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Earthworm Biomarkers as Tools for Soil Pollution Assessment

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1. Introduction

Soil pollution has enormously increased during the last decades due to the intensive use of biocides and fertilizers in agriculture, industrial activities, urban waste and atmospheric deposition. Its occurrence is related to the degree of industrialization and intensity of chemical usage. Soil pollution causes decrease in soil fertility, alteration of soil structure, disturbance of the balance between flora and fauna residing in the soil, contamination of the crops, and contamination of groundwater, constituting a threat for living organisms.

The most diffusive chemicals occurring in soil are heavy metals, pesticides, petroleum hydrocarbons, polychlorobiphenyl (PCBs), dibenzo-p-dioxins/dibenzofurans (PCDD/Fs). Heavy metals from anthropogenic sources are widely spread in the environment and most of them finally reach the surface soil layers. Heavy metals can enter the soil from different sources, such as pesticides, fertilizers, organic and inorganic amendants, mining, wastes and sludge residues (Capri & Trevisan, 2002). In contrast to harmful organic compounds, heavy metals do not decompose and do not disappear from soil even if their release to the environment can be restricted (Brusseau, 1997). Therefore, the effects of heavy metal contamination on soil organisms and decomposition processes persist for many years. Pesticides are widely used in agriculture for counteracting insects, fungi, rodents or other animals living in or on the crops. They are either directly applied to soil to control soil borne pests or deposited on soil as run off from foliar applications and their concentrations are high enough to affect the soil macro-organisms (Bezchlebova et al., 2007). The pesticides most widely used in the past have been organochlorine pesticides, characterized by high hydrophobicity and persistence. Currently, they have been replaced by less persistent compounds. Organophosphates have become the most widely used pesticides today. They are used for pest control on crops in agriculture and on livestock, for other commercial purposes, and for domestic use. Due to their water solubility, the organophosphate residues in agricultural practices are capable of infiltrating through soil into surface water. As a consequence of their wide diffusion they have been detected in food, ground and drinking water, and natural surface waters (Dogheim et al., 1996; Garrido et al., 2000). Soil pollution by petroleum hydrocarbons usually originates from spills or leaks of storage tanks during fuel supply and discharge operations. Petroleum hydrocarbons include aliphatic and aromatic compounds; some of them are known or suspected human carcinogens, and are classified as priority pollutants. PCBs are persistent soil contaminants due to their hydrophobicity and resistance to biodegradation (Weber et al., 2008). They can be released into the environment from poorly maintained hazardous waste sites that contain PCBs, illegal or improper dumping of PCB wastes, such as transformer fluids, leaks or releases from electrical transformers containing PCBs, and disposal of PCB-containing consumer products into municipal or other landfills not designed to handle hazardous waste. PCBs are also currently released into the environment by municipal and industrial incinerators from the burning of organic wastes. PCDD/Fs are chemically very stable and are highly hydrophobic compounds. PCDD/Fs have a high affinity to organic matter and have limited mobility unless transported in association with particulate organic matter. However, these compounds are bioaccumulative, can be found in the terrestrial food chain, and have been reported to impact the biota at the higher trophic levels. Recently, the attention of the scientific community focused on emerging contaminants in the soil, such as pharmaceuticals, endocrine disruptors, personal care products, surfactants, flame retardants. They are currently not included in routine monitoring programmes, but may be candidates for future regulation depending on research on their toxicity, potential effects on the environment and occurrence in the environmental compartments.

Due to the increasing concern about chemical contamination of soil there is an increasing interest in the scientific community and international agencies for soil pollution monitoring and assessment. The traditional approach to soil pollution assessment, based on the analysis of the concentrations of pollutants in the soil and comparison with specific threshold values, does not provide indication of deleterious effects of contaminants on the biota. It neglects several essential aspects such as toxicity of chemicals not included in the selection of contaminants to be analyzed, interactive effects (synergism and antagonisms) of pollutants on biota and bioavailability. Bioavailability refers to the fraction of a contaminant that is taken up by an organism from the environmental media (i.e., through both passive and active routes), and directly influences toxicity (Smith et al., 2010). Bioavailability of pollutants in soil to terrestrial invertebrates and plants can be influenced by some characteristics of the soil such as pH, cation exchange capacity, and organic matter content (Bradham et al., 2006; Spurgeon et al., 2006; Criel et al., 2008). The influence of a single factor on pollutant bioavailability is usually site-, chemical-, and soil-specific. For this reason, it is difficult to model pollutant bioavailability based on total concentration and soil characteristics alone. The best integrators of these complex effects are the exposed organisms themselves.

For these reasons, new biological approaches to soil monitoring, such as the measurement of biochemical and cellular responses to pollutants (i.e. biomarkers) on organisms living in the soil (bioindicators), have become of major importance for the assessment of the quality of this environmental compartment (Kammenga et al. 2000). Soil invertebrates may represents good sentinel organisms of soil chemical pollution because they are in direct contact with soil pore water or food exposure, in contrast to many vertebrates that are indirectly exposed through the food chain (Kammenga et al., 2000). Among soil invertebrates earthworms are relevant organisms for soil formation and organic matter breakdown in most terrestrial environments. Because of their particular interactions with soil, earthworms are significantly affected by pollution originated on intensive use of biocides in agriculture, industrial activities, and atmospheric deposition. Hence, earthworms as been proved as valuable bioindicators of soil pollution (Lanno et al., 2004).

The aim of the chapter is to review the use of molecular and cellular biomarkers in earthworms as early and sensitive indicators of soil pollution and stress.

2. Earthworm as bioindicator organisms of soil pollution

Earthworms are very important organisms for soil formation and organic matter breakdown in most terrestrial environments and traditionally they have been considered to be convenient indicators of land use impact and soil fertility. They contribute to pedogenesis and soil profile, affect the physical, chemical and microbiological properties of soil (Barlett et al., 2010) and contribute to improve soil fertility. In particular earthworms may increase mineralization and humification of organic matter by food consumption, respiration, and gut passage (Lavelle & Spain 2001). They may indirectly stimulate microbial mass and activity as well as the mobilization of nutrients by increasing the surface area of organic particles and by their casting activity (Emmerling & Paulsch 2001). Moreover, their burrowing activities significantly contribute to increase water infiltration and soil aeration.

The role of earthworms in the decomposition of organic matter and subsequent cycling of nutrients has raised the interest of their use as indicator organisms for the biological impact of soil pollutants. This in turn has lead to a large body of work on earthworm ecotoxicology (Spurgeon et al., 2003). In addition, earthworms manipulation is relatively simple; this facilitates the measurement of different life-cycle parameters e.g. growth and reproduction, as well as accumulation and excretion of pollutants, and biochemical responses. Thus, earthworms are suitable organisms for soil ecotoxicological research.

Because of their particular interactions with soil, they are significantly affected by pollutants reaching the soil system. Earthworms can be exposed to contaminants in various ways. Firstly, living in the soil they are in direct contact with soil pore water and therefore with pollutants therein dissolved. The earthworm skin is extremely permeable to water (Wallwork, 1983) and it represents a main route for contaminant uptake (Jajer et al., 2003; Vijver et al., 2005). Secondly, these organisms ingest large amounts of soil, therefore they are continuously exposed to contaminants adsorbed to solid particles through their alimentary tract (Morgan et al., 2004). Chemicals uptake via the dermal route can be directly related to the pore water concentration. Vijver et al. (2005) measured the contribution of each pathway for heavy metals uptake in the earthworm *Lumbricus rubellus.* The authors concluded that the dermal route is the main uptake route for metals and that pore water uptake via ingestion does not significantly contribute to metal accumulation.

Earthworms are able to accumulate various organic and inorganic contaminants (Morrison et al., 2000) present in the soil. Previous studies under both field and laboratory conditions demonstrated that earthworms bioaccumulate certain metals (such as Cd, Cu, Zn and Pb) from soils. They accumulate efficiently and tolerate high tissue metal concentrations using a variety of sequestration mechanisms (Peijnenburg, 2002; Andre et al., 2009). Earthworms appear to have well-developed trafficking and storage pathways for heavy metals, particularly for essential trace metals such as Cu and Zn (Morgan & Morgan, 1999). Metals are primarily accumulated within the posterior alimentary canal of the earthworm when inhabiting heavy metal contaminated sites. This part of the body includes the intestine and the related chloragogenous tissue that separates the absorptive epithelia from the coelomic cavity (Andre et al., 2009). The chloragogenous tissue is composed of pedunculated cells and its main functions are synthesis of haemoglobin, homeostasis of cation composition in the blood and coelomic fluid, maintenance of a balanced pH level, storage of nutrients and waste, and uptake and detoxification of toxic cations (Jamieson, 1992). Therefore, chloragogenous tissue represents a major metal sink (Morgan et al., 2002).

Bioaccumulation is species-specific (Heikens et al., 2001; Hendrickx et al., 2004; Nahmani et al., 2007) and is influenced by the physicochemical properties of the pollutants and of the environmental scenario (Vijver et al., 2005). In fact it depends on factors such as metal speciation and concentration (Heikens et al., 2001; Hobbelen et al., 2006; Spurgeon et al., 2006; Nahmani et al., 2007), soil type and characteristics (Hendrickx et al., 2004; Kizilkaya, 2005; Hobbelen et al., 2006; Spurgeon et al., 2006), temperature (Olchawa et al. 2006), and exposure duration (Nahmani et al. 2007). Bioaccumulation in earthworms can be expresses as Biota to- Soil Accumulation Factor (BSAF) (Cortet et al., 1999), calculated by the following formula: BSAF = metal content in earthworm/total metal content in soil. In Tab.1 the BSAF values calculated for several soil pollutants in two earthworms species is reported.

	Cd	Zn	Cu	Pb	DDTs	PCBs	PBDEs
A. caliginosa	6.18–17.02	1.95–7.91	0.27–0.89	0.08–0.38	-	-	-
L. rubellus	3.64–6.34	1.5–6.35	0.29–0.87	0.04–0.13	1.48–1.70	1.09–2.76	1.99–5.67
Ref.	Dai et al. 2004				Vermeulen et al., 2010		

Table 1. Biota-to-soil accumulation factors (BSAF) in earthworms

Earthworms serve as a major food source for numerous animals, in particular, amphibians, reptiles, birds and mammals. Therefore, bioaccumulation of chemical contaminants by earthworms implies the risk of the transfer of pollutants to higher trophic levels (Marino et al., 1992).

The importance of earthworms in testing the adverse effects of chemicals on soil organisms has been recognised by several environmental organisations and, as a result, a set of standard test guidelines are available. For official guidelines soil toxicity assessment must be addressed by at least two-three different assays, thus combining plant assays and earthworm assays. During the early 1980s, acute toxicity tests for earthworms, based on mortality and grow rate, were developed jointly by the European Union (EU) and by the Organization for Economic Cooperation and Development (OECD) (OECD, 1984). They were later implemented with chronic toxicity tests based on reproduction rate measurement (OECD, 2004).

Several standardized tests for assessing the toxicity of contaminated soil to earthworms exist (OECD 1984, 2004), but these methods remain relatively costly and time-consuming. For instance, the acute earthworm toxicity test requires 14 days (OECD 1984), and since it only measures lethality it may be insufficient for predicting long-term population fitness following chronic exposure to contaminated soil (Whitfield et al., 2011). Chronic test, based on the inhibition of earthworm reproduction (OECD 2004), provides a more ecologically relevant sub-lethal endpoint than lethality, but it requires a longer exposure period for accurate assessment (50 days). In recent years there is a growing interest for increasing the knowledge on molecular and cellular responses of earthworms to pollutants as biomarkers of pollutant exposure and effect to be used in soil monitoring and assessment programmes (Beliaeff & Burgeott, 2002; Handy et al., 2003). With respect to standard toxicity tests the biomarker approach can offer more information about the organism's stress response to individual toxicants and mixtures (Kammenga et al., 2000; Scott-Fordsmand & Weeks, 2000; Hankard et al., 2004; Svendsen et al., 2004) and a tool in monitoring field populations where the assessment of conventional endpoints is difficult to be applied.

Most studies on earthworm biomarkers has been conducted on *Eisenia spp.* while other earthworm species remain less investigated. *Eisenia fetida* is the standard testing organism used in terrestrial ecotoxicology due to its rapid life cycle and simple rearing in the laboratory. The ecological relevance of *Eisenia spp.* as bioindicator organism in soil monitoring based on biomarker approach has been recently questioned (Sanchez-Hernandez, 2006) because they are epigeic species, forming no permanent surface burrows on the soil surface, feeding on decaying organic matter, while in most cases contaminants occur at soil depths where these earthworms are not found. *Eisenia fetida* is a North European litter-dwelling species inhabiting the soil surface, living primarily in sites rich in organic matter (Jänsch et al., 2005) such as compost heaps, manure piles, or sewage sludge, and thus unlikely to be present naturally in agricultural soils or contaminated land sites (Spurgeon et al., 2002). The study of anecic species (i.e. *Lumbricus terrestris*), that forms temporary deep burrows and comes to the surface to feed, and endogeic species (i.e. *Aporrectodea caliginosa*), which build complex lateral burrow systems through all layers of the upper soil rarely coming to the surface, could be more ecologically relevant. In fact these species can be exposed to pollutants present not only in the soil surface but also in the soil deeper layer, providing an integrated response to soil pollution.

3. Biomarkers: Definition

A biomarker is defined as a ''biochemical, cellular, physiological or behavioural variations that can be measured in tissue or body fluid samples, or at the level of whole organisms, to provide evidence of exposure and/or effects from one or more contaminants'' (Depledge, 1994). The effects of contaminants at lower levels of biological organization (e.g. biochemical, cellular, physiological) in general occur more rapidly than those at higher levels (e.g., ecological effects) and therefore may provide a more sensitive early warning of toxicological effects within populations. Potentially, any alterations in any of the molecular, cellular, biochemical, and physiological processes occurring within an organism following pollutant exposure could be used as biomarkers.

Biomarkers, in general, may be classified into biomarkers of exposure, and biomarkers of effect (Chambers et al., 2002). The former indicate that an organism has experienced exposure to a pollutant, and offer an early signal for exposure to micropollutants. They can provide qualitative and quantitative estimates of exposure to various compounds. However, the change in these biomarkers may not be predictive of the degree of adverse effects either on the organism or in the population. Biomarkers of effect are associated specifically with the toxicant's mechanism of action and are sufficiently well characterized to relate the degree of biomarker modification to the degree of adverse effects (Chambers et al., 2002). Biomarker of exposure and biomarkers of effect can differently contribute to environmental assessment. Biomarkers of exposure may have the potential to offer an alternative to some chemical analyses or to measure effects of short-lived chemicals as well as giving a more biologically relevant indication of exposure (Hagger et al., 2006). On the other hand, biomarkers of effect can shed light on qualitative aspects of hazard identification by both demonstrating that a hazard is occurring and elucidating the mechanisms responsible (Chambers et al. 2002). These biomarkers can provide insights into both the causal factors of the hazard and its ecological consequences, depending on the degree of specificity of the biomarker with the pressure and the degree to which its expression to higher order effects is understood. The specificity of biomarkers to pollutants ranges from highly specific biomarkers to not specific biomarkers such as immune system impairment or DNA damage. Therefore, the use of a suite of biomarkers with a range of specificity is an important aspect of environmental monitoring based on the biomarkers. Although all biomarker types can provide useful information about exposure or effects of pollutants on living organisms, certain criteria have been developed to address the selection of the most useful and relevant to use for pollutant impact assessment in environmental monitoring. These include whether a biomarker is sensitive, whether it responds in a dose- or time dependent manner to the toxicant (Walker et al., 1998), including its transient and biochemical memory (how long after exposure the response lasts), whether the variability in biomarker response due to natural variation is known (i.e., season, temperature, sex, weight, age) (Hagger et al., 2006). To provide an accurate toxicity assessment, biomarkers should responds to a pollutant in a dose-dependent manner over a concentration range of pollutant that is environmentally meaningful. The validity of any biomarker depends on its ability to precisely separate anthropogenic stressors from the influence of natural variability. Finally, biomarkers that can be linked to important biological processes and for which a correlation between the observed responses and deleterious effects at the individual or population/community level is established are considered of relevance in environmental assessment.

4. Biomarkers in earthworms

Overall, the use of biomarkers in earthworm is becoming increasingly important for the evaluation of effects of contaminants on soil organisms. Acetylcholinesterase, metallothionein, biotransformation enzymes and antioxidant defences are among the most used biomarkers due to their crucial role in the neurocholinergic transmission and in cell homeostasis preventing toxic action of chemicals (Sanchez-Hernandez, 2006; Novais et al 2011). However, the research of novel biomarkers in earthworms is receiving increasing attention for its potentiality in soil pollution monitoring and assessment.

4.1 Metallothioneins

Metallothioneins (MTs) are low-molecular-weight cystein-rich metal-binding proteins that are involved in homeostasis of essential metals like Cu and Zn and detoxification of non essential metals such as Ag, Cd and Hg (Costello et al., 2004; Amiard et al., 2006). In addition to their function as metal chelators, MTs act as free radical scavengers (Min, 2007). They contain 25%–30% cysteine, but few aromatic or histidine residues. Vertebrate and invertebrate metallothioneins contain two unique metal-thiolate clusters determined by the presence along the sequence of the protein of metal chelating Cys-X-Cys sequences, where X can be any amino acid other than cysteine. The MT protein is dumbbell-shaped, and the polypeptide backbone is wrapped around the metal thiolate core, forming the scaffold for two domains, designated α and β, separated by a short linker region. Induction of MTs by metal exposure has been detected in a wide variety of organisms including earthworms (Stürzenbaum et al., 2001). For example a significant induction of MT proteins was observed in different earthworm species such as *Lumbricus rubellus*, *Eisenia fetida*, *Eisenia andrei* exposed to cadmium (Calisi et al., 2009; Demuynck et al., 2006; Ndayibagira et al., 2007; Brulle et al., 2007), or in *Lumbricus mauritii* exposed to Pb and Zn contaminated soil (Maity et al., 2011) and in *Lumbriucus terrestris* exposed to cadmium, copper and mercury (Calisi et al., 2011a). It is known that earthworms share a high tolerance to heavy metal exposure

(Stürzenbaum et al., 1998) also thanks to the fundamental contribution of these metal-binding proteins. In *Lumbricus terrestris* Calisi et al (2011b) found the major concentration of MT in the postclitellar portion of the animal body with respect to the preclitellar part. This result is in agreement with the hymmunoistochemical localization of MT in the intestine and chloragogenous tissue previously reported by Stürzenbaum et al. in *Lumbricus rubellus* (2001).
Although the amino acid sequences of more than 50 invertebrate MT and MT-like proteins have already been determined, little is known about the biochemical properties of earthworm MTs. So far, only 5 MT genes of earthworms have been cloned from *Lumbricus castaneus*, *Eisenia fetida*, *Lumbricus rubellus*, and *Lumbricus terrestris* (Gruber et al., 2000; Liang et al., 2009) whose expression is differentially regulated by different heavy metals (Sturzenbaum et al., 1998, 2001). Metallothionein induction is one of the mostly utilized biomarker in earthworms and is applied as early biomarker of exposure to heavy metals in soil monitoring.

4.2 Acetylcholinesterase

Acetylcholinesterase (AChE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine. AChE is the target site of inhibition by organophosphorus and carbamate pesticides. In particular, organophosphorus pesticides inhibit the enzyme activity by covalently phosphorylating the serine residue within the active site group. They irreversibly inhibit AChE, resulting in excessive accumulation of acetylcholine, leading to hyperactivities and consequently impairment of neural and muscle system. Acetylcholinesterase represents the main cholinesterase in earthworms (Rault et al., 2007). Its activity has been identified and biochemically characterised only in a few earthworm species (Caselli et al., 2006). According to Rault et al. (2007) and Calisi et al. (2011b), the highest concentration of AChE activity was found in the pre-clitellar part of the animal and suggests a main role of this enzyme in functioning of the dorsal brain localized near the prostomium. Rao et al. (2003) and Rao & Kavitha (2004) found a time-dependent AChE inhibition in *Eisenia fetida* exposed to chlorpyrifos and azodrin, two organophosphate pesticides, in the standardized paper contact test. Calisi et al. (2009) reported a significant inhibition (about 45 %) of AChE activity in *Eisenia fetida* after two weeks of exposure to the carbamate methiocarb added into the soil at the maximal concentrations recommended in vineyards (EEC, 2001). Moreover, Calisi et al (2011b) observed that in *Lumbricus terrestris* high percentage inhibition of AChE activity by pesticide exposure was not paralleled by a corresponding high mortality value (higher than) as observed in birds and mammals (see Tab.2), where AChE inhibition higher than 50% of normal is referred to be irreversible and regarded as being in the lethal range (for a review see Lionetto et al., 2010).

ACHE inhibition (%)	Mortality (%)	Species	Ref.
70	30	Earthworm *(Lumbricus terrestris)*	Calisi et al., 2011b
70	100	Mammals and birds	Walker, 1998

Table 2. AChE inhibition and mortality in earthworm and higher vertebrates

The lower sensitivity of animal survival to AChE inhibition compared to vertebrates was also recently documented in other earthworm species (Rault et al., 2008) and suggests that the toxic action of pesticide on earthworms can involve also other molecular or cellular target beyond AChE.

Recently, the potential of some metallic ions, such as Hg^{2+}, Cd^{2+}, Cu^{2+} and Pb^{2+}, to depress the activity of AChE of fish and invertebrates, *in vitro* and or *in vivo* conditions has been demonstrated in several studies (for a review see Lionetto et al., 2010). On the contrary in *Lumbricus terrestris* (Calisi et al., 2011b) and *Eisenia fetida* (Calisi et al., 2009) AChE activity was unaffected by copper sulphate exposure.

AChE inhibition in earthworms is presently regarded as giving early warning of adverse effects of pesticides (Booth & O'Halloran, 2001), and consistently included among the batteries of biomarkers employed for early assessments of pollutant impact on wildlife in terrestrial ecosystems. However, concerning AChE in earthworms only a few pesticides in use have been tested against relatively few earthworm species both in laboratory tests and under field conditions (Rao et al., 2003; Calisi et al., 2009; Scott-Fordsmand &Weeks, 2000; Rao & Kavitha, 2004; Gambi et al., 2007). As pointed out by Scott-Fordsmand & Weeks (2000) and by Sanchez-Hernandez (2006) the potential use of AChE in earthworms as biomarker of pesticide exposure has not been sufficiently explored.

4.3 Biotransformation enzymes

In their habitat earthworms can be exposed to a variety of plant alkaloids, PAHs and pesticides known to be inducers of detoxification responsible enzymes. In eukaryotes, detoxification of organic compounds usually occurs in two phases. Phase I detoxification processes involve the cytochrome P450 enzyme system and results in the introduction of a functional group, such as hydroxyl or sulphonyl, to non-polar compounds. In some cases the metabolites of phase I reactions are more toxic than the parent compound. Phase II detoxification enzymes, such as glutathione S-transferase (GST), attach a large polar, water-soluble moiety to the products of phase I metabolism to promote excretion and elimination of the toxicant.

The presence of cytochrome P450 was demonstrated in *Lumbrucus terrestris* by Liimaitainen & Hänninen (1982), but only the occurrence of the monooxygenase activity benzoxy-resorufin-Odealkylase (BenzROD) but not of other phase I enzymes was proven (Berghout et al., 1991). However, induction of CYP1A in earthworms has demonstrated to be quite difficult to be measured because of interference from endogenous pigments (Liimatainen & Hänninen, 1982) and the identification of non-inducible forms of cytochrome P450 (Milligan et al., 1986). Achazi et al. (1998), by utilizing ethoxy-, pentoxy- and benzoxyresorufin as substrates for monooxygenase activity, demonstrated pentoxy-resorufin-Odealkylase (PentROD) and BenzROD activities in *Eisenia fetida* microsomes, but exposure of the animals for up to four weeks to 100 mg fluoranthene or benzo[a]pyrene kg-1 soil (dry weight) did not induce significant changes in the activity of these monooxygenases. The same authors demonstrated the presence of etoxy-resorufin-Odealkylase (EROD) and PentROD activities in *Eisenia crypticus* but failed to demonstrate an induction of these activities following xenobiotic exposure. On the other hand short-term exposure to benzo[a]pyrene by feeding reduced the EROD activity significantly by 45%, but did not affect PentROD activity. After long-term (8 weeks) exposure to benzo[a]pyrene in the agar–agar medium EROD activity was not changed but PentROD was decreased to zero (Achazi et al., 1998).

Glutathione transferases (GSTs) form a ubiquitous superfamily of multi-functional dimeric enzymes (w50 kDa) with roles in phase-II detoxification. GSTs neutralise a broad range of xenobiotics and endogenous metabolic by-products via enzymatic glutathione conjugation, glutathione-dependent peroxidase activity or isomerisation reactions (Hayes et al., 2005). Several studies have demonstrated the sensitivity of earthworm GST to metals and pesticide exposure (Aly & Schröder, 2008; Maity et al., 2008; Lukkari et al., 2004; Saint-Denis et al., 2001; Booth et al., 2000). Recently, transcriptome approaches in the earthworm *Lumbricus rubellus* highlight GSTs as responders to several classes of pollutants including inorganic (cadmium, copper), organic (fluoranthene) and agrochemicals (atrazine) (Bundy et al., 2008; Owen et al., 2008). LaCourse et al. (2009) demonstrated *Lumbricus rubellus* to possess a range of GSTs related to previously known GSTs from other taxa including nematodes and humans, with evidence of tissue-specific isoforms, activity, location, the ability to detoxify products of cellular toxicity and potential response to pollution. This study combined sub-proteomics, bioinformatics and biochemical assay to characterise the *Lumbricus rubellus* GST complement as pre-requisite to initialise assessment of the applicability of GST as a biomarker.

4.4 Antioxidant enzymes

The exposure to either organic or inorganic pollutants is known to induce oxidative stress in the cells. A by-products of the metabolism of xenobiotics is the production of free radicals, on the other hand exposure to metals leads to the generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl ($OH\cdot$) radicals (Dazy et al., 2009). In order to scavenge ROS and avoid oxidative damage on biological macromolecules (lipids, proteins or DNA), cells protect themselves using enzymes and small molecular-weight antioxidants, such as glutathione (Valavanidis et al., 2006). Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase are important enzymatic antioxidants in the response to oxidative stress: superoxide dismutase metabolizes the superoxide anion (O_2^-) into molecular oxygen and H_2O_2, which is then deactivated by catalase, thus preventing oxidative damage. The glutathione reductase enzyme also plays an important role in cellular protection by reducing glutathione in the oxidized form (GSSG) to GSH (reduced and active form). Several studies (Liu et al., 2010) indicate that exposure to either organic or inorganic pollutants are able to induce a stress response in the antioxidant enzymes, suggesting their potential application as general biomarkers for assessing effects of pollutants in terrestrial ecosystems at early stages and with low concentrations. However, the dose-dependent and time-dependent response of antioxidant enzymes to pollutant exposure is sometimes complex and a better understanding of their behaviour in stress condition is needed for their application in monitoring and assessment programmes. For example Liu et al. (2010) demonstrated that exposure to toluene, ethylbenzene and xylene in earthworms (*Eisenia fetida*) induced a bell-shaped change in superoxide dismutase and catalase activities with a tendency of inducement firstly and then inhibition with increasing concentrations of the pollutants. Moreover, Wu et al (2011) found superoxide dismutase to be induced during the early period of phenanthrene exposure while with longer exposure times its activity decreased.

4.5 Cellular biomarkers on coelomocytes

Earthworm coelomic fluid is particularly interesting from a toxicological perspective for the development of novel cellular biomarkers. It can transport pollutants throughout the exposed organism and its cells (coelomocytes) are involved in the internal defence system

(Cooper et al., 2002; Reinhart & Dollahan, 2003; Engelmann et al., 2004). The coelomocyte population is comprised of amoebocytes originating from mesenchymal lining of the coelom (Hamed et al., 2002) and eleocytes (chloragocytes) sloughed into the coelomic fluid from the chloragogen tissue surrounding the intestine and blood vessels (Affar et al., 1998). Thank to the important role played by coelomocytes in the animal physiology, any impairment of their functioning can alter the health of the entire organism. Five cell types were observed by Calisi et al. (2009) in *Eisenia fetida* celomic fluid, corresponding to the previously described coelomocyte cell types (Valembois et al., 1985): leukocytes type I (basophilic) and II (acidophilic), granulocytes, neutrophils, and eleocytes.
The most recent cellular biomarkers standardized on earthworm coelomocytes are summarized in Tab.3.

Biomarker	Type	Analytical technique	Species	Ref.
Eleocyte riboflavin concentration	General biomarker of exposure	Flow cytometry	*Dendrodrilus rubidus*	Plytycz et al., 2007
Lysosomal membrane stability	General biomarker of exposure and effect	Neutral red retention assay	*Lumbricus spp. Eisenia spp. Aporrectodea caliginosa*	(for review see Sanchez-Hernandez, 2006)
Granulocyte morphometric alteration	General biomarker of exposure and effect	Diff Quick® stain	*Eisenia fetida Lumbricus terrestris*	Calisi et al 2009 Calisi et al. 2011b
Gene expression	Specific biomarkers of exposure	Real-Time PCR	*Eisenia fetida*	Brulle et al., 2010

Table 3. Cellular biomarkers on earthworm coelomocytes

Eleocytes are characterized by the presence of granules (chloragosomes), showing a high autofluorescence derived from riboflavin stored in (Cholewa et al., 2006, Plytycz et al., 2007) and from other fluorophores, putatively including lipofuscins (Cygal et al., 2007, Plytycz et al., 2009). Riboflavin storage was detected in all earthworm species studied, either in chloragocytes localised in chloragogen tissue of *Lumbricus spp.* and *Aporrectodea spp.* or in freely floating eleocytes (Plytycz et al., 2006). This suggests that riboflavin plays an important role in immunity of lumbricid worms, as it does in vertebrates (e.g. Verdrengh & Tarkowski, 2005). The amount of riboflavin in eleocytes is species-specific (Plytycz et al., 2006) and changes in response to environmental factors, including metal pollution, in a metal- and species-specific manner (e.g. Kwadrans et al., 2008, Plytycz et al., 2009). It was proposed as general biomarkers of exposure to environmental pollutants.
The most investigated coelomocyte alteration is represented by lysosomal membrane stability used as an indicator of chemical exposure and associated biological effects (Svendsen et al., 1996; Maboeta et al., 2002; Svendsen et al. 2004). Responses of the lysosomal system are generally thought to provide a first answer to pollutant exposure in a wide variety of animals including earthworms, since injurious lysosomal reactions frequently

precede cell and tissue pathology (Moore et al., 2006). The neutral red retention assay (NRRA) has been successfully applied for the *in vivo* evaluation of lysosomal membrane stability in earthworm coelomocytes (Svendsen et al., 1996; Weeks & Svendsen, 1996; Scott-Fordsmand et al., 1998; Svendsen et al., 2004, Gastaldi et al., 2007). The quantification of this biomarker is based on the time at which 50% of the cells, previously incubated with neutral red, show sign of lysosomal leaking (the cytosol becoming red and the cells rounded), as evaluated by microscopic observations. Several studies have been demonstrated that lysosomal membrane destabilization is a useful predictor of adverse effect on lifecycle parameters such as survival, reproduction, and growth (Sanchez-Hetnandez 2006).

Recently Calisi et al. (2009, 2011b) demonstrated pollutant-induced morphometric alterations in both *Eisenia fetida* and *Lumbricus terrestris* granulocytes with possible applications as sensitive, simple, and quick biomarker for monitoring and assessment applications (Calisi et al., 2009; Calisi et al., 2011b). Granulocyte morphometric alterations were determined by image analysis on Diff-Quick® stained cells (Calisi et al., 2009; Calisi et al., 2011b). The rapid alcohol-fixed Diff-Quick stain is widely utilised in clinical and veterinary applications for immediate interpretation of histological samples. It was successfully applied to earthworm coelomocyte staining (Calisi et al., 2009). Granulocytes appeared as large cells with broad pseudopodial processes; they were filled with numerous acidophilic granules, presumably corresponding to the lysosomal compartment. They are the cell type mainly involved in phagocytosis (Engelmann et al., 2002; Cooper & Roch, 2003). A considerable enlargement of granulocytes was observed in copper sulphate exposed earthworms with respect to control group. The enlargement was quantified by measuring the area of 2D digitalised granulocyte images. The same effect was observed also when the animals were exposed to xenobiotics, such as the pesticide carbamate methiocarb. Either copper sulphate or methiocarb exerted the same effect on granulocyte dimension. In general, cell swelling can result from the impairment of mechanisms regulating intracellular osmolarity, such as alteration in protein catabolism and/or amino acid and ion transport across cell membrane and it is often an indication of cell damage or metabolic alterations. Heavy metals and pesticides are known to interfere with a wide range of metabolic functions and membrane transport mechanisms (Lionetto et al., 1998; Scott Fordsman & Weeks, 2000; Sanchez-Hernandez, 2006). This could result in an increase of intracellular osmolyte content, followed by osmotic influx of water and cellular swelling. Therefore, granulocyte enlargement could be the resulting integrated effect of the impairment of several cellular functions by different classes of toxic chemicals and can be related to manifestations of sublethal injury due to pollutant exposure. Moreover, in either copper sulphate or methiocarb exposed animals the increase in the granulocyte dimension was accompanied by cell rounding with loss of pseudopods. This effect could be ascribed to toxic chemical-induced reduction of the microfilament and microspine number. This result can be assigned at alteration on actin or tubulin cytoskeletal components by either copper or methiocarb. The cytoskeleton has been demonstrated to be an intracellular target of heavy metals and xenobiotic such as pesticides and polycyclic aromatic hydrocarbon (Gomez-Mendikute & Cajaraville, 2003). The cytoskeleton has also been shown to play a role in cell volume regulation (Pedersen et al., 2001). Therefore, a possible pollutant induced alteration of cytoskeletal components could contribute to the observed morphometric alterations of earthworm granulocytes.

A pollutant induced increase in the cell size was previously documented in the granulocytes of *Mytilus galloprovincialis* (Calisi et al., 2008) following cadmium exposure. In earthworms granulocyte enlargement was similar in the two species investigated, suggesting the potential application of this response in several earthworm species. Due to the important immunological role of granulocytes, which mediate many of the innate immune responses in earthworms (Cooper et al., 2002), the observed adverse effects of pollutants on these cells may increase the susceptibility of animals to diseases and reduce their survival ability. In fact, the immune system is extremely vulnerable to injury by chemical pollutants. Major changes in the immune system can be expressed in considerable morbility and even mortality of the organisms involved. Therefore, early subtle alterations in some of the components of the immune system can be used as early indicators of altered organism health. Pollutant induced granulocyte enlargement in *Lumbricus terrestris* was consistent with alterations at the organism level, such as mortality and reduced reproduction rate, suggesting a possible link to organism health impairment. Compared to the other biological responses to pollutant, granulocyte enlargement showed high percentage variation, very similar to the values of specific biomarkers (such as MT induction in copper exposure and AChE inhibition in methiocarb exposure). This result pointed out the high sensitivity of the granulocyte enlargement with respect to other general standardized biomarkers, such as lysosomal membrane stability, and indicated its possible applications as a sensitive, simple, and quick general biomarker for monitoring and assessment applications (Calisi et al., 2009; Calisi et al. 2011b) to be included in a multibiomarker strategy. It demonstrates several of the necessary characteristics for successful application as an effective biomarker in monitoring and assessment programs. This includes an evaluation of pollutant-induced stress at the cellular level in an easy, sensitive, and inexpensive way. Moreover, it provides a sensitive generalized response to pollutants that can integrate the combined effect of multiple contaminants present in the soil.

Earthworm coelomocytes have been recently exploited for trascrittomic studies to identify genes whose expression varies during metal exposure (Brulle et al., 2010). Brulle et al. (2008) identified and assayed (by Real-Time PCR (RTPCR)) 3 transcripts that were significantly elevated in coelomocytes when *Eisenia fetida* was exposed to a metalliferous field soil from the vicinity of a Pb-smelter. These were Cd-MT, and two hitherto unstudied earthworm immunity biomarkers (lysenin, and a transcript identified as coactosin-like protein, CLP). The lysenin is a haemolytic protein, produced in coelomocytes. CLP is a member of the ADF/cofilin group of actin-binding proteins which support the activity of the 5-lipoxygenase (5-LO), an enzyme of central importance in cellular leukotriene synthesis, which are key mediators of inflammatory disorders in vertebrates.

4.6 Genotoxicity biomarkers

Coelomocyte are also interesting for ecotoxicological research and application being the cells of choice for the assessment of the genotoxic effect of pollutants on earthworms. Many pollutants in soil either metals or POPs can alter both the structure and integrity of DNA. Since DNA damage may result in severe consequences for individuals and species, it is considered as an important indicator to be used in the assessment of earthworm health (Reinecke & Reinecke 2004). However, so far there have been only a few studies which used earthworms for assessing the genotoxicity of field-contaminated soils (Button et al., 2010; Espinosa-Reyes et al., 2010; Klobučar et al., 2011; Quiao et al., 2007). The single cell gel electrophoresis (or comet assay) and micronucleus test are two most extensively used methods in the detection of genotoxicity of chemicals in the environment. Compared to other assays, they are sensitive, rapid and easy to handle. Comet assay measures DNA damage in single cells, as single- and double-strand breaks, alkali-labile sites, oxidative DNA base damage (Cotelle & Ferard, 1999). The comet assay technique involves embedding cells in agarose gel on microscope slides and lysing with detergent and high salt. Slides are then soaked in an alkaline solution to allow cleavage of DNA at alkali labile sites. During electrophoresis under alkaline conditions, cells with damaged DNA display increased migration of DNA from the nucleus towards the anode. Broken DNA migrates further in the electric field, and the cell then resembles a 'comet' with a brightly fluorescent head and a tail region which increases as damage increases. The degree of migration is related to DNA damage (Lee & Steinert 2003). The Comet assay presents various advantages, because of its sensitivity for detecting low levels of DNA damage in single cells and the relative ease of application (Tice et al., 2000). The Comet assay has been demonstrated to be effective in determining DNA damage levels in the coelomocytes of earthworms exposed to genotoxic compounds, both in vivo and in vitro, in several studies (Reinecke & Reinecke, 2004; Fourie et al., 2007; Di Marzio 2005; Bonnard et al., 2009). Dose-dependent DNA damage in earthworm coelomocytes has been demonstrated *in vivo* for chromium (Manerikar et al., 2008), cadmium (Fourie et al., 2007) nickel (Reinecke & Reinecke, 2004; Bigorgne et al., 2010) and arsenic (Button et al 2010).

Besides comet assay the micronucleus test has emerged as one one of the most powerful methods for assessing chromosome damage (both chromosome loss and chromosome breakage) accumulated during lifespan of the cell in vertebrates and invertebrates. A micronucleus is formed during cell division. It may arise from a whole lagging chromosome or an acentric chromosome fragment detaching from a chromosome after breakage which do not integrate in the daughter nuclei. Sforzini et al (2010) provided the first step of validation of this test on earthworm (*Eisenia andrei*) cells.

4.7 Haemoglobin oxidation

Changes in haematology are reported to be early warning signals of the toxic effects of pollutants in vertebrates (Bowerman et al. 2000; Dauwe et al. 2006; Rogival et al. 2006), but they are poorly explored in invertebrates and for comparison in earthworms. Earthworms have a closed circulatory system The blood contains haemoglobin which is a large extracellular hemoprotein flowing in a closed circulatory system. In spite of the fundamental role of this respiratory pigment in earthworm physiology, little is known about its sensitivity to environmental pollutants. Recently Calisi et al (2011a) demonstrated heavy metal (cadmium, copper, mercury) exposure to significantly induce changes in either Hb concentration or its oxidation state in the earthworm *Lumbricus terrestris*. Exposure to heavy metals (10^{-5}-10^{-3} M for Cd, 10^{-4}-10^{-3} M for Hg, and 10^{-4}-10^{-2} M for Cu) was found to increase blood Hb concentration. The observed effects were seen at concentrations in the order of 65 (for Cu) to 200 (for Hg) mg/l, below the LC50 value for heavy metal exposure previously observed in earthworms (Neuhauser et al., 1985). Further studies are needed to demonstrate if the observed effect is due to a metal induced increased expression of Hb protein and/or to a reduced degradation of the molecule. In addition to changes in the Hb concentration, heavy metals showed a dramatic effect on the oxidation state of the respiratory pigment. A strong dose-dependent increase of blood methemoglobin (MetHb) percentage was observed following 48 h exposure with the highest Hb oxidation sensitivity to mercury, followed by cadmium and copper. The role of trace metals in the generation of free radical mediated oxidative stress is known. This could account for the Hb oxidation observed in the earthworms during metal exposure. In addition, a direct action of metals on the earthworm haeme group cannot be excluded. In fact, copper is a known direct-acting methemoglobin-producing agent in humans directly converting Fe(II) to Fe(III) in a two-stage reaction (Smith & Reed 1993, French et al. 1995). Compared to other biological responses to heavy metals, such as the known metallothionein induction, MetHb increase showed a higher sensitivity. In fact, the lowest concentration able to significantly increase MetHb concentration was 10^{-8} M for mercury, 10^{-7} M for cadmium and 10^{-6} M for copper while 10^{-5} M was the concentration of each metal able to significantly induce metallothionein increase in the same species and in the same exposure conditions. Moreover, it is interesting to observe that MetHb formation was very suitable for routine application in monitoring assessment in terms of measurable biological response. In fact, it showed a very high percentage variation following heavy metal exposure, being about ten fold higher compared to Mt induction. Future studies will be addressed to evaluate if the observed response is specific for heavy metal exposure or represents a biomarker of general health of earthworms in polluted sites. In any case it demonstrated to be a suitable biomarker of exposure/effect to be included in a multibiomarker strategy in earthworm in soil monitoring assessment.

5. Earthworm biomarker relevance for soil pollution monitoring and assessment

Earthworm biomarkers represent useful tools in soil monitoring and assessment as an early warning of adverse ecological effects (Sanchez-Hernandez, 2006; Rodriguez-Castellanos & Sanchez-Hernandez, 2007). As indicated by Sanchez-Hernandez (2006) four types of approaches can be performed in soil pollution monitoring : 1) biomarker analysis on native earthworm populations; 2) use of transplanted organisms in *in situ* exposure bioassays; 3) exposure of a selected earthworm population to the environmental medium (soil) in laboratory standardized conditions; 4) simulated field studies. The use of natural population offers the advantage of an ecologically more relevant approach to environmental monitoring and assessment. In addition the usefulness of native organisms arises mainly when studying pollutant long-term effects that may be emphasized in organisms from natural populations. In fact it is difficult to extrapolate effects from spiked soils to field soils, when these are already polluted for a long time. The bioavailability of pollutants in comparable soil types polluted in the field or spiked in the lab is different (Smolders et al., 2003). Soil characteristics, e.g. pH, organic matter and clay content (Peijnenburg et al., 2002) also play an important role in determining the bioavailability of pollutants. Using native earthworm populations for biomarker analysis integrates the bioavailability of pollutants, exposure pathways and temporal aspect of exposure (Spurgeon et al., 2002; Sanchez-Hernandez, 2006). However, so far only few studies have explored the potentiality of the biomarker approach to native earthworms, if compared with studies on aquatic environments. For example Laszczyca et al. (2004) found spatial and temporal variation of AChE and antioxidant enzymes in three natural earthworm population (*Aporrectodea caliginosa, Lumbricus terrestris and Eisenia fetida*) collected from meadow sites along a 32 km long transect from a Zn/Pb ore mine and a smelter metallurgic complex. Lukkari et al (2004b) documented an increase in the response of three biomarkers (metallothionein, cytochrome P4501A, glutathione transferase) along a 4 km long transect from an area contaminated by a steel smelter. The response of the three biomarkers was positively correlated with decreasing distance from the steel smelter. Moreover, Svendensen et al (2003) demonstrated the validity of using NRRA in biological impact assessment along gradients of contamination. The authors collected earthworms (*Lumbricus castaneus*) at the site of a large industrial plastics fire in Thetford, UK along a 200 m transect leading from the factory perimeter fence. NRRA response was positively correlated with decreasing distance from the factory. While metal residues in soil and earthworms were found to be highly elevated close to the factory perimeter and to rapidly drop to background levels within the first 50 m of the transect, the NRRA values were significantly different from the NRRA determined in control animals also in the sourrounding forest along the transect. This results shows that NRRA determination represents a more sensitive indicator of pollutant exposure along a contamination gradient with respect to the analytical metal residue determination. Most of the available studies have been carried out on earthworm population inhabiting areas contaminated with heavy metals (Button et al., 2010). However, in some cases the employment of native earthworms to determine soil toxicity (particularly in studies of long-term exposure) is complicated by the fact that some populations appear to have developed a resistance to metals in soil (Spurgeon & Hopkin, 2000).

In the case of transplanted organisms earthworms can be collected from a population at one location and translocated to the monitoring sites. This approach provides the advantage of ensuring comparable biological samples, reducing the variability of results usually encountered in field sampling programmes. Using caged organisms in biomonitoring studies, as well as in related research, makes it easier to standardize the results and to compare control organisms to animals collected from potentially polluted sites.

The application of the biomarker approach to a selected earthworm population exposed to the test soil in laboratory standardized conditions offers a complementary approach to standard toxicity tests (i.e., mortality and reproduction rates) to investigate the effects of contaminant toxicity on living organisms at earlier stages and lower concentrations (Lukkari et al. 2004; Gastaldi et al. 2007; Schreck et al. 2008). Appropriate biomarkers may be applied in standardized bioassays to provide evidence of the cause-effect relationship between soil contaminants and toxic effects in the individuals. Biomarkers can give a contribution in acute bioassays as a measurement of the bioavailable and bioactive fraction of contaminants and in chronic bioassays as sublethal endpoints (Sanchez-Hernandez, 2006). For example, many studies have reported that the lysosomal destabilization linearly correlate with the bioavailable fraction of heavy metals in the soil (Sanchez-gHernandez, 2006). In addition, NRRA was demonstrated to be more sensitive to Cu exposure than reproduction rate (Scott-Fordsmand et al., 2000). The need to develop biomarkers for earthworms to supplement standard toxicity tests has been widely discussed by Van Gestel & Weeks (2004) and Sanchez-Hernandez (2006).

Simulated field studies offer the opportunity to study the effects of pollutants on the biomarker responses in the organisms under the influence of multiple environmental variables (for review see Sanchez-Hernandez, 2006). They can be carried out in microcosm or mesocosm. Soil microcosm experiments are carried out in laboratory scale, under standardized ambient conditions, while mesocosm experiments are carried out in field scale and are functionally closer to the real environmental scenarios.

For the potential of the biomarker approaches to be realized in soil monitoring and assessment a crucial aspect need to be clearly addressed. Ideally, the degree to which the magnitude of the biological response relates to the dose of exposure should be known, enabling severity of the exposure to be clearly assessed. In general, the extent to which a molecular or cellular response occurs is generally related to the dose of chemical received. Nevertheless, exposure to low doses may produce no effects because of the presence of a threshold level of effect, variable for each responses. In other cases where the threshold level is exceeded, protective mechanisms may mask the effects, such as the induction of metallothionein. In addition contaminated soil typically contains a complex mixture of contaminants that often interact and the organisms are exposed to multiple chemicals and multiple stresses which can confound a simple dose-response curve. It is important, however, to note that although it is useful to understand the toxic responses of contaminants and how they alter with dose and over time it is harder to quantify these responses especially in field conditions in terms of dose-response. It is well understood that no one biomarker has been validated as unique tool of detecting specific pollutant exposure and effects. The biological response of an organisms to pollutant exposure can be various because of the variety of pollutants that may be present in the environment. Thus, a suite of biomarkers is required to be effectively applicable in a biomonitoring programme. By using a suite of biomarkers future attempts should be made to try and develop a quantitative biomarker index that could simplify the complex biological alterations measured by multiple biomarkers into a single, predefined quality class.

6. Conclusions

During the last years, earthworm biomarkers have become increasingly relevant for the evaluation of contaminants effects on soil organisms However, the application of the biomarker approach to soil pollution monitoring, compared to aquatic environment monitoring, is recent and some aspects need to be further evaluated. First, it is necessary to identify and characterise appropriate sentinel earthworm species to be used as field collected organisms, in order to provide a quick assessment of soil pollution. Second, earthworm biomarkers studies have been mostly conducted for heavy metals. Thus, developing biomarkers of exposure/effects to a wider range of chemicals of concern for soil pollution constitutes a major demand. Third, there is a growing interest for increasing the knowledge of biological responses of earthworms to pollutants in order to standardize a suite of sensitive and reliable biomarkers for the detection of the pollutant induced stress syndrome in soil organisms. Earthworm physiological fluids, such as coelomic fluids and blood, offer an interesting field for exploitation of novel sensitive non destructive biomarkers, including cytological, biochemical and trascriptomic parameters. Thus, granulocyte morphometric alteration has been recently demonstrated as a suitable general biomarker of effect that could be included in a multibiomarker strategy. It provides a sensitive generalized response to pollutants that can integrate the combined effect of multiple contaminants present in the soil. Finally, earthworm biomarkers have been scarcely investigated under field conditions. The most studies on earthworm biomarkers have been carried out in laboratory condition, but only few studies are available which used native earthworm populations for assessment of polluted soils. Hence, there has been a growing interest in field studying earthworm biomarkers and validating their effectiveness in the field conditions as an early warning of adverse ecological effects. This represents an attractive field of research in the light of the growing interest in the use of earthworm biomarkers as valuable tools for soil pollution monitoring and assessment.

7. References

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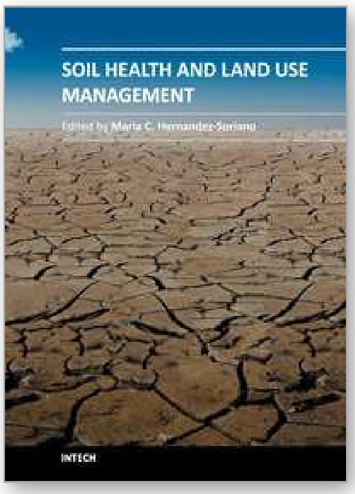

Soil Health and Land Use Management

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Soils play multiple roles in the quality of life throughout the world, not only as the resource for food production, but also as the support for our structures, the environment, the medium for waste disposal, water, and the storage of nutrients. A healthy soil can sustain biological productivity, maintain environmental quality, and promote plant and animal health. Understanding the impact of land management practices on soil properties and processes can provide useful indicators of economic and environmental sustainability. The sixteen chapters of this book orchestrate a multidisciplinary composition of current trends in soil health. Soil Health and Land Use Management provides a broad vision of the fundamental importance of soil health. In addition, the development of feasible management and remediation strategies to preserve and ameliorate the fitness of soils are discussed in this book. Strategies to improve land management and relevant case studies are covered, as well as the importance of characterizing soil properties to develop management and remediation strategies. Moreover, the current management of several environmental scenarios of high concern is presented, while the final chapters propose new methodologies for soil pollution assessment.