedures. Additionally, compared to metal extractions, a far higher proportion of extractions have been validated to organisms other than plants. A good review of different approaches is given by Ehlers and Loibner (2006) and readers are referred to that paper for details.

As with metals a number of physical-chemical (extraction) procedures have been developed to aid in the prediction of the bioavailable concentration of organic contaminants. These procedures provide knowledge about the extent of contaminant retention in soils, within shorter periods and for lower budgets and give more precise information on soil constituents being responsible for the sequestration of hydrophobic organic contaminants in soil compared to bioassays. Tab. 16.3 provides an outline of some of the principle studies reported in the literature in which chemical solvents were used to evaluate bioavailability / bioaccessibility of organic contaminants. The most straightforward extractions involve shaking soil with a weakly polar (e.g., methanol-water, *n* butanol, ethanol) or non-polar (e.g., hexane) liquid. Kelsey et al. (1997) and Tang and Alexander (1999) report good correlations between extractable concentrations of atrazine, phenanthrene, anthracene, fluoranthene and pyrene and uptake by earthworms and plants and breakdown by bacteria, but found no universal extractant that produced good correlations for all chemicals and organisms tested.

Tab.6.3: Outline of some principal studies that employed chemical solvents to evaluate bioavailability of organic contaminants. Taken from Jensen and Mesman (2006).

Contaminant	**Solvent**	**Bioassay**	**Operation**	**Comments**
Atrazine Phenanthrene (Kelsey et al.	Methanol/water, n-Butanol	Earthworm uptake and degradation	25 ml solvent and 10 g solid. Shaking for 2 h	Methanol/water best predictor for atrazine whereas n-butanol was best for

1997)				phenanthrene.
DDT, DDE, DDD PAH (mixture) (Tang et al. (1999, 2002))	THF Ethanol	Earthworm uptake	15-20 ml solvent and 1 g soil, 10 sec of mixing	Good correlation with earthworm accumulation.
Anthracene, Fluoranthene, Pyrene (Tang and Alexander (1999))	n-Butanol Propanol Ethyl acetate	Plant retention, earthworm uptake and microbial degradation.	25 ml solvent and 1-2 g soil, 5 sec of mixing.	Reasonable correlation with bioassays.
Phenanthrene Pyrene Chrysene (Liste and Alexander (2002))	n-Butanol	Earthworm uptake and microbial degradation	15 ml extractant, 5-10 g soil, mixing: 5 sec (worm) or 120 sec (degradation)	Applicable for bioavailability prediction.

In recent years, biomimetic extractions have become increasingly common to assess bioavailability of organic contaminants in soils. Among biomimetic extractions of hydrophobic organic contaminants, two distinctive lines of approaches can be distinguished, i.e. those that are equilibrium-based and negligibly change the partitioning of a contaminant between the solid phase and the pore water, and those that deplete all contaminants released from the solid phase into the pore water, within a certain time period.

Non-depleting procedures measure chemical activity of a contaminant in soil, whereas depleting procedures measure the bioaccessible fraction of a contaminant. Examples of equilibrium based procedures are negligibly depleting solid phase micro extraction (nd-SPME) (see details later), extraction with poly-oxymethylene strips, triolein-embedded cellulose acetate membrane, and hollow fibre supported liquid membrane. Depleting extraction procedures include, for example, Tenax beads and β-cyclodextrin. The later biomimetic extractions are based on the principle that organisms predominantly take up contaminants from the pore water and thereby temporarily deplete the pore water, which again is rapidly replenished with contaminants desorbing from the solid phase.

Reid et al. (2000) propose shaking soil with an aqueous solution of hydroxypropyl-□-cyclodextrin (HPCD), a large molecule with a hydrophilic exterior surface and a hydrophobic cavity within the molecule. Reid et al. (2000) report good correlation between HPCD extractable polycyclic aromatic hydrocarbons (PAHs) and biodegradation of PAHs (and thus bioavailability) by microorganisms. Cuypers et al. (2002) also report good correlations. An alternative approach to assess bioavailability/bioaccessibility has been the use of C18 membrane disks, which are placed in the soil and then removed and purged. The amounts of contaminant extracted have shown good correlations between, for example, extractable DDT, DDE, DDD and earthworm uptake (Tang et al. 1999), extractable PAH and earthworm uptake (Tang et al. 2002) and extractable PAH and PCB and earthworm uptake (Krauss and Wilcke 2001). Beads made from Tenax TA have also been suggested as an extractant (Cornelissen et al. 1998). A slurry of soil, Tenax TA beads and salt solution is shaken for a specified period of time and then the Tenax TA beads (which float or stick to the vessel walls) are removed and extracted for organic contaminants. Morrison et al. (2000) report good correlations between Tenax TA extracted DDT, DDE and DDD and earthworm tissue concentrations, but poor correlations for dieldrin. Cuypers et al. (2002) report good correlations between Tenax extractable PAH and biodegradation of PAHs. Ten Hulscher et al. (2003) also report good correlations between extractable PAHs and uptake in earthworms, though the nature of the relationship varied with exposure media. Results reported by De La Cal et al. (2008) suggest that 48 h of Tenax extraction is needed as proxy for the bioaccessible fraction of highly hydrophobic organic contaminants like polybrominated diphenyl ethers, DDT, and DDT metabolites. Finally, a proposed procedure for measuring bioavailability using a solid phase extractant is “Solid-phase micro-extraction with negligible depletion” (nd-SPME) which involves inserting a fibre thinly coated in an organic compound such as poly(dimethysiloxane) (PDMS) and polyacrylate into the soil, leaving it there for a period of time and then removing it and extracting organic contaminants from the fibre. Van der Wal et al. (2000) report good correlations between accumulation of HCB, telodrin, dieldrin and PCBs in earthworms and extractions using SPME.

16.4.2. Modelling the bioavailability of contaminants

16.4.2.1 Metals and metaloids

Models can be split into two varieties, mechanistic models (i.e. those with a theoretical basis) and empirical models (i.e. those which are correlations). An example of a model based on theory is the *biotic ligand model*.

The biotic ligand model is used to predict the toxicity of contaminants to target organisms. It was initially designed for aquatic systems (e.g., Di Toro et al. 2001), but more recently has also been developed for soil systems (e.g., Allen et al. 2008, Lock et al. 2007, Thakali et al. 2006a, 20006b, Van Gestel and Koolhaas 2004, Steenbergen et al. 2005). The majority of chemical extractions that are used as proxies for bioavailability are correlated with the metal concentration in an organisms tissue. In contrast, the biotic ligand model is used to predict a toxicological endpoint, be that root elongation, earthworm or springtail reproduction, respiration et cetera. Thus biotic ligand models have great potential for the assessment of ecological risks related to contaminated sites, as they determine not just whether a contaminant is bioavailable, but also whether that contaminant will have a toxic effect. Although from a contaminated site Risk Assessment perspective the toxicity component of biotic ligand models is their most valuable component and it is almost impossible to separate the bioavailability and toxicity components, the theory behind the biotic ligand model is important as an approach to modelling bioavailability and is described below.

The theory underlying the biotic ligand model is that free metal ions react with binding sites at the organism – pore water interface and the fraction of binding sites occupied by the metal of interest governs the toxic response. There is competition for these binding sites between the contaminant of interest and other ions present such as H^+, Mg^{2+} and Ca^{2+}. Thus the bioavailability component of the model comprises determining the free metal ion activity in the pore water and determining the partition coefficient between the pore water and the binding sites of the organism (the biotic ligand). Free metal ion activities are either measured directly with an ion specific electrode (e.g. Steenbergen et al. 2005), or calculated using a chemical speciation program such as WHAM (Windemere Humic Acid Model) VI (e.g. Thakali et al. 2006a, b; Tipping 1998). The partition coefficients are derived by experiments in which toxic effects and ion activities are measured with one variable, e.g., activity of the metal of interest or pH being varied whilst other variables are kept constant (see De Schamphelaere and Janssen 2002). It is important to note that the above approach is different from simply correlating metal free ion activity with the metal concentration in the organism of interest. Sometimes such correlations exist, but not always as competition exists between the metal of interest and other ions for the exchange sites on the biotic ligand.

Empirical models generally measure the tissue concentration of a metal in the organism of interest and a host of soil properties, such as pH, bulk metal concentration, pore water metal concentration, dissolved organic carbon, concentration of Fe and Al oxyhydroxides, concentration of clay minerals, etcetera. Multiple linear regression techniques are then used to derive a predictive relationship for tissue concentrations. A large number of such models exist in the literature for earthworms and were recently reviewed by Nahmani et al. (2007b). The majority of these models take the form:

$$\text{Log } M_{ew} \approx a * \text{Log } M_s + b \qquad \text{(Eq. 16.8)}$$

where:

M_{ew} = concentration of metal in the earthworm (mg kg^{-1})

M_s = concentration of metal in the soil (mg kg^{-1})

The problem with these models (be they for earthworms or other organisms) is that they are hardly ever, if at all, validated with independent data sets, largely due to the lack of appropriate data. Exposure periods, for example, may differ between experiments, different species of test organisms may have been used, a variable present in the regression may not have been measured in another study, et cetera. Thus, their applicability to different sites and soils is always open to question. One of the few studies in which relationships were derived and validated with independent data is that of Sample et al. (1999). In this study 26 data sets were used to derive regression equations and six data sets to validate the data. The best regression coefficients were obtained when metal body burden was regressed against soil concentration and (usually) soil Ca-concentration. Including pH in the regressions occasionally improved the predictive power of the equations. Nahmani et al. (Submitted) applied a selection of these equations and those derived by Neuhauser et al. (1995) and Peijnenberg et al. (1999) to their own data set that they had used to determine rates of metal uptake. The equations of Sample et al. (1999) were found to best predict tissue metal concentrations.

16.4.2.2 Organic contaminants

As with metals, for organic contaminants a distinction can be made between statistical models linking accumulated contaminants to extractable concentrations or pore water contents, and more mechanistically based modelling approaches like the one reported by Jager (1998) for estimating bioconcentration in earthworms.

Three conceptual frameworks provide the basic concepts for modelling bioavailability of contaminants. The first conceptual framework is the concept of chemical equilibrium in which chemical activities (or fugacities) are the driving factor for transport and distribution processes, including passive uptake of contaminants by biota (MacKay et al. 1992). The fugacity concept dictates chemical fugacities to be similar across biological membranes and explains observed variability in uptake patterns for organisms for which active uptake (like feeding and ingestion of solid soil particles) is of importance. Similarly, the concept explains why deviations from pore water uptake are often observed for highly hydrophobic contaminants (i.e., with log-transformed values of the octanol-water partition coefficient > approximately 5). Within the fugacity concept, Reichenberg and Mayer (2006) identified two complementary aspects of bioavailability of organic contaminants, these being the accessible quantity and the chemical activity that is to be deduced from this quantity as related to the physico-chemical conditions of the soil.

The second conceptual framework is the concept of equilibrium partitioning theory (EPT) in which chemical activities in the pore water are assumed to drive uptake and effects (Van Gestel 1997). The equilibrium partitioning concept is schematically given in Figure 16.2. EPT assumes that the major distribution processes in the soil compartment, i.e., between soil - pore water - biota, can be described or predicted from simple physico-chemical properties such as the lipophilicity of the organic contaminant and the relative amount of binding sites in the soil or fat content of the biota. The EPT can be used to predict body residues and/or toxicity in soil dwelling species on the basis of data generated with aquatic organisms assuming that either pore water is the only route of exposure or that additional uptake pathways are proportional to pore water uptake, and that aquatic species generally have the same overall sensitivity distribution as terrestrial species. Within the basic EPT concept it is explicitly realized that the morphology, physiology and behaviour of biota dominate actual uptake and effects.

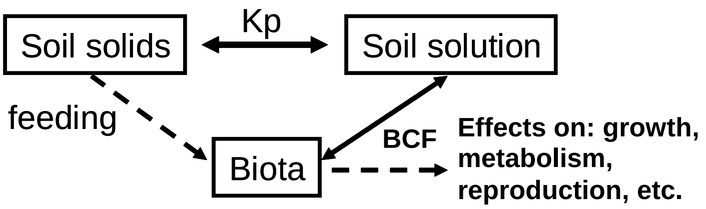

Fig. 16.2: Schematic representation of the equilibrium partitioning theory (EPT) (Kp = partitioning coefficient, BCF = bioconcentration factor).

The third and most general concept is the general concept of bioavailability advocated by the ISO-working group on bioavailability (ISO/DIS 2006, Harmsen 2007). This group established a general bioavailability scheme for both organic and inorganic contaminants.

It should be noted that the fugacity concept and the concept of equilibrium partitioning share many communalities and in fact, Reichenberger and Mayer (2006) added another concept: the thermodynamic concept of the chemical potential. In practical terms, the concepts have in common that it is the concentration of the contaminant in the pore water that is to be used as the basis for modelling uptake and effects of contaminants.

A model developed by Belfroid (1995) in which estimates of accumulation of organic contaminants by earthworms (inputs: bioconcentration in water, sorption and elimination constants, rate of soil ingestion, and uptake efficiency) were generated, showed that in most cases uptake from pore water is the dominant uptake route. However, for contaminants with Log Kow exceeding 5, oral uptake may contribute significantly: about 10 % for soils with an average organic matter content of 3 %, and about 50 % for soils with an organic matter content of 20 % (Belfroid et al. 1995). This implies that at maximum, the modelled concentration of organic contaminants exceeds the EPT prediction by a factor of 2. As reported by Jager (2003), this is similar to the differences found in general when comparing values for the bioaccumulation factor (BAF) and the bioconcentration factor (BCF). The BAF and BCF represent one of the most simplified single-compartment models for bioaccumulation, predicting partitioning between exposure medium and biota. Both BCF and BAF are generally calculated as the ratio, at equilibrium, of internal biota concentration to exposure concentration, with BCF being related to accumulation in organisms arising from water only, and BAF to accumulation from water and dietary sources (McGeer et al. 2003). In general, BAF is derived from measurements in the field and BCF is more readily measured under laboratory conditions.

Further support, although not for a soil inhabiting species, is obtained from experiments with *Lumbriculus variegatus*. Sormunen et al. (2008) showed that these sediment-ingesting worms have access to an additional bioavailable fraction of PCBs on top of the pore water pool that was especially evident when pore-water concentrations of PCB 77 approached the solubility limit. Thus, feeding may modify the bioavailable fraction in a way that cannot be explained by simple equilibrium partitioning models.

In laboratory tests, the validity of the EPT has been confirmed for various organisms like earthworms, enchytraeids, and nematodes. Almost the same evidence has been found for collembolans and isopods, while no prediction was possible for snails due to specific uptake mechanisms. Thus, evidence exists that pore water is the main uptake route for the above-mentioned organism taxa, at least for contaminants with a log $K_{ow} < 6$. For contaminants with a log $K_{ow} > 6$ other routes, e.g., via feeding, become more relevant for the same organisms.

16.5 Concluding remarks

Bioavailability is an important concept when considering the ecological risks posed by contaminated sites. Legislators are moving in the direction of incorporating procedures of bioavailability into regulations regarding contaminated sites. In particular concepts of bioavailability are important in higher tier Risk Assessments. Whilst bioavailability, and related terms such as bioaccessibility, can be precisely defined, the impact that soil properties have on soils and the multitude of organisms found at contaminated sites means that a “one size fits all” precise definition of bioavailability only exists on a philosophical level. Any given contaminant will have differing bioavailabilities to different organisms in different soils. With all this potential variability it is not surprising that a range of different methods for measuring and calculating bioavailability exist. The chemical methods which exist for predicting bioavailability are validated for specific conditions, but can not hope to be universally applicable. Similarly, models have been developed which work well for specific conditions, but are inevitably organism-specific. Thus when applying proxies for bioavailability, it is important to bear in mind the conditions for which that proxy was developed. At present it is the case that the best method to determine contaminant concentrations and effects in organisms is to measure them. Chemical extractions are probably currently the better option for predicting longer term bioavailability providing that the drawbacks of extractions are clearly stated. However, in the future calculations will replace chemical extractions as the method of choice for predicting bioavailability. In terms of using bioavailability measures to support contaminated site ecological Risk Assessment and risk management, legislators must identify key organisms, with the support of ecotoxicologists, that they wish to protect, for example earthworms as an example of a major soil invertebrate. Once key species have been identified there is cause to be optimistic that suitable methods for assessing bioavailability either exist or can be developed. A key need for legislators is not additional and new methods for measuring bioavailability, but a series of validation studies using existing methods.

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