Journal of Novel Applied Sciences
Available online at www.jnasci.org
©2015 JNAS Journal-2015-4-2/125-134
ISSN 2322-5149 ©2015 JNAS

Influence of heavy metals on earthworms, soil microbial community and mammals

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ABSTRACT: The definition of heavy metals varies in the scientific literature. However, the most wide definition consider heavy metals as all of those metals whose atomic weight is more than that of hydrogen. According to another definition, a heavy metal is a metal, whose density is above 5 g/cm3. Earthworms play an import role in soil ecosystems, since they contribute to organic matter incorporation and decomposition, excavation of burrows and production of casts. A decrease in earthworm abundance caused by soil heavy metal contamination may diminish these ecological functions. Mammals used as a source of biomarkers should have a high abundance in urban areas, a low rate of migration and be limited to a small space to ensure the detection of local phenomena according to the variety of local sources in cities. Soil microbial community structure and activity depend to a large extent on the status of their soil habitat. Within this habitat, soil organisms are eating, respiring, competing, cooperating, and responding to changes in their immediate environment. Indeed, the majority of the microbial community may be dormant at any given time in most soils, ready to respond as conditions for a particular group become favorable.

Keywords: biomarkers, Phospholipid fatty acid, Metabolic quotient, Vermicomposting.

INTRODUCTION

Humans have converted one-quarter of the Earth's surface to cultivated systems, largely by changing native ecosystems to arable lands within the past 50 years (Millennium Ecosystem Assessment 2005). Agricultural expansion and intensification is likely to accelerate as the world human population grows, and soil functions at all levels will be affected, including the structure and functions of soil microbial communities. An important area of study is community-level responses to land-use changes rather than effects particular species or groups such as arbuscular mycorrhizal fungi or nitrogen-fixing bacteria, which are well known to play key roles in agroecosystems. It is well established that the conversion of native ecosystems to agricultural uses can strongly affect microbial community structure, composition and diversity. For example, conversions of tropical forest to plantations (Waldrop . 2000) have been found to engender distinct soil microbial community structures, and agricultural intensification has been reported to decrease microbial diversity (Steenwerth . 2006). Additionally, the type of land management practices used in agro ecosystems also affects microbial community structure and function through a variety of different mechanisms. Numerous studies have documented changes in microbial community structure resulting from physical disturbance, especially tillage (Frey . 1999; Guggenberger . 1999). Tillage represents a severe disturbance to fungi by severing hyphal connections. However, no-till systems favour the development of fungi as compared with bacterial community components (Kennedy and Schillinger 2006, Minoshima . 2007). Conversions to agriculture and attendant cultivation practices also alter microbial communities through changes to temperature, soil moisture (through irrigation and alteration of soil structure), and other physical parameters. Land-use changes alter soil microbial community structure through changes in carbon availability and quality, pH (Cookson . 2007), nutrient availability, or other chemical parameters. For example, studies comparing agroecosystems and natural systems report that adding nitrogen decreases the relative abundance of fungi by comparison to bacteria (Bardgett . 1999 , Bradley . 2006). Seghers . (2004) found that nitrogen fertilizers decreased populations of methanotrophs and root endophytes in the bulk soil microbial community. They also found differential effects of organic fertilizers versus inorganic, which was consistent with other studies. For example, Cookson . 2007 and Wander . (1995) reported that manure-amended plots showed less diverse populations of microorganisms than cover cropped soil, but that microbial biomass was more metabolically active

(Wander . 1995). Ulrich . (2008) found that manure applications led to an increase in the population densities of cellulytic bacteria within the soil microbial community (see chapters elsewhere in this book). Fungal-to-bacterial ratios are commonly measured as indicators of microbial community structure, and the relative proportions of fungi are increased by no-till practices, crop rotations, and use of cover crops (Six . 2006).

Heavy metals

The definition of heavy metals varies in the scientific literature. However, the most wide definition consider heavy metals as all of those metals whose atomic weight is more than that of hydrogen. According to another definition, a heavy metal is a metal, whose density is above 5 g/cm3 (Anon., 1964; Lapedes, 1974; Sherlock, 1985). Heavy metal toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems of our modern society. Heavy metals are natural components of the Earth's crust and they enter into the biosphere both naturally and as a result of human activities; mostly from industry, traffic and in purities of fertilizers. The metals are predominantly transferred as molecules or particulate matter via the atmosphere, mostly over distances. The amount of anthropogenic ally derived heavy metals has increased continuously since the beginning of the industrial revolution but public awareness and concern associated with their environmental and health risks have risen sharply in the last decades. One focal point of metal emissions is congested urban areas where there is a high density of industry and traffic (Komarnicki ., 2005). The soil inherits heavy metals from its parent's materials. Some soils have been found to have a high background of some heavy metals, which are toxic to plants and wildlife, i.e. these soils contain extremely high concentrations of these elements in the parent materials (He ., 2005).

Primary sources pollution of heavy metals

Primary sources of pollution are from the burning of fossil fuels, mining and melting of metallic ferous ores, municipal wastes, fertilizers, pesticides, and sewage sludge (Peng ., 2006; Xiong, 1998). The pollution of surface waters is significant, though on a local scale. However. the heavy runoff associated with perennial rainfall may mitigate the impact. Several small and heavy industries involved in activities such as battery and paint manufacturing, petroleum refining, cement and ceramic production, steel production, are now being located haphazardly, mostly near metropolitan centres. No centralized sewage systems exist, and the industrial effluents from the factories are usually discharged untreated into streams, lagoons, open drains and other water bodies. This has resulted in high, although not alarming, levels of lead, cadmium and copper in some localized areas. Studies of heavy metal contents of surface waters at Ibadan show that levels of Pb may reach 50 J.Lg/litre(Mombeshora ., 1983). In waters of the Lagos lagoon, Pb levels exceeding 120 J.Lg/litrehave been obtained (Olade unpublished data). It is a common practice to dump solid municipal waste into streams traversing urban areas. Because of the natural acidity (pH) of these waters and oxidation, heavy metal contaminants are being released into stream waters. Although surface water pollution has often been assumed to be a minor problem in developing countries of tropical humid regions, it has become clear that our freshwater resources are limited and care should be taken to protect them. There is an urgent need to conduct environmental impact assessment studies before industries are located, so as to ameliorate environmental pollution from point sources. The most common heavy metal contaminants are: Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni) and Zinc (Zn) (Lasat ., 2001; USEPA, 1997).

Cleaning of polluted areas

Unlike organic compounds, metals cannot be degraded, and their cleanup requires their immobilization and toxicity reduction or removal. In recent years, scientists and engineers have started to generate cost effective technologies which includes use of microorganisms/ biomass or live plants for cleaning of polluted areas (Kuzovkina , 2004; Qui ., 2006). Growing plants can help contain or reduce heavy metal pollution. This is often called phytoremediation (EPA, 1988). It has the advantage of relatively low cost and wide public acceptance (Schnoor, 1997). It can be less than a quarter of the cost of excavation or in situ fixation. Phytoremediation has the disadvantage of taking longer to accomplish than other treatment . Plants can be used in different ways. Sometimes a contaminated site is simply revegetated in a process called phytostabilization. The plants are used to reduce wind and water erosion that spread materials containing heavy metals. In one example, grass or tree buffers could reduce sediment loss from the chat piles at a contaminated site in Galena, Kansas, anywhere from 18% to 25% , If all of the ground could be vegetated, sediment loss could be cut by approximately 70% (Green . 1997). Excavation and physical removal of the soil is perhaps the oldest remediation method for contaminated soil. It is still in use at many locations, including residential areas contaminated with lead in southwestern Missouri. Advantages of excavation include the complete removal of the contaminants and the relatively rapid cleanup of a contaminated site (Wood, 1997).

Earthworms play an import role in soil ecosystems

Earthworms play an import role in soil ecosystems, since they contribute to organic matter incorporation and decomposition, excavation of burrows and production of casts. A decrease in earthworm abundance caused by soil heavy metal contamination may diminish these ecological functions (Lee, 1985; Edwards and Bohlen, 1996). Earthworms are relatively localized animals and therefore in field studies their limited mobility makes them very suitable for monitoring the impact of contaminants (Pankakoski ., 1994). Generally, earthworms can be used as an indicator of soil impairment because their abundance and biomass decrease with soil contamination (Belotti, 1998; Spurgeon ., 1999; Lukkari ., 2004). Earthworms are the docile creatures of soil ecology. Earthworms are also called the 'ecosystem engineers' (Lavelle ., 1997; Hale ., 2005) as they have great potentiality to change soils and plant communities. Earthworms are macro fauna commonly found in the tilled soils, grasslands and other agro ecosystem. About 800 genera and 8000 species of earthworm are recorded in the world (Edward, 2004) which belongs to the order Oligochaeta. Many authors agree on the beneficial role of earthworm in the soil but few reviewers and researchers point to the negative effects (Agarwal ., 1958; Rose and wood, 1980) of introduced earthworm in the soil fertility and crop production. Barios . (1987) reported the positive effects of earthworms in nutrient mineralization and release of nutrients in the soil system. Three major types of earthworms found in the soil ecosystem; which are classified as a) Epigeic, b) Endogeic and c) Anecic (Bouche, 1972).

Earthworms are good biomarkers

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 ., 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. In the context of bio monitoring, the earthworms are good biomarkers to predict and to monitor the total-soil metal concentration and provide early warning signals of potential danger to the biota (Morgan ., 1988; Heikens ., 2001; Burgos ., 2005). However, there is also evidence that the accumulated levels in earthworms do not consistently reflect the metal contamination level of the soil. In acidic sandy soils, cadmium can accumulate in earthworms to a considerable extent, even though the soil contamination level may be rather low. Earthworms accumulate much more lead from contaminated acidic sandy soils than from soils which have been limed (Ma, 1987 and 1989).

The relationships between earthworms and heavy metal concentrations

In a study carried out in the U.K. (Morgan ., 1988) earthworms (Lumbricus rebellus and Dendrodrilus rubidus) were sampled from uncontaminated and known metal-contaminated sites. Significant positive correlations were found between the earthworms and 'total' (conc. nitric acid-extractable) soil cadmium, copper, lead and zinc concentrations. The relationships were linear, and the accumulation patterns for both species were similar as far as a single metal was concerned, even though there was a species difference in the mean metal concentrations. Earthworm cadmium concentration exceeded that of the soil, home to the worms; in contrast, the earthworm lead levels were lower than the soil lead concentration in all but one (an acidic, low soil calcium) site. Soil pH coupled with a cation-exchange capacity and soil calcium had a major influence on lead accumulation and cadmium accumulation may be suppressed in extremely organic soils (Morgan ., 1988). Heavy metal concentrations in organic rich soils close to the emission sources (1, 2, and 4 km) were high enough to have harmful effects on earthworms and their environments. In general, diversity, total numbers, and biomass of earthworms increased with increasing distance from the emission sources. Positive correlations between metal concentrations in the earthworms and those in the soils were observed (Heikens ., 2001; Lukkari ., 2004). Generally earthworms increase the mobility and availability of metals and metalloids in soils (Sizmur and Hodson, 2009). This can result in greater concentrations of metals leaching out of the soil into ground water (Tomlin ., 1993) or greater uptake into plants (Ma ., 2003; Yu ., 2005; Wang ., 2006) and soil animals (Currie ., 2005; Coeurdassier ., 2007). In addition to this, earthworms may reduce the efficiency of soil remediation by mobilising recalcitrant metals (Udovic ., 2007). The mechanisms for earthworms increasing metal mobility and availability are unclear, but may involve changes in microbial populations, pH, dissolved organic carbon or metal speciation (Sizmur and Hodson, 2009).

Mammals used as a source of biomarkers

Mammals used as a source of biomarkers should have a high abundance in urban areas, a low rate of migration and be limited to a small space to ensure the detection of local phenomena according to the variety of local sources in cities (Wren, 1986; Fliclinger and Nichols, 1990). This is the case of the mole (Talpa europaea L.) which lives to an age of up to 6 years (Lodal and Grue, 1985). In general, insectivores accumulate more potentially harmful metals like cadmium and lead than other investigated, predominantly herbivorous, small mammal species (Wren, 1986; Ma ., 1991). One disadvantage, however, lies in the mole’s restricted occurrence to particular soil types (Oppermann, 1968; Milner and Ball, 1970; Funmilayo, 1977 and 1979). In Finnish rural areas, the concentrations of cadmium, copper, zinc, molybdenum and lead in the liver and kidneys of moles, Talpa europaea L. (Insectivora) were lower in juveniles except for zinc in the liver, which was lower in adults. If the animals were divided according to their age (0–6 years), cadmium and molybdenum concentrations in the liver increased significantly with age, while concentrations of copper, zinc and chromium tended to decrease. Female moles had higher lead concentrations than males, especially adult females, which also had lower levels of copper in their liver than the adult males. Moles in the metropolitan area of Helsinki clearly differed from those in rural areas in that the concentrations of heavy metals in these moles were higher (especially for the most toxic metals: cadmium, lead and mercury), and their body weight was lower (Pankakoski ., 1993). The accumulation of heavy metals in moles reflects the bioavailability of these metals to earthworms (Ma ., 1989; Pankakoski ., 1993; Komarnicki ., 2000). However, accumulated levels in earthworms and moles do not consistently reflect the metal contamination level of the soil. In acidic sandy soils cadmium can accumulate in earthworms to a considerable extent, and critical levels of cadmium toxicity in moles which eat the worms can be exceeded even though the soil contamination level may be rather low. Earthworms and moles also accumulate more lead from contaminated acidic sandy soils than from soils which have been limed. At the same level of soil contamination, lead can exceed critical levels of toxicity in moles living in acidic sandy soils, while the animals reveal no elevated tissue levels if they live around limed sites (Ma, 1987).

Vermicomposting as a cleanup technique

One of the methods is the usage of earthworms to clean up the soil from various pollutants, such as heavy metals, by the process of vermincomposting (Bianchina, 2009; Chhotu and Fulekar, 2009). Vermicomposting represents an excellent treatment method for contaminated soils, not only for waste reduction but also to recondition the soil. Vermicomposting constitutes a special form of composting in which earthworms metabolize and excrete a mixture of soil and organic matter. In the digestive system of these worms, microorganisms transform organic species (proteins, nucleic acids, fats, carbohydrates, etc.) into more stable products in the process of vermicompost (Bianchina, 2009). In addition, earthworms are able to clean up the soil from various pollutants, and are able to accumulate heavy metals on their bodies from the soil too. The process of vermicomposting is used to treat heavy metal contaminated soil through bioaccumulation and conversion to non-toxic forms (Jain and Singh, 2004). Vermicomposting offers a solution to tonnes of organic agro-wastes that are being burned by farmers and to recycle and reuse these refuse to promote our agricultural development in more efficient, economical and environmentally friendly manner. The role of earthworms in organic solid waste management has been well established since first highlighted by Darwin (1881) and the technology has been improvised to process the waste to produce an efficient bio-product vermicompost (Kale ., 1982; Ismail, 1993, Ismail, 2005). Epigeic earthworms like Perionyx excavatus, Eisenia fetida, Lumbricus rubellus and Eudrilus eugeniae are used for vermicomposting but the local species like Perionyx excavatus has proved efficient composting earthworms in tropical or sub-tropical conditions (Ismail, 1993; Kale, 1998). The method of vermicomposting involving a combination of local epigeic and anecic species of earthworms (Perionyx excavatus and Lampito mauritii) is called Vermitech (Ismail, 1993; Ismail, 2005). The compost prepared through the application of earthworms is called vermicompost and the technology of using local species of earthworms for culture or composting has been called Vermitech (Ismail, 1993). Vermicompost is usually a finely divided peat-like material with excellent structure, porosity, aeration, drainage and moisture holding capacity (Edwards, 1982, 1988). The nutrient content of vermicompost greatly depends on the input material. It usually contains higher levels of most of the mineral elements, which are in available forms than the parent material (Edwards and Bohlen, 1996).

Vermicomposting improves different properties of soil

Vermicompost improves the physical, chemical and biological properties of soil (Kale, 1998). There is a good evidence that vermicompost promotes growth of plants (Lalitha ., 2000) and it has been found to have avourable influence on all yield parameters of crops like wheat, paddy and sugarcane (Ismail, 2005). Vermiculture is the culture of earthworms and vermicast is the fecal matter released by the earthworms (Ismail, 2005). Many agricultural industries use compost, cattle dung and other animal excreta to grow plants. In today’s society, we are faced with the dilemma of getting rid of waste from our industries, household etc. In order for us to practice effective waste management we can utilize the technology of vermicomposting to effectively manage our waste. This process allows us to compost the degradable materials and at the same time utilize the products obtained after composting to enhance crop production and eliminate the use of chemical fertilizers. As indicated by Ansari and Ismail (2001), the application of chemical fertilizers over a period has resulted in poor soil health, reduction in produce, and increase in incidences of pest and disease and environmental pollution. In order to cope with these trenchant problems, the vermin-technology has become the most suitable remedial device (Edwards and Bohlen, 1996; Kumar, 2005).

Effect of earthworms on soil pH

During their feeding process, earthworms change various properties of ingested soil, including the pH, which is kept in a neutral range even if the bulk soil pH is below 6.0 (Edwards and Bohlen, 1996; Mrsic, 1997). Soil pH is a significant factor affecting the mobility and bioavailability of heavy metals in soil, hence the importance of determining its change due to earthworm activity. The pH of L. rubellus casts produced in non-remediated soil (6.69) was not different ($p < 0.05$) from the slightly acidic soil itself (6.64), while in E. fetida casts, the pH was significantly higher (6.99). The pH values measured in casts produced by both earthworm species in remediated soil were significantly lower for L. rubellus and E. fetida, respectively compared to the pH of the rest of the soil. To our knowledge, no other data for the pH of earthworm casts produced in remediated soil are available in the scientific literature. However, the significant change of pH towards neutral values in E. fetida casts in both remediated and non-remediated soil is congruent with the results of Wen . (2004) for soils with E. fetida activity. Changes in pH value could be attributed to the activity of the earthworm’s calciferous glands or to their alkaline urine (Salmon, 2001). However, the exact mechanisms are still unclear. Our results indicate the active role of earthworms in regulation of soil pH. This should be considered in after-remediation risk assessments. It has already been reported that earthworm gut conditions modify the mobility of metals due to pH change and thus favour their assimilation (Weltje, 1998).

Earthworms and organic solid waste management

In recent years, disposal of organic wastes from various sources like domestic, agriculture and industrial has caused serious environmental hazards and economic problems. Burning of organic wastes contributes tremendously to environmental pollution thus, leading to polluted air, water and land. This process also releases large amounts of carbon dioxide in the atmosphere, a main contributor to global warming together with dust particles. Burning also destroys the soil organic matter content, kills the microbial population and affects the physical properties of the soil (Livan and Thompson, 1997). It has been demonstrated that earthworms can process household garbage, city refuse, sewage sludge and waste from paper, wood and food industries (Kale ., 1982; Senapati and Dash, 1982; Muyima ., 1994; Edwards and Bohlen, 1996; Ismail, 2005). In tropical and subtropical conditions Eudrilus eugeniae and Perionyx excavatus are the best vermicomposting earthworms for organic solid waste management (Kale, 1998). The use of earthworms in composting process decreases the time of stabilisation of the waste and produces an efficient bio-product, i.e., vermicompost. Organic farming system is gaining increased attention for its emphasis on food quality and soil health. Vermicompost and vermiculture associated with other biological inputs have been actually used to grow vegetables and other crops successfully and have been found to be economical and productive (Ismail, 2005; Ansari and Ismail, 2008; Ansari and Jaikishun, 2011). In this regard, recycling of organic waste is feasible to produce useful organic manure for agricultural application. Compost is becoming an important aspect in the quest to increase productivity of food in an environmentally friendly way. Compost is becoming an important aspect in the quest to increase productivity of food in an environmentally friendly way. Vermicomposting offers a solution to tonnes of organic agro-wastes that are being burned by farmers and to recycle and reuse these refuse to promote our agricultural development in more efficient, economical and environmentally friendly manner. Both the sugar and rice industries burn their wastes thereby, contributing tremendously to environmental pollution thus, leading to polluted air, water and land. This process also releases large amounts of carbon dioxide in the atmosphere, a main contributor to global warming together with dust particles. Burning also destroys the soil organic matter content, kills the microbial population and affects the physical properties of the soil (Livan and Thompson, 1997). Therefore organic farming helps to provide many advantages such as; eliminate the use of chemicals in the form of fertilizers/pesticides, recycle and regenerate waste into wealth; improve soil, plant, animal and human health; and creating an ecofriendly, sustainable and economical bio-system models (Ansari and Ismail, 2001a, b).

Soil microbial community structure

Soil microbial community structure and activity depend to a large extent on the status of their soil habitat. Within this habitat, soil organisms are eating, respiring, competing, cooperating, and responding to changes in their immediate environment. Indeed, the majority of the microbial community may be dormant at any given time in most soils, ready to respond as conditions for a particular group become favorable (Stenström . 2001). Soil microbes are primary mediators of organic matter decomposition and nutrient cycling, and thus play a key role in maintaining function and sustainability of terrestrial ecosystems. Microbial biomass and basal respiration, as important indicators of soil microbial community size and activity, have been included in current soil monitoring concepts because of their sensitive responses to environmental changes and anthropogenic disturbance (Filip, 2002). Generally, soil microbial features show clear seasonal changes due to the fluctuation of soil temperature and moisture, and crop development (Debosz ., 1999; Mandal ., 2007). However, seasonal changes of microbial biomass and activity in surface and subsurface soils have often shown inconsistent trends, strongly depending on the particular ecosystems (Chen ., 2005). The soil habitat is perhaps best envisioned as a complex matrix with pores and soil aggregates of differing sizes (Sylvia . 2005). Certain bacteria and fungi tend to congregate in the soil immediately adjacent to plant roots (the rhizosphere), where they may feed off the sugars that plant roots exude or actually physically associate with the plant root system and exchange sugars and nutrients in a (usually) mutualistic relationship (mycorrhizas). The soil community and its habitat are influenced by an interconnected web of variables that differ among ecosystems, making each ecosystem somewhat unique in its microbial community (Wixon and Balser 2009). Across the globe, as with vegetation, community structure is perhaps most influenced by soil temperature and moisture (Sylvia . 2005), though it changes with the seasons (e.g. Lipson 2007), and is strongly affected by soil acidity or alkalinity (pH). Within a given ecosystem, depth in soil is a primary consideration for microbial habitat, and many key habitat characteristics (e.g. oxygen levels, availability of food and nutrients) change through the soil profile. Carbon availability (and often quality) declines, as does overall microbial biomass (Fig. 1). Soil structure, such as particle size fractions and stable aggregates also change with depth and impact the soil biological habitat (Van Gestel . 1996; Ranjard . 2000; Sessitsch . 2001; Poll . 2003). Few studies have considered, however, the importance of both soil parent material and resultant soil texture (Ulrich and Becker 2006; Rasmussen . 2007). Soil organic carbon (SOC) is the largest terrestrial component of the global carbon budget (Jobbágy and Jackson 2000). Worldwide, the top 1 m of soil contains two to three times more carbon than the amount stored in all aboveground vegetation (Brady and Weil 2002). Studies of soil carbon and microbial communities often concentrate on the upper 20–30 cm of soil, as this is considered to be the most biologically active portion of the soil profile (Fierer . 2007; Jobbágy and Jackson 2000; Veldkamp . 2003; Baisden and Parfitt 2007; Goberna . 2006). However, the majority of carbon in soil occurs below 20 cm, and thus by discounting lower depths we are missing up to 50–65% of the carbon (Jobbágy and Jackson 2000). As a result, many current ecosystem models of land-use and climate change inadequately model carbon turnover and microbial communities because they disregard the carbon stocks and biological activities of deeper soil horizons (Baisden and Parfitt 2007). Generally, most microbial community studies down the soil profile have occurred in grasslands and agricultural lands (Fierer . 2007; Lavahun . 1996; Blume . 2002; Taylor . 2002; Allison . 2007).

The impacts heavy metal levels on soil microbial communities

The impacts of elevated heavy metal levels on the size and activity of natural soil microbial communities have been well documented. Field studies of metal contaminated soils have demonstrated that elevated metal loadings can result in decreased microbial community size (Jordan and LeChevalier 1975; Brookes and McGrath 1984; Chander and Brookes 1991; Konopka . 1999) and decreases in activities such as organic matter mineralization (Chander and Brookes 1991) and leaf litter decomposition (Strojan 1978). Remediation strategies for metal contaminated soils seek to reduce the biological impact of the metals by removing the metals or reducing their bioavailability. Soils at a field site which was contaminated with heavy metals due to the operation of a zinc smelter were remediated by surface application of sewage sludge. This remediation program resulted in increases in microbial community size and total heterotrophic activity, suggesting a recovery of the microbial community (Kelly and Tate 1998). However, measures of total microbial community size or activity focus on the community as a whole, and questions remain as to how metal contamination and remediation affect specific populations within soil microbial communities. It has been demonstrated that changes in specific microbial populations can occur even when total microbial community size remains unchanged (Pennanen 2001). Therefore, it has been suggested that measures of microbial community structure may be more sensitive to disturbance than assays which focus on general microbial processes or overall community size (Kennedy and Smith 1995). Collins and Stotzky (2001) stated that microorganisms interact with metals in various ways: many metals are essential to microorganisms, because they are electron acceptors or cofactors in enzymes, whereas other metals are toxic. Leita . (1995) studied influence of Pb, Cd, and Tl on microbial biomass survival and activity during a laboratory incubation of soil. In comparison to uncontaminated soil, the microbial biomass C decreased sharply in soil contaminated with Cd and Tl, whereas the addition of Pb did not have any significant inhibitory effect on the level of microbial biomass C. Heavy metals influenced microorganisms by affecting their growth, morphology, and biochemical activities.

Estimate the size of the microbial biomass in soil.

A variety of methods exists to estimate the size of the microbial biomass in soil. Of these methods, the most simple and rapid is substrate (glucose) induced respiration (SIR), which stimulates a maximal respiratory response from the soil biomass, measured conduct metrically as CO2 evolution, and methods currently available are those involving direct counting in which microorganisms can be variously stained, and relates this respiration to biomass C (Anderson and Domsch, 2000). Soil microbial biomass is a living pool containing 1- 5% of the soil organic matter (Jenkinson & Ladd, 1981; Sparling, 1992), excluding root, meso- and macro-fauna. Its activity and often fast turnover impact soil characteristics affecting its quality by conduction of biochemical transformation of organic matter being a source or a sink of plant nutrients (De-Polli & Guerra, 1999; Franzluebbers, 2002, Haubensak ., 2002; Hargreaves ., 2003). Microbial biomass determinations may indicate changes in the soil organic matter before they can be detected by measuring total soil carbon (Jenkinson & Ladd, 1981; Powlson ., 1987) making possible its use as an indicator of early changes in soil organic matter content (Costantini ., 1996; Cosentino ., 1998). Belowground phenomena have been of increasing interest due to its role on agricultural production, soil genesis and nutrient cycling, greenhouse gases mitigation and emissions, bioremediation, biological control of plant pest and diseases, plant growth promoting factors, source of organisms for industrial use. Those aspects are to some extension mediated by the soil microbial biomass. The basic concept of treating the entire soil microbial population as a single entity (Powlson, 1994) was proposed by Jenkinson (1966). Soil microbiota treated as a living mass in a “black box” is to some extension a holistic approach when it considers the behavior of this large and important pool, but it is reductionist when refers its huge biodiversity to a single mass measurement. However, this biodiversity is not to be neglected because it may account for the large spatial variability of the microbial biomass measurements.

Isolation-based techniques

Several researchers, using isolation-based techniques, have demonstrated that heavy metal contamination can cause shifts in microbial populations (Barkay . 1985; Doelman . 1994; Gingell . 1976; Roane and Kellogg 1996). However, isolation-based techniques are limited as they provide information on only a small component of the microbial community since only a small percentage of soil microbes are culturable (Ward . 1990).

Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acid (PLFA) analysis is a procedure which is useful for evaluating microbial community structure. Changes in PLFA profiles are indicative of changes in the overall structure of microbial communities (Frostegrd . 1996) and “signature” PLFAs can provide information on specific groups of microorganisms present in a community (Frostegrd . 1993a). PLFA analysis offers an advantage over isolationbased techniques because it avoids the selectivity inherent in the isolation of microorganisms (Cavigelli . 1995). Previous work has shown that metal contamination can result in shifts in PLFA profiles for soil microbial communities (Pennanen . 1996; Griffiths . 1997).

Metabolic quotient

Ecophysiological indices (metabolic quotients) are generated by basing physiological performances (respiration, growth/death, carbon uptake) on the total microbial biomass per unit time. Any environmental impact which will affect members of a microbial community should be detectable at the community level by a change of a particular total microbial community activity which can be quantified (qCO2, etc.) (Anderson, 2003). The ratio of biomassCto soil organicC(Cmic:Corg) reflects the contribution of microbial biomass to soil organic carbon (Anderson and Domsch, 1989). It also indicates the substrate availability to the soil microflora or, in reverse, the fraction of recalcitrant organic matter in the soil; in fact this ratio declines as the concentration of available organic matter decreases (Brookes, 1995). The qCO2 (the community respiration per biomass unit or the metabolic quotient) has been widely used in literature and is originally based on Odum’s theory of ecosystem succession. Although its reliability as a bioindicator of disturbance or ecosystem development has been recently criticised by some authors, it is recognized to have valuable application as a relative measure of howefficiently the soil microbial biomass isutilizing C resources and the degree of substrate limitation for soil microbes (Wardle and Ghani, 1995; Dilly and Munch, 1998).

REFERENCES

Anderson JPE and Domsch KH. 2000. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem., 10: 215-221.

Anon. 1964. Encyclopaedia of Chemical Science. Princeton, Van Nostrand, pp. 533.

Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS and Davies WJ. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Funct Ecol 13(5):650–660

Belotti E. 1998. Assessment of soil quality by means of a field survey. Applied Soil Ecology 10: 51–63.
Bianchina JN. 2009. Development of a flowsystem for the determination of Cd in fiel alcohol using vermicompost as bioabsorbent. Talanta, 78: 333-336.
Boonyapookana B, Parkpian P, Techapinyawat S, Delaune RD and Jugsujinda A. 2005. Phytoaccumulation of lead by Sunflower (Helianthus annus), Tobacco (Nicotiana tabacum), and Vetiver (Vetiveria zizanioides), Journal
Bradley K, Drijber R and Knops J. 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biol Biochem 38(7):1583–1595
Burgos MG, Winter C, Sturzenbaum SR, Randerson PF and Morgan AJ. 2005. Cu and Cd effects on the earthworm Lubricus rubellus in the laboratory: multivariate analysis of relationships between exposure, biomarkers, and ecologically relevant parameters. Environmental Science and Technology 39(6): 1757–1763.
Chhotu DJ and Fulekar MH. 2009. Phytoremediation of heavy metals: Recent techniques. Afr. J. Biotechnol., 8(6): 921-928.
Ciura J, Poniedzialek M, Sekara A and Jedrszezyk E. 2005. The possibility of using crops as metal phytoremediants, Polish Journl of Environmental Studies, 14, 17-22.
Cookson W, Osman M, Marschner P, Abaye D, Clark I and Murphy D. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biol Biochem 39(3):744–756
Cookson W, Osman M, Marschner P, Abaye D, Clark I and Murphy D. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biol Biochem 39(3):744–756
Edwards CA and Bohlen PJ. 1996. Biology and Ecology of Earthworms 3rd edition, Chapman & Hall, London.
Edwards CA and Bohlen PJ. 1996. Biology and Ecology of Earthworms, third ed. Chapman & Hall, London.
Filip Z. 2002. International approach to assessing soil quality by ecologically related biological parameters. Agric. Ecosyst. Environ. 88, 169–174.
Flickinge REL and Nichols JD. 1990. Small mammal populations at hazardous waste disposal sites near Houston, Texas, USA. Enviromental Pollution 65: 169–180.
Funmilayo O. 1977. Distribution and abundance of moles (Talpa europaea L.) in relation to physical habitat and food supply. Oecologia 30: 277–283.
Funmilayo O. 1979. Food consumption, preferences and storage in the mole. Acta Theriologica 24: 379–389.
Glab T and Kulig B. 2008. Effect of mulch and tillage system on soil porosity under wheat (*Triticum aestivum*). Soil Till. Res. 99, 169–178.
Gong W, Yan X, Wang J, Hu T and Gong Y. 2009. Long-term manuring and fertilization effects on soil organic carbon pools under a wheat-maize cropping system in North China Plain. Plant Soil 314, 67–76.
Guggenberger G, Frey SD, Six J, Paustian K and Elliott ET. 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci Soc Am J 63(5):1188–1198
He ZL, Yang XE, Stoffela PJ. 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology 19 (2-3): 125–140.
Heikens A, Peijnenburg WJGM and Hendriks AJ. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113(3): 385–393.
Heikens A, Peijnenburg WJGM and Hendriks AJ. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113(3): 385–393.
Hu N, Lou YL, Zhang XK, Liang WJ and Liang L. 2010. Effects of conservation tillage on the composition of soil exchangeable base. Chin. J. Appl. Ecol. 21, 1492–1496 (in Chinese with English abstract).
Jain K and Singh J. 2004. Modulating of fly ash induced genotoxicity in vica faba by vermi-composting. Ecotoxicol. Environ. Saf. 59: 89-94.
Jenkinson DS, Brookes PC and Powlson DS. 2004. Measuring soil microbial biomass. Soil Biol. Biochem. 36, 5–7.
Ji S and Unger PW. 2001. Soil water accumulation under different precipitation, potential evaporation, and straw mulch conditions. Soil Sci. Soc. Am. J. 65, 442–448.
Jordán A, Zavala LM and Gil J. 2010. Effects of mulching on soil physical properties and runoff under semi-arid conditions in southern Spain. Catena 81, 77–85.
Kennedy A, Schillinger W. 2006. Soil quality and water intake in traditional-till vs. no-till paired farms in Washington’s Palouse region. Soil Sci Soc Am J 70(3):940–949
Komarnicki GJK. 2000. Tissue, sex and age specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (Talpa europaea L.) in a central urban area, Chemosphere 41: 1593–1602.
Komarnicki GJK. 2005. Lead and cadmium in indoor air and the urban environment. Environmental Pollution 136: 47–61.
Kuzovkina YA, Knee M and Quigley MF. 2004. Cadmium and copper uptake and translocation in five Willow (Salix L.) species, International Journal of Phytoremediation, 6, 269-287.
Lapedes DN.1974. Dictionary of scientific and technical terms. N.Y. McGraw Hill, pp.674.
Lasat MM. 2002. Phytoextraction of toxic metals: A review of biological mechanisms, J. Environ. Qual., 31, 109-120.
Lee K and Foster RC. 1991. Soil fauna and soil structure. Aust. J. Soil Res., 29: 745-776. Livan, M.A and W. Thompson (1997). NARI Annual Report.
Lee KE. 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use, Academic Press, Sydney.
Lodal J and Grue H. 1985. Age determination and age distribution in populations of moles (Talpa europaea) in Denmark. Acta Zoologica Fennica 173: 279–281.

Lukkari T, Taavitsainen M, Vaisanen A and Haimi J. 2004. Effects of heavy metals on earthworms along contamination gradients in organic rich soil. Exotoxicology and Environmental Safety 59(3): 340–348.
Lukkari T, Taavitsainen M, Vaisanen, A and Haimi J. 2004. Effects of heavy metals on earthworms along contamination gradients in organic rich soil. Exotoxicology and Environmental Safety 59(3): 340–348.
Ma WC. 1987. Heavy metal accumulation in the mole, Talpa europea, and earthworms as an indicator of metal bioavailability in terrestrial environments. Bulletin of Environmental Contamination and Toxicology 39(6): 933–938.
Mandal A, Patra AK, Singh D, Swarup A and Masto RE. 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activites in soil during crop development stages. Bioresour. Technol. 98, 3585–3592.
Marinari S, Masciandaro G, Ceccanti B and Grego S. 2000. Influence of organic andmineral fertilizers on soil biological and physical properties. Bioresour. Technol. 72, 9–17.
Martin A. 1991. Short- and long-term effects of the endogeic earthworm Millsonia anomala (Omodeo) (Megascolecidae, Oligochaeta) of tropical savannas on soil organic matter. Biol. Fertil. Soils., 11: 234-238.
MIDI. 1995. Sherlock microbial identification system operating manual: version 5. MIDI, Newark, Del.
Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: synthesis [Internet]. http://www.millenniumassessment.org/documents/document.356.aspx.pdf
Milner C and Ball DF. 1970. Factors affecting the distribution of the mole (Talpa europaea) in Snowdonia (North Wales). Journal of Zoology 162: 61–69.
Minoshima H, Jackson L, Cavagnaro T, Sanchez-Moreno S, Ferris H and Temple S. 2007. Soil food webs and carbon dynamics in response to conservation tillage in California. Soil Sci Soc Am J 71(3):952–963
Morgan JE and Morgan AJ. 1988. Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils. Environmental Pollution 54(2): 123–38.
Morgan JE and Morgan AJ. 1999. The accumulation of metals (Cd, Cu, Pb Zn and Ca) by two ecologically contrasting earthworm species (Lumbricus rubellus and Aporrectodea caliginosa): implications for ecotoxicological testing. Applied Soil Ecology 13, 9e20.
Mrsic N. 1997. Soil fauna (translated from Slovenian language). Tehniska zalozba Slovenije, Ljubljana.
Muyima NYO, Reinecke AJ and Viljoen-Reinecke SA. 1994. Moisture requirements of Dendrobaena veneta- a candidate for vermicomposting. Soil Biol. Biochem., 26: 973- 976.
Nooren CAM, Van Breeman N, Stoorvogel JJ and Jongmans AG. 1995. The role of earthworms in the formation of sandy surface soils in a tropical forest in Ivory Coast. Geoderma., 65: 135-148.
Olson HW. 1928. The earthworms of Ohio. Ohio Biol. Surv. Bull., 17: 47-90.
Oppermann J. 1968. Die Nahrung des Maulwurfs (Talpa europaea) in unterschiedlichen Lebensräumen. Pedobiologia 8: 59–74.
Pabin J, Lipiec J, Włodek S and Biskupski A. 2004. Effect of different tillage systems and straw management on some physical properties of soil and on the yield of winter rye in monoculture. Int. Agrophys. 17, 175–181.
Pankakoski E, Hyvärinen H, Jalkanen M and Koivisto I. 1993. Accumulation of heavy metals in the mole in Finland. Environmental Pollution 80(1): 9-16.
Pankakoski E, Koivisto I, Hyvärinen H, Terhivuo J and Tahka KM. 1994. Experimental accumulation of lead from soil through earthworms to common shrews. Chemosphere 29(8): 1639–1649.
Pennanen T. 2001. Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH a summary of the use of phospholipid fatty acids, Biolog and H- 3-thymidine incorporation methods in field studies. Geoderma 100:91–126
Pennanen T, Frosetgrd A, Fritze H and Bth E. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal polluted gradients in coniferous forests. Appl Environ Microbiol 62:420–428.
Peters RW and Shem L. 1992. Use of chelating agents for remediation of heavy metal contaminated soil. In: Vandegrift, G.E., Reed, D.T., Tasker, I.R. (Eds.), Environmental Remediation Removing Organic and Metal Ion Pollutants. American Chemical Society, Washington, pp. 70e84.
Pichtel J, Vine B, Kuula-Vaisanen P and Niskanen P. 2001. Lead extraction from soils as affected by lead chemical and mineral forms. Environmental Engineering Science 18, 91e98.
Piearce TG, Oates K and Carruthers WJ. 1990. A fossil earthworm embryo (Oligochaeta) from beneath a late bronze age midden at Potterna, Wiltshire, UK. J. Zool. Land., 220: 537-542.
Pramoth A. 1995. Vermiwash-A potent bio-organic liquid “Ferticide”. M.Sc., dissertation, University of Madras. 29 pp.
Reddy MV and Pasha M. 1993. Influence of rainfall, temperature and some soil physicochemical variables on seasonal population structure and vertical distribution of arthworms in two semi- and tropical grassland soils. Int. J. Biotech., 37: 19-26.
Rivero VC, Masedo MD and De la Villa RV. 2000. Effect of soil properties on zinc retention in agricultural soils. Agrochimica 43, 46e54.
Roane TM and Kellogg ST. 1996. Characterization of bacterial communities in heavy metal contaminated soils. Can J Microbiol 42:593–603
Ruby MV, Davis A, Schoof R, Eberla S and Sellstone CM. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environmental Science and Technology 30, 422e430.
Salmon S. 2001. Earthworm excreta (mucus and urine) affect the distribution of springtails in forest soils. Biology and Fertility of Soils 34, 304e310.

Satchell JE. 1983. Earthworm microbiology. In: Earthworm ecology: From Darwin to Vermiculture. (Satchell, J. E. ed.), Chapman and Hall, London, UK. pp: 351-364.
Schnoor JL. 1997. Phytoremediation, University of Lowa, Department of Civil and Engineering, 1-62.
Senapati BK and Dash MC. 1982. Earthworms as waste conditioners. Indust. Eng. J., 11: 53-58.
Sherlock JC. 1985. Heavy metal residues. Gibson, G.G., Walker, R. (Eds.), Food toxicology real or imaginary problems? pp. 271–280.
Shipley AE. 1970. In: The Cambridge Natural History. (Harmer, S. F. and Shipley, A. E. eds.). Codicote, England.
Shuster WD, Subler S and McCoy EL. 2000. Foraging by deep-burrowing earthworms degrades surface soil structure of a fluventic Hapludoll in Ohio. Soil & Tillage Research., 54: 179-189
Six J, Frey SD, Thiet RK and Batten KM. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70(2):555–569
Sizmur T and Hodson ME. 2009. Do earthworms impact metal mobility and availability
Spurgeon DJ and Hopki SP. 1999. Seasonal variation in the abundance, biomass and biodiversity of earthworms in soils contaminated with metals emissions from a primary smelting works. Journal of Applied Ecology 36: 173–183.
Spurgeon DJ and Hopkin SP. 1999. Tolerance to zinc in populations of the earthworm Lumbricus rubellus from uncontaminated and metal-contaminated ecosystems. Archives of Environmental Contamination and Toxicology 37, 332e337.
Steenwerth K, Jackson L, Carlisle E and Scow K. 2006. Microbial communities of a native perennial bunchgrass do not respond consistently across a gradient of land-use intensification. Soil Biol Biochem 38(7):1797–1811
Strojan CL. 1978. Forest leaf litter decomposition in the vicinity of a zinc smelter. Oecologia 32:203–212
Su DC and Wong JWC. 2004. Selection of Mustard Oilseed Rape (Brassica juncea L.) for Phytoremediation of cadmium contaminated soil, Bull.Environ. Contam. Toxicol., 72, 991-998.
Tomlin AD, Shipitalo MJ, Edwards WM and Protz R. 1995. Earthworms and their influence on soil structure and infilteration. In: Earthworm ecology and biogeography in North America. (Hendrix, P.F. ed.), Lewis Publishers, Chelsea. pp: 159-184.
USEPA. 1997. Cleaning Up the Nation’s Waste Sites: Markets and Technology Trends, United States Environmental Protection Agency EPA/542/R-96/005, Office of Solid Waste and Emergency Response, Washington, DC.
Valle JV, Moro RP, Garvin MH, Trigo D and Diaz Cosin DJ. 1997. Annual dynamics of the earthworms Hormogaster elisae (Oligochaeta, Hormogastridae) in Central Spain. Soil Biol. Biochem., 29: 309-312.
Wagner-Riddle C, Gillespie TJ and Swanton CJ. 1996. Rye mulch characterization for the purpose of microclimatic modelling. Agric. For. Meteor. 78, 67–81.
Waldrop MP, Balser TC and Firestone MK. 2000. Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32(13):1837–1846
Wander M, Hedrick D, Kaufman D, Traina S, Stinner B and Kehrmeyer S. 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. Plant Soil 170(1):87–97
Ward DM, Weller R and Bateson MM. 1990. 16srRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345:63–65
Weltje L. 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. Chemosphere 36, 2643e2660.
White DC, Davis WM, Nickels JS, King JD and Bobbie RJ. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51–62
Wood TG. 1974. The distribution of earthworms (Megascolecidae) in relation to soils, vegetation and altitude on the slopes of Mt. Kosciusko, Australia. J. Anim. Ecol., 43: 87-106.
Wren CD. 1986. Mammals as biological monitors of environmental metal levels. Environmental Monitoring and Assessment 6: 127–144.
Zaller JG and Arnone JA. 1999. The earthworms and soil moisture effects on the productivity and structure of grassland communities. Soil Biol. Biochem., 31: 517- 523.
Zelles L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35:275–294