Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 1, p. 85-101, 2015
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Effect of vermicomposting on microbial biomass in contaminated soil by heavy metals

**Mohammad Lakzayi[1], Hossein Moradi[2], Ebrahim Sabbagh[1], Khashayar Rigi[1*]**

[1]*Department of plant Production, Khash Branch, Islamic Azad University, Khash, Iran*

[2]*Higher Educational Complex of Saravan, Iran*

Article published on January 05, 2015

**Key words:** Soil quality, Phytoremediation, soil basal respiration Soil organic matter.

**Abstract**

Declining soil quality (SQ) is emerging as an environmental and economic issue of increasing global concern as degraded soils are becoming more prevalent due to intensive use and poor management, often the result of over-population. Pressing problems such as erosion, compaction, acidification, organic matter losses, nutrient losses and desertification reduce agricultural production capacity. SQ decline severely impacts the environment and agricultural viability, and thus ecosystems and the population's health, food security, and livelihoods. Tests to monitor air and water quality have been standardized and widely adopted internationally. Earthworms, which improve soil productivity and fertility, have a critical influence on soil structure. Earthworms bring about physical, chemical and biological changes in the soil through their activities and thus are recognised as soil managers. Soil organic matter (SOM) plays an important role in maintaining the productivity of tropical soils because it provides energy and substrates, and promotes the biological diversity that helps to maintain soil quality and ecosystem functionality. SOM directly influences soil quality, due to its effect on soil properties.

***Corresponding Author:** Khashayar Rigi ✉ al.sobhkhiz22@gmail.com

## Introduction

*Soil microbial community*

The degradation of ecological environments has accelerated in the last century because of population increase. Accordingly, strategies for controlling soil erosion and recovering fragile ecosystems have been proposed. Among them, vegetation is reportedly the most effective and useful (Hou *et al.* 2002). However, determining the effect of restoration is often limited to vegetation indicators such as plant diversity and coverage (Mummy *et al.* 2002), soil physicochemical properties, microbial biomass, as well as enzyme activities (Eaton *et al.* 2008; Fu *et al.* 2010; Wang *et al.* 2011). The soil microbial community is also an inherent factor in determining the biogeochemical cycles and organic matter turnover in soils (Harris 2009). Thus, investigating the soil microbial community composition is important in explaining the soil ecological processes during vegetation succession. The structural composition of a soil microbial community can be evaluated by using phospholipid fatty acids (PLFAs). Given that phospholipids are rapidly degraded following cell death, PLFAs can reliably reflect the microbial community. Many researchers have used PLFAs to study the variations in soil microbial community composition during vegetation successions. For instance, McKinley (2005) reported that the prairie age is the most important environmental factor in determining the PLFA microbial community composition in native North American prairie grassland. Studies on the primary succession of a brown coal mine deposit found similar results (Baldrian *et al.* 2008). Other studies suggested that abiotic soil parameters such as organic matter content, C/N ratio, and pH have a more significant effect on the soil microbial PLFA biomass and microbial community composition than the soil age (Merila *et al.* 2002; 2010; Welc *et al.* 2012). Moore *et al.* (2010) studied the long-term soil microbial community dynamics in subsurface soil horizons and discovered that older subsurface soils have a lower microbial community biomass, a higher fungal proportion, and a different community structure than younger subsurface soils. The soil texture, vegetation species, and meteorological factor also reportedly contribute substantially to studies on different ecosystems (Bach *et al.* 2010). However, few studies have evaluated the changes in microbial community composition during the natural restoration of a disturbed field, specifically in the Loess Plateau in China, one of the areas in the world that are most seriously affected by soil and water erosion.This article is review and the aims are influence of humic acid, vermicomposting and earthworms on some microbial indices in contaminated soil.

*Municipal wastewater sludge (MWS)*

The major benefits of municipal wastewater sludge (MWS) application are; increased supply of major plant nutrients; provision of some of the essential micronutrients (e.g., Zn, Cu, Mo, and Mn) and; improvement in the soil physicochemical properties, i.e. better soil structure, increased water holding capacity, and improved soil water transmission characteristics, etc. Toxic compounds such as heavy metals (HMs) could compromise the beneficial use of MWS. On the other hand, the MWS its use in agriculture is associated with health risks because of presence of pathogens (Toze, 2006), metallic contaminants like Cu, Ni, Cd, Cr, Zn, etc. and toxic organo-compounds. Soil microbial content and activity have a variety of properties that can affect changes in metal speciation, toxicity and mobility, as well as mineral formation or dissolution or deterioration. Amendment of MWS led to significant increase in heavy metals e.g., Pb, Cr, Cd, Cu, Zn and Ni concentrations of soil (Singh and Agrawal, 2007). Soil microbial activity has a great potential as an early and sensitive indicator of stress in soil. Excessive accumulation of heavy metals in agricultural soil through wastewater irrigation may cause soil contamination and affect food quality and safety (Rahman *et al.*, 2012). The bioavailable pool of metals, estimated as free ion activities, decreased with the increasing occurrence of metal-organic matter complexes (Hernandez-Soriano *et al.*, 2013). It is suggested that the metals affected microbial biomass

(MB) and activities by behaving synergistically or additively with each other. Although soils had higher MB and activities than the background soil, due to higher OM content, the ratios of microbial parameters/organic carbon (OC) indicated that inhibition of microbial growth and activities had occurred due to metal stress (Bhattacharyya *et al.*, 2008).

*Microbial biomass*

Microbial biomass is the living portion of soil organic matter, which is also affected by alterations in the soil environment. The microbial biomass comprises 1-3 % of total soil carbon and up to 5 % of total soil nitrogen (Horwath & Paul, 1994). Since it is composed of bacteria, fungi, actinomycetes, micro algae and microfauna, environmental alterations affect physical, chemical and biological soil properties, modifying microbial biomass composition. Therefore, microbial biomass also has been considered a sensitive bioindicator of soil conditions (Doran *et al.*, 1994; Horwath & Paul, 1994; Me *et al.*, 2000; Manlay *et al.*, 2000; Chen *et al.*, 2003; Feng *et al.*, 2003; Schloter *et al.*, 2003; Chen *et al.*, 2004; Perez *et al.*, 2004; Spedding *et al.*, 2004; Perez *et al.*, 2005).

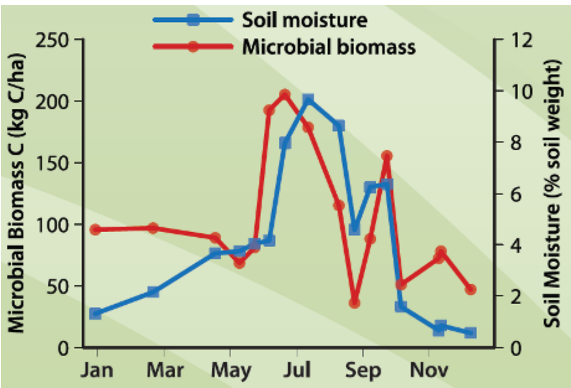


**Fig. 1.** Microbial biomass carbon over a year from a soil near Meckering, WA.

On the other hand, Manlay *et al.* (2000) studied the relationship of biomass and soil nematode communities and did not observe any correlations between these two categories of soil quality indicators. However, the authors considered that the failure in correlation was due to the fact that termites and ants consumed too much roots, and not many roots were left for natural nutrient cycling.

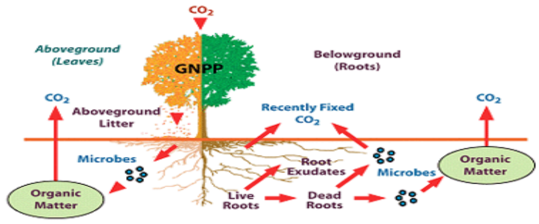


**Fig. 2.** Pathways of carbon cycling for plant allocation to leaves and roots. Belowground, plant C cascades through several reservoirs - live roots, dead roots, microbial biomass, and organic matter - each with their own mean residence times and respiratory losses. Some C is rapidly cycled in respiration or exudates. G/NPP = gross/net primary productivity; both gross and net are represented.

Generally, soil microbial biomass can be limited by soil moisture under both dry and wet conditions. Exposure of microbial communities to fluctuating moisture may lead to selection for organisms better adapted to these conditions. Although some researchers studied the effect of dry/wet cycles on soil microbial communities under different farming systems (Lundquist *et al.*, 1999a) or on available carbon sources in the laboratory (Lundquist *et al.*, 1999b), there is a gap to quantify impacts of flooding duration and type of soil on microbial diversity in floodplain soils, both in the laboratory and in the field. Soil microbial biomass, both a source and sink of available nutrients for plants, plays a critical role in nutrient transformation in terrestrial ecosystems (Singh *et al.* 1989). Any changes in the microbial biomass may affect the cycling of soil organic matter. Thus, the soil microbial activity has a direct influence on ecosystem stability and fertility (Smith *et al.* 1993). Generally, microbial biomass can offer a means in assessing the soil quality in different vegetation types (Groffman *et al.* 2001).

*Phospholipid fatty acids (PLFAs)*

Phospholipid fatty acids (PLFAs) are major constituents of the cell membranes of all microorganisms (Vestal and White, 1989). The large variety of PLFAs present in living organisms and extracted from soil may provide a unique fingerprint of the viable microbial community of a given soil, at a given time (Bossio and Scow, 1998; Lundquist *et al.*, 1999a). Using multivariate statistical analyses, this variation in fatty acid (FA) composition between microorganisms can be exploited, revealing differences between microbial communities (Bossio *et al.*, 1998; Macalady *et al.*, 2000; Pennanen, 2001). Certain PLFAs are related to functional groups of organisms and appear promising in their use as biomarkers for these organism groups (Vestal and White, 1989). Zelles (1999) proposed that the classification of PLFAs into a number of fractions should simplify the evaluating procedure and improve the assessment of soil microbial communities. The PLFA method can provide information on a variety of microbial characteristics, such as biomass, physiology, taxonomic and functional identity, and overall community composition (Green and Scow, 2000).

*Soil organic carbon (SOC)*

Soil organic carbon (SOC) is a key component of the soil-plant ecosystem and is closely associated with soil properties and processes, nutrient buffering and supply, as well as emission and storage of greenhouse gases (Kasel and Bennett, 2007; Yang *et al.*, 2009; Wu and Cai, 2012).

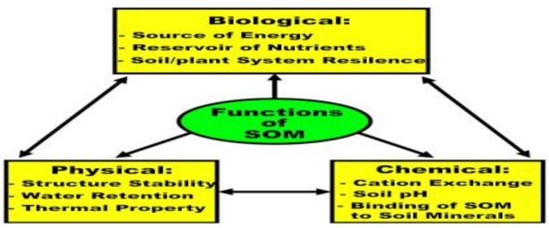


**Fig. 3.** Function of soil organic matter.

The SOC is an important factor affecting soil quality (Nsabimana *et al.*, 2004). Besides being a source and sink of nutrients for plants, the SOC has an important function in the carbon (C) cycle, accounting for the major terrestrial pool of this element.

*The main factor determining SOC content*

Many studies have suggested that land use change is the main factor determining SOC content because of its effects on soil aggregates (Yang *et al.*, 2009), microbial activity, and biogeochemical cycles (Nsabimana *et al.*, 2004). These biogeochemical changes are directly linked to the future productivity and stability of SOC (Pandey *et al.*, 2010). In recent decades, the effect of land use change from natural forest to agricultural land and plantation has prompted ecologists to focus their attention on SOC, microbial properties, and microbial activity of soils (Yang *et al.*, 2009; Ye *et al.*, 2009). The SOC losses often occur when converting from natural to agricultural ecosystems, which is due to the reduction of organic matter inputs, the decrease of physical protection of SOC and the changes in soil moisture and temperature regime exacerbated decomposition rates (Zhang *et al.*, 2007). The SOC is composed of diverse fractions varying in their degree of decomposition, recalcitrance, and turnover rate (Huang *et al.*, 2008). Microbial biomass is the most active fraction of soil organic matter, typically comprising 1%–5% of the total organic matter content (Nsabimana *et al.*, 2004). Given its high turnover rate, soil microbial biomass can be used as a potential early and sensitive indicator of SOC changes (Cookson *et al.*, 2007; Huang and Song, 2010).

*Soil quality*

The clearing of natural vegetation for agriculture leads to alterations in soil properties and, in several cases, to environmental degradation (Bewket & Stroonijdeb, 2003; Muniz *et al.*, 2011). This phenomenon calls for a concept of soil quality, which is the capacity of a soil to function within the limits of an ecosystem, interacting positively with the environment external to that ecosystem (Larson & Pierce, 1991). Declining soil quality (SQ) is emerging as an environmental and economic issue of increasing global concern as degraded soils are becoming more prevalent due to intensive use and poor management, often the result of over-population (Eswaran *et al.*, 2005). Pressing problems such as erosion, compaction, acidification, organic matter losses, nutrient losses and desertification reduce agricultural production capacity. SQ decline severely impacts the environment and agricultural viability, and thus ecosystems and the population's health, food security, and livelihoods. Tests to monitor air and water quality have been standardized and widely adopted internationally (Riley, 2001). However, although an estimated 65% of the land area worldwide is degraded (FAO, 2005), no standardized SQ tests exist currently, especially for use in the tropics (Winder, 2003).

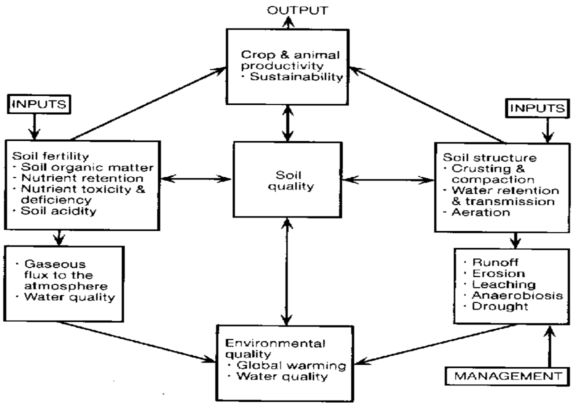


**Fig. 4.** Soil quality in relation to soil properties, soil processes, and environmental quality.

The World Soils Agenda developed by the International Union of Soil Scientists lists as the first two agenda items 1) assessment of status and trends of soil degradation at the global scale and 2) definition of impact indicators and tools for monitoring and evaluation(Hurni *et al.*, 2006). There is clearly a need for international standards to measure SQ. These could be useful for agricultural research and extension agencies, non-governmental organizations, governments and farmers to better understand, implement and monitor sustainable.

A soil with good quality fulfills a number of functions (carbon storage, water reservoir, nutrient cycling, growth medium, among others), while simultaneously protecting other environmental components and sustaining human health (Doran & Parkin, 1994). In this sense, assessing the soil quality is one of the main tasks of soil scientists (Basher, 1997). Soil quality includes an inherent and a dynamic component (Carter, 2002).

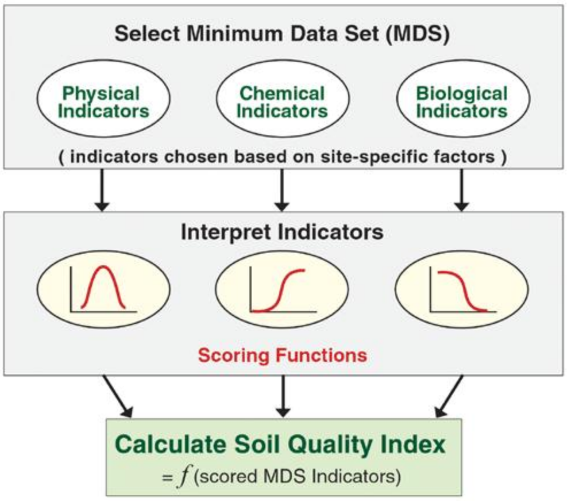


**Fig. 5.** Calculate of soil quality.

The former is an expression of the soil forming factors, documented by soil surveys as expressed by land capability classification. Dynamic SQ, however, refers to the condition of soil that is changeable in a short period of time largely due to human impact and management (Carter, 2002).

The SQ concept encompasses the chemical, physical and biological soil characteristics needed to support healthy plant growth, maintain environmental quality, and promote human and other animal health (Doran *et al.*, 1994). With farmer and lay audiences, the term "soil health" is often preferred when referring to this dynamic SQ concept as it suggests a holistic approach to soil management (Idowu *et al.*, 2007). New regulations have catalyzed a proliferation of various indicators and "environmental report cards" for assessing vulnerability and improvement towards sustainability (Riley, 2001). Indicator suitability can be judged by several criteria, such as relevance, accessibility to users, and measurability (Nambiar *et al.*, 2001). Criteria and thresholds for relevant indicators must then be set by which to assess performance level relative to a standard (Manhoudt *et al.*, 2005).

*Earthworms*

Earthworms are terrestrial invertebrates belonging to the Order Oligochaeta, Class Chaetopoda, Phylum Annelida, which have originated about 600 million years ago, during the pre-Cambrian era (Piearce *et al.*, 1990). Earthworms occur in diverse habitat, exhibiting effective activity, by bringing about physical and chemical changes in the soil leading to improvement in soil fertility. An approach towards good soil management, with an emphasis on the role of soil dwellers like earthworms, in soil fertility, is very important in maintaining balance in an ecosystem (Shuster *et al.*, 2000). The role of earthworms in soil formation and soil fertility is well documented and recognised (Darwin, 1881; Edwards *et al.*, 1995; Kale, 1998; Lalitha *et al.*, 2000).

*Earthworms play an important role*

Earthworms play an important role in the decomposition of organic matter and soil metabolism through feeding, fragmentation, aeration, turnover and dispersion (Shuster *et al.*, 2000). Earthworms were referred by Aristotle as "the intestines of earth and the restoring agents of soil fertility" (Shipley, 1970). They are biological indicators of soil quality (Ismail, 2005), as a good population of earthworms indicates the presence of a large population of bacteria, viruses, fungi, insects, spiders and other organisms and thus a healthy soil (Lachnicht and Hendrix, 2001).

The role of earthworms in the recycling of nutrients, soil structure, soil productivity and agriculture, and their application in environment and organic waste management is well understood (Edwards *et al.*, 1995; Tomlin *et al.*, 1995; Shuster *et al.*, 2000; Ansari and Ismail, 2001a,b; Ismail, 2005; Ansari and Ismail, 2008; Ansari and Sukhraj, 2010). Earthworms play an important role as of soil "ecosystem engineers" to maintain soil fertility and in soil conservation. Through their activity they influence soil properties that determine its functional characteristics. Through their feeding, burrowing and casting habits earthworms enhance the incorporation of organic matter into soils and stimulate the formation of macro aggregates that impacts on soil structure as well as on the population of soil organisms (Guggenberger *et al.*, 1996; Blanchart *et al.*, 1997, Bossuyt *et al.*, 2005; Pérès *et al.*, 2010; Briones *et al.*, 2011). Activity of earthworms has a such an impact on important regulatory functions like those associated with the soil water balance, of biological population, nutrient cycling and soil organic carbon cycles that determine important ecosystem services like carbon sequestration and water supply and therefore the ability to sustain agricultural productivity (Derouard *et al.*, 1997; Bronick and Lal, 2005; Ilstedt *et al.*, 2007; Bhardwaj *et al.*, 2011). However, these roles may depend on (i) the type of ecological categories, namely epigeic, endogeic and anecic species (Bouché, 1977); and (ii) their functional attributes (e.g. compacting vs decompacting). Earthworms of different ecological groups prefer different habitats and feed on different food resources. Their effect on soil structure through the type and amounts of burrows created and type and amount of cast produced will depend on the group (Pérès *et al.*, 2010).

Epigeic earthworm species such as Eisenia foetida (Lumbricidae), for instance, incorporate litter material into the mineral soil thereby making it available to all kinds of soil organism to enter the soil food web, and hereby increasing soil porosity (Monroy *et al.*, 2011). Anecic species mix plant fragments and mineral particles ingested during their burrowing through the soil and feeding on the surface and stimulate humification and the formation of stable organic-mineral compounds. Consequently, they increase soil macroporosity and enhance water infiltration. Endogeic earthworm species primarily consume soil and associated humified organic matter in the upper layer of the mineral soil. However, their effect on soil physical properties depends on the organic matter content in soils and on cast types, because of the selective feeding on organic materials, low assimilation efficiency and depending on body size, (Blanchart *et al.*, 2004; Jiménez and Decaëns, 2004).

*Effect of earthworms on soil quality*

Earthworms, which improve soil productivity and fertility (Edwards *et al.*, 1995), have a critical influence on soil structure. Earthworms bring about physical, chemical and biological changes in the soil through their activities and thus are recognised as soil managers (Ismail, 2005).

*Effect of earthworms on chemical properties of soil*

Earthworms bring about mineralization of organic matter and thereby release the nutrients in available forms that can be taken up by the plants (Edwards and Bohlen, 1996). Organic matter that passes through the earthworm gut is egested in their casts, which is broken down into much finer particles, so that a greater surface area of the organic matter is exposed to microbial decomposition (Martin, 1991). Earthworms have major influences on the nutrient cycling process in many ecosystems (Edwards and Bohlen, 1996). These are usually based on four scales (Lavelle and Martin, 1992), during transit through the earthworm gut, in freshly deposited earthworm casts, in aging casts, and during the long-term genesis of the whole soil profile. Earthworms contribute nutrients in the form of nitrogenous wastes (Ismail, 2005). Their casts have higher base-exchangeable bases, phosphorus, exchangeable potassium and manganese and total exchangeable calcium. Earthworms favour nitrification since they increase bacterial population and soil aeration. The most important effect of earthworms may be the stimulation of microbial activity in casts that enhances the transformation of soluble nitrogen into microbial protein thereby preventing their loss through leaching to the lower horizons of the soil. C: N ratios of casts are lower than that of the surrounding soil (Bouché, 1983). Lee (1983) summarised the influence of earthworms on soil nitrogen and nitrogen cycling.

*Vermicomposting*

Vermicomposting is the biological degradation and stabilization of organic waste by earthworms and microorganisms to form vermicompost. This is an essential part in organic farming today. It can be easily prepared, has excellent properties, and is harmless to plants. The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization. These rapidly convert the waste into humus-like substances with finer structure than thermophilic composts but possessing a greater and more diverse microbial activity. Vermicompost being a stable fine granular organic matter, when added to clay soil loosens the soil and improves the passage for the entry of air. The mucus associated with the cast being hydroscopic absorbs water and prevents water logging and improves waterholding capacity. The organic carbon in vermicompost releases the nutrients slowly and steadily into the system and enables the plant to absorb these nutrients. The soil enriched with vermincompost provides additional substances that are not found in chemical fertilizers (Kale, 1998). 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 *et al.*, 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).

*Soil organic matter (SOM)*

Soil organic matter (SOM) plays an important role in maintaining the productivity of tropical soils because it provides energy and substrates, and promotes the biological diversity that helps to maintain soil quality and ecosystem functionality. SOM directly influences soil quality, due to its effect on soil properties (Wendling *et al*., 2010). Once soil is cultivated for agricultural production, especially in the tropics and the semi-arid regions, SOM is rapidly decomposed due to modifications in conditions such as aeration, temperature, and water content (Ashagrie *et al*., 2007). This can affect many soil functions that are either directly or indirectly related to SOM, due to its capacity to retain water and nutrients. Although the breakdown rate of SOM can be faster in the tropics, regular inputs of organic amendments can promote a buildup of SOM (Follett, 2001). Soils with low natural fertility and reduced water availability have been brought into production by means of high technology strategies including irrigation and the intensive use of fertilizers and pesticides. These management practices are very common in the tropics and can contribute to negative changes in soil quality, which should be investigated and monitored in order to reduce environmental impacts of agricultural activities as well as to promote a more sustainable use of soils. Due to the complexity of SOM compounds, the relationship between SOM characteristics and land use is poorly understood. Hence, knowledge of different pools of SOM should render the impacts of management practice more measurable. Soil organic matter labile fractions have been used instead of total SOM as sensitive indicators of changes in soil quality (Bayer *et al*., 2002; Haynes, 2005), due to the many important interactions of these components in the soil system. Non-humic substances are labile compounds with relatively rapid turnover in soil, since they are readily utilized as substrates by soil microorganisms (Schmidt *et al*., 2011). Humic substances are more stable organic matter compounds, which make up a significant portion of the total soil organic C and N (Lal, 1994; Milori *et al*., 2002). Humic substances can improve soil buffering capacity, increase moisture retention, and supply plants with available micronutrients. Moreover, these compounds can also bind metals, alleviating both heavy metal toxicity and metal deficiency in soils (McCarthy, 2001). Humin (H) is the insoluble fraction of humic substances; humic acid (HA) is the fraction that is soluble under alkaline conditions; and fulvic acid (FA) is the fraction that is soluble under both alkaline and acidic conditions (Sutton and Sposito, 2005). The chemically reactive and refractory nature of humic substances contributes to their persistence in soils (Kiem and Kogel-Knabner, 2003; Rovira and Vallejo, 2007), as well as to their important role in nutrient flows through ecological systems, and C emissions to the atmosphere (Lal, 2006). The relationship between concentrations of humic and fulvic acids (HA/FA ratio) is indicative of the potential mobility of C in the soil system. In general, sandy soils present higher ratios due to the solubility and selective loss of fulvic acids during the decomposition of SOM. The (HA + FA)/humin ratio indicates the degree of illuviation of SOM (Benites *et al*., 2003). Changes in field management practices can alter the chemical properties of soil humic substances (Moraes *et al*., 2011). Spaccini *et al*. (2006) reported a progressive decrease in humic substance concentrations in soils that were converted from forest to arable farming. Such decrease is commonly attributed to microbial oxidation of the organic materials previously protected in the soil aggregates destroyed by cultivation. Most studies report a reduction in SOM and in its fractions when forest is converted to other land uses (Navarrete and Tsutsuki, 2008).

*The Measurement of Soil Organic Matter*

In the laboratory, soil organic carbon is usually measured to estimate the amount of soil organic matter. This is because it is the most reliable and easy method to estimate soil organic matter. However to convert the soil organic carbon measurement to soil organic matter it is necessary to convert the soil organic carbon reading based on the assumed percentage of carbon in the organic matter. This can vary and the conversion can range from 1.72 to 2.0 depending on the source of the materials for the soil organic matter. In practice the value of 1.72 is used (Nelson and Sommers 1996; Baldock and Skjemstad 1999). For more precise scientific work only the values of soil organic carbon are used. Soil carbon is measured by oxidation using wet or dry methods (Baldock and Skjemstad 1999). The wet method uses a chemical agent such as dichromate but this method can vary depending on whether samples are heated. In the original method (Walkley and Black 1934) the samples were not heated and there is incomplete oxidation of the soil organic carbon. With heating this method (Heanes 1984) generally does oxidise the soil organic carbon completely although some of the coarser carbonised carbon may resist oxidation (Baldock and Skjemstad 1999). In dry oxidation the soil is heated in oxygen and all carbon, organic and inorganic, is converted to $CO_2$. The amount of $CO_2$ is analysed usually spectroscopically. The result must be corrected for inorganic carbon such as carbonates or these must be removed by acid treatment before the sample is analysed (Baldock and Skjemstad 1999). The dry oxidation method is currently the preferred method for most samples, although where there are large amount of carbonate present, the wet oxidation method may still have some advantages (Schmidt *et al.* 2012). A review of how effective the different methods of measuring soil organic matter and soil carbon are at detecting different organic materials was undertaken by Conyers *et al* (2011). The general conclusion was that the dry oxidation method and Heanes method (1984) were the most reliable across a wide range of materials.

*Phytoremediation*

Phytoremediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to decrease their toxicity (Salt *et al.* 1998). This technique has many advantages compared with other remediation procedures – low economic costs and the possibility of being applied to soils, causing a minimum environmental impact. Addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente *et al.* 2005). The effect of organic matter amendments on heavy metal bioavailability depends on the nature of the organic matter, their microbial degradability, salt content and effects on soil pH and redox potential, as well as on the particular soil type and metals concerned (Walker *et al.* 2003, 2004).

*Determining the fungal and bacterial biomass in soil*

Fungi and bacteria are drivers of major soil processes such as carbon and nutrient cycling (Gregorich *et al.* 1997; Milne and Haynes 2004). Changes in their abundance and functioning may be linked to soil sustainability, fertility and crop productivity (Bardgett *et al.* 1999; Beare 1997; Feng *et al.* 2004). The high functional and species diversity of soil fungi and bacteria make quantifying their relative contribution to soil biomass challenging. Several approaches are in use for determining the fungal and bacterial biomass in soil, and each one has its own merits and disadvantages. The direct counting method (Bloem *et al.* 1995) requires higher manipulative skills and a longer time commitment. The indirect methods such as phospho-lipid fatty acid, ergosterol and DNA-based fingerprinting (Grant and West 1986; van Elsas *et al.* 1998; Zelles *et al.* 1992) may overestimate the microbial biomass as there are no ways of discriminating dead and living components during analysis. The rRNA-amplification methods that assess metabolically active microbial fractions require expensive instrumentation (Pennanen *et al.* 2004). The SIR method, first developed by Anderson and Domsch (1975) which has since been substantially improved, is relatively quick and inexpensive. It is an accurate measure of the biomass of the active soil microbial communities as it measures their respiration directly. The method uses a microbial 'booster', usually glucose, and a fungal and bacterial inhibitor separately and in combination to suppress the activity of those microbial fractions. Even though it is simpler, the SIR method has to be locally calibrated because the interaction between microorganisms and inhibitors may dictate the experimental outcome. Different microorganisms respond differently to various biocides (Ingham and Colman 1984); non-target inhibition is characteristic with certain biocides (Tremaine and Mills 1987). Moreover, Ingham *et al.* (1986) and Stamatiadis *et al.* (1990) have proposed that some soil microorganisms may use biocides directly as nutrient sources. Previous research has shown Captan (N-[Trichloromethylthio]-4-cyclohexene-1,2-dicarboximide), a broad spectrum fungicide, and the prokaryotic inhibitor Oxytetracycline hydrochloride, to be effective biocides to use in fungal bacterial ratio assessments in agricultural soils (Bailey *et al.* 2002; 2003).

*Rate of respiration in soil*

Most microorganisms in the soil are dormant (Jenkinson and Ladd, 1981), so their rate of respir- ation is low. However, their respiration can be stimu lated by adding an easily decomposable substrate. Respiration may then rapidly increase to a maximum and remains at a constant rate for more than 4 h (Drobnik, 1960). Glucose is commonly used as a sub- strate because most soil microorganisms can readily utilize it as a carbon source (Stotzky and Norman, 1961). Anderson and Domsch (1978) suggested that the initial maximal respiration rate induced by glucose was proportional to the size of the original soil microbial biomass. The quantity of glucose added to achieve a maximal initial respiration rate varies greatly, depending on soil physical and chemical properties. Thus, Anderson and Domsch (1978) suggested that the quantity of glucose required to elicit the maxi- mal respiration should be determined for each soil.

*Substrate-induced respiration (SIR)*

The substrate-induced respiration (SIR) rate is strongly influenced by soil water content (Wardle and Parkinson, 1990a). To avoid the in fence of water content on the SIR measurement, West and Sparling (1986) added glucose rather than glucose powder to ensure excess water in all cases. For reliable SIR measurement it is most important that the glucose should be distributed evenly throughout the soil. Adding glucose solution appears not only to give the best distribution of glucose in soil, but is also analytically very convenient. However, it is not certain that both approaches measure the same par ammeters under such die rent conditions. For example, West and Sparling (1986) pointed out that a large amount of $CO_2$ may be dissolved in soil solution when the soil pH is greater than 6.0. Similarly, Sparling and West (1990) reported that, above about pH 6.5, $CO_2$ measured by GC may be underestimated because $CO_2$ is retained in solution, whereas retention is small above this pH. One of the perceived advantages of SIR is the measurement of the contribution of bacterial and fun- gal biomass to substrate-induced $CO_2$ respiration through coupling with antibiotics. Apparently successful measurements have been reported with this method in arable (Anderson and Domsch, 1973a,b, 1975), grassland (West, 1986; Wardle and Parkinson, 1990b) and rhizosphere soils (Nakas and Klein, 1980) and in plant residues (Beare *et al.*, 1990). However, when Ross *et al.* (1981) measured $O_2$ uptake following glucose addition they found no selective inhibition of $O_2$ uptake by streptomycin and cyclohexi-mide. Unsuccessful application of this method has also been reported by West (1986) in an arable soil of low biomass content, and by Bewley and Parkinson (1985) in an organic soil.

*Plants can endure high level of metals in the soil*

Plants can endure high level of metals in the soil with the help of some extrinsic mechanisms, which helps to reduce the absorption of metals by plant roots (Michalak, 2006). Amongst the mechanism, mycorrhizal action helps to augment plants' tolerance towards heavy metals (Turnau *et al.*, 2005) and can be exploited in remediating soils contaminated with heavy metals (del Val *et al.*, 1999). A mycorrhizal fungus is a symbiotic association in and on the roots of a host plant. Arbuscular mycorrhiza fungi (AMF) are the most common group of mycorrhizal fungi, which are obligatory (Javaid, 2007; Javaid *et al.*, 2007; Javaid & Riaz, 2008) and they are found in more than 80% of land plant families (Smith & Read, 2008). The fungus is supplied with soluble carbon sources by the host plants, whereas the fungus provides the host plant with a better ability to take up water and nutrients from the soil (Entry *et al.*, 2002; Javaid, 2009).

*Heavy metals*

At least 20 metals are classified as toxic with half of them emitted into the environment that poses great risks tohuman health [Akpor & Muchie, 2010]. The common heavy metals like Cd, Pb, Co, Zn and Cr are phytotoxic at both low concentration as well as very high concentration are detected in waste water especially the urban lakes water ecology [Divya *et al.*, 2012]. Recently, there has been an increasing interest in using biological indicators and remediators such as plants for soil, air and water pollution [Ruiz & Velasco, 2010). Aquatic macrophytes are widely distributed in various wet environments, from fresh to salt water [Bonanno & Guidice, 2010] and they play an important role in heavy metals cycling in the wetlands due to uptake, storage, and release processes. Phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach most suitable for developing countries [Ghosh & Singh, 2005]. The soil at the landfill may become toxic and poison because of presence of higher concentration of heavy metal (M.S Li *et al.*, (2007)). Even though there is some metal that good to the soil which is may come from dumping waste, (such as nitrate that can act as fertilizer to the soil), but most of the soil will accepted more type of dangerous heavy metal in the soil that will lead to the negative effects. This situation will make the soil have more dangerous and toxic heavy metal rather than good heavy metal. After a long time, waste that decomposed at the landfill will make the soil become polluted and may contain large amount of heavy metal. There are some of heavy metal can react with rain rich oxygen to form sulphur and then produces macromolecule of sulphides. These hazardous molecules will influences the quality of atmosphere thus may affect health and life of animal and human being that closed to the landfill. Usually after the landfill is closed, that placed is usually reconstructed as recreational places or residential area. A lot of problems will occur if the soil becomes porous and not stablebecause of the presence of heavy metal in the soil. Even though there are some heavy metal that may good for the soil, but most of heavy metal that come from industrial waste are toxic and dangerous to human. This issues then will make the reconstructed area are not safe and not suitable for human lives (Javaid, 2007; Javaid *et al.*, 2007; Javaid & Riaz, 2008).

*Effect of Heavy Metals*

A research done by M.S Li *et al.*, (2007) also done in China, large amount of heavy metal also found in Guangxi, South China cause by mining process and decomposition of coal in landfill soil. Mining, in particular the metal ore extraction is the second source of heavy metal (especially manganese) contamination in soil after sewage sludge. Even though there are some perception that told manganese are non-toxic metal element, but the fact is exposure too much to manganese may cause health damage such as Parkinson-like symptoms. Manganese ore also can accompany with other heavy metal mixture such as Pb, Zn, Cd, Ni, Co and Fe and extraction of this combine element will lead to release of more toxic metals into the environment.

*Effect of heavy metals on soil basal respiration and soil microbial metabolic quotient ($qCO_2$)*

Mineralization of organic carbon to $CO_2$ commonly known as “soil respiration” is a good index of the total activity of microflora involved in organic matter decomposition (Anderson, 1982). Therefore, soil respiration has been the most studied parameter on the effects of metals on microbial activities in soil (Baath, 1989). The basal respiration (Rb), apart from reflecting the rate of mineralization of soil organic carbon, reflects the respiratorynactivity of its microorganisms, which biodegrade organic compounds in the soil (Anderson and Domsch, 1978) and is closely related to soil environmental quality (Yeates, 1994). Heavy metals may reduce soil respiration by forming complexes with the substrates or by killing the microorganisms (Landi *et al.*, 2000). The metabolic quotient, i.e., the ratio of basal respiration to microbial biomass, indicating how efficiently the microbial biomass is utilizing available carbon for biosynthesis, is a measure of microbial response to disturbance and has been considered a sensitive ecophysiological indicator of heavy metal-induced stress in soil (Anderson and Domsch, 1990; Wardle and Ghani, 1995). Our results demonstrated that qCO2 increased markedly with increasing heavy metal concentration and was negatively correlated with soil microbial biomass, but qCO2 was significantly positively correlated with total Cu, EDTAextractable Cu, total Zn, EDTA-extractable Zn, total Pb, and EDTA-extractable Pb (Table 3), indicating a shifting of energy from growth to maintenance in an ecosystem and changes of soil quality affected by heavy metals. Chander and Brookes (1991b) reported less biomass formation from labeled substrate and higher qCO2 values in heavy metal-contaminated soils compared to normal soils. Biomass synthesis is less efficient under heavy metal stress and biomass reduction in heavy metal-contaminated soils is mainly due to inefficient biomass synthesis. Although a plausible explanation, a high microbial qCO2 in metal-contaminated soil is in itself no proof of either a higher maintenance-energy requirement or lower substrate- utilization efficiency. The microbial maintenance energy requirement is defined as the energy required for other functions than growth and is most accurately determined in energy-limited chemostat cultures from the dependence of the yield on the specific growth rate (Giller *et al.*, 1998). Therefore qCO2 can serve as an important indicator of soil quality and be closely related to soil pollution.

*Humic acids*

Humic acids are characterized as a heterogeneous natural resource, ranging in colour from yellow to black, having high molecular weight, and resistance to decay. Humic acid, as a commercial product contains 44-58% C, 42-46% O, 6-8% H and 0.5-4% N, as well as many other elements (Larcher, 2003; Lee and Bartlette, 1976). It improves soil fertility and increases the availability of nutrient elements by holding them on mineral surfaces.

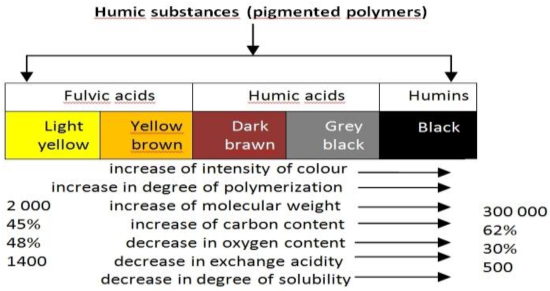


**Fig. 6.** Chemical properties of humic substances.

The humic substances are mostly used to remove or decrease the negative effects of chemical fertilizers from the soil and have a major effect on plant growth, as shown by many scientists (Linchan, 1978; Ghabbour and Davies, 2001; Pal and Sengupta, 1985). Humic acid has an essential role in agricultural processes. It increases cation exchange capacity and enhances soil fertility, converting the mineral elements into forms available to plants (Stevenson, 1994). Humic substances lead to a greater uptake of nutrients into the plant root and through the cell membrane (Yılmaz, 2007; Tipping, 2002; Kulikova *et al.*, 2005).

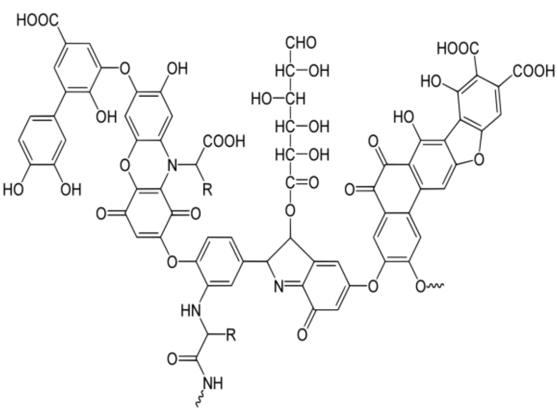

**Fig. 7.** Example of a typical humic acid, having a variety of components including quinone, phenol, catechol and sugar moieties.

Humic acids show a sponge-like tampon character in the wide pH scale, its activity may be changed by various pH levels but neutralizes soil pH, so many trace elements become available to the plant (Yılmaz, 2007). Humic substances can break the bonds between phosphate and the iron ions in between acid soils and in calcium and iron ions in alkaline soils (Stevenson, 1994). The available studies have revealed correlations between the root growth and development and the uptake of some nutrients. For instance, humic acid caused increases in length and dry weight of maize plant roots, and enhanced the uptake of nitrogen, phosphorus, K+, Ca2+, Cu2+, Mn2+, $Zn^{2+}$ and $Fe^{3+}$ (Eyheraguibel *et al.*, 2008). Humic substances increased root length in Helianthus annuus L. (Kolsarıcı *et al.*, 2005), in maize roots, and uptake of micronutrients such as Zn2+, Fe3+, Mn2+ and Cu2+ (Sharif *et al.*, 2002), as well as root dry weight in tomato and cucumber (Atiyeh *et al.*, 2002); in ryegrass, humic substances stimulated root development and enhanced nitrogen, K+, Cu2+ and Mn2+ content (Bidegain *et al.*, 2000); and increased root fresh and dry weight (Dursun *et al.*, 1999). According to Adani *et al.* (1998) commercial humic acid affected tomato root fresh and dry weights of tomato as well as iron content, depending on the source of the humic acid. The two concentrations (20 and 50 mg/L) of humic acid, resourced from fertiliser, caused iron to increase to 113%, and 123% whereas humic substance derived from leonardite increased iron content to 135% and 161% in tomato roots.

*The role of humic acid*

The role of humic acid is well known in controlling, soil-borne diseases and improving soil health and nutrient uptake by plants, mineral availability, fruit quality, etc (Mauromicale *et al.*, 2011). Humic acid based fertilizers increase crop yield (Mohamed *et al.*, 2009), stimulate plant enzymes/hormones and improve soil fertility in an ecologically and environmentally benign manner (Mart, 2007; Sarir *et al.*, 2005). Several research workers highlighted the positive benefits of humic acid application on higher plants (Vasudevan *et al.*, 1997; Ashraf *et al.*, 2005; Susilawati *et al.*, 2009). Humic acids also reduce toxic effects of salts on monocots (Masciandaro *et al.*, 2002) and dicots (Ferrara *et al.*, 2001), including soybean, wheat (Ozkutlu *et al.*, 2006), rapeseed (Keeling *et al.*, 2003), forage, turnip (Albayrak, 2005) and mustard (Duval *et al.*, 1998). Enhanced nutrient uptake by plants as a result of humic acid application is also well established (Aydin *et al.*, 1999; Day *et al.*, 2000; Mackowiak *et al.*, 2001; Sharif *et al.*, 2004). Likewise, the increased yield is also observed in many crops due to humic acid application, including potato (Grady and Tina, 1999), brassica (Peng *et al.*, 2001; Vetayasuporn, 2006), tomato, onions and other leafy vegetables (Grady and Tina, 1999; Erik *et al.*, 2000).

*Cadmium (Cd)*

Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedel-Koska and Doran, 2000). They present a risk for primary and secondary consumers and ultimately humans. Due to high $Cd^{2+}$ mobility in the soil-plant system it can easily enter food chain and create risk for humans, animals, plants and the whole environmental of our modern society (Pinto *et al.* 2004). A part of agricultural soils all over the world is slightly to moderately contaminated by cadmium (Cd) due to extended use of superphosphate fertilizers, sewage sludge application as well as smelters dust spreading and atmospheric sedimentation (Thawornchaisit and Polprasert, 2009). According to Wagner (1993), Cd concentration in soil solution of uncontaminated soils is in the range of 0.04-0.32 µM, while moderately polluted soils contain 0.32-1.00 µM. In soil, containing more than 35 µM Cd in the soil solution, only species with Cd tolerance are capable of surviving. Cadmium has been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition, and water imbalance (Benavides *et al.*, 2005). Cadmium produces alterations in the functionality of membranes, decreases chlorophyll content, and disturbs the uptake and distribution of macro- and micro-nutrients in plants (Ramon *et al.*, 2003).

**References**

**Anderson J, Domsch H.** 1975 Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology. **21,** 314-322.

**Andrews SS, Karlen DL, Cambardella CA.** 2004. The soil management assessment framework: A quantitative soil quality evaluation method. Soil Science Society of America Journal . **68,** 1945-1962.

**Bach E, Baer S, Meyer Six J.** 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biology and Biochemistry. **42,** 2182-2191.

**Badalucco L, Pomaré F, Grego S, Landi L.** 1994. Activity and degradation of streptomycin and cycloheximide in soil. Biology and Fertility of Soils. **18,** 334-340.

**Bailey V, Smith J, Bolton H.** 2002. Fungal-to-bacterial ratios in soils investigated for enhanced carbon sequestration. Soil Biology and Biochemistry. **34,** 997-1007.

**Bailey V, Smith J, Bolton H.** 2003. Novel antibiotics as inhibitors for the selective respiratory inhibition method of measuring fungal: bacterial ratios in soil. Biology and Fertility of Soils. **38,** 154- 160.

**Baldrian P, Trogl J, Frouz J, Snajdr J, Valaskova V, Merhautová V, Cajthaml T, Herinková J.** 2008. Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining. Soil Biology and Biochemistry. **40,** 2107-2115.

**Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC.** 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils. **29,** 282-290.

**Benavides M, Gallego S, Tomaro M.** 2005. Cadmium toxicity in plants. Braz journal of Plant Physiology. **17,** 21-34.

**Bittelli M, Flury M, Campbell G, Nichols E.** 2001. Reduction of transpiration through foliar application of chitosan. Agricultural and Forest Meteorology. **107(3),** 167-175.

**Bloem J, Bolhuis PR, Veninga MR, Wieringa J.** 1995. Microscopic methods for counting bacteria and fungi in soil. In 'Methods in applied soil microbiology and biochemistry'. (Eds K Alef, P Nannipieri).Biology and Fertility of Soils. **12,** 162-174.

**Bossio DA, Scow KM.** 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied and Environmental Microbiology. **61,** 4043–4050.

**Carter MR.** 2002. Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. Agronomy Journal. **94,** 38-47.

**Chen C, Blumfied M.** 2003. Soil microbial biomass during the early establishment of hoop pine plantation: seasonal variation and impacts of site preparation. Forest Ecology and Management. **186,** 213-225.

**Chlopecka A, Adriano DC.** 1997. Influence of zeolite, apatite and Fe-oxide on Cd and Pb uptake by crops. The Science of the Total Environment journal. **207,** 195-206.

**Clemente R, Waljker J, Bernal P.** 2005. Uptake of heavy metals and As by Brassica Juncea grown in a contamination soil in Arnalcollar (Spain): The effect of soil amendments. Environmental Pollution. Agronomy Journal. **136,** 46-58.

**Coq S, Barthes GB, Oliver R, Rabary B, Blanchart E.** 2007. Earthworm activity affects soil aggregation and organic matter dynamics cultivated kiwifruit orchard. Applied Soil Ecology. **49,** 158–166.

**Derouard L, Tondoh J, Vilcosqui L, Lavelle P.** 1997. Effects Of earthworm introduction on soil processes and plant growth. Soil Biology and Biochemistry. **29,** 542–545.

**Doran JW, Coleman DC, Bezdicek DF, Stewart BA.** 1994. Defining Soil Quality for a Sustainable Environment. SSSA Special Publication Soil Science Society of America. earthworm species. Agriculture, Ecosystems and Environment. **103,** 329–342.

**Eaton JM, McGoff NM, Byme KA, Leahy P, Kiely G.** 2008. Land cover change and soil organic carbon stocks in the Republic of Ireland 1851–2000. Journal of Climate Change. **91,** 317–334.

**ElDuweini, AK, Ghabbour SI.** 1965. Population density and biomass of earthworms in different types of Egyptian soils. Soil Biology and Biochemistry. **2,** 271-287.

**Ernst G, Henseler I, Felten D, Emmerling C.** 2009. Decomposition and mineralization of energy crop residues governed by earthworms. Soil Biology and Biochemistry. **41,** 1548–1554.

**GilotC, Lavelle P, Ganry F.** 1996. Effects of a tropical geophageous earthworm Millsonia anomala on some soil characteristics, on maize-residue decomposition and on maize production in Ivory Coast. Applied Soil Ecology. **4,** 201–211.

**Grant WD, West AW.** 1986. Measurement of ergosterol, diaminopimelic acid and glucosamine in soil: evaluation as indicators of microbial biomass. Journal of Microbiological Methods. **6,** 47-53.

**Green CT, Scow KM.** 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal. **8,** 126–141.

**Guggenberger G, Thomas RJ, Zech W.** 1996. Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community. Applied Soil Ecology. **3,** 263–274.

**Hoffman GK, Austin GS.** 1994. Agricultural materials: soil additives; In: D.D. Carr, Senior Editor, Industrial Rocks and Minerals, 6th Edition. Society of Mining, Metallurgy and Exploration. Microbial Ecology journal . **12,** 89-96.

**Jordan D, Miles RJ, Hubbard VC, Lorenz T.** 2004. Effect of management practices and cropping systems on earthworm abundance and microbial activity in sanborn field : a 115-year-old agricultural field. Fresenius Environmental Bulletin.. **48(2),** 99-110.

**Kalka J, Miksch K, Grabinska-Sota E, Zbrog A.** 2002. The effects of pyrethroid insecticides on earthworm Eisenia fetida. Fresenius Environmental Bulletin. **11(2),** 114-117.

**Langan AM, Shaw EM.** 2006. Responses of the earthworm *Lumbricus terrestris* (L.) to iron phosphate and metaldehyde slug peelet formulations. Applied Soil Ecology. **34,** 184-189.

**Lofs-Holmin A.** 1983. Influences of agricultural practices on earthworm (Lumbricidae). Acta Agriculturae Scandinavica . **33,** 225-234.

**Lydy MJ, Linck SL.** 2003. Assessing the impact of triazine herbicide on organophosphate insecticide toxicity to the earthworm Eisenia fetida. Archives of Environmental Contamination and Toxicology. **45(3),** 343-349.

**Mackay AD, Kladivko J.** 1985. Earthworms and rate of breakdown of soybean and maize residues in soil. Soil Biology and Biochemistry. **17(6),** 851-857.

**Mahajan S, Kanwar SS, Sharma SP.** 2007. Long-term effect of mineral fertilizers and amendements on microbial dynamics in an alfisol of western Himalayas.Indian Journal of Microbiology. **47(1),** 85-89.

**Marhan S, Scheu S.** 2005. The influence of mineral and organic fertilizers on the growth of the endogeic earthworm Octolasion tyrtanum (Savigny). Pedobilogia. **49(3),** 239-249.

**Marinissen JCY.** 1992. Population dynamics of earthworms in a silt loam soil under conventional and integrated arable farming during two years with different weather patterns. Soil Biology and Biochemistry. **24(12),** 1647-1654.

**Morowati M.** 2000. Histochemical and histopathological study of the intestine of the earthworm (Pheretima elongata) exposed to a field dose of the herbicide glyphosate. The Environmentalist. **20(2),** 105-111.

**Schmidt O, Clements RO, Donaldson G.** 2003. Why do cereal–legume intercrops support large earthworm populations? Applied Soil Ecology. **22(2),** 181-190.

**Scullion J, Ramshaw GA.** 1987. Effects of various manorial treatments on earthworm activity in grassland. Biological Agriculture & Horticulture. **4,** 271-281

**Senapati BK, Dash MC.** 1984. Functional role of earthworms in decomposer subsystem. Tropical Ecology. **25(2),** 54-73.

**Tindaon F, Benckiser G, Ottow JCG.** 2011. Side effects of nitrification on non-target microbial processes in soils. Journal of Tropical Soils. **16(1),** 7-16.

**Zaman M, Matsushima M, Chang SX, Inubushi K, Nguyen L, Goto S, Kaneko F, Yoneyama T.** 2004. Nitrogen mineralization, N2O production and soil microbiological properties as affected by longterm applications of sewage sludge composts, Biological Fertile. Soils. **40,** 101–109.

**Zmora-Nahum S, Markovitch O, Tarchitzky J, Chen Y.** 2003. Dissolved organic carbon (DOC) as a parameter of compost maturity, Soil Biol journal. **37,** 120- 143.

**Hoogerkamp M, Rogaar H, Eijsackers H.** 1983. Effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. In: Earthworm ecology, from Darwin to Vermiculture. (Satchell, J. E. eds.), Chapman and Hall, London. Chinese Journal of Applied Ecology. **12,** 85-105.

**Ilstedt U, Malmer A, Verbeeten E, Murdiyarso D.** 2007. The effect of afforestation on water infiltration in the tropics: A systematic review and meta-analysis. Forest Ecology and Management. **251,** 45–51.

**Laossi KR, Noguera DC, Decaens T, Barot S.** 2011. The effects of earthworms on the demography of annual plant assemblages in a longterm mesocosm experiment. Pedobiologia. **54**, 127–132.

**Li Y.** 2009. The effect of Cd on mycorrhizal development and enzyme activity of Glomus mosseae and Glomus intraradices in Astragalus sinicus L. Chinese Journal of Applied Ecology. **75,** 894–899

**Lingua G, Franchin C, Todeschini V, Castiglione S.** 2008. Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. Chinese Journal of Applied Ecology. **153,** 137–147

**Lundquist EJ, Jackson LE.** 1999b. Wet-dry cycles affect dissolved organic carbon in two California agricultural soils. Soil Biology and Biochemistry. **31,** 1031–1038.

**Lundquist EJ, Scow KM, Jackson LE, Uesugi SL, Johnson CR,** 1999a. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biology and Biochemistry. **31,** 1661–1675.

**Macalady JL, Mack E, Nelson DC.** 2000. Sediment microbial community structure and mercury methylation in mercurypolluted Clear Lake, California. Applied and Environmental Microbiology. **66,** 1479–1488.

**Maire N, Borcard D, Laczkó E, Matthey W.** 1999. Organic matter cycling in grassland soils of the Swiss Jura mountains: biodiversity and strategies of the living communities. Soil Biology and Biochemistry. **31,** 1281–1293.

**MANLAY R, CADET J.** 2000. Relationships between abiotic and biotic soil properties during fallow periods in the Sudanian zone of Senegal. Applied Soil Ecology. **14,** 89-101.

**Mariotte C A, Hudson G, Hamilton D, Neilson R, Boag B, Handley L L, Wishart J, Scrimgeour C M, Robinson D.** 1997. Spatial variability of soil total C and N and their stable isotopes in an upland Scottish grassland. Plant and Soil sciences. **196,** 151-162.

**McKinley V L, Peacock A D, White D C.** 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry. **37,** 1946-1958.

**Michalak A.** 2006. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. Polish J. Environ. Stud. **15,** 523–530

**Moore J, Macalady J L, Schulz M S, White A F, Brantley S L.** 2010. Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. Soil Biology and Biochemistry. **42,** 21-31.

**Nsabimana D, Haynes R J, Wallis F.** 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology, **26(2),** 81–92.

**Nsabimana D, Haynes R J, Wallis F M.** 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology. **26(2),** 81–92.

**Wang B, Liu G, Xue S, Zhu B.** 2011. Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the Loess Plateau. Environmental Earth Sciences. **62,** 915-925.

**Welc M, Bünemann E K, Fließbach A, Frossard E, Jansa J.** 2012. Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. Soil Biology and Biochemistry. **49,** 184-192.

**Lezhi B, Zucong C,** 2012. Key variables explaining soil organic carbon content variations in croplands and non-croplands in Chinese provinces. Chinese Geographical Science. **22(3),** 1–9.

**Yang Y, Guo J, Chen G.** 2009. Effects of forest conversion on soil labile organic carbon fractions and aggregate stability in subtropical China. Plant and Soil. **323(1),** 153–162.