Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 6, p. 213-231, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Influence of earthworms and humic acid on some microbial indices in a Pb contaminated soil

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Article published on December 14, 2014

Key words: Compost, vermicomposting, lignocellulose, plumbum.

Abstract

Increasing human population pressure has decreased the availability of arable land and it is no longer feasible to use extended fallow periods to restore soil fertility. The fallow period which would have restored soil fertility and organic carbon is reduced to lengths that cannot regenerate soil productivity leading to the non-sustainability of the farming systems. Microbiological and biochemical soil properties are very reactive to small changes occurring in management practices. Therefore, it is possible to use them in a basic analysis for evaluating the effects of the application of different sources and amount of organic matter on soil characteristics during experimental trials. The chemical and physical stability of the compost determines the shelf-life and applicability of compost for various uses. Stable compost is one that shows an advanced degree of organic matter decomposition with resistance to further decomposition. Lignocellulose is the major structural material of plant bodies and constitutes the enormously important bio renewable resource used to make building materials, paper, textiles and many polymer derivatives. Heavy metals have sensitive influence on microbial community structure in soil, which ultimately lead to the changes of microbial amounts microbial activities including enzymes. Vermicomposting has been widely identified as one of the potential activity to reduce the quantity of solid waste that need to be sent to the landfills. Vermicomposting is classified as biological treatment under intermediate treatment technologies of solid waste management.

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Introduction

Increasing human population pressure has decreased the availability of arable land and it is no longer feasible to use extended fallow periods to restore soil fertility. The fallow period which would have restored soil fertility and organic carbon is reduced to lengths that cannot regenerate soil productivity leading to the non-sustainability of the farming systems (Nandwa, 2001). High population densities have necessitated the cultivation of marginal lands that are prone to erosion hence enhancing environmental degradation through soil erosion and nutrient mining. As a result, the increase in yield has been more due to land expansion than to crop improvement potential (FAO, 2003). During the second half of the 20th century, many energy-consuming agricultural practices were adopted as part of the modern scientific approach to achieve higher yields. Such practices were also encouraged by the large availability of cheap fuel. Heavy tillage, frequent weed control, abundant fertilization and surface water movement across large fields by pumping were keystones of the dominant production paradigm. Plow-based soil cultivation, in particular, has become so common in mainstream modern agriculture that the term “tillage” is widely used as a synonym for “agriculture” (Dick and Durkalski, 1997). Nevertheless, continuous soil disturbance through cultivation and particularly through soil inversion has lead to the degradation of soil structure, soil compaction, and decreased levels of organic matter in soil. This, in turn, has caused a wide range of environmental impacts, including soil degradation, water and wind erosion, eutrophication, increased carbon emissions released from the soil due to the use of high energy-consuming machinery, and an overall reduction in beneficial soil organisms and mammals. As soil accumulated over the eons, it provided a medium in which plants could grow. In turn, plants protected the soil from erosion. The agricultural activity of humans has been disrupting this relationship. Climate change has also exacerbated the problems of degradation and variability as rainfall events have become more erratic with a greater frequency of storms (Osborn *et al.*, 2000). Conservation agriculture systems using a low level of external inputs have become more important worldwide in the last few years since they promote the preservation of natural resources, reducing ecosystem degradation, both natural and agroecosystems (Mäder *et al.*, 2002; Francis and Daniel, 2004). Within this context, organic agriculture promotes the use of agronomic practices and alternative technologies in accordance with the socioeconomic and ecological conditions of the zone where the productive system is located, in particular as it refers to fertilization, which is usually carried out by applying organic materials such as manure, plant residues, or compost, much of which are produced inside the same farm (Shannon *et al.*, 2002). Applying organic materials to crop soil not only generates a better nutritional state, but furthermore, positively influences other properties, such as soil particles aggregation, water holding capacity and aeration (Pagliai *et al.*, 2004), contributing to generating high production, even with a low or nil application of fertilizers. Another important contribution to soil quality improvement using conservation practices based on the use of organic substances like compost is the beneficial effect on growth, diversity, and activity of diverse groups of rhizospheric microorganisms that promote plant growth (Oehl *et al.*, 2004; Gosling *et al.*, 2006). This article is review and motivation and aims of the study were influence of earthworms and humic acid on some microbial indices in a Pb contaminated soil.

Soil organic carbon (SOC)

Optimum management of the soil resource for provision of goods and services requires the optimum management of organic resources, mineral inputs and the soil organic carbon (SOC) pool (Vanlauwe, 2004). The importance of SOC has increased interest and research on its build up in the soil–plant system with current emphasis on conservation tillage. SOC can play an important role and its maintenance is an effective mechanism to combat land degradation and increase future food production. Various farm practices have been employed to build SOC stocks in

West Africa. Crop (CR) residue application as surface mulch can play an important role in the maintenance of SOC levels and productivity through increasing recycling of mineral nutrients, increasing fertilizer use efficiency, and improving soil physical and chemical properties and decreasing soil erosion. However, organic materials available for mulching are scarce due to low overall production levels of biomass in the region as well as their competitive use as fodder, construction material and cooking fuel (Lamers and Feil, 1993). Windmeijer and Andriesse (1993) found levels of SOC for equatorial forest, Guinea savanna and Sudan savanna to be 24.5, 11.7, and 3.3 g kg1, respectively, and showed positive correlation with both N and P (Table 1).

Table 1. Carbon stocks and other fertility indicators of granitic soils in different agro-ecological zones in West Africa.

AEZ	pH (H_2O)	OC (g kg^{-1})	Total N (g kg^{-1})	Total P (mg kg^{-1})
Equatorial forest	5.3	24.5	1.6	628
Guinea savanna	5.7	11.7	1.39	392
Sudan savanna	6.8	3.3	0.49	287

Source: Windmeijer and Andriesse (1993).

Biological fertility

Microbiological and biochemical soil properties are very reactive to small changes occurring in management practices. Therefore, it is possible to use them in a basic analysis for evaluating the effects of the application of different sources and amount of organic matter on soil characteristics during experimental trials. Microorganisms, e.g. bacteria, fungi, actinomycetes and microalgae, play a key role in organic matter decomposition, nutrient cycling and other chemical transformations in soil (Murphy *et al.*, 2007). Since organic carbon (C) is utilized for energy by decomposer microorganisms, its fate is to be either assimilated into their tissues, released as metabolic products, or respired as carbon dioxide (CO2). The macronutrients N, P and sulfur (S), present in the organic chemical structures, are converted into inorganic forms. Subsequently, they are either immobilized and used in the synthesis of new microbial tissues or mineralized and released into the soil mineral nutrient pool (Baldock and Nelson, 2000). For assimilation by microorganisms of decomposing organic residues, the N has to be assimilated in an amount determined by the C/N ratio of the microbial biomass. More specifically, the amount of N required by the microorganisms is 20 times smaller than that of C. If there are both a low concentration of easily decomposable C compounds and a larger N quantity in respect to that required by the microbial biomass, there will be net N mineralization with release of inorganic N. On the contrary, Corbeels *et al.* (1999) found that if the amount of N present in the residues is smaller than that required by the microbial biomass, further inorganic N will need to be immobilized from the soil to complete the decomposition process. It is difficult to distinguish between the direct and the indirect effects of an amendment on the behavior of soil microorganisms. In soils amended with compost or other raw organic materials, even in association with mineral fertilizer N, autochthonous microbiological activity and growth can be stimulated. However, different authors (Ros *et al.*, 2006a; Kaur *et al.*, 2008) suggest that a direct effect from microorganisms introduced with the compost is detectable. Several long-lasting experiments have demonstrated that soil biological properties, such as microbial biomass C, basal respiration and some enzymatic activities, are significantly improved by compost treatments. This is particularly evident in the upper layers of the soil because of the added labile fraction of organic matter, which is the most degradable one (Zaman *et al.*, 2004; Ros *et al.*, 2006a, b; Tejada *et al.*, 2006, 2009). Since generally the composts are slowly decomposed in the soil, the continuous release of nutrients can sustain the microbial biomass population for longer periods of time, compared with mineral fertilizers (Murphy *et al.*, 2007). In fact, an interesting residual effect of composts on the microbial activity has often been observed in many experimental seasons after their application, which also results in a longer availability of plant nutrients. Ginting *et al.* (2003), for example, found 4 years after the last application of compost and manure that the residual effects resulted in 20 to 40% higher soil microbial biomass C compared with the N fertilizer treatment.

Municipal Solid Waste Compost

Soils intensively affected by human activities might present special features such as mixed horizons, foreign materials and thin topsoil (Short *et al.*, 1986; Civeira and Lavado, 2008). Normally, these soils are poor in organic matter (OM) (e.g., < 1%) and fertility with reductions in their most important physical properties, such as structural stability and water retention. Eventually, these characteristics might have a detrimental effect on plant growth and submit this particular environment to erosion processes (Vetterlein and Hüttl, 1999; Scharenbroch *et al.*, 2005). Consequently, deteriorated soils in populate cities do not tolerate agricultural or recreational uses and turned these environments into places with low probability of community progress. Due to urban soils present different characteristics compared to agricultural ones, their intrinsic properties and rehabilitation techniques have not yet been sufficiently relieved (Larson and Pierce, 1991; Scharenbroch *et al.*, 2005). In recent decades, the application of organic wastes from different origins (manure, sewage sludge and municipal organic wastes) to degraded soils is a practice globally accepted to recover, replenish and preserve OM, fertility and vegetation (Vetterlein and Hüttl, 1999; Civeira and Lavado, 2008). Before application to soils, organic wastes should been stabilized using composting techniques. The use of composted organic wastes produces changes in soil physical, chemical and biological properties and can enhance plant growth after its application. However, the influence of C rich materials, like municipal organic wastes compost, on soil physical, chemical and biological properties depends upon several factors: amount and components of added organic materials, soil type and weather conditions (Unsal and Ok, 2001; Drozd, 2003). As pointed out by Giusquiani *et al.* (1995) and Drozd (2003) the use of composts from municipal solid wastes (MSW) improves the restoration of degraded soils and allows an appropriate final disposition of such materials, solving a major environmental and economic problem generated in the cities.

Nutrient Management

Compost is made from a variety of feedstock's such as plants materials from agricultural and vegetable gardens, animal waste (manure), municipal solid waste, yard waste (garden and park trimmings), domestic and commercial food waste, and municipal and industrial waste water processing sludge (bio solids). Green waste is organic wastes primarily consisting of fresh plant material, though domestic and commercial food waste is often included under this classication (Oregon Department of Environmental Quality, 2001). Green waste contains appreciable amounts of nitrogen, phosphorus, and mineral nutrients (Raj and Antil, 2011; Amlinger *et al.*, 2003) and has low C/N ratios. Feedstocks such as hay, paper, cardboard, and dry plant leaves contain primarily carbon, oxygen, and hydrogen and have high C/N ratios, such waste is called brown waste. Compost made from green waste usually contains large amounts of nitrogen and phosphorus. Composting therefore plays an important role in nutrient cycling. Currently composted use as nutrient sources in farmlands, public and private gardens, parks, highway embankments, and landscaping (Goyal *et al.*, 2005; Zmora-Nahum *et al.*, 2005; Raj and Antil, 2011).

Maturity and Stability of compost

The chemical and physical stability of the compost determines the shelf-life and applicability of compost for various uses. Stable compost is one that shows an advanced degree of organic matter decomposition with resistance to further decomposition (Mondini *et al.*, 2003; Wichuk and McCartney, 2010). A stable compost shows steady values of a number of indices like respiration rates (Wu *et al.*, 2000), microbial count and biomass, organic matter content, C/N ratio, and storage temperature (Ba *et al.*, 2007; Wichuk and McCartney, 2010). A related term that is also used, sometimes interchangeably, is the maturity of compost. Many authors consider compost maturity from the viewpoint of how binomial the compost is to plants, as determined by indicators like germination rates, plant growth (biomass formation) assays, and residual content of phytotoxic compounds, like pesticides and low molecular weight organic acids (Iannotti *et al.*, 1993; Wu *et al.*, 2000). For example, measurement of microbial respiration and oxygen uptake rates are often used to monitor the composting process and assess compost maturity (Lasaridi and Stentiford, 1998; Wu *et al.*, 2000; Borken *et al.*, 2002; Boulter-Bitzer *et al.*, 2006; Scaglia *et al.*, 2007; Kalamdhad *et al.*, 2008; Tejada *et al.*, 2009; Soriano-Disla *et al.*, 2010; Komilis and Kanellos, 2012). Maturity and stability of compost can also be assessed by determining changes in chemical structure of the compost over time. Tang *et al.*, 2006 used solid-state 13C NMR, respiratory qui none proles (amount of quinone enzymes), elemental composition, and germination indices to determine changes in compost characteristics during the maturing period (up to 18 months). The results indicate that the initial microbial biomass that had developed during the thermophiles stage was maintained for 2 to 4 months, but then a decrease in carbon content, C/N ratio, microbial biomass, microbial variety, and phytotoxicity was observed. Common indicators of compost maturity and stability are germination rates (phytotox-icity) and amounts of soluble organic matter (humic and fulvic acids), which indicate degree of humectations. Bustamante *et al.*, 2010 used the germination index (percentage of germinating seeds in a compost sample of a given age compared to a control growth medium) and the amount of dissolved organic matter from compost extracts to assess compost maturity.

Soil organic matter

The soil organic matter (SOM) plays an essential role in soil biogeochemical processes (Bot and Benites, 2005). Thus, a productive and healthy soil must present a balance among SOM protection and soil biological functioning (Wander, 2004).

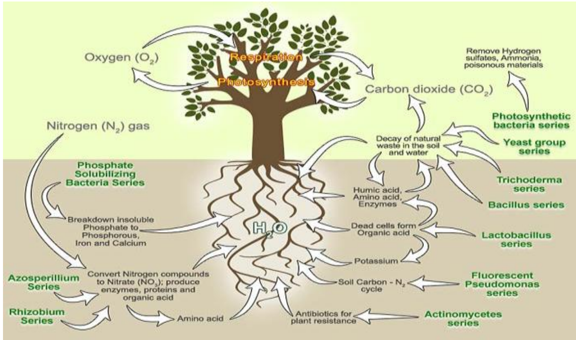

Fig. 1. Microorganisms in soil and how it effects plant growth.

However, the prediction of organic matter dynamics in soil is hampered by the complexity of SOM distribution and chemical composition (Foereid *et al.*, 2012). The integration of organic inputs in the physicochemically defined organic pools in soil (Six *et al.*, 2002) and their effect on native organic matter has been described to vary with land use, soil physicochemical properties (Strong *et al.*, 2004; Denef and Six, 2005); and composition of the organic inputs (Kimetu and Lehmann, 2010). The term soil organic matter refers to all organic substances in the soil: plant and animal residues, substances synthesized through microbial and chemical reactions and biomass of soil micro-organisms. The processes responsible for the stabilization of SOM constitute an essential component of global biogeochemical cycles (Lehmann *et al.*, 2007). Total soil organic matter might not be the best indicator to assess changes in soil quality at these sites, as it often takes many years before significant changes are detected in the quantity and quality of soil organic matter (Hassink *et al.*, 1997; Gong *et al.*, 2009). However, different fractions of the soil organic matter may respond differently. For example, the light fraction organic matter, which can be isolated from soil organic matter by flotation, is a plant-like fraction with high C concentration (Tan *et al.*, 2007) and has a relatively short turnover time in the soil (Gregorich *et al.*, 1996).

Thus, this fraction is generally considered to be a highly dynamic soil property, and may therefore provide an early indicator of management-induced changes in soil quality (Six *et al.*, 2002; Murage *et al.*, 2007; Bending and Turner, 2009; Wang *et al.*, 2009).

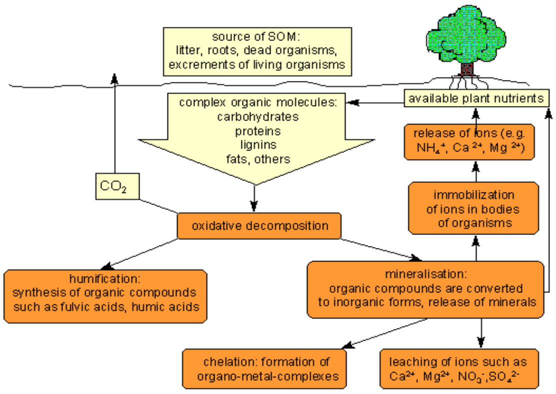

Fig. 2. Transformation of soil organic matter within soil.

Research suggests that repeated applications of organic amendments, such as animal manures, biosolids, and composted materials, can increase soil organic matter level (Darby *et al.*, 2006; Tester, 1990), even in the southeastern U.S., where the organic matter oxidation is rapid (Ozores-Hampton *et al.*, 1998). Soil organic matter has many known benefits, such as increased water holding capacity, improved soil aeration (through reduced bulk density), and increased soil fertility (Tester, 1990). Increasing soil organic matter can also enhance microbial activity, which can improve plant nutrient uptake and suppression of certain plant diseases (Darby *et al.*, 2006; Stone *et al.*, 2003; Vallad *et al.*, 2003). Producer reliance on inorganic fertilizers to meet crop nutrient requirements does nothing to enhance soil organic matter levels. In addition, fields are commonly left fallow between crop cycles allowing the loss of organic matter as topsoil is eroded by wind or water. There are also many references in the literature that support the idea that F. oxysporum can be suppressed using composted materials (Gordon and Martyn, 1997; Noble and Conventry, 2005). However, evidence that suppression occurs in a field environment is limited. A majority of the research related to compost and disease suppression has been done in a controlled greenhouse environment using peat-based potting mixes in pots (Aldahmani *et al.*, 2005; Rose *et al.*, 2003).

Stability of Organic Matter

The physical stabilization is the preferential location of OM in the soil structure which results in lower access to OM by soil micro-organisms. Thus, integration of OM in soil aggregates reduces the availability of OM for microbial transformation (Six *et al.*, 2002). The biochemical stabilization is a selective enrichment of organic compounds, and refers to the inherent recalcitrance of specific organic molecules against degradation by microorganisms and enzymes. Thus, compounds like lignin, lipids and polyphenols will remain more stable in the soil matrix compared to more labile compounds like polysaccharides and proteins (Six *et al.*, 2002; Kögel-Knabner *et al.*, 2008).

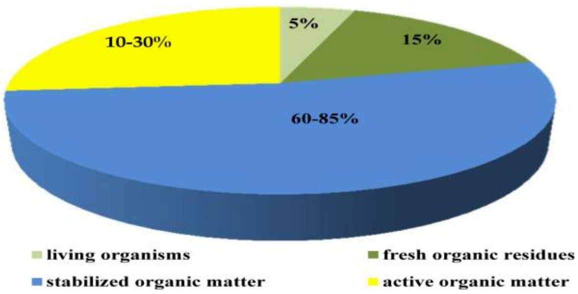

Fig. 3. Composition and distribution of fractions of soil organic matter.

The chemical stabilization involves all intermolecular interactions between organic and inorganic substances leading to a decrease in availability of the organic substrate due to surface condensation and changes in conformation, i.e., sorption to soil minerals and precipitation. The chemical stabilization of SOM results mainly from the interaction of SOM with minerals and metal ions. These interactions include organo-mineral associations such as complexation of organic substances with polyvalent cation bridges, weak hydrophobic interactions (Van der Waals and H-binding) and sorption of SOM to soil minerals (von Lützow *et al.*, 2006; Jastrow *et al.*, 2007). Therefore, some authors have pointed clay fraction as an inhibitor of SOM decomposition

(Kleber *et al*., 2007). For instance, Merckx *et al.* (1985) described that the stabilization of C and N in soils is positively correlated to the content of clay and silt. Moreover, other authors have indicated that the specific type of clay present in the soil, i.e. clay mineralogy, is most relevant for the capability of a particular soil to stabilize OM (Sollins *et al*., 1996; Denef and Six, 2005). Consequently, it might be adequate to evaluate specific surface and surface reactivity of soil minerals as predictors of OM stabilization rather than clay content (Baldock and Skjemstad, 2000).

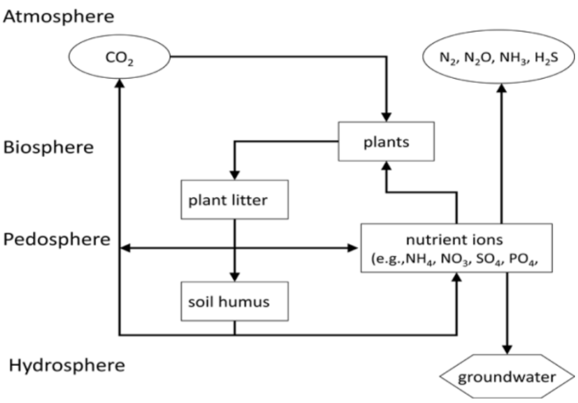

Fig. 4. Conceptual model of soil organic matter decomposition.

Human impact on soil micro-organisms

Human impact on soil micro-organisms by changes in land use may lead to adverse effects on soil processes, which is the driving force behind many research activities. Important and typical research objectives have been land-use conversion (Ayanaba *et al.* 1976; Luizao *et al.* 1992; Basu and Behera 1993; Prasad *et al.* 1995; Gijsman *et al.* 1997; Motavalli *et al.* 2000; Dinesh *et al.* 2004a; Bossio *et al.* 2005; Nogueira *et al.* 2006), crop rotation (Wick *et al.* 1998; Oberson *et al.* 2001; Bu¨nemann *et al.* 2004; Izquierdo *et al.* 2003; Adeboye *et al.* 2006; Vineela *et al.* 2008), tillage (Salinas-Garcı´a *et al.* 2002; Balota *et al.* 2004; Agele *et al.* 2005), agroforestry (Mazzarino *et al.* 1993; Tornquist *et al.* 1999; Marschner *et al.* 2002), and conversion of rainforest to plantation (Behera and Sahani 2003; Dinesh *et al.* 2003; Adachi *et al.* 2006).

Organic matter in tropical soils

The maintenance of soil fertility and soil quality is especially important in tropical regions (Bationo *et al.* 2007). Since temperature controls many processes in soil, especially those mediated by soil micro-organisms, the higher temperature in tropical regions leads to faster turnover rates of microbial biomass and soil organic matter in comparison to temperate climatic conditions (Jenkinson and Ayanaba 1977; Diels *et al.* 2004; see Chap. 1), shortening the time taken for ecosystems to respond to changes in management practices and increasing the risk of permanent damage (Cerri *et al.* 2003). In contrast to the nearly constant air temperature, humidity and soil water content are characterized by extreme changes between dry and rainy seasons under tropical savannah (Gijsman *et al.* 1997; Andersson *et al.* 2004; Ndiaye *et al.* 2004) and tropical monsoon climatic conditions (Srivastava and Singh 1991; Manna *et al.* 2007; Vineela *et al.* 2008). Only under equatorial rainforest climatic conditions is microbial activity not limited throughout the year by humidity and soil water content (Henrot and Robertson 1994; Cleveland *et al.* 2003; Li *et al.* 2006a,b). Tropical equatorial rainforests have been subjected to heavy logging and agricultural clearance throughout the twentieth century and up to the present day, and the area covered by rainforests around the world is rapidly shrinking (Luizao *et al.* 1992; Henrot and Robertson 1994; Adachi *et al.* 2006). In these equatorial areas, agricultural land-use systems are new and may not be sustainable (Motavalli *et al.* 2000; Dinesh *et al.* 2003; Bossio *et al.* 2005). In other tropical regions, especially those with tropical monsoon climatic (Witt *et al.* 2000) conditions, sustainable agricultural land-use systems have existed for several hundreds of years.

Lignocellulose

Lignocellulose is the major structural material of plant bodies and constitutes the enormously important biorenewable resource used to make building materials, paper, textiles and many polymer derivatives. At the nanoscale lignocellulose is a highly versatile composite of three complex biopolymers, namely, crystalline nm-scale fibrils of cellulose which are linked together by less-ordered polysaccharides (such as xylans) and embedded in lignin, a complex and heterogeneous phenolic macromolecule. Despite its huge economic importance, many aspects of lignocellulose structure and formation remain shrouded in mystery.

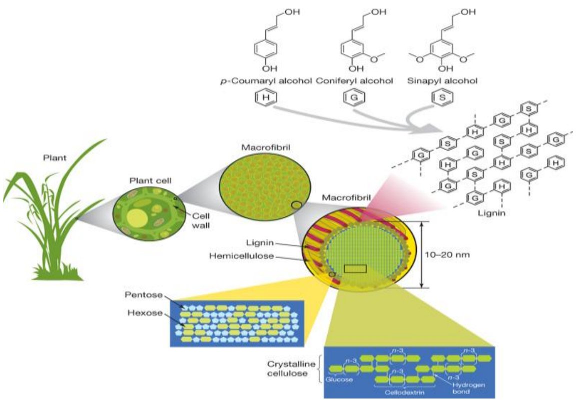

Fig. 5. Whatever the source is, lignocellulose serves as raw material in many different processes.

For instance, little is known of the details of how the cellulose-synthesizing nano-machine at the cell surface links simple sugar molecules into long strands and extrudes them at the cell surface in such a way that they make a strong, insoluble and highly inert crystalline fibril. In addition to its current economic importance as a biomaterial, lignocellulose is also the largest store of renewable solar energy on Earth (Atiyeh *et al.*, 2000b)

Earthworm

It is well established that earthworms have beneficial physical, biological and chemical effects on soils and many researchers have demonstrated that these effects can increase plant growth and crop yields in both natural and managed ecosystems (Edwards and Bohlen, 1996; Edwards, 1998). These beneficial effects have been attributed to improvements in soil properties and structure (Kahsnitz, 1992), to greater availability of mineral nutrients to plants (Gilot, 1997), and to increased microbial populations and biologically active metabolites such as plant growth regulators (Tomati and Galli, 1995; Doube *et al.*, 1997).

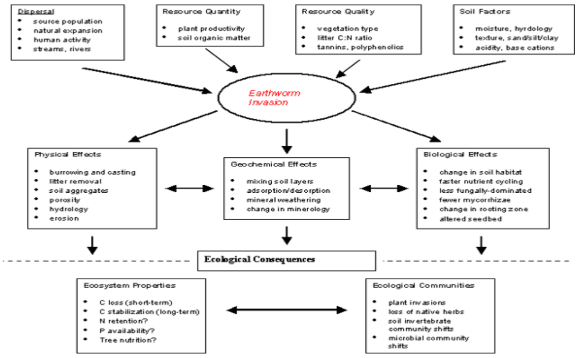

Fig. 6. Factors that influence earthworm invasions, the three main categories by which invasion influences ecological systems, and the consequences of invasion for ecosystem processes and ecological communities. From Bohlen *et al.* (2004a).

In recent years, the applied use of earthworms in the breakdown of a wide range of organic residues, including sewage sludge, animal wastes, crop residues, and industrial refuse, to produce vermicomposts has increased tremendously (Mitchell *et al.*, 1980; Reinecke and Venter, 1987; Edwards and Neuhauser, 1988; Chan and Griffiths, 1988; Hartenstein and Bisesi, 1989; Haimi, 1990; van Gestel *et al.*, 1992; Dominguez and Edwards, 1997; Edwards, 1998; Kale, 1998). The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances with a finer structure than composts but possessing a greater and more diverse microbial activity, commonly referred to as vermicomposts (Elvira *et al.*, 1996, 1998; Atiyeh *et al.*, 2000b). Several earthworm biomarkers have been developed (Scott- Fordsmand and Weeks, 2000) and applied (Aamodt *et al.*, 2007; Reinecke and Reinecke, 2007). Earthworms play a major role in the functioning of the soil ecosystem by participating in organic matter cycles and modifying soil structure (Edwards and Bohlen, 1996). They can therefore function as a key species. Furthermore, they are in direct contact with the soil porewater and consequently with the bioavailable contaminants in the soil. The first alterations caused by xenobiotics in organisms may be found at the subcellular level, e.g., in the lysosomes (Moore, 1985). The neutral red retention time (NRRT) is a commonly used nonspecific biomarker technique that measures changes in lysosomal membrane stability, especially the increased membrane permeability in response to stress. The NRRT provides a rapid and sensitive indication of response to altered environmental conditions. The NRRT assay, originally developed for marine organisms and fish (Lowe *et al.*, 1992; Lowe and Pipe, 1994) has been modified for Earthworms by Weeks and Svendsen (1996). Although effects at the biomarker level may be indicative of disturbances at higher levels, e.g. populations or communities (Weeks *et al.*, 2004), only a few examples are available of such a link between biomarker level responses and effects on the functioning of earthworms in ecosystems (Maboeta *et al.*, 2003; Spurgeon *et al.*, 2005).

Vermicomposting

Vermicomposting has been widely identified as one of the potential activity to reduce the quantity of solid waste that need to be sent to the landfills. Vermicomposting is classified as biological treatment under intermediate treatment technologies of solid waste management (Department of Local Government 2006) which also consists of physical processing and thermal treatment. In general, vermicomposting includes physical and biochemical processes when the use of earthworms is able to break down the organic elements of household waste including kitchen waste and coffee grounds. By using Lumbricus rubellus, this process tolerance in mesophilic temperature range; 35°C to 40°C with moisture content between 40% to 50% and pH between in neutral range; 7 (Sharma *et al.* 2005) identified as cost effective and natural alternative method. Furthermore, only short duration is needed to accomplish; 2 to 32 days of vermicomposting (Wang *et al.* 2007).

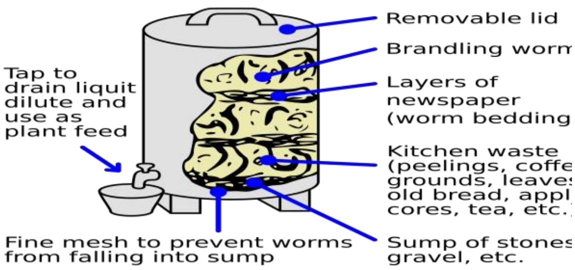

Fig. 7. Diagram of a household-scale worm composting bin.

Vermicomposting is also a process of bio-oxidation and stabilization of organic matter involving the join action of earthworms and microorganisms. The presence of earthworms doubles the rate of carbon loss due to joint action of earthworms and microorganism activity that accelerates the mineralization of C (Aira *et al.* 2007). This results in faster decomposition process to convert organic substances to inorganic substances. According to Edwards and Lofty (1972), about 5 to 10% of ingested material is absorbed into the earthworms' tissue for growth and metabolic activity and the rest is excreted as vermicast. The mixture of vermicast with mucus secretion of the gut wall and microbes transformed it into a value added material; vermicompost which is high in nutrient elements contents (Nagavallemma *et al.* 2004).

Earthworm species suitable for vermicomposting

Earthworms represent the major animal biomass in most terrestrial temperate ecosystems (Edwards & Bohlen, 1996). Indeed, more than 8,300 species of earthworms have been described (Reynolds & Wetzel, 2010), although for the great majority of these species only the names and morphologies are known, and little is yet known about their biology, life cycles and ecology. Different species of earthworms have different life histories, occupy different ecological niches, and have been classified, on the basis of their feeding and burrowing strategies, into three ecological categories: epigeic, anecic and endogeic (Bouché 1977). Endogeic species (soil feeders) forage below the surface soil, ingest high amounts of mineral soil and form horizontal burrows. Anecic species (burrowers) live in deeper zones of mineral soils, ingest moderate amounts of soil, and feed on litter that they drag into their vertical burrows. And, epigeic earthworms (litter dwellers and litter transformers) live in the soil organic horizon, in or near the surface litter, and mainly feed on fresh organic matter contained in forest litter, litter mounds and herbivore dungs, as well as in man-made environments such as manure heaps. These latter species, with their natural ability to colonize organic wastes; high rates of consumption, digestion and assimilation of organic matter; tolerance to a wide range of environmental factors; short life cycles, high reproductive rates, and endurance and resistance to handling show good potential for vermicomposting (Domínguez & Edwards, 2010b).

Temperature suitable of Earthworm for product vermicomposting

Earthworms have fairly complex responses to changes in temperature. Neuhauser *et al.* (1988) studied the potential of several earthworm species to grow in sewage sludge, and they concluded that all these species have a range of preferred temperatures for growth, ranging between 15°C (59°F) and 25°C (77°F). In their studies, cocoon production was more restricted by temperature than growth, and most of the cocoons were laid at 25°C (77°F). Edwards (1988) studied the life cycle and optimal conditions for survival and growth of E. fetida, D. veneta, E. eugeniae, and P. excavatus. Each of these four species differed considerably in terms of response and tolerance to different temperatures. The optimum temperature for E. fetida was 25°C (77°F), and its temperature tolerance was between 0°C (32°F) and 35°C (95°F). Dendrobaena veneta had a rather low-temperature optimum and rather less tolerance to extreme temperatures. The optimum temperatures for E. eugeniae and P. excavatus were around 25°C (77°F), but they died at temperatures below 9°C (48.2°F) and above 30°C (86°F). Optimal temperatures for cocoon production were much lower than those more suitable for growth for these species. Temperatures below 10°C (50°F) generally result in reduced or little feeding activity; and below 4°C (39.2°F), cocoon production and development of young earthworms ceases completely. In extreme temperature conditions earthworms tend to hibernate and migrate to deeper layers of the windrow for protection. Earthworms can also acclimate to temperature in autumn and survive the winter, but they cannot survive long periods under freezing conditions unless they are in protective cells. The unfavorable effect of high temperatures (above 30°C (86°F)) on most species of earthworms is not entirely a direct effect because these warm temperatures also promote chemical and microbial activities in the substrate, and the increased microbial activity tends to consume the available oxygen, with negative effects on the survival of earthworms (Edwards, 1988).

Leonardite

“Leonardite” and “humate” are loosely used terms covering a variety of naturally occurring lithologies with high humic acid content, including weathered (oxidized) lignite, sub-bituminous coal and a variety of carbonaceous rocks, such as mudstones, shales and claystones (Kohanowski, 1957 and 1970; Hoffman *et al.*, 1993). Leonardite is a natural organic material through the decomposition process for more than 70 million years. This organic material is considered as an oxidized form of lignite that occur at shallow depths, overlying more compact coal in a coal mine (Stevenson 1979). Leonardite cannot be used as fuel because of its low heating content. Although undesirable as fuel, its high content of humic acid (HA) (which ranges from 30 to 80%), may make it useful as a soil amendment and organic fertilizer. These raw materials are used mainly as soil conditioners; however they also have applications in wood stains, drilling fluid additives and as binder in iron pelletizing (Hoffman *et al.*, 1993). To be of economic interest, raw humate or leonardite should contain a sufficient concentration of humic acid. New Mexico’s humate typically contains 12 to 18% humic acid, but materials used for drilling fluid applications typically contain more than 65% humic acid (Hoffman *et al.*, 1993). Humates are generally mined by front-end loaders, stockpiled to reduce water content, then crushed and screened (Hoffman and Austin, 1994). Materials with high humic acid content may be further processed for use as a component in water-soluble wood stains, as a drilling fluid additive (Odenbaugh and Ellman, 1967, Roybal *et al.*, 1986), as binder in iron ore pellets, and as lignite briquettes. Humic and fulvic acids are usually used in agricultural production and are widely known as having agronomic potential (Ece *et al.*, 2007). Humic substances (humic and fulvic acids), the major components of soil organic matter, are mostly used to eliminate adverse effects of chemical fertilizers and decrease soil pH (Chen and Aviad, 1990; Akıncı *et al.*, 2009; Katkat *et al.*, 2009).

Humic acid

Humic acid (HA) is a complex organic molecule with high molecular weight ranging from approximately 5,000–100,000 Daltons. It is dark brown or black, soluble in alkaline solution and insoluble in acidic condition (Fulcrum Health Limited 2004). The role of HA in improving agricultural soils is well established, especially in soils with low organic matter (Pettit 2002). Humic acid has long been used in enhancing crop productivity and soil fertility. It also plays an important role in human health and animal husbandry (ENVIROMATE TM 2002). Many reports on its medicinal values have also been published (Anon. 1999; ENVIROMATE TM 2002). The market for humate is expanding. Humic acid products mainly as plant growth enhancers and as an ingredient in fertilizer products are widely distributed throughout the world. The largest markets are in Europe (Germany, UK, Switzerland, Spain and Italy), North America (USA, Canada) and also in Asia (Anon. 2004).

Malaysia imports almost all its humic acid (HA) requirements in solid as well as in liquid forms. Most common high purity commercial HA are in the form of K, Na, and Ca humates while unprocessed HA of varying quality from naturally occurring low rank and oxidized coals are also found in the market. Improve the chemical properties of the soil and biologically stimulate plant growth Humic acid contains many functional chemical groups that help to physically modify and improve the chemical properties of the soil and biologically stimulate plant growth (Burdick 1965; Anon. 2003).

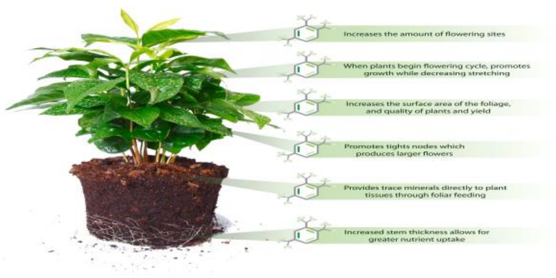

Fig. 8. Humic acid role in improving soil quality and plant growth.

These functional groups including the aromatic backbone and amines (R-NH2) cause HA to be biologically active. The oxygen containing functional groups (carboxyl, phenol, hydroxyl and ketone) tend to increase the cation exchange capacity (CEC) of the soil. Traditionally, HA is extracted from lignite, brown coals and humified organic materials. Earlier work on extracting humic acids from peat with alkaline and sodium pyrophosphate solution had been attempted (Gracia *et al.* 1993). The amount of extractable HA from humic substances and its chemical characteristics depend on several factors. These include the types of organic material from which HA is extracted, temperature, grain size, frequency of extraction, the extracting agent and its strength and drying procedure. Extracting HA under elevated temperature greatly enhanced HA recovery (Sasaki and Oyamada 1966; Asing *et al.* 2004).

Heavy metals

A soil pollution assessment becomes very complex when different sources of contamination are present and their products are variably distributed. In these cases the spatial variability of heavy metal concentrations in soils is basic information for identifying the possible sources of contamination and to delineate the strategies of site remediation. A detailed appraisal of the characteristics of urban soils points out that soils in urban and suburban areas are frequently disturbed and subjected to mixing, filling and contamination with inorganic components and organic residue (Craul, 1985). Heavy metals have sensitive influence on microbial community structure in soil, which ultimately lead to the changes of microbial amounts microbial activities including enzymes (Wang *et al.*, 2008). Soil enzyme activities are known as sensors because of its sensitive to any natural and anthropogenic disturbance occurring in the soil ecosystem. The soil biological characteristics are more dynamic and more sensitive than physicochemical properties, so they are recognized as bio-indicators of soil quality (Khan *et al.*, 2010). Assessment of heavy metal polluted soil quality by microbiology indicators has become a hot spot in current soil biology field (Pan and Yu, 2011). Albeit the development of scientific and industrial technology has provided not only a large number of benefits to the society, it has also generated different kinds of several undesirable environmental pollutants including heavy metals. Heavy metals are being generated by different kinds of industries and reaching in the soil through industrial effluent. The quality and concentration of the heavy metals varies from industry to industry and has a direct concern with nature of the product. Heavy metals are the stable metals or metalloids whose density is greater than 4.5 g/cm3, namely Pb, Cu, Ni, Cd, Zn, Hg and Cr etc. They are stable and cannot be degraded or destroyed, and therefore they tend to accumulate in soils and sediments. There are several sources of heavy metals in the environment: 1) air which contains mining, smelting and refining of fossil fuels, production and use of metallic commercial products and vehicular exhaust, 2) water having domestic sewage, sewage and industrial effluents, thermal power plants and atmospheric fallout and 3) soil like – agricultural and animal wastes, municipal and industrial sewage, coal ashes, fertilizers, discarded manufacture goods and atmospheric fallout. Soil pollution with heavy metals has become a critical environmental concern due to its potential adverse ecological effects. Heavy metals occur naturally at low concentrations in soils. However, they are considered as soil contaminants due to their widespread occurrence, acute and chronic toxicity. These metals are extremely persistent in the environment. They are non-biodegradable, non-thermo-degradable and thus readily accumulate to toxic levels. Since they do not break down, they might affect the biosphere for a long time. It is known that heavy metals form an important polluting group. They have not only toxic and carcinogenic effect but also tend to accumulate in living organisms. The irrigation of wastewater (industrial, municipal and house hold), sewage-sludge and dumped solid wastes on soils has been widespread in agricultural areas. The heavy metals also occur naturally, but rarely at toxic level. Heavy metals come from local sources mostly from the industries (non-ferrous industries, power plants, iron, steel and chemical industries), agriculture (irrigated with polluted water, use of mineral fertilizers especially phosphates, contaminated manure, sewage sludge and pesticides containing heavy metals), from waste incineration, burning of fossil fuels and road-traffic. Researchers have made an important contribution both in India and abroad to investigate the impact of effluent storage / irrigation / drainage on soil related with heavy metal contamination and their accumulation in the plant system and lastly in the product. Aydinalp and Marinova (2003) observed the soil of Turkey –Bulgaria for the presence of heavy metals and found their higher values indicating metallic soil pollution. However, these heavy metals were found in minimum levels in the plants grown in that specific area. Mustafa *et al.* (2006) made an extensive study on impact of irrigation of sewage along the main discharge channel of Konya, Iran and found increased concentration of heavy metals such as Zn, Cu, Cr, Mn, Cr, Ni, Pb and Cd in fertile soil and showed negative effect on plant system. Mishra and Tirpathi (2008) studied heavy metals contamination of soil and their bioaccumulation in the agricultural products especially vegetables irrigated with treated waste water in Varanasi, India and found that metals concentration of Cd-3.4, Cr-56.3, Pb-123.5, Zn-122.3

and Cu-77.8 mg/kg each in soil in the selected sites . Rattan *et al.* (2005) studied the long term impact of irrigation with sewage effluents on heavy metal content in soil, crops and ground water.

Plumbum (Pb)

Lead (Pb), from the Latin plumbum, has an atomic number of 82 and an atomic weight of 207.19. While lead is the 36th most abundant element, it is the most abundant heavy metal in the earth's crust with an average concentration of 13 mg kg-1 (Brown and Elliott, 1992; Nriagu, 1978). The relatively low Pb concentration in soil solutions confirms reports of lead's low mobility among the heavy metals (Kabata-pendias and Pendias, 1984). Natural mobilization of lead into the environment occurs principally from the erosion of lead-containing rocks and through gaseous emissions during volcanic activity (Waldron, 1980).Plumbum (Pb) is a toxic heavy metal which causes a serious threat to humans and the environment as a result of wastewater irrigation, sludge applications, solid waste disposal, automobile exhaust, and industrial waste dumping (Khan *et al.*, 2008; Cheng *et al.*, 2002). Ionic lead ($Pb2^{+}$) is the dominant form of lead in the soil and groundwater. However, $Pb4^{+}$ occasionally can be found in some highly oxidized soils. With the exception of a few sporadic measurements in urban air, marine fish, and in human brains there is relatively little information available on organometallic lead compounds (Nriagu, 1978). Because lead enters the soil in various complex compounds, its reactions may differ widely between geographic areas. Lead is found in soils most commonly as galena (PbS) and in smaller quantities as cerrusite (PbCO3), anglesite (PbSO4) and crocoite (PbCrO4). Lead added to soil may react with available soil anions such as SO42-, PO43-, or CO32- to form sparingly soluble salts (Waldron, 1980). Compounds such as lead carbonate [Pb3 $(OH)_2(CO3)_2$] and chloropyromorphite [Pb5(PO4)3Cl] provide the least soluble inorganic salts at near neutral pH's. There are several other mechanisms by which lead may be immobilized in soils, such as complexation by soil organic matter (humic and fulvic acids), which are then adsorbed onto soil solids, or by ion exchange at sites on the solid material in the soil. These mechanisms may facilitate attachment of substantial amounts of lead, but cannot account entirely for the low mobility of lead in soils (Harrison and Laxen, 1981). Pb pollution in the atmosphere in the city mainly comes from leaded petroleum and most of the Pb from vehicle emissions was deposited to the soil. It has been demonstrated repeatedly that heavy metals could have toxic effects on soil biological processes and long-term adverse impacts on the health of soil ecosystems (Bhattacharyya *et al.*, 2008).

Fungi

True' fungi are ubiquitous in the environment and fulfil arange of important ecological functions, particularly those associated with nutrient and carbon cycling processes in soil (Christensen, 1989). Despite this, our understanding of soil fungal community diversity and functioning remains poor relative to that of soil bacterial communities, and it is not uncommon for articles that purport to review aspects of 'soil microbial ecology' to consider only bacteria (e.g. Hattori *et al* ., 1997; Ogram, 2000; Kent and Triplett, 2002). A major contributing factor has been the tendency of mycologists to rely upon culture-based methods in ecological investigations of soil fungi. The limitations of these approaches have frequently been highlighted (e.g. Zak and Visser, 1996; Bridge and Spooner, 2001), and the data provide only a selective, and invariably biased, window on diversity. A critical factor in advancing bacterial ecology has been the widespread adoption of culture-independent methods such as analyses of DNA and/or RNA extracted directly from soil (for review, see Ranjard *et al* ., 2000). Exploitation of the variation within 16S rRNA gene sequences of different bacterial species, in combination with the application of molecular techniques, has driven a bacterial ecology revolution over the last decade or so, significantly increasing our understanding of the diversity and functioning of bacteria in a range of environments. While molecular methods have been used in many investigations of soil fungi, for the most part, these have been used to aid the identification of isolated fungi or to investigate fungi in discrete units such as ectomycorrhizal root tips or Glomalean spores (reviewed by Horton and Bruns, 2001; Clapp *et al* ., 2002). As such, they provide little information regarding the distribution, diversity and activities of fungal mycelia in soil. Similar limitations are encountered in the use of phospholipid fatty acids (PLFAs) as, although they have been widely used (for review, see Zelles, 1999), there are a limited number of fungal-specific markers, and they only provide an estimate of total fungal biomass in soil.

Carbon availability index (CAI) and Basal respiration (BR)

In afforestation programs, such as those carried out in semiarid areas, in which organic amendments are needed to improve soil fertility and its capacity for supporting plant development, soil basal respiration is a very important parameter. It has been widely used as an index of soil microbial activity (Nannipieri *et al*., 1990). The ratio of BR to SIR was used to give a quantitative measure of carbon availability (Parkinson and Coleman, 1991), or carbon availability index (CAI) based on the fact that the only difference between BR and SIR was the addition of available carbon in SIR. Microbial respiration was not limited by available carbon if the ratio, or CAI, was close to 1, and was limited by available carbon when CA1 was less than 1. The concept of CA1 here is related to the concept of metabolic quotient for COz (qCOz) (Anderson and Domsch, 1985, 1993). If the microbial biomass component in qCO2 is determined by the SIR method (e.g. Anderson and Domsch, 1978) and if the conditions in determining the BR and the SIR are set to be the same, qCOz can be linearly correlated with CA1 because the SIR method is based on the linear regression with microbial biomass values obtained by using the fumigation-incubation method (Jenkinson and Powlson, 1976). But they are different in several aspects. CAI, an index of carbon availability without any unit, is different from qCo2 which is basal respiration rate (e.g. mg CO* -C h-') per unit of microbial biomass (e.g. mgC). Secondly, most of the qCOz values reported in the literature were calculated using BR values measured after a pre-incubation or conditioning time ranging from 15 to 48 h; whereas the BR values in this study were determined without pre-incubation. CA1 is mainly used to assess the carbon availability to microbial populations in a soil sample at the time of the measurement. Whereas maize experiment. If the destructive sampling did not release significant amounts of available C, these results support the proposition (Helal and Sauerbeck, 1986) that the microbial activities in the immediate rhizosphere (i.e. rhizoplane and rhizosphere soil) are not limited by the available carbon source. However, they do not support the assumption (Newman and Watson, 1977; Darrah, 1991a.b) that the amount of available carbon is the sole controlling factor for microbial growth in the rhizosphere.

Microbial biomass carbon (MBC)

In recent years, interest in knowing the soil microbial biomass C (Cmic) has increased due to the role played by microorganisms in the fertility of soil (Paul and Voroney, 1989). This parameter has been used as an index for comparing natural and degraded systems (Ross *et al*., 1982). As shown in Tables 3 and 5, ORG was more efficient than mycorrhizal treatments (M or F) for enhancing soil microbial biomass. However, the highest values of Cmic were obtained with the combination of treatments, direct mycorrhizal inoculation and organic amendment. The positive effect on microbial biomass observed in the amended soils is due to a direct (microbial growth in ORG, Pascual *et al*., 1997) and indirect effect (improvement of plant growth). The MBC, is major energy source for microorganisms, is comprised of living microorganisms which are helpful for aggregate formation and nutrient conservation (Watts *et al*., 2005). MBC was analyzed by using the chloroform fumigation and extraction method, and the result was calculated using Kc of 0.38 (Vance *et al*., 1987).

Metabolic quotient (qCO2)

The metabolic quotient (qCO2) is an index of ecosystem stress (Insam and Domsch, 1988; Anderson and Domsch, 1993). In general, three years after the starting of the experiment, the values of qCO_2 in amended soils were smaller than in unamended soils It is logical to infer that from the beginning the amended soils have high qCO_2values due to the organic matter incorporated with the organic waste (Pascual *et al.*, 1998). As time elapses, organic matter becames more stable; the ecosystem matures and thus the qCO_2 decreases. A combination of these two parameters as in the case of the specific respiration or metabolic quotient (*q*CO_2), which expresses the amounts CO_2-C produced per unit biomass and time, could be a more sensitive indicator of both permanent (stress) and temporary (disturbance) environmental changes. This indicator has been successfully used to assess soil microbial activity and to detect disturbance or stress of the soil microbial biomass due to the external input of organic matter and as a microbial stress indicator (Anderson & Domsch, 1990; Sánchez-Monedero *et al.*, 2004). It can be interpreted as an index of "microbial efficiency", since it is a measurement of the energy required to maintain metabolic activity in relation to the energy necessary for synthesizing biomass (Fernandes *et al.*, 2005; Gibbs *et al.*, 2006). Hence, soils with a higher *q*CO_2 are commonly regarded as under stress (Anderson & Domsch, 1990).

Effect of organic waste on microbial communities in the soil

Microbial activity is of great importance for biological and biochemical soil processes because it directly influences the transformation of nutrients and organic compost. It is also qualitatively and quantitatively associated with the presence of extracellular hydrolytic enzymes which are important in the process of decomposition and mineralization of organic matter (Kiss *et al.*, 1975; Nakas *et al.*, 1987; Martens *et al.*, 1992; Ross & Cairns, 1992; Elliott *et al.*, 1993). The most important general indicators of soil microbial activity are microbial biomass C and soil respiration, while specific indicators are related to the activity of extracellular hydrolytic enzymes such as phosphatase and β–glucosidase, involved in nutrient ycling (Gil-Sotres *et al.*, 2005). The evaluation of biological and biochemical soil properties and β– glucosidase activity has been suggested because of their relationship to the soil C cycle and the sensitivity of these indicators to detect changes resulting from agricultural management practices (Nannipieri *et al.*, 1990; Dick & Tabatabai, 1993; Gil-Sotres *et al.*, 2005; Lagomarsino *et al.*, 2009). The activity of phosphomonoesterase enzymes, such as acid and alkaline phosphatases, has been widely studied because of its importance in organic P mineralization, releasing orthophosphates that are readily assimilated by plants and soil microorganisms (Sylvia *et al.*, 1999). Microbial communities in the soil are enhanced and stimulated by the addition of organic waste, especially due to the presence of readily available nutrients and C compounds. In general, organic waste has high levels of macronutrients such as N, P, K, Ca (Aita *et al.*, 2007; Giacomini *et al.*, 2009), and micronutrients such as B, Zn and Mn. Since the application of organic waste can change biological and biochemical indicators, studies are needed to measure the effect of this practice on soil (Martens, 2000; Ros *et al.*, 2003; Tejada *et al.*, 2006). Although several studies have shown that compost can improve soil by promoting appropriate biological activity and improving nutrient availability and soil structure (soil particle aggregation) (Pascual *et al.*, 1999a; Ros *et al.*, 2003; Crecchio *et al.*, 2004), few studies show the effect of household compost application on soil microbial and enzymatic activity. These effects on soil physical and chemical properties of management affect the microbial biomass and important processes such as decomposition of organic matter and mediation of nutrient availability to plants. The microbial biomass drives nutrient mineralization and is a small but labile source of major plant nutrients (C, N, P and S) (Jenkinson and Ladd 1981; Dick 1992). Also, microbial biomass can be an early indicator of changes in soil management compared to total organic C and N, which are unresponsive over short periods (Powlson and Jenkinson 1981; Carter 1986; Powlson *et al.* 1987; Saffigna *et al.* 1989). Thus, microbial biomass can be used to determine the level of degradation of the soil (Smith and Paul 1990; Doran and Parkin 1994; Brookes 1995; Sparling 1997). Although there has been considerable research on the effects of soil and crop management on soil microbiology in temperate regions (Carter 1986; Follet and Schimel 1989; Saffigna *et al.* 1989; Fauci and Dick 1994; Franzluebbers *et al.* 1995; Miller and Dick 1995; Mendes *et al.* 1999) there is relatively little information in tropical/subtropical environments, particularly for highly weathered soils (Alvarez *et al.* 1995; Guerra *et al.* 1995; Balota *et al.* 1998; Rheinheimer *et al.* 2000).

substrate-induced respiration (SIR)

A variety of methods exist 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 conductimetrically as CO2 evolution, and relates this response to biomass C (Anderson and Domsch, 1978). Unfortunately, application of the SIR method is restricted to moist soils, as a basic requirement is a soil water content sufficient to dissolve the added substrate. A lack of water may also reduce microbial respiration in some soil samples (Orchard and Cook, 1983). In this study we propose an improvement to the SIR method which alleviates both these problems: glucose is dissolved in water prior to addition to soil and added at a ratio of 2 ml glucose solution to 1 g equivalent oven-dry weight of soil (West, 1986). Data are presented which assess modifications to the original method, including use of sealed vessels to contain soil suspensions and use of gas chromatography to measure CO2 respiration, and interference from viable root fragments in the assay. The improved SIR method is recalibrated with biovolume-derived biomass carbon estimates of grassland and arable soils, previously incubated at a wide range of water contents.

Effect of earthworms on metal mobility

Lukkari *et al.* (2006) gave evidence that earthworms increase the extractability of Cu and Zn in their faeces, but decrease the overall extractability of metals in the bulk earthworm inhabited soil. This indicates that there are probably at least two separate conflicting mechanisms by which earthworms impact metal mobility. Earthworms burrow and create casts that have elevated concentrations of extractable trace elements (Sizmur *et al*., 2011a). In addition, they also release mucus into the soil solution which may decrease the solubility of metals (Sizmur *et al*., 2010). Mucus is produced in greater quantities during copulation (Edwards and Bohlen, 1996) and so this effect would be observed to a greater extent in experiments where two or more earthworms are incubated in each test vessel.

Effect of compost and humic acid on microbial activity in pollution soil by heavy metals

Different trace elements bind with organic compounds to varying degrees and behave differently to changes in soil pH. Therefore, the impact of compost or earthworm additions on the solubility of trace elements depends not only on the changes in soluble organic carbon and pH, but also on the chemistry of the element in question. Copper and Pb both bind strongly with organic carbon and therefore their solubility is much affected by changes in soluble organic compounds (McBride *et al*., 1997). Zinc, however, does not bind so strongly with organic carbon and so its solubility is relatively more affected by changes in pH (McBride, 1994). The solublity of Cu, Pb and Zn is increased with decreasing pH because these elements are cationic (McBride *et al*., 1997), but As solubility is decreased with decreasing pH because As forms an oxy-anion in solution and binds to positively charged soil surfaces such as iron oxyhydroxides (Masscheleyn *et al*., 1991). However, the total concentration of metal extracted in the shoots was in both cases higher than in the control. Other materials such as pig manure vermicompost can also be used to improve plant yield and assist phytoremediation, as demonstrated by Wang *et al.* (2012) in an experiment using Cd as target heavy metal and *Sedum alfredii* as phytoremediator. Indeed, the use of organic amendments has numerous applications, for example, Siebielec and Chaney (2012) have demonstrated the effectiveness of biosolids compost in the rapid stabilization of Pb and Zn and revegetation of military range contaminated soils increasing tall fescue growth by more than 200 %. There is an abundance of reports in the literature about amendments, such as lime and compost being used to reduce the bioavailability of heavy metals (Komárek *et al.*, 2013) and thus having the potential to be combined with phytoremediators (de Abreu *et al.*, 2012). Humic acids (HA) are organic compounds naturally present in water and soil. They form three-dimensional structure molecules, containing aromatic nuclei with oxygen and nitrogen heterocycles. In the side chains, bound to an aromatic nucleus, hydroxyl, carbonyl, carboxyl, amine and sulfhydryl functional groups are present (MacCarthy 2001). Due to different HA structures, the content of functional groups and various qualities (colloidal, spectral, electrochemical and ion exchange) their considerable adsorption capacity is assumed (Klocking 1994). Chelators, such as humic substances, could be used for increasing the solubility of metal cations, and thus their bioavailability to plants. The term humic substance refers to a category of naturally occurring organic materials found in soils, sediments, and natural waters. They result from the decomposition of plants and animal residues (MacCarthy, 2001). Humic acids are those parts of humic substances, which are not soluble in water under acidic conditions, but becomes soluble and extractable at higher pH values. Humic acids contain acidic groups such as carboxyl and phenolic OH functional groups (Hofrichter and Fakoussa, 2001), therefore, provides organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals (Lagier *et al.*, 2000).

References

Adachi M, Bekku YS, Rashidah W, Okuda T, Koizumi H. 2006. Differences in soil respiration between different tropical ecosystems. Appl Soil Ecol. **34,** 258–265

Adeboye MKA, Iwuafor ENO, Agbenin JO .2006. The effects of crop rotation and nitrogen fertilization on soil chemical and microbial properties in a Guinea Savanna Alfisol of Nigeria. Journal of plant Soil sciences . **281,** 97–107

Aira M, Monroy F, Dominguez J. 2007. Eisenia fetida (Oligochaeta: Lumbricidae) Modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microbial Ecology journal. **54,** 662-671.

Akınci S, Buyükkeskin T, Eroglu A, Erdogan BE. 2009. The effect of humic acid on nutrient composition in broad bean (Vicia faba L) Roots Not. Soil Tillage Research journal. **1,** 81–87.

Anderson T, Domsch KH. 1993. The metabolic quotient for CO2 (qCO2) as a speci®c activity parameter to assess the effects of en- vironmental conditions, such as pH, on the microbial biomass of the soil. Soil Biology & Biochemistry. **25,** 393±395.

Asawalam DO. 2006. Influence of cropping intensity on the production and properties of earthworm casts in a leucaena alley cropping system. Biology and Fertility of Soils. **42,** 506-512.

Baker GH, Barrett VJ, Carter PJ, Cayley JWD, Saul GR. 1993. The influence of fertilizer on the abundance and diversity of earthworms in pastures in western Victoria. Proceedings of the 7th Australian Agronomy Conference. **12,** 312-315.

Basu S, Behera N. 1993. The effect of tropical forest conversion on soil microbial biomass. Biology Fertil Soils. **16,** 302–304.

Behera N, Sahani U. 2003. Soil microbial biomass and activity in response to Eucalyptus plantation and natural regeneration on tropical soil. Ecology journal Management. **174,** 1–11

Bohlen PJ, Parmelee RW, Allen MF, Ketterings QM. 1999. Differential effects of earthworms on nitrogen cycling from various Nitrogen-15-Labeled substrates. Soil Science Society of America Journal. **63,** 882-890.

Borken W, Muhs A, Beese F. 2002. Application of compost in spruce forests: ects on soil respiration, basal respiration and microbial biomass, Forest Ecology Management. **159,** 49-58

Bunemann EK, Schwenke GD, Zwieten LV. 2006. Impacts of agricultural inputs on soil organisms-a review. Australian Journal of Soil Research. **44(4),** 379-406.

Capowiez Y, Berard A. 2006. Assessment of the effects of imidacloprid on the behaviour of two earthworm species (Aporrectodea nocturna and Allolophora icterica) using 2D terraria. Ecotoxicology and Environmental Safety. **64(2),** 198-206.

Capowiez Y, Rault M, Costagliolia G, Mazzia C. 2005. Lethal and sublethal effects of imidacloprid on two earthworm species (Aporrectodea nocturna and Allolobophera icterica). Biology and Fertility of Soils. **41(3),** 135-143.

Cheng SP, Grosse W, Karrenbrock F, Thoennessen M. 2002. Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Soil Biology & Biochemistry. **18(3),** 317-325.

Civeira G, Lavado RS. 2006. Organic matter addition effect on some hydrological properties in a degraded urban soil. Soil Biological journal. **24,** 123-130.

Haynes RJ, Naidu R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cycling in Agroecosystems. **51,** 123–137.

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. Pedobiologia. **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.