Earthworms and Their Use in Eco(toxico)logical Modeling

Willie J.G.M. Peijnenburg and Martina G. Vijver

Abstract A healthy terrestrial food web is essential for the sustainable use of soils. Earthworms are key species within terrestrial food webs and perform a number of essential functionalities like decomposition of organic litter, tillage and aeration of the soil, and enhancement of microbial activity. Chemicals may impact the functions of the soil by directly affecting one or more of these processes or by indirectly reducing the number and activity of soil engineers like earthworms. The scope of this chapter is on the assessment and modeling of the interactions of chemicals with earthworms and the resulting impacts. It is the aim of this contribution to provide a general review of the research that were undertaken to increase our understanding of the underlying processes.

Chemicals may induce a variety of adverse effects on ecosystems. Chemical speciation, bioavailability, bioaccumulation, toxicity, essentiality, and mixture effects are key issues in assessing the hazards of chemicals. Although it is possible to group chemicals with regard to their fate and effects, a plethora of chemical and biological processes affects actually occurring effects. These effects are usually modulated by (varying) environmental conditions. Using the basic processes underlying the uptake characteristics and the adverse effects of organic pollutants and metals on earthworms as an illustration, an overview will be given of the interactions between the chemistry and biology of pollutants, mostly at the interface of biological and environmental matrices. The impact of environmental conditions on uptake and toxicity of chemicals for soil dwelling organisms will explicitly be accounted for. The environmental chemistry of organic compounds and metals, as well as the resulting methods for assessing chemical availability are assumed as tokens and the emphasis is thus on the biological processes that affect the fate and effects of contaminants following interaction of the earthworms with the bioavailable fraction.

Keywords Oligochaetes · Physiology · Pollutants · Uptake · Accumulation modeling · Effect modeling

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1 Earthworms: Relevance, Preferences, and Interactions

1.1 Earthworms and Their Environmental Relevance

Soils are used for a large number of strongly varying purposes, including agriculture, forestry, gardening, and playing fields. A healthy terrestrial food web is essential for the sustainable use of soils for these and other purposes. The soil food web is the set of organisms that work underground to help sustain the essential functions of soil. There are billions of organisms that make up the soil food web. These include bacteria, fungi, protozoa, nematodes, arthropods, and earthworms. Each type of organism plays an important role in keeping the soil healthy. Earthworms take a special place in this respect as not only they eat about every other particle in the soil, but also when they eat they leave behind "castings" which are high in organic matter and plant nutrients, and are a valuable fertilizer. By actively adding earthworms to the soil, soils get in a better condition and their fertility is further improved.

Widely respected ecologists like Darwin and Righi were among the first scientists to recognize the importance of species in general and earthworms in particular. During 40 years of active research on endangered earthworms in tropical areas, Righi published about 100 papers on earthworm taxonomy, physiology, ecology, and biogeography: see for instance Fragoso et al. [1] for a review on the influence of Righi on tropical earthworm taxonomy. It was Charles Darwin [2] who considered earthworms as one of our planet's most important caretakers. "I doubt," he said, "whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creatures." Darwin was the first to describe how earthworms tilled the soil, swallowing and ejecting soil as castings, or worm manure. He estimated that an acre of garden soil could contain over 50,000 earthworms and yield 18 tons of organic castings per year (scientists now figure worms can number over one million per acre). Darwin's naturalist approach and his long-term experience in observing the behavior of different animals helped him distinguish various possible "functions" of earthworms. He briefly alluded to different functional groups of worms:

1. Deep burrowing and shallow burrowing species
2. Large-compact and small-granular casters
3. Litter and soil feeders

These characteristics are among the most important characteristics currently used in various functional classifications of the soil fauna and earthworms. The most widely used recent functional classifications are those of Bouché [3], Lee [4,5], and Lavelle [6]. These classes generally include three main groups (endogeic, anecic, and epigeic earthworms) that are defined on the predominant habitat of a species. Although these three subgroups have been proposed, some earthworm species do not seem to fit into any particular category or, rather, fit in between proposed categories (e.g., epi-endogeic and endo-anecic). Other earthworm's classifications include those of Lavelle [7] and Lavelle et al. [8], into ecosystem engineers and litter transformers, and of Blanchart et al. [9] into compacting and decompacting species. These schemes attempt to integrate knowledge on feeding habits and functional significance of earthworms in the soil. Darwin's contributions in this area deal primarily with the influence of earthworms on soil physical processes, although he also touches upon the selection and processing of particular leaf litters.

Earthworms move through the soil creating tunnels, and thus areas that can be filled by air and water. Fields that are "tilled" by earthworm tunneling can absorb water at a rate 4–10 times that of fields without earthworm tunnels. This reduces water runoff, restores groundwater, and helps store more water for dry spells. Burrowing also helps nutrients enter the subsoil at a faster rate and opens up pathways for roots to grow into. During droughts the tunnels allow plant roots to penetrate deeper, to reach the water they need to thrive. Earthworms help to keep the soil healthy by moving organic matter from the surface into the soil. By speeding up the breakdown of plant material, earthworms also speed up the rate at which nutrients are recycled back to the plants. Earthworms are thus an essential part of the soil food functioning. Without them, all the organic matter would build up on the soil surface.

The capability of changing the soil structure by preferential feeding on organic material by earthworms was the basis for vermiculturing of organic-matter-rich waste materials. Together with bacteria, earthworms are the major catalyst for decomposition in a healthy vermicomposting system, although other soil species also play a contributing role: these include insects, other worms, and fungi/molds. Vermicompost is a nutrient-rich, natural fertilizer and soil conditioner. The earthworm species (or composting worms) most often used are Red Wigglers (*Eisenia fetida*) or Red Earthworms (*Lumbricus rubellus*). These species are commonly found in organic-rich soils throughout Europe and North America and especially prefer the special conditions in rotting vegetation, compost, and manure piles. To benefit from their active stimulation of soil processes, earthworms nowadays are commercially available. Mail-order suppliers or angling (fishing) shops keep earthworms in bred and composting worms are sold for vermicomposting practices and sold as bait. Small-scale vermicomposting is well-suited to turn kitchen waste into high-quality soil, where space is limited. Thanks to the pioneering work of Dr. Clive Edwards [10] in the area of vermicomposting that this technique is now widely applicable to generate soil structure and soil quality enhancing compost. Vermicomposts can also be used in pollutant bioremediation for organic contaminants and heavy metals as the microbial degradation of the organic pollutants is accelerated dramatically and the heavy metals become irreversibly bound into the humic materials that are formed, so that they are not available to plants. Dr. Zharikov's research [11] into methods of soil purification revealed that earthworms are also capable of enhancing the cleaning of the contaminated soils by stimulating the growth of microorganisms that breakdown the contaminants.

As there is no doubt that the earthworm can be of major benefit to a healthy soil ecosystem, it is important to understand the key role of earthworms in many biogeochemical cycles and in soil development as related to the impacts of land uses. This is particularly true in relation to restoration of damaged ecosystems and to preventive maintenance to avoid damage.

1.2 Earthworms and Their Preferences

Environmental factors that provide the most dominant impacts on earthworm populations are moisture, temperature, and pH, on top of food resource quantity and quality. Soil moisture affects earthworm abundance, activity patterns, and thus geographic distribution. Earthworms tend to dig deeper or even tend to go into a diapauses during periods of prolonged drought. During rainy periods earthworm species tend to surface to escape from drowning. Soil temperature influences seasonal activity, limiting earthworms during warm and cold periods. Soil pH often is cited as a limiting factor on earthworm distributions. For instance, the best studied group (European Lumbricidae) generally does not inhabit soils with pH below 4.0. Other taxa tolerate lower pH values, including some Pacific coast native species (pH 3.1–5.0; [12]), thereby indicating that soil acidity might be less limiting for certain earthworm species than for others.

Soil climate determines the periods of earthworm activity. Within a habitat type, variations in soil climatic factors occur (because of slope, aspect, soil particle size distribution, and drainage characteristics) that result in variation in earthworm activity period and earthworm abundance. A forested habitat probably has a relatively buffered soil climate compared to the more exposed grasslands and agricultural land. Grassland temperature and moisture regimes are probably more extreme and could accentuate the effects of slope, soil properties, and other site characteristics. An agricultural cycle having long periods of bare ground could further intensify the impact of weather on earthworms.

The quantity and quality of food influences earthworm abundance. Food sources are all types of organic matter. Organic matter may render the soil strongly acidic, could be rich in digestibility reducing compounds, or could have a high carbon-to-nitrogen ratio. These qualities tend to reduce earthworm populations. Lack of organic matter is generally a significant limiting factor for earthworms. The fact that most agricultural soils are depleted of organic matter, likely accounts for lower abundance of earthworms in agricultural land or recently abandoned cropland.

1.3 Earthworms and Essential Elements

Natural and man-made chemical substances may severely interfere the natural fluctuations in earthworm populations in specific habitats. Availability or the lack of essential nutrients on the one hand shapes natural ecosystems, whereas on the other hand excess amounts of bioavailable nutrients and micropollutants reduce the natural abundance of species and affect the natural ecosystem functioning. This observation was the basis for the concept of optimal concentration of essential elements (OCEE). This concept was among others proposed to account for metal-specific aspects of essentiality and homeostasis.

A first attempt to account for the metal-specific aspects of essentiality and homeostasis was achieved by the optimal concentration of essential elements-no risk area

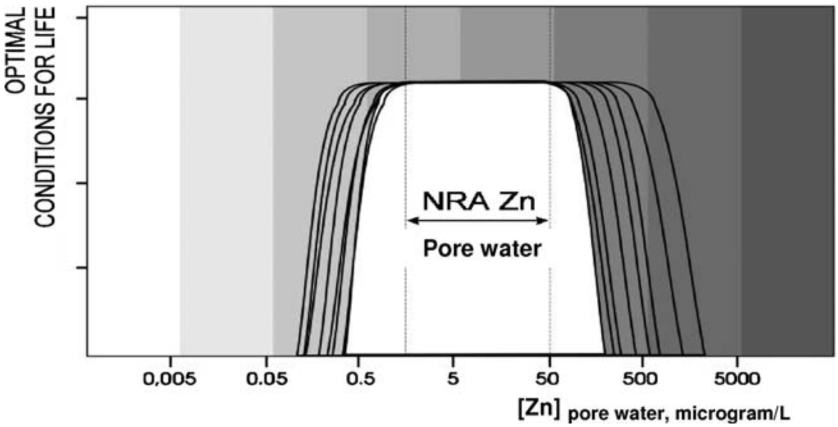

Fig. 1 Hypothetical presentation of the OCEE curves of all individual organisms in a given environment. The *inner envelope* of these *curves* represents the no risk area (NRA) for that given environment in which all organisms are protected from both toxicity and deficiency (adopted from [13])

concept (OCEE-NRA) based upon the assumption that all OCEEs for all individual organisms belonging to a certain habitat type (ecoregions) are centered on the natural essential element (metal) background concentration typical for that habitat. Figure 1 gives a schematic representation of the OCEE-NRA concept: at low nutrient levels, adverse effects are observable related to lack of nutrients; increased levels of essential elements induce toxicity. Furthermore, research results indicated that the sensitivity of the toxicity response of an organism to an essential metal is a function of the essential element concentration in which it was cultivated. Acclimatization explains the decrease in sensitivity at higher background concentrations in the culture medium. The recognition and demonstration that organisms do belong to different OCEE-NRAs underscore the relevance of this concept and have lead to the fundamentals of the metallo-region concept. The major technical difficulties for the integration of the OCEE-NRA concept into regulatory frameworks for environmental risk assessment are the spatial and temporal variability in natural background levels as well as the variability in physicochemical conditions influencing metal bioavailability and toxicity.

Apart from agriculturally oriented studies on optimal levels of essential elements, relatively little quantitative information is available on deficiency levels of most nutrients for earthworms.

1.4 Earthworms and Pollutants

As earthworms ingest large amounts of soil or specific fractions of soil (i.e., organic matter), they are continuously exposed to contaminants through their alimentary surfaces [14]. Moreover, several studies have shown that earthworm skin is a significant route of contaminant uptake as well [15–17]. Toxic substances and excess nutrients are accumulated and subsequently exert adverse effects by a variety of interactive modes of action, both with regard to the mechanisms of uptake and the mechanisms of toxicity. Whereas interactions with organic micropollutants are strongly modulated by organic carbon pools in the soil and in the fat tissue, uptake and effects of metals are modulated by interactions between the various soil and pore water constituents. Soil constituents serve in this sense as capacity controlling factors modulating the bioavailable pool whereas pore water parameters like pH, dissolved organic carbon, and macronutrients like Ca/Mg/Na serve as intensity-controlling factors as they modulate actually occurring effect.

It is the aim of this chapter to exemplify the use of earthworm as a key species in soil toxicity testing. Based on ecological considerations, the objective of this contribution is to give a general overview on the accumulation of chemicals by earthworms and the toxic effects exerted due to interactions of these animals with micropollutants. Providing an in-depth discussion of the basic phenomena underlying accumulation and adverse effects is not the primary aim. Instead, a short overview will be provided of the approaches used in testing assessing and modeling bioaccumulation and ecotoxicity.

2 Earthworms as Model Organism

2.1 Bioindicators for Chemical Stress

Bioindicators are used as representatives of parts of ecosystems or of one or more functions [18]. The basic consideration of the use of biomarkers is that living organisms provide the best reflection of the actual state of ecosystems and of changes therein. These measures can be done on either structure or functioning of ecosystems. For both type of measurements, oligochaete are generally regarded as highly suitable bioindicators. Their importance in the structure of ecosystems can be explained because they are an ecologically dominant invertebrate group. Moreover, earthworms occur in many different soils from temperate to tropical areas. Also their importance in food chains, with earthworms being a food source for many organisms such as birds and mammals, has implicated that many ecological studies have focused on studying the ecology and ecotoxicology of the earthworm. Thereupon, most oligochaetic species are easy to handle and to culture under laboratory settings [19]. Respecting this, earthworm species are often used as test organisms to determine the effect and accumulation of chemicals from soil [19–23]. Due to their behavior and morphology, earthworms are in close contact with the aqueous and solid phases of the soil. From experimental studies it could be concluded that for both inorganic [17] and organic [16] contaminants earthworms are exposed to pollutants in the soil mainly via the pore water. Most oligochaetic species are not extremely sensitive to low levels of chemicals [24, 25]. The chemical composition of their body is fairly constant, which facilitates the understanding of the mechanisms of toxicity. Their internal organization is not highly complex, and possesses strongly differentiated organs. Moreover, it is described very well in literature [18].

2.2 *Ecophysiology of Earthworms*

Oligochaete worms have a thick mucus layer that surrounds the epidermis [26], through which respiration and the excretion of waste products occur. This mechanism makes the earthworms sensitive to water loss. The digestive interior of oligochaete species is well investigated [27]. There is evidence that the uptake of food via the gut is not a heterogeneous process during the gut passage. During ingestion mucus is mixed with the food. In the first part of the digestive system of an oligochaete, calciferous glands actively release Ca^{2+} in the gut contents. The crop is used for storage of the gut content, before mechanical grinding and digestion in the gizzard. The gizzard opens up into the intestine, which forms the largest part of the alimentary canal. Gut conditions in the final part of the digestive system (the intestine) are actively regulated by excretion of $NH_4{}^+$. A typhlosole (see Fig. 2), a dorsal infolding of the gut epithelium effectively increasing the internal surface, is present along the anterior and mid intestine, thereby also increasing the secretory and absorptive surface areas. The pH along the entire digestive tract is quite constant between 6 and 7, and the digestion is driven by enzymes [28]. The gut pH is often higher than the bulk soil pH, especially in earthworms inhabiting acid soils.

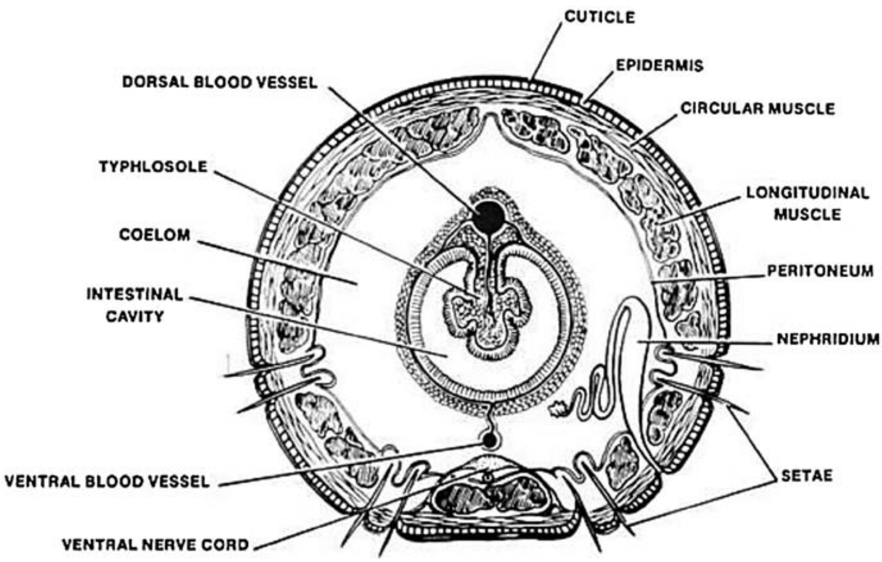

Fig. 2 Cross section of the posterior body cavity of earthworms

The largest part of the body burden is bound in the chloragogenous tissue [29] located around the digestive tract (see Fig. 3). The cells of this tissue (chloragocytes) contain many chloragosomes, including calcium granula (type A) and sulfur-rich granules (type B). All granulum types are likely to play a role in the homeostasis of essential elements but also for detoxification of chemicals that entered the body. The resorption capacity of the digestive tract is most efficient in the posterior alimentary canal.

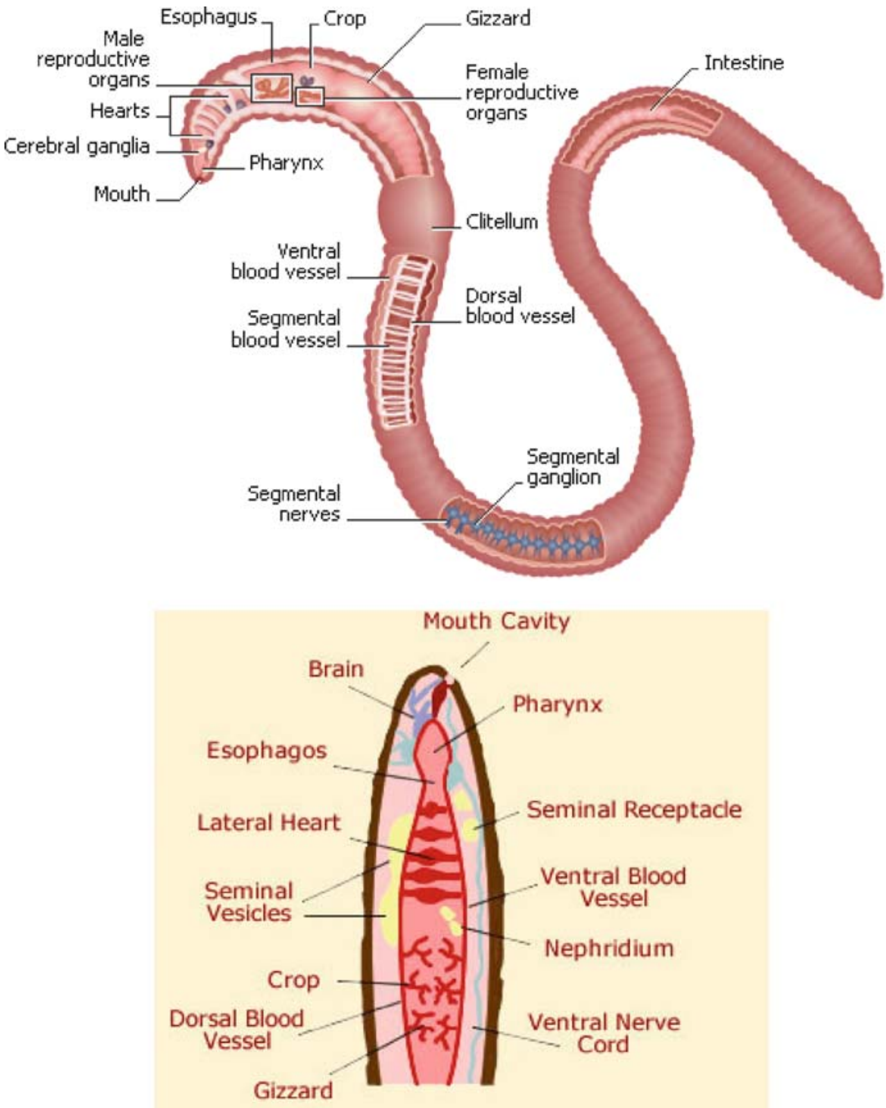

Fig. 3 Schematic of the anatomy of earthworms

3 Accumulation

Biological uptake of most synthetic (hydrophobic) organic contaminants occurs by simple passive diffusion across a cell membrane. Membrane carriers are not involved and the biological effect of organic contaminants is often (but surely not always) characterized by narcosis, implying that the extent of adverse effect of organic contaminants is proportional to the value of the octanol–water partition coefficient. In contrast, as metals generally exist in strongly hydrated species, they are unable to traverse biological membranes by simple diffusion. In general, the interaction of metals with organisms is somehow related to a liquid phase, according to the principles of the Free Ion Activity Model (FIAM) [30]. The mechanisms can be described as follows:

1. Advection or diffusion of the metal from the bulk solution to the biological surface
2. Diffusion of the metal through the outer "protective layer"
3. Sorption/surface complexation of the metal at passive binding sites within the protective layer, or at sites on the outer surface of the plasma membrane
4. Uptake of the metal (transport across the plasma membrane)

Membrane transport occurs by facilitated transport, usually passive (i.e. not against a concentration gradient), and necessarily involves either membrane carriers or channels. The chemical binds to the carrier protein and is carried through the membrane by a process that requires no cellular energy. There is some specificity to the carrier protein binding, and so the process is applicable only for selected chemicals. Transport of essential metals is for instance facilitated by carriers or pores specific to the element, although metals are also transported on carriers designed for elements of similar physicochemical characteristics.

3.1 BCFs and BAFs

The terms "Biota Concentration Factors" (BCFs) and "Bioaccumulation Factors" (BAFs) can be defined as similar words and are both used to quantify to which extent chemicals are transported from the exposure medium into organisms. By definition, the higher the BCF value, the more chemicals are taken up and the higher the potential risk regarding adverse effects on the organism itself and at higher trophic levels. Most studies report relationships between internal and external concentrations (BCF) where steady state is assumed [31, 32]. An extended overview of BCFs in earthworms for organic chemicals is given by Jager [33], whereas Sample et al. [34] developed and tested uptake factors and regression models for uptake factors for metals in earthworms. The bioconcentration factors found for PCBs were between 7,200 (low mol. PCB) and 126,000 (high mol. PCB). BCFs for chlorobenzenes in earthworms ranged from 12 to 4,000. Pesticides display widely varying BCF values: ranging from less than 1 (for instance Aldicarb: 0.7) to over 5,000

(Lindane). In general, BCFs increase with hydrophobic properties of organic chemicals albeit that especially biotransformation may lower apparent BCF values. BCF values are known to be species and soil dependent. As an example Kelsey et al. [35] determined the BAF in four field-weathered soils for an epigeic species *Eisenia fetida*, an anecic species *Lumbricus terrestris*, and an endogeic species *Aporrectodea caliginosa*. The epigeic species had BCFs that were approximately tenfold higher than those for the other species. With regard to contaminant-residence time, the BAF for *E. fetida* was lower in weathered soils relative to that in freshly amended soils, but age of p,p'-DDE did not significantly alter the BAF for *A. caliginosa* [35]. The biota-soil accumulation factors (BSAFs) observed for individual PAHs in field-collected earthworms (*A. caliginosa*) were up to 50-fold lower than the BSAFs predicted using equilibrium-partitioning theory [36].

An overview of BCFs in earthworms for inorganic chemicals is given by Janssen et al. [31]. Bioaccumulation factors varied between metals. The BCF of As ranged from 0.1 to 3, Cd ranged from 1 to 203, Cr ranged from 0.03 to 0.5, Cu ranged from 0.2 to 8, Ni ranged from 0.07 to 0.6, Pb ranged 0.005 to 1.3, and Zn ranged from 0.1 to 18. In general, BCFs for metals decrease with higher exposure concentrations [37]. The same inverse relationship was found in aquatic systems between bioaccumulation factors and, trophic transfer factors and exposure concentrations [38].

A general finding is that BCFs decline with increasing pollutant concentration in soil. The uptake and adverse effects of chemicals to earthworms can be modified dramatically by soil physical/chemical characteristics, yet expressing exposure as total chemical concentrations does not address this problem. Bioavailability can be incorporated into ecological risk assessment during risk analysis, primarily in the estimation of exposure. However, in order to be used in the site-specific ecological risk assessment of chemicals, effects concentrations must be developed from laboratory toxicity tests based on exposure estimates utilizing techniques that measure the bioavailable fraction of chemicals in soil, not total chemical concentrations [39]. The final and most difficult task in any assessment is to relate body residues to levels known, or suspected, to be associated with adverse biological responses. To address this, physiological knowledge on chemical distribution over the body should be combined with the knowledge on accumulation. Paracelsus stated in 1564 that "What is there that is not poison? Solely the dose determines that a thing is not a poison" [40]. We should add to this statement that also the biological significance of accumulation is of importance [41].

3.2 More Compartments

Earthworms are able to accumulate organics to a great extent. The ability to deal with high levels of accumulated organics can be ascribed to the manifestation of organics to bind to fatty tissues [42–45]. Bioaccumulation of organics also can be ascribed using multiple compartments, although two compartments are usually sufficient.

Earthworms are also able to accumulate metals to a great extent. The ability to deal with high levels of accumulated metals can be ascribed to the slow turnover of the tissues in which metals accumulate. Morgan et al. [46] found distinct differences in the distribution of various metals throughout the earthworms' body, whereby the sequestration on chlorogocytes played a dominant role, resulting in different patterns of tissue accumulation [47] and different tolerances [48]. Metals such as Cd and Cu are predominantly bound to metal-binding proteins [49] and with these proteins, the metal moves through the body to organs and tissues in which it is deposited in inorganic forms. Cd was retrieved in high amounts from the nephridia and to a lower extent from the body wall of earthworms [50]. Pb is found in waste nodules located in the coelomic fluid [51]. The granulas contain many essential and nonessential chemicals. For instance Cd preferentially binds to sulfur-rich granules instead of oxygen-rich granules, and hence is found in the type B granules, also called cadmosomes.

A pragmatic method to describe and quantify the internal sequestration of metals is found in Vijver et al. [41, 52].

3.3 How to Perform Experiments for Optimal Results

Dynamic biological measures of bioavailability – thus the rate at which organisms take up contaminants from the environment – are the best and according to the latest scientific state-of- the-art on how to derive indicators of bioavailability [53]. Actual uptake and elimination fluxes are very difficult to measure. A pragmatic solution to overcome this problem is to measure body burdens as a function of time in an organism exposed to the medium tested. Parameter estimation is done by curve fitting the accumulation data. In the most simple case, the exposure concentration is constant, and as soon as the organism is exposed, internal concentrations are increasing [53, 54].

By this way an accumulation curve can be fitted according to the following general equation (most simple form):

$$Q = C_0 + (a/k)\mathrm{e}^{-kt}. \qquad (1)$$

In this equation, Q is the amount of chemical accumulated at equilibrium or at steady-state conditions; C_0 the initial body burden; a the uptake flux; k the elimination rate constant; and t is the time.

Exposure of organisms under fluctuating external conditions, as is the common case in reality, can also be modeled. This is done by taking into account the kinetics of the bioavailable fraction of the chemical for a specific organism. For instance, in the case of biotransformation of the contaminant being taken up by the earthworm or in case of cocoon production, (1) transforms into [55]:

$$Q = (a_1 C_0)/(k_2 - k_0) \times (\mathrm{e}^{-(k_0 t)} - \mathrm{e}^{-(k_2 t)}). \qquad (2)$$

In (2) a_1 is the uptake rate constant, k_0 the rate constant for degradation of the chemical in the medium, and k_2 is the elimination rate constant.

The common experimental set up in order to measure accumulation is often to expose relatively large numbers of earthworms, divided over a number of jars, to a soil. At different time intervals, earthworms are sacrificed and measured for their body burden. It is preferred to measure more frequently over time instead of more replicates at the same exposure time. Especially within the initial stage of the exposure and thus during initial uptake of the chemicals by the earthworms, many samples with a small time interval should be taken. The sampling strategy should be according a log-scale, with fewer measurements at the end than at the beginning in order to accurately capture initial uptake kinetics.

Accumulation is the net effect of uptake and the ability of the organism to eliminate a chemical once it has entered the body. Estimation of uptake in the presence of simultaneous elimination is improved significantly if the uptake is followed by an elimination phase without uptake, because this will yield a better estimate of the elimination rate constant, and consequently also a better estimate of the uptake parameter. Therefore, experiments usually involve an uptake phase and an elimination phase, simply by transferring the organism to a clean medium after a certain period. This situation can easily be performed when artificially spiked soils are used for the accumulation testing. However, when using natural contaminated field soils, in most cases it is difficult to find an uncontaminated field soil with similar characteristics as the contaminated field soil. Subsequently an appropriate elimination phase is difficult to test. An alternative technique allowing for the quantification of uptake and turnover kinetics in biota is isotopic labeling. The main advantage of this technique is that it overcomes the problem of selecting an unpolluted reference site and that it is nondestructive for the exposed organisms. Hence the biological variation of accumulation can be studied for single species. Moreover, it overcomes detection limitations within the body burden of earthworms, and allows insight into essential metal uptake even in the presence of highly regulated body concentrations.

3.4 Alternative Measures of Bioaccumulation

Alternatives to assess bioaccumulation without the direct measurement of internal concentrations in organisms or effects on earthworms are the use of mimic techniques (see for an overview of these techniques [56]). The use of passive sampling devices (PSDs) is an example of these kinds of mimic techniques which are potentially direct chemical indicators for assessing the bioavailability of chemicals. PSDs are constructed in several forms but often consist of lipophilic material within a semipermeable membrane, mimicking biological membranes. Exposure of biota to chemicals is assessed this way, and the techniques account for aging and mobility of chemicals in the matrix [57]. The results of Awata et al. [57] showed that concentrations as determined in the PSD were in good agreement with accumulation data in the earthworms as measured after exposure in contaminated soils. Uptake rates and maximum concentrations in PSDs were observed to positively correlate with uptake rates and maximum concentrations in earthworms for both of the soil types studied (sandy loam and silt loam). These results indicate that PSDs may be used as a surrogate for earthworms and provide a chemical technique for assessing the availability of aged chemical residues in soil. Similar findings were reported by Van der Wal et al. [58], who concluded that measuring concentrations of hydrophobic chemicals using polydimethylsiloxane solid phase microextraction (which is a kind of PSD) is a simple and reliable tool to estimate bioaccumulation in biota exposed to soil. The opposite was been concluded by Bergknut et al. [59], who showed a distinct difference between evaluated PSD techniques and bioaccumulation in earthworms. Generally, there were larger proportions of carcinogenic PAHs (4–6 fused rings) in the earthworms compared to the concentrations as found with the mimic techniques. In cases that the exposure media (e.g. soils) were heterogeneous, the PSDs had no predictive capacity.

From the information provided above it may be concluded that it will be difficult to develop a single and universally applicable chemical method that is capable of mimicking biological uptake, and thus estimating the bioavailability of chemicals. In some cases, a strong numerical relationship of bioaccumulation of chemicals with biomimetic techniques is reported; in other cases no such correlation is found. This general finding is related to the fact that accumulation by living organisms like earthworms is more dynamic than can be simulated by chemical means. Only in those cases where chemical interactions overrule organism-specific ecological impacts (like feeding behavior, regulation of body concentrations by active uptake and/or elimination, and biotransformation), a strong correlation between uptake and biometry may be found.

4 Toxicity

4.1 Toxicity Testing

4.1.1 General

Earthworms are frequently used as part of batteries of indicator species to test the effects of pollutants on ecosystems. A wide array of substrates (including artificial substrates like OECD soils – a mixture of sand, kaolinite clay, peat, and $CaCO_3$ to adjust pH), test designs, and endpoints are exploited and guidelines have been designed to standardize the assessment of adverse effects on earthworms. Apart from laboratory testing, terrestrial model ecosystems (TMEs; [60]), field enclosures, and field testing [61] are employed to increasingly mimic actually occurring effects in the field. Testing data are employed to derive models capable of predicting effects at various levels of integration, varying from simple linear regression equation based on soil or pore water characteristics up till advanced concept taking account of the specific interactions of chemicals with earthworms. The species most commonly tested in a laboratory setting are the compost worms *Eisenia andrei* and *E. fetida* as more field-relevant species like *L. rubellus* and *A. caliginosa* are difficult to rear.

A general distinction that is often made when performing earthworm testing is between acute (i.e., short exposure time) and chronic testing. For some chemicals, like for copper, this difference is often artificial as the acute-to-chronic-effect ratios are close to 1. As a rule of thumb, exposure times up till 14 days are considered to represent acute testing. Exposure times in field testing may exceed various seasons and last even for several generations of animals.

4.1.2 Biomarkers of Exposure and Toxicity

Apart from the commonly studied endpoints discussed below, the use of biological responses other than reproduction, growth, and mortality to estimate either exposure or resultant effects has received increased attention [62–65]. Biomarkers are typically biochemical changes that are induced following exposure to a contaminant. Biological responses are possible at the molecular, subcellular, and cellular level. A major reason for the interest in biomarkers is the limitation of the classical approach in ecotoxicology in which the amount of chemical present in an animal or plant is related to adverse effects on the classical endpoints. Bioavailability and toxicity differ, however, in laboratory tests compared to those observed in the field, and multiple toxicants are typically present simultaneously under field conditions. Also, only a few of the conventional endpoints can be assessed in in situ experiments. Biomarkers have the potential to circumvent the limitations mentioned as they respond only to the biologically available fraction of a pollutant, independent of mitigating effects of soil characteristics.

In order for a biomarker, or a battery of biomarkers, to be useful in effective assessment of chemicals to earthworms, a number of key features apply [66]:

1. The marker must be identified in the species of interest.
2. Knowledge is required on the range of toxic compounds that elicit a biomarkers response.
3. To estimate the magnitude of the chemical stress, a dose–response relationship between the biomarker response and the bioavailable concentration of the chemical is desirable.
4. Possibilities to link biomarkers responses to higher levels of biological hierarchy are desirable. For a biomarker to be of more use than an indicator of exposure, a correlation between the observed responses and deleterious effects at the individual or populations/community level should be established. A subcellular biomarker may, for example, act as an early warning of effects at population level.
5. For a biomarker to be useful in the field, any response should have a low inherent variability with a known (preferably: a low) dependence on physiological and physiochemical conditions. Among others, the induction time and the persistence

of a biomarker response should be known in order to estimate the likelihood and significance of detecting a response in field samples.

Up till now, various biomarkers have been developed and have been applied with varying amounts of success. The most important categories include:

1. *DNA alterations induced by reactions of contaminants with genotoxic properties*. The most common reactions are adduct formation (covalent binding of the contaminant or its metabolite to DNA), strand breakage, base exchange, and increased unscheduled DNA synthesis. Limited information is available on the environmental significance of DNA alteration at higher levels, the natural variability, and the persistence in time of DNA adducts.
2. *Induction of metal-binding proteins*. Heavy metals entering earthworms at concentrations exceeding the metabolically required metal pool may be bound and detoxified by binding to metallothionein and other metal-binding proteins. Although the role of metallothionein and other metal-binding proteins is not fully understood, these proteins are thought to be involved in the intracellular regulation of essential and nonessential metal levels in tissues. Apart from limited attempts on Cd, no studies have been undertaken to establish dose–response relationships for induction of metal proteins. Links to higher levels and natural variability also require more attention before this type of biomarker is suited for quantifying exposure and/or metal toxicity.
3. *Inhibition of enzymes*. Inhibition of cholinesterases is the most common studied biomarker of exposure of earthworms to carbamate and organophosphorus pesticides. Cholinesterases are used for the transmission of nerve signals and contaminants can cause a depression in cholinesterases activity. Depression of cholinesterases activity may well depend on the metabolic compounds rather than the parent compound and just a few studies have reported on natural variability of the cholinesterases activity in earthworms. On the other hand, inhibition of cholinesterases activity in earthworms was shown to be dose dependent in both coelomic fluid and in nerve tissue [67].
4. *Lysosomal membrane integrity*. Lysosomes are a morphological heterogeneous group of membrane-bound subcellular organelles that catabolize organelles and macromolecules. A change in lysosomal membrane stability is thought to be a general measure of stress. At the subcellular level, the lysosomal system has been identified as a particular target for toxic effects of contaminants. The neutral red retention time (NRR) is used to investigate lysosomal stability and for just a few chemicals a dose–response relationship was obtained thus far. Few studies have been concerned with the natural variability of the lysosomal membrane stability and with the establishing links with higher levels like reproductive output and mortality [68]. Aquatic studies have indicated that lysosomal response can also be induced by nonchemical stressors such as osmotic shock and dietary depletion.
5. *Immunological responses building upon the fact that the immune system is the main defense of an earthworm against invasion of foreign material and biological agents*. A wide range of chemicals has been shown to be capable of affecting

the immune system, which in severe cases may quickly result in morbidity and death. Sublethal changes in special compartments of the immune system occur first and provide early indications of toxic effects. The immunological system is known for its flexibility and adaptability and it has been observed for earthworms that the immunological depression returns to normal levels quickly after removal of the earthworms from the source of exposure. Relatively few studies have dealt with the impact of chemicals on the immune system of earthworms, and dose–response relationships as well as linkage to higher levels of effects are rarely available.

Although some biomarkers provide a forewarning of adverse effects resulting from exposure of earthworms to contaminants, more work is needed to understand the limitations of the use of biomarkers. Thus, for biomarkers to be of use as early warning tools, more effort is needed in linking biomarker responses at the subcellular and cellular levels with effects at population level under natural conditions.

4.2 The Kinds of Effects Commonly Measured

4.2.1 Laboratory Testing

Laboratory tests play an important role in earthworm testing. The endpoints mortality, reproduction, and change of body weight are standardized and well described in widely accepted guidelines for testing of chemicals [69–71]. Other endpoints like behavior, morphological changes, and physiological changes are reported occasionally, but they are not evaluated in a standardized way. All tests include a validity criterion for effects in the control, like mortality not to exceed 10%.

Mortality is usually expressed by means of LC_{50}, the dose at which 50% of the animals die. Although extrapolation of laboratory-derived test results to the field is not straightforward, this endpoint is highly relevant for the field. The performance of the standardized test is usually checked by occasional testing of a reference compound like chloracetamide in case of testing of organic pesticides.

Reproduction too is of high relevance for the field. Various endpoints may be considered, including number of cocoons, hatchability of cocoons, number of juveniles, weight of juveniles, and time needed for the juveniles to reach sexual maturation. Juvenile numbers in the control and the coefficient of variation following duplication are important validity criteria. The best way to do reproduction testing is by establishing a full dose–response relationship and subsequently evaluating the no observed effect concentration (NOEC) or the effect concentration (EC_x) at which a specific percentage of reduction of reproduction is deducible.

Body weight change is less clearly defined in testing protocols and may be interpreted in different ways. Ring tests have shown that reproducibility of body weight change is sufficient, but an inverse relationship between reproduction and body weight change was found: animals that rapidly gain weight do not reproduce at the same time and the mechanisms influencing this process are not yet fully understood.

Care should be taken in the evaluation of body weight changes when mortality occurs, as mean body weight changes may be obscured by differences in sensitivity among animals of different size and weight. This problem is less relevant when body weight change is expressed as the change in overall biomass, thus including the mortality endpoint.

Independent of the endpoint and the test duration, behavior of the earthworms is a factor complicating the interpretation of the test results. Prolonged burrowing time, prolonged crawling on the soil surface, flaccidity, hardened test animals, and color changes either may directly affect the testing results or may be an indicative of more delicate effects. A test approach that is recently getting increased attention deals with the ability of earthworms to avoid contaminated soil. This ability can act as an indicator of toxic potential in a particular soil [72] and has the potential to be used as an early screening tool in site-specific risk assessment. Avoidance tests are becoming more common in soil ecotoxicology because they are ecologically relevant and have a shorter duration time compared with standardized soil toxicity tests. Soil properties like quantity and quality of soil organic matter, texture, and soil pH can, however, modify the avoidance response, and obviously the impact of soil properties needs to be properly considered when interpreting results of avoidance tests with earthworms.

4.2.2 Terrestrial Model Ecosystems and Field Enclosures

To facilitate extrapolation of laboratory-derived testing results toward the field, TMEs and field enclosures are used to more realistically simulate field conditions [61]. Experiments with model ecosystems offer several advantages compared to field studies and simple laboratory setups. Though limited in size they bear complex biotic and abiotic interactions. The parameters under investigation can be easily modulated, environmental conditions can be controlled, and in contrast to field tests it is possible to study effects of chemicals while avoiding uncontrolled distribution of residues and metabolites within the biosphere. Despite the complexity of TMEs and field enclosures, they can be sufficiently replicated in order to establish an appropriate statistical plot design. Model ecosystems described in the literature differ in many features. This concerns size, soil structure (intact soil core vs. homogeneous filling), organisms (natural community vs. selected taxa), and the exposure site (field vs. laboratory). Thus model systems differ notably in their similarity to field conditions.

The extrapolation from model ecosystem experiments to the field situation is to be more feasible than from laboratory experiments. Experiments measuring microbial activity and availability of macronutrients showed for instance that field enclosures (exposition in the field) are more reliable in resembling the field situation than indoor TMEs (exposition in the laboratory) [73]. Nonetheless, a noncritical transfer of results from model systems to the field is not acceptable and a sound validation with appropriate field studies is recommended [74]. Different experiments with various types of model systems have been conducted and published [75–77]. The objectives of most studies were (1) to analyze the fate of chemicals, (2) to study their direct effects on organisms, (3) to validate mathematical models, and (4) to measure secondary, indirect effects on the ecosystem [78].

Recently TMEs were discussed for regulatory purposes in the environmental risk assessment of industrial chemicals, biocides, and plant protection products within the European Union [79]. Annex IV of the EU-Directive 91/414/EC [80] concerning the placing of plant protection products on the market lists the conditions (thresholds) which demand a scientific verification of laboratory effect studies with soil organisms under field conditions. Referring to Annex II, Sect. 8.4, the authors [79] conclude that TMEs are considered to be an important tool for risk assessment if they resemble conditions in the field. Thus it is apparent that a yet poorly considered objective of TME studies should concern the comparability of TME and field results.

A wide array of endpoints is potentially assessed in TMEs and field enclosures. This includes the endpoints common in laboratory testing as well as feeding activity, burrowing behavior, and avoidance testing.

4.2.3 Field Testing

Conditions in the field are highly variable and may change drastically over episodes of less than 1 day (like the day/night cycle, deposition of rain and/or snow, strongly increased temperatures in the top layer during periods of sunshine, as well as longer lasting episodes of flooding and drought). Adverse effects on earthworms are typically assessed at the species and community level in terms of abundance and population densities, and maturity. Often, biomarkers are applied to identify previous exposure and body burdens are used as indicators of effective exposure. No standardized assessment methods of field effects are available, let alone validated models to extrapolate across soils.

4.3 Factors Affecting Toxicity Test Results

Standardized toxicity testing is conducted under fixed biotic and environmental conditions that allow comparison of results among testing laboratories and facilitate interpretation of the findings. However, increased standardization inherently hinders extrapolation of test results toward the field. To improve understanding of specific differences between laboratory testing and field effects, the factors affecting differences in effective exposure and actually occurring effects in the laboratory settings and in the field need intense investigation.

An obvious factor that is of relevance in comparing test results is the test species used vs. species common in the field. Compost worms are commonly used in laboratory testing, probably due to the relative ease of culturing compost worms by means of organic rich material like dung. As noted before, typical field worms like *L. rubellus*, *L. terrestris*, *A. caliginosa*, and *Aporrectodea rosea* do not reproduce easily. This raises the question of typical differences in sensitivity toward chemical across compost worms and typical soil worms. Spurgeon and Hopkins [21] observed that although *E. fetida* was less sensitive to zinc than *L. rubellus* and *A. rosea*, the difference in toxicity was no more than a factor of 2 and was within-test variability. Heimbach [81] on the other hand observed larger differences in earthworm sensitivity to earthworms, up to a factor of 10 between *E. fetida* and *L. terrestris*.

Field populations of earthworms typically consist of a mixture of adults, subadults (nonclitellate worms), juveniles, newly hatched animals, and cocoons. Particularly severe effects of contaminants on any life stage could have severe effects on populations. On the other hand, laboratory testing is typically carried out with adult worms only. Typically, juveniles are more sensitive to toxicants than adult worms; Spurgeon and Weeks [82] showed for instance a difference of a factor of 1.9 between toxicity of zinc to juvenile and adult worms.

Exposure time is an important factor in extrapolating toxicity test results. This is especially true for chemicals (most notably metals) that display slow uptake and elimination kinetics. Typical maximum exposure times in laboratory testing of about 28 days are often too low to reach equilibration of metal levels in the organisms. This is especially true for nonessential metals as internal concentrations of essential elements are usually regulated within well-defined limits. The aspect of test duration therefore requires specific attention in extrapolating test results.

Weather conditions are another factor to consider, albeit that data on the effect of temperature and humidity on earthworm sensitivity are scarce. The most common earthworm species in the field are typically least sensitive to contaminants at temperature conditions in between 10 and 15°C.

Soil properties and pretreatment conditions are probably the most dominant factors impacting the sensitivity of earthworms. In case of metals, soil pH is a dominant factor in this respect. In general, a decrease of pH will increase metal levels in the pore water and hence toxicity, albeit that hydrogen ions are protective of metal toxicity. Soil sorption sites like organic matter and clay strongly modulate toxicity. Criel et al. [83] studied for instance the effect of soil characteristics on the toxicity of copper to terrestrial invertebrates, and performed chronic toxicity tests with *E. fetida* in 19 European field soils. Toxicity values varied largely among soils with 28d EC_{50} (concentrations causing 50% effect) ranging from 72.0 to 781 mg Cu kg^{-1} dry weight. Variation in copper toxicity values was best explained by differences in the actual cation exchange capacity (CEC) at soil pH. Using the obtained regression algorithms, the observed toxicity could – in most cases – be predicted within a factor of two.

The effect of pretreatment is most significantly related to aging of the contaminants prior to testing. Longer aging times greatly decrease toxicity for both organic chemicals and metals.

4.4 How to Model Toxicity

The example given above of Criel et al. [83] of modeling metal toxicity across a series of field soils is a nice illustration of the current state of the art. As opposed to the aquatic compartment, the interplay between the biotic and abiotic factors modulating toxicity is not yet well understood. Consequently, models for predicting toxicity toward earthworms across a wide array of soils and soil types are virtually lacking. This is especially the case for metals.

4.4.1 Organic Compounds

In case of hydrophobic organic chemicals that act strictly according to the general mechanism of polar narcosis, competition for sorption of the contaminant between the soil organic matter and the organic matter of the earthworm has been the basis for establishing the critical body residue concept (CBR) and the translation of CBRs toward critical concentrations in any of the environmental compartments, assuming on the one hand that the total body concentration of a nonpolar narcotic organic contaminant is proportional to the concentration at the target or receptor of toxicity, while on the other hand assuming that (1) the fat tissue is the main storage compartment for hydrophobic organic chemicals and (2) the fat tissue behaves similarly to the abiotic organic phases present in the system.

McCarty and Mackay [84] showed that CBRs for polar narcotics are indeed fairly constant. The latter two assumptions imply that the CBR or a specific effect level (EC_x, with x being the extent of adverse effect) is proportional to the octanol–water partitioning coefficient of the chemical:

$$\log \mathrm{CBR} \text{ or } \log \mathrm{EC}_x \approx a(\log K_{\mathrm{ow}}) + b. \tag{3}$$

Karickhoff et al. [85] were one of the first authors to show the equilibrium concept of partitioning of organic compounds by reporting that K_{ow} is proportional to the compound-specific organic-carbon normalized partition coefficient (K_{oc}). Subsequently it is K_{oc} that may be used to predict not only the degree of chemical partitioning between water and the sediment or soil organic carbon, but also the baseline-toxicity of hydrophobic organic chemicals in a specific medium varying in organic carbon content:

$$K_{\mathrm{oc}} = K_{\mathrm{d}}/f_{\mathrm{oc}}, \quad \text{with } K_{\mathrm{d}} = C_{\mathrm{w}}/C_{\text{solid phase}} \tag{4}$$

and

$$\log \mathrm{CBR} \text{ or } \log \mathrm{EC}_x = a(\log K_{\mathrm{oc}})f_{\mathrm{oc}} + b. \tag{5}$$

Although the study was not carried out with earthworms, Paumen et al. [86] recently cautioned that even minute changes in the chemical structure (in this case isomers and metabolites) of a toxicant may induce unpredictable (isomer) specific toxicity, not only emphasizing the need of chronic toxicity testing to gain insight into long-term effects but also elegantly showing the limitations the CBR concept.

Van Gestel and Ma [87] combined information on exposure routes (pore water) and toxicity data of chlorinated aromatics for two earthworm species (*E. andrei* and *L. rubellus*) in four (chlorophenols and chloroanilines) and two (chlorobenzenes) soils to derive quantitative structure activity relationships (QSARs) that may be used to predict toxicity of chloroaromatics in additional soils. The QSARs are based on lipophilicity of the test compounds, expressed in terms of their log K_{ow}. It was noted by these authors that both earthworm species are not equally sensitive to chlorobenzenes and chloroanilines, *E. andrei* is more sensitive than *L. rubellus* to chlorophenols and toxicity data of chlorosubstituted anilines, phenols, and benzenes are in close agreement with data on toxicity for fish.

A similar conclusion was drawn by Miyazaki et al. [88] for acute toxicity of chlorophenols for *E. fetida*. A different exposure modality was used by these authors to derive QSARs as the worms were exposed on filter paper wetted with a solution of the individual chlorophenols.

4.4.2 Metals

For many metals, it is the free ionic form that is most responsible for toxicity. This is despite the fact that strictly speaking, metals may be taken up via various exposure pathways and in a complexed state, bound to a number of ligands of varying binding capacity and varying binding strength. The FIAM is used to explain the relationship between speciation in the external environment and bioavailability to the organisms [30]. The FIAM produces speciation profiles of a metal in an aquatic system and provides insight into the relative bioavailabilities of the different forms of metal as well as the importance of complexation. The basic assumption underlying the FIAM is that adverse effects are proportional to the activity of the free metal ion in solution, or in the case of soils – the pore water. Although it has been shown that other species might also contribute to metal uptake and metal toxicity, most evidence supports the FIAM.

There is, however, an increasing body of evidence becoming available, showing that the toxicity caused by the free metal ion is modulated by a number of chemically induced competing processes. This observation was the basis for the development of Biotic Ligand Models (BLMs). BLM theory on the one hand incorporates the impact of water chemistry (most notably pH and DOC) on metal speciation, whereas the model on the other hand quantifies the assumption of competition between the major cations like Ca^{2+}, Mg^{2+}, Na^{+}, and H^{+}, and free metal ions for binding sites at the organism–water interface may result in a decreased toxicity of the free metal ion [89]. In some cases it is taken into account that other metal species have the potential to contribute to toxicity, like complexes with OH^{-} and $CO_3{}^{2-}$ ions and organic metabolites in case of Cu. BLMs include all these aspects and are, therefore, gaining increased interest in the scientific as well as the regulatory community. In fact, the BLM concept, now developed for Cu, Ni, Ag, and Zn, is considered as

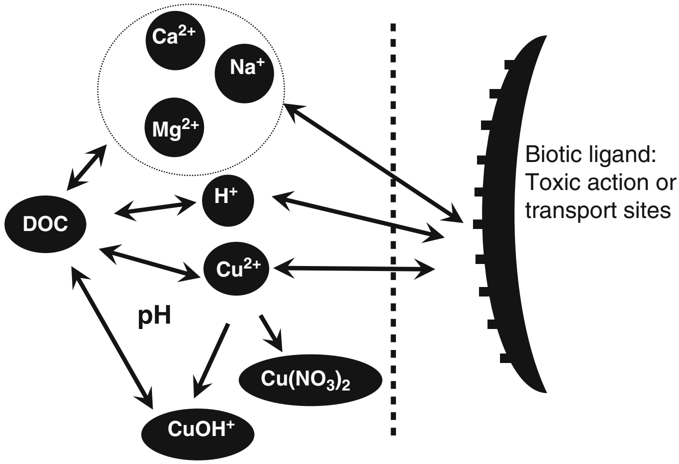

Fig. 4 Schematic overview of the processes underlying the Biotic Ligand Concept for metal toxicity, in this case copper. The *left-hand side* of the scheme depicts pore water constituents that affect copper speciation, the *right-hand side* depicts the interaction of the free copper ion with the biotic ligand of the earthworm (in this case the epidermis) as affected by competition with competing ions like $Ca^{2+}/Na^{+}/Mg^{2+}/H^{+}$

the currently most practical technique to assess the ecotoxicity of metals on a site-specific basis. Therefore, the BLM concept is now being approved in the EU. A schematic representation of the BLM concept is given in Fig. 4.

A basic assumption of the BLM is that metal toxicity occurs as the result of metal ions reacting with binding sites at the organism–water interface, represented as a metal–biotic ligand (metal–BL) complex. The concentration of this metal–BL complex is proportionally related to the magnitude of the toxic effect, independent of the physical–chemical characteristics of the test medium. Hence, the acute toxicity of a trace metal to an organism can be calculated when metal speciation, the activity of each cation in solution, and the stability constant for each cation to the BL(s) for the organism are known. BLMs have recently been developed for copper toxicity to earthworms [90]. Paquin et al. [89] provided a historical overview of the fundamentals of BLMs.

5 Conclusions

Species-specific morphological, physiological, and behavioral aspects basically determine the contribution of potential uptake pathways of nutrients and natural and anthropogenic contaminants. Intraspecies (especially including short-term weather deviations) and interspecies variances (like size and ecological preferences) will most likely modify the actual contribution of potential exposure pathways, thus modifying actually occurring adverse effects.

Earthworms are ubiquitous ecosystem engineers and litter transformers that are essential for maintaining a healthy soil ecosystem. They inhabit virtually all soil layers while they tend to move upward and downward the soil profile in response to variations in the water table. Earthworms have been studied for various decades and their intra- and interspecies variances are fairly well understood. It may be concluded that earthworms are suited organisms for ecotoxicity studies and indicator organisms for the assessment of potential risks:

1. The uptake routes of chemicals are clear, with a dominant contribution of uptake of pollutants via the pore water. For hydrophobic chemicals with log $K_{\mathrm{OW}} >$ approximately 6, ingestion of food and soil particles may induce additional uptake of micropollutants.
2. The magnitude of accumulation of chemicals is rather high and earthworms are therefore suited for assessing potentially bioavailable fractions and resulting adverse effects. Compartment modeling may be used to quantify accumulation as a function of time.
3. Earthworms are well suited for assessing adverse effects:
   (a) A number of toxicity endpoints (like mortality, reproductive success, growth) may relatively easily be deduced, whereas earthworms are not specifically more sensitive or less sensitive for the majority of chemicals. Van Gestel and Ma [87] found for instance that toxic effects of chlorinated aromatics are similar for earthworms and fish.
   (b) Because of their ease of cultivation and their ubiquitous nature, earthworms have frequently been the topic of study and effect and accumulation data are relatively abundant for comparative purposes and for inter- and intrasystem extrapolation.
   (c) It has been shown that it is possible to derive QSARs for predicting effects of chemicals on various earthworm species.

On the other hand it should be noted that most effect and accumulation assays haven typically been carried out in a laboratory setting. Field studies varying from TMEs [75] up till analysis of population parameters are scarce. Field studies at all levels of ecological hierarchy would be well suited for extrapolation and validation of models generated on the basis of laboratory data and would provide important tools for assessing ecosystem health.

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