Chapter 11

# Dual Role of Vermicomposting in Relation to Environmental Pollution

## Detoxification and Bioremediation

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## INTRODUCTION

In recent years, bioremediation has become common practice in the restoration of contaminated soils. Phytoremediation and biostimulation are the most popular strategies used for the bioremediation of contaminated soils, although they have some limitations. Biostimulation involves the addition of nutrients and other supplementary chemicals to increase native populations of microorganisms in an attempt to accelerate the biodegradation of environmental contaminants (Tyagi et al. 2011, Megharaj et al. 2011). Nutrients can be applied in the form of inorganic fertilizers, oleophilic fertilizers and organic waste (e.g., animal manure) (Tyagi et al. 2011); however, the application dose and nutrient ratios must be optimal to have significant impacts on the pollutant degradation rate (Megharaj et al. 2011).

In the last two decades, vermicomposting has emerged as an eco-friendly technology for improving the physicochemical and biological properties of degraded

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soils as well as to increase crop yield (Edwards et al. 2010). The final product of vermicomposting, i.e., vermicompost, is a finely divided, porous peat-like material with several beneficial properties related to soil function: high water-holding capacity, high contents of humic substances and nutrients, well-established bacterial and fungal communities, and large loads of extracellular enzymes. Although vermicompost has several characteristics suitable for mitigating soil pollution, it has scarcely been investigated in relation to bioremediation.

This chapter provides up-to-date knowledge on two lines of active research in vermicomposting technology: (1) the use of vermicompost to remove hazardous chemicals from contaminated raw materials (detoxification), and (2) the impact of vermicompost for remediating contaminated soils (bioremediation). The chapter is divided into four sections. The first section provides an overview of the functional features of the vermicomposting process. The second section describes the main findings regarding the detoxification capacity of vermicomposting to degrade organic contaminants and to immobilize metals present in agroindustrial and municipal waste. The third section reports several important examples that demonstrate the potential use of vermicompost in the degradation and immobilization of pesticides and metals in soil. The microbiota and organic ligands (e.g., humic substances) present in vermicompost are the main features that account for this bioremediation capability. Moreover, recent studies suggest that vermicompost contains extracellular detoxifying enzymes (e.g., carboxylesterases, peroxidases and laccases), thus indicating possible new lines of research related to the bioremediation potential. The final section will suggest new lines of future research in the area of vermicomposting for bioremediation purposes.

## Functional Processes of Vermicomposting

Intensive agriculture, which is characterized by conventional tillage, massive and repeated fertilization, high pesticide input and low plant diversity, has a negative impact on soil biodiversity (Tsiafouli et al. 2015, Steffen et al. 2015). In this context, organic or ecological agriculture has emerged as an alternative form of cropping that promotes natural soil processes and seeks to reduce the negative impacts on soil biota (Mäder et al. 2002). However, this type of sustainable agriculture does not reach the productive standards of intensive agriculture (Seufert et al. 2012), and novel eco-friendly strategies are therefore required to increase crop yield and maintain soil fertility and biodiversity. Although soil biological dynamics are fundamental for the balance and productivity of natural ecosystems, this fact is still ignored in managed ecosystems, such as agricultural ecosystems (Bender et al. 2016).

Soil biodiversity is lower in intensive agriculture than in organic agriculture or natural ecosystems (Tsiafouli et al. 2015). The reduced biodiversity implies a lower functional capacity to resist environmental stressors than in soil systems with a high biodiversity (Bender et al. 2016). Moreover, high-diversity communities are more productive and sustainable than low-diversity ones. Therefore, the loss of some species or functional groups in intensively managed agricultural systems will lead to greater maladaptation in ecosystem functioning than observed in natural ecosystems. Plant productivity depends on aboveground interrelationships of different functional groups, and soil microbial consortia provide key ecosystem functions (Putten et al. 2013). The importance of soil microbial communities for plant growth and the development of plant communities is demonstrated in studies in which the soil microbiome is manipulated (Chaparro et al. 2012), and in field studies involving the inoculation of different soils ranging from those in highly productive ecosystems to degraded soils (van de Voorde et al. 2012, Wubs et al. 2016). Therefore, soil biodiversity is a key concept in sustainable agriculture, as well as in the bioremediation of polluted soils.

The production and use of vermicompost, a known organic fertilizer that contains a well-established microbial community, may be a promising eco-friendly strategy for promoting soil fertility by supplying both nutrients and microbial communities to the soil. The most common organic materials used for vermicomposting are animal manures, biosolids generated in wastewater treatment plants, biosolids derived from industries dealing with any type of organic material (e.g., olive mill waste and dairy industry waste), the organic fraction of municipal solid waste, and a wide range of animal and plant residues.

Vermicomposting consists of the biooxidative decomposition of dead organic matter in a mesophilic environment (< 30ºC) created by the cooperative actions of detritivore earthworm species (e.g., *Eisenia fetida*, *E. andrei*), microorganisms and, to a less extent, other members of the soil fauna decomposer community. During the vermicomposting process, the physical, microbiological and biochemical properties of the organic matter are greatly modified, thus stabilized (Aira et al. 2006). Earthworms are key drivers of the process because of the significant contribution that they make to the fragmentation of organic debris and dispersion of microorganisms. Likewise, earthworms play a significant role in microbial activity via comminution of organic matter and enhancement of the surface area available for microbial attack (Aira et al. 2007), and via grazing directly on microbiota (Aira et al. 2007, Domínguez et al. 2017). Through these and other specific activities, earthworms enhance the efficiency of the microbial communities and their turnover rates, thus substantially increasing the substrate decomposition rates. In addition to microbes, earthworms also affect other soil biota either directly, through the ingestion of protozoa and microfauna present in the detrital food webs, or indirectly, by modifying the availability of resources for these organisms (Edwards et al. 2010).

The nutritional, microbial and enzymatic enrichment of vermicompost is achieved by a long-term process including two successional stages: an active stage and a maturation stage.

(1) The active stage is characterized by the feeding activity of the earthworms, which contributes to modifying the physical and chemical properties of the feedstock as well as its microbial composition (Aira et al. 2006, 2007). During this stage, earthworms are continuously moving and burrowing into the substrate, thereby contributing to its oxygenation and homogenization which, in turn, accelerates its decomposition. On the other hand, the feeding activity of earthworms also alters the physicochemical and biological properties of the feedstock as it passes through their guts. During this gastrointestinal transit of organic matter, many digestive enzymes produced by the earthworms themselves and symbiont

microorganisms boost organic matter decomposition. These diverse extracellular enzymes, break down a wide variety of organic molecules, including cellulose and phenolic compounds. Because of the high content of organic carbon, these digestive enzymes become stable by binding to the organic complexes of casts (Domínguez et al. 2017, Sanchez-Hernandez and Domínguez 2017).

(2) The maturation stage starts once earthworms move away from the processed substrate, and new microbial communities take over the further decomposition of more recalcitrant molecules (Aira et al. 2007). During the maturation stage, the cast-associated processes most closely associated with the presence of material not processed by the transit through the earthworm gut and with physical modification of the egested material contribute to the further decomposition of the substrate. Therefore, vermicompost reaches an optimal stage in terms of its biological properties, marked by its capacity to promote plant growth and reduce plant diseases. The duration of both vermicompost stages are highly variable; thus while the active stage depends on the earthworm species, their population dynamics and the environmental conditions, the maturation stage depends on the efficiency of the active stage (Edwards et al. 2010).

## Detoxification: Cleaning Contaminated Organic Waste

The use of vermicompost to promote soil fertility requires that this nutrient-rich and microbiologically active organic amendment meets a series of quality standards that involve microbiological and physicochemical parameters. Thus, vermicompost must be free of potentially hazardous contaminants such as metals, hydrocarbons, pesticides, pharmaceuticals, cosmetics and other contaminants typically found in agroindustrial and municipal organic residues. In this context, many studies have considered the capacity of the vermicomposting process to remove toxic metals and organic contaminants.

### *Metals*

The use of the term "heavy metal" in environmental sciences continues to be a matter of intensive debate in the scientific literature (Pourret and Bollinger 2018), and its use is discouraged because the definition is vague and misleading (Duffus 2002, Hodson 2004, Hübner et al. 2010). Therefore, in this chapter, we have opted to use the term "metal" (or "metalloid" in the case of As, At, B, Ge, Po, Sb, Se, Si, and Te), as suggested by Chapman (2007).

The industrial and municipal waste commonly used in vermicomposting may contain important amounts of metals, and the concentrations in the final product (vermicompost) may pose a serious threat to soil function when used as a soil amendment. A recent review by Swati and Hait (2017) summarizes the range of metal concentrations in industrial, municipal and domestic organic waste as well as the standards for composts that are adopted in many countries. As pointed out by these authors, the standards for the environmentally safe use of compost are based on total metal concentrations, and the chemical speciation of metals in the environment is ignored. Chemical speciation can be defined as the formation of multiple chemical forms in which a metal exists in the environment (Wright and Welbourn 2002). Metal speciation is highly dependent on fluctuating environmental variables such as pH, organic matter content, exchange capacity, temperature and moisture, which ultimately affect metal bioavailability and toxicity in both the vermicompost itself and in the vermicompost-amended soil.

Chemical speciation affects the metal concentration in vermicompost. Changes in metal speciation affect bioaccumulation by earthworms and facilitate sorption of the metal on to organic ligands in the vermicompost. Many studies have demonstrated that vermicomposting significantly reduces the available fraction of several metals and metalloids such as As, Cu, Cd, Cr, Ni, Pb and Zn, irrespective of the earthworm species and type of raw material used (Singh and Kalamdhad 2013, Sahariah et al. 2015, Goswami et al. 2016, Lv et al. 2016, He et al. 2016). However, the reduction in metal availability does not necessarily indicate a lack of toxicity. Thus, toxicity testing with vermicompost should be adopted as a complementary measure prior to its use as a soil amendment. For example, the study by Vašíčková et al. (2016) clearly shows why toxicity assessment of vermicompost is highly recommended. These researchers compared the toxicity of As-contaminated sludges after three different types of treatment, i.e., composting, vermicomposting and mixing with soil. The results of ecotoxicity tests using *Folsomia candida* (reproduction test), *Enchytraeus crypticus* (reproduction test), and *Lactuca sativa* (root elongation test) demonstrated that vermicomposting As-contaminated sludge increased its toxicity relative to the other two procedures, although vermicomposting greatly reduced the available fraction of As. According to the authors, the unexpected toxicity may be due to ammonia, among other potentially toxic by-products generated during vermicomposting (Vašíčková et al. 2016).

Obviously metals cannot be mineralized as organic compounds. Therefore, the only rational way to reduce their concentrations in the vermicompost, relative to those in the feedstock, is for them to be accumulated in earthworms. Many studies have demonstrated the capacity of earthworms to accumulate metals (Swati and Hait 2017), which is favoured by the induction of metal-binding proteins such as metallothioneins (Goswami et al. 2016). Surprisingly, however, the same studies have documented a slight increase in the total metal concentrations of the vermicompost relative to the feedstock (Bakar et al. 2011, Yadav and Garg 2011, Lv et al. 2016). The marked differences in organic matter content and quality between the organic waste and the final vermicompost probably explain the increase in metal concentrations in the vermicompost. Vermicompost is a humic-rich material (Elvira et al. 1998, Goswami et al. 2016), and humic substances facilitate the formation of metal-humus complexes which, in turn, result in higher metal contents than in the initial organic residues (He et al. 2017). Indeed, the study by Goswami et al. (2016) suggests such a chemical relationship. These researchers observed higher metal concentrations in the vermicompost than in compost, with the former having average humic acid carbon contents ranging between 0.1 and 0.51%, whereas the contents in compost varied between 0.02 and 0.25%.

In summary, these examples illustrate that vermicompost obtained from metal-contaminated feedstock may increase the environmental risk of soil contamination due to over-supply of metals. In this context, novel vermicomposting strategies (e.g., periodic removal of composting earthworm population by fresh earthworm population) should be adopted to take advantage of the high capacity of earthworms to accumulate metals.

## *Organic Pollutants*

Agroindustrial and municipal organic wastes usually contain a large variety of organic pollutants such as polycyclic aromatic hydrocarbons, polychlorinated and polybrominated biphenyls, pesticides, pharmaceuticals and personal care products. This chemical cocktail poses a challenge to vermicomposting because both earthworms and microflora may be negatively affected by exposure to mixtures of pollutants. Accordingly, monitoring of the toxic effects during vermicomposting of contaminated raw materials is recommended in order to evaluate the viability of the decomposing process and, if appropriate, to propose corrective measures. Most vermicomposting studies measure changes in the earthworm population (individual density and reproduction rate) and, occasionally, changes in microbial activity and biomass relative to the potential toxicity of the feedstock. For example, plant waste containing residues of the neonicotinoid imidacloprid had a significant impact on *E. fetida* during vermicomposting for 15 weeks. Although a pesticide concentration of 2 mg/kg did not kill the earthworms, they did not produce cocoons (Fernández-Gómez et al. 2011). Similarly, degradation of the antibiotic oxytetracycline and its metabolites was monitored during the decomposition of chicken manure by a first thermophilic composting phase (20 d), followed by a second vermicomposting (7 wk) phase with the earthworm species *E. fetida* (Ravindran and Mnkeni 2017). The highest rate of degradation of this pharmaceutical took place during the composting phase (30–80% reduction compared to the initial concentration), whereas degradation rates of 15–40% occurred in the vermicomposting phase. The high temperature typically reached during composting (50–70ºC) probably accounted for oxytetracycline degradation. However, it is not clear whether a single vermicomposting phase would have been as effective as composting for degrading this antibiotic. Although high temperatures are not generated during vermicomposting, the higher microbial activity may have a similar impact on oxytetracycline persistence. Villalobos-Maldonado et al. (2015) suggested that the cooperation between earthworms and microorganisms may have the same outcome as composting. These researchers demonstrated that vermicomposting reduced the initial concentration of the recalcitrant contaminant decachlorobiphenyl by 80–95% during incubation for 3 months with *E. fetida* and a mixture of peat moss and rabbit excrement as organic feedstock.

Although the aforementioned studies have demonstrated that vermicomposting reduces the concentration of organic pollutants, the toxic effects on earthworms, microorganisms and extracellular enzyme activities, which are the main drivers of organic matter decomposition, have received little attention. Moreover, understanding the ability of earthworms and microorganisms to adapt to feedstocks containing environmental contaminants demands future research. This toxicological data would help in the selection of the most appropriate conditions (earthworm species, organic waste mixture, pre-treatment of raw material, and so on) in the vermicomposting of contaminated-feedstocks.

## Bioremediation: Cleaning Contaminated Soils

The aim of vermicomposting is to obtain a stabilized, nutrient-rich material for use as a soil amendment. Most studies concerning vermicomposting technology have provided knowledge about the following issues: the impact of the feedstock characteristics on the chemical and microbiological properties of the final vermicompost, the functional role of earthworms in the oxidative decomposition of organic matter, and the effects of vermicompost on plant growth (Edwards et al. 2010). By comparison, the application of vermicomposting in the field of bioremediation has received less attention, even though vermicompost is a nutrient- and microbial-rich material. The high contents of organic matter and nutrients undoubtedly promote soil microbial proliferation (biostimulation). Likewise, vermicompost is a microbiologically active substrate, and as such, the addition of vermicompost is a means of inoculating the soil with non-native microorganisms that may participate in the degradation of organic contaminants (bioaugmentation). In the following two subsections, we will identify the main effects of vermicompost in soil contaminated by metals and organic pollutants.

### *Metals*

In previous sections, we have highlighted that one of the main impacts of the vermicomposting process on the chemical speciation of metals is the reduced availability, probably due to the formation of metal-humic complexes. However, the immediate question arising is whether vermicompost has the same effect on metals present in soil when used as an amendment.

Although the chemical characteristics of vermicompost suggest that it will be an excellent sorbent substrate for metals, thereby reducing their bioavailability and toxicity in soil, very few studies have examined the potential applications of vermicompost in the remediation of metal-contaminated soils. For example, Zhu et al. (2017) suggested that vermicompost can efficiently retain toxic metals from the soil solution. In this study, a series of kinetic assays of adsorption and desorption were performed with different $Pb^{+2}$ and $Cd^{+2}$ solutions to compare the metal binding capacity of both cow manure and the vermicompost derived from this waste material. Although the adsorption isotherm kinetics of these metals differed, vermicompost was better for retaining both metals. The functional capacity of vermicompost was suggested to be the main cause of binding metals from water. Use of Fourier transform infrared spectroscopy revealed that the cow manure-derived vermicompost displayed functional groups such as –OH (aliphatic alcohol), –COOH (aromatic compounds), and bonds such as C=O and C–O (carbonates and aliphatic alcohol) and P–O (phosphates), thus explaining the high functional capacity of vermicompost to bind $Pb^{+2}$ and $Cd^{+2}$ (Zhu et al. 2017). In a similar study, Singh and Kaur (2015) studied the potential of a cow manure-derived vermicompost to remove metals from industrial effluents. Using a simulated bio-filter, these authors demonstrated that vermicompost retained $Cu^{+2}$ and $Zn^{+2}$ from beverage plant, paper mill and distillery effluents. With the same aim, He et al. (2017) examined the kinetics of $Pb^{+2}$ and $Cd^{+2}$ adsorption on a vermicompost generated from sewage sludge. This vermicompost displayed a high capacity to adsorb both metals (individually or together), although the maximal adsorption capacity was lower for $Cd^{+2}$, probably because of competition with $Pb^{+2}$ for the binding sites. Together, these examples suggest that vermicompost may be used as a suitable and complementary filter-like support for the removal of potentially toxic metals from contaminated water. However, it remains to be elucidated whether such an environmental service can also be achieved in metal-contaminated soils. Moreover, in order to gain a deeper insight into the use of vermicompost for remediating metal-contaminated soils, some aspects related to the type and surface functionality of vermicompost must be investigated: the mechanism of chemical interaction between metal and vermicompost, the impact of environmental variables, such as soil pH, moisture and dissolved organic carbon on the capacity of vermicompost to sorb metals, as well as the effect of aging on the capacity of vermicompost to retain metals.

Vermicompost can act as a metal adsorbent. For example, in a laboratory experiment using metal-contaminated soils, Hoehne et al. (2016) examined the capacity of vermicompost to immobilize Cd, Cr, and Pb. Using a sequential extraction procedure, these researchers measured the different chemical forms in which these metals could be distributed in the soil. Moreover, the chemical analysis was accompanied by a phytoremediation experiment in which three-black oat (*Avena strigosa*) was used to verify whether vermicompost altered the chemical speciation of metals, thus favoring their phytoextraction. Doses of 50, 75 and 100% vermicompost in metal-contaminated soil increased the bioavailable fraction of Cd and Cr, which was corroborated by the metal concentrations measured in the three-black oat. Although the application of vermicompost facilitated the phytoextraction, marked metal-specific differences that depended on the dose of vermicompost were observed. The vermicompost doses of 25% (for Cr- and Pb-contaminated soils) and 50% (for Cd-contaminated soil) were suggested to be suitable for mobilizing metals from soil and thus improving phytoextraction (Hoehne et al. 2016). By contrary, the study by Wang et al. (2018) showed that the available Cd fraction in soil contaminated by this metal was slightly lower in the presence of vermicompost, biochar or a mixture of both amendments. However, all treatments were regularly irrigated with water of different pH during 2 months in an attempt to simulate the impact of acid rain on the adsorptive capacity of these amendments to immobilize Cd. Although the results of the study clearly showed that vermicompost and biochar reduced the available fraction of Cd, the complex experimental design did not allow the researchers to determine whether the vermicompost itself was able to alter the chemical speciation of Cd in non-water saturated soils. Despite these promising findings demonstrating the qualities of vermicompost as an efficient adsorbent of metals, the understanding of the impact of vermicompost on historically metal contaminated soils remains limited.

In the aforementioned studies, the test soils were experimentally spiked with metals (generally as metal salts); however, aging of metals in the soil may affect the capacity of vermicompost to immobilize metals. For example, Abbaspour and Golchin (2011) suggested that this type of aging effect was critical for the use of vermicompost to remediate contaminated soils. The addition of manure-derived vermicompost to a soil collected from a Pb-Zn mine area had little impact on metal speciation after an incubation period of 6 months. Similarly, Fernández-Gómez et al. (2012) concluded that the addition of two different types of vermicompost to a soil under *Trifolium repens* (planted for purposes of phytoremediation) did not improve the capacity of this plant species to remove Ni, Pb and Cd from soil, even in the presence of arbuscular mycorrhizal fungi. However, the addition of vermicompost had a beneficial impact on the growth of *T. repens* and soil enzyme activity (Fernández-Gómez et al. 2012), suggesting that vermicompost may be used to alleviate the toxicity of contaminated soils on other biological components with a more significant role in bioremediation such as plants and microorganisms.

### *Organic Pollutants*

Vermicompost has attractive properties as a substrate for the bioremediation of contaminated soils. Three of these properties play an important role in the bioremediation capacity: the high organic matter content, microbial abundance and diversity, and the existence of pollutant-detoxifying exoenzymes.

Vermicompost is an organic carbon-rich substrate. Although the total amount of organic matter in the final vermicompost is lower than in the original feedstock, the content of humic substances are higher. These humic substances facilitate the formation of metal-humic complexes, as well as the adsorption of organic contaminants with a high partitioning coefficient between soil organic carbon and the soil solution (Log $K_{OC}$). The functional significance of this interaction was highlighted by Fernández-Bayo et al. (2007), who evaluated the capacity of spent grape marc-derived vermicompost to adsorb the insecticide imidacloprid from soil. The adsorption of imidacloprid was higher in the vermicompost-amended soils than in vermicompost-free soils, and was higher in soils with a low organic carbon content. The absence of a direct relationship between imidacloprid adsorption and the organic carbon content of soil led these researchers to confirm the importance of vermicompost in this adsorptive process, which was attributed to the lignocellulosic nature of the vermicompost (Fernández-Bayo et al. 2007). Indeed, a comparison of the potential of three types of vermicompost (elaborated from spent grape marc, biosolid vinasse and olive-mill waste) to bind the herbicide diuron showed that the vermicompost with the highest lignin content, i.e., spent grape marc-derived vermicompost, displayed the highest adsorption capacity (Fernández-Bayo et al. 2009). Similarly, the sorptive properties of vermicompost also explained the reduction in the leaching potential of diuron, imidacloprid and the metabolites of these in an experimental soil column with vermicompost on top (Fernández-Bayo et al. 2015). Nevertheless, the intrinsic properties of contaminants determine the efficacy of vermicompost as an adsorptive substrate. For example, PAHs are also efficiently retained by vermicompost, although the sorption process seems to depend on the number of benzene rings in the molecule (Dores-Silva et al. 2018), probably due to the positive relationship between the adsorption capacity (Log $K_{OC}$) and the number of benzene rings in the PAH molecule.

Vermicompost is also a microorganism-rich substrate, and the pollutant biodegradation rate is therefore expected to increase in vermicompost-amended soils. The bioaugmentation effect has been addressed in several studies. For example, Di Gennaro et al. (2009) demonstrated that the microbial communities present in soils historically contaminated with PAHs changed after addition of a vermicompost generated from olive-mill waste. The naphthalene dioxygenase activity was higher in these soils than in vermicompost-free soils, thus indicating why naphthalene was degraded at a higher rate in the vermicompost-amended soils than in vermicompost-free contaminated soils. Perhaps more interestingly, spiking the vermicompost with naphthalene induced expression of biodegradation indicator genes in the native microbiota of the vermicompost (Di Gennaro et al. 2009), suggesting that it is possible to stimulate potential contaminant degraders in the vermicompost before it is used for bioaugmentation purposes (Castillo et al. 2014, Castillo Diaz et al. 2016).

Vermicompost contains a significant fraction of extracellular enzymes involved both in the nutrient cycling (e.g., phosphatases, β-glucosidases, cellulase, protease and ureases) and in the metabolism of organic pollutants (e.g., laccases, peroxidases and carboxylesterases). During vermicomposting, the microbiota changes greatly relative to that in the original raw material (Aira et al. 2007, Gómez-Brandón et al. 2011), and the final vermicompost contains a high diversity of bacteria and fungi (Anastasi et al. 2005). Changes in the activity of many enzymes is also observed during vermicomposting as a consequence of microbial foraging. Therefore, vermicompost contains a high load of extracellular enzymes, which are stabilized by organic matter. Recent studies performed in our laboratory have reported the existence of carboxylesterase activity in vermicompost derived from different types of organic waste (Sanchez-Hernandez and Domínguez 2017, Domínguez et al. 2017). The enzyme activity is involved in the metabolism of synthetic pyrethroid and anticholinesterase (organophosphorus and carbamate) insecticides in animals (Sogorb and Vilanova 2002, Wheelock et al. 2005), plants (Gershater and Edwards 2007, Gershater et al. 2007), microorganisms (Bornscheuer 2002, Singh 2014) and soil (Sanchez-Hernandez et al. 2017, Sanchez-Hernandez et al. 2018). In the particular case of organophosphorus pesticides, carboxylesterase-mediated detoxification involves the formation of a stable enzyme-pesticide complex by the direct interaction between the organophosphorus molecule and the active site of the enzyme (Sogorb and Vilanova 2002). Some laboratory experiments with vermicompost carboxylesterase suggest that this enzyme irreversibly binds the organophosphorus chlorpyrifos-oxon, thus acting as a molecular scavenger of this class of pesticides (Domínguez et al. 2017, Sanchez-Hernandez and Domínguez 2017).

In order to assess the enzymatic bioremediation capacity of vermicompost, we performed a laboratory study to explore the stability of vermicompost carboxylesterase activity in response to physical stress (desiccation and heat shock) applied to the vermicompost. We also assessed the potential of these esterases to bind organophosphorus pesticides in dried vermicompost. In this preliminary study, the carboxylesterase activity was measured in suspensions of cow manure-derived vermicompost in water (1:50, w/v, rotating mixing for 30 min at 25ºC). The esterase activity decreased slightly over two weeks during which the vermicompost was air-dried, but the enzyme lost between 48% and 66% of the initial activity during longer (21 days) air-desiccation treatments (Fig. 1A). In addition, heating vermicompost to 40ºC (24 h), 50ºC (24 h) and 90ºC (6 h) showed the high stability of this esterase activity, which was probably due to an interaction with the high organic matter content of vermicompost (Fig. 1B). Indeed, it is well known that a high proportion of the total enzyme activity in the soil, for instance, corresponds to extracellular enzymes that are stabilized by association with soil organo-mineral complexes (Nannipieri et al. 1996). This association protects and stabilizes the enzymes against physicochemical (e.g., temperature- and protease-induce degradation) and biological stressors (e.g., microbial foraging). Because vermicompost is rich in both organic matter and microorganisms, it can also be assumed that enzyme activities in vermicompost are mainly extracellular in location and microbial in origin (released as exoenzymes). On the other hand, soil carboxylesterases are generally glycoproteins, and it has been postulated that the stability of the enzyme in the soil matrix is due to the interaction between the carbohydrate residue of the enzyme and the organic matter and clays (Satyanarayana and Getzin 1973). A comparable interaction could be assumed in the case of vermicompost, which is rich in organic matter.

Early studies indicated a high level of carboxylesterase activity in vermicompost (Sanchez-Hernandez and Domínguez 2017, Domínguez et al. 2017). The existence of these enzymes suggests that vermicompost provides binding sites for organic contaminants of chemical nature (e.g., humic substances) and of enzymatic nature. Indeed, the carboxylesterase activity of cow manure-derived vermicompost was sensitive to *in vitro* inhibition by chlorpyrifos-oxon, dichlorvos and paraoxon-methyl in a dose-dependent manner (Fig. 2). The sensitivity to organophosphorus insecticides was confirmed by spiking both wet and air-dried vermicompost with 10 μg/g chlorpyrifos-oxon; the carboxylesterase activity in both types of vermicompost was significantly inhibited (58% of control activity) 2 days after the spiking (Fig. 3). Interestingly, in these preliminary trials, the response of vermicompost carboxylesterase to desiccation, heat stress and pesticide exposure strongly depended on the substrate used in the enzyme assay. Therefore, and as suggested by others (Wheelock et al. 2005), the use of more than one model substrate is highly recommended for studying the dynamics of vermicompost carboxylesterase activity against environmental stressors.

Together, these findings suggest that vermicompost contains a significant level of carboxylesterase activity that can bind organophosphorus pesticides, thus boosting the bioremediation capacity of vermicompost in relation to organic contaminants. In this context, the addition of vermicompost to the topsoil could act as a molecular and biochemical barrier to reduce contaminant transportation and thus facilitate its

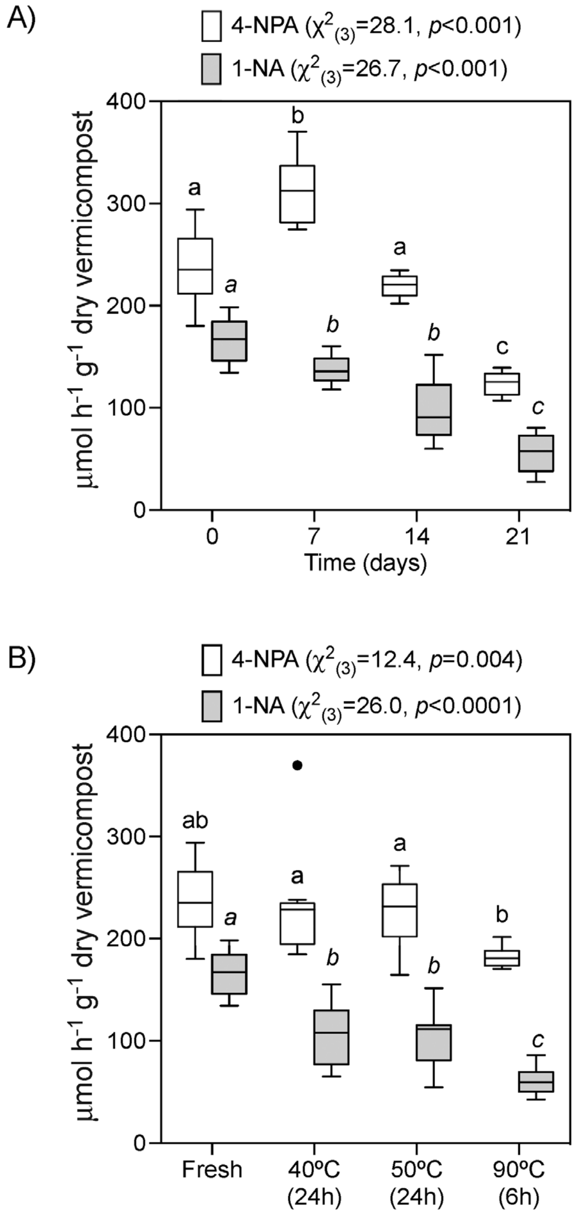



**Fig. 1.** Stability of carboxylesterase activity in air-dried vermicompost derived from cow manure (graph A) and treated for thermal denaturing of proteins (graph B). The enzyme activity was assayed in suspensions of vermicompost in water with two different substrates, i.e., 4-nitrophenyl acetate (4-NPA) and 1-naphthyl acetate (1-NA), as described in Sanchez-Hernandez et al. (2017). Tukey box plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers) of 10 samples. Significant differences between treatments are indicated by different letters (normal typeface for 4-NPA and italics for 1-NA) after Kruskal-Wallis test followed by a post hoc Mann-Whitney test ($p < 0.05$).

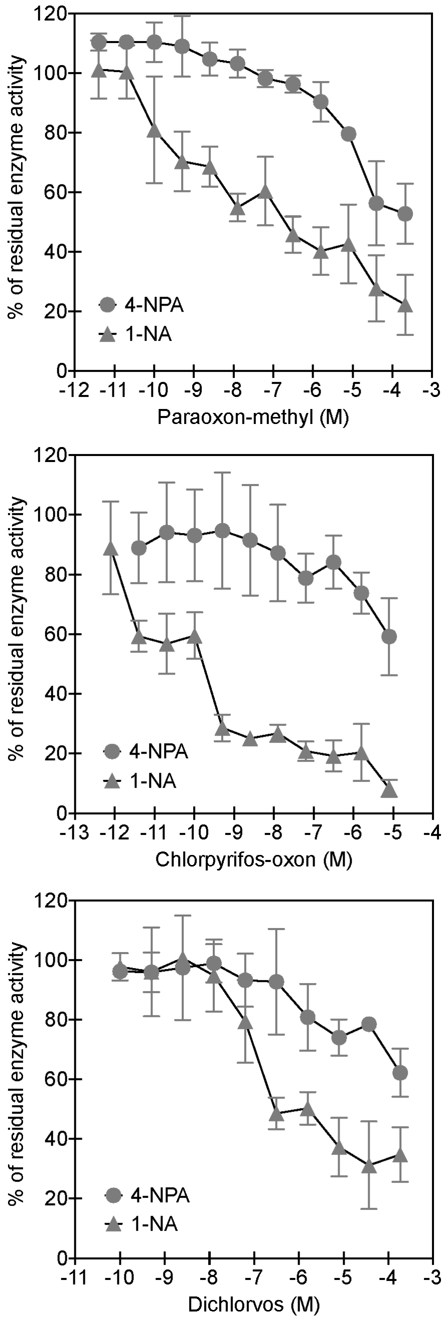
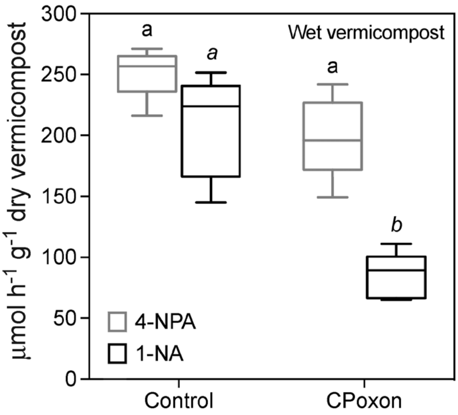



**Fig. 2.** *In vitro* dose-response relationships between carboxylesterase inhibition and the molar concentration of organophosphorus insecticides (30-min incubation at 20ºC). Symbols represent the mean and standard deviation of three independent incubations. Substrates for carboxylesterase assay as in Fig. 1.



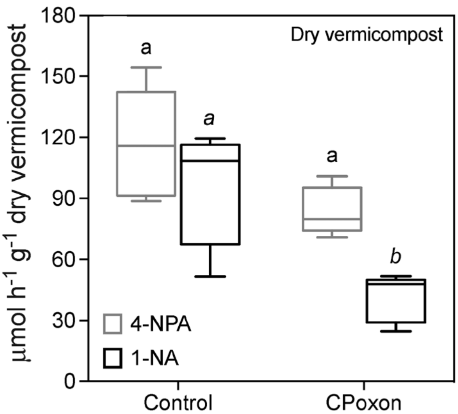




**Fig. 3.** Effect of chlorpyrifos-oxon (CPoxon) treatment (10 µg/g dry mass; incubated thermostatized chamber for two days at 15ºC and continuous dark) on the activity of carboxylesterase in wet (82% $H_2O$, w/v) and dry (8% $H_2O$, w/v) vermicompost. Tukey box plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers) of 5 samples. Significant differences between treatments are indicated by different letters (normal typeface for 4-NPA and italics for 1-NA) after Kruskal-Wallis test followed by a post hoc Mann-Whitney test ($p < 0.05$).

biodegradation. Although significant advances have been made in different research laboratories, the use of vermicompost for the bioremediation of contaminated soils, or as a preventive measure to avoid the impact of pesticides on soil function remains to be validate.

## Concluding Remarks and Knowledge Gaps

Vermicomposting is a low cost, simple and eco-friendly technology for generating value-added products (vermicompost) that can be used as fertilizers and as reactive substrates for the effective removal of soil contaminants. In this context, vermicomposting technology provides a dual role in protecting the environment from chemical stressors. First, the joint function of microorganisms and earthworms during the decomposition of organic matter provides a unique scenario for the removal of pollutants from agroindustrial and municipal waste (detoxification capability). Vermicomposting alters the chemical speciation of metals and thus decreases their bioavailability. In addition, the accumulation of metals by earthworms contributes to reducing the concentration of metals in the final vermicompost. This bioaccumulation process also occurs with organic contaminants present in the agroindustrial and municipal waste used as feedstock. However, in the case of organic pollutants, biodegradation is the main route of dissipating pollutants. Second, vermicompost is a microbiologically and enzymatically active substrate with a large abundance of organic ligands (e.g., humic substances), both characteristics of which are ideal for exploitation in the biostimulation and bioaugmentation of contaminated soils.

Nevertheless, the dual role of vermicomposting demands future research, particularly when new families of contaminants challenge the current technology for reclaiming wastewater and solid waste. Nowadays, the so-called emerging pollutants (pharmaceuticals, personal care products, flame retardant chemicals, etc.), as well as nanomaterials and micro(nano)plastics are commonly detected in sewage sludges (Mahon et al. 2017, Vicent et al. 2013, Hurley and Nizzetto 2018, Boix et al. 2016). In the following subsections, we suggest two lines of research aimed at increasing the understanding of the potential applications of vermicomposting, and vermicompost, in environmental pollution.

### *Toxicity of Vermicomposting Feedstock to Earthworms and Microorganisms*

To date, most vermicompost studies have dealt with changes in earthworm population dynamics (number of adults, juvenile and cocoons per $m^3$ substrate) and in microbial communities, in relation to the physicochemical properties of feedstock. However, very few studies have considered the toxicity of the raw material, particularly agroindustrial and municipal waste. Particular attention has been given to the impact of metals on earthworms, which is often assessed in terms of bioaccumulation (Yadav and Garg 2011). The induction of metal-binding proteins (e.g., metallothioneins) in *E. fetida* and *Lampito mauritii* during vermicomposting, thus favoring the accumulation of metals in the earthworm tissues, has been described (Goswami et al. 2014, Goswami et al. 2016). Apart from these studies, no data are available on the molecular mechanisms involved in toxicity and tolerance of earthworms to contaminated feedstock. The lack of information is more evident in relation to the microbiota involved in vermicomposting.

Fungi are well represented during the vermicomposting process. For example, Anastasi et al. (2005) isolated up to 142 entities in vermicompost obtained from a mixture of 70% dung from cows, poultry and other animals and 30% plant debris with *Lumbricus rubellus* as the composting earthworm species, compared to 118 entities isolated from composting plant debris for 6 months. In addition, a laboratory experiment by Aira et al. (2006) demonstrated that fungal communities increased during vermicomposting of fresh pig slurry using *E. fetida*. Because fungi are important in the bioremediation of contaminated soils, they should be considered key biological entities in the decomposition of organic pollutants present in raw material, or in the immobilization of metals. Indeed, fungi have multiple systems for immobilizing or accumulating metals (e.g., complexation with glomalin, sorption to cell wall chitin and chitosan, binding to siderophores, storage in vacuoles, complexation with metallothioneins and phytochelatins, formation of organometals, among others), and for degrading organic contaminants (e.g., exoenzymes) (Harms et al. 2011). The role of fungi in removing contaminants from feedstock during vermicomposting should be investigated in the coming years as a possible detoxifying function of the process. In this respect, many ecotoxicological approaches and methods (ranging from biomarker measurements to standardized toxicity testing) could be exploited in the assessment of the feedstock toxicity during vermicomposting.

### *Vermicomposting in Microplastic Research*

The terms "microplastic" (0.1 µm–5 mm size) and "nanoplastic" (< 0.1 µm) refer to particles of synthetic and semisynthetic materials, which are generated by fragmentation and degradation of polymer-based materials or are engineered as supplements in cosmetic products and other goods (Ng et al. 2018). Nowadays, micro(nano)plastics are pollutant entities of global concern in all environmental compartments and ecosystems. Municipal wastewater treatment plants are an important source of micro(nano)plastics in the environments. Sewage sludge is often applied to farmland and probably represents the major source of micro(nano)plastics to agricultural soils (Mahon et al. 2017). Because sewage sludge is often used as a feedstock in vermicomposting, the questions arise as to whether vermicomposting could reduce the occurrence of micro(nano)plastics in the final vermicompost and whether vermicomposting could be used as potentially means of producing plastic degraders. The previously mentioned study by Anastasi et al. (2005), which examined the bacterial and fungal diversity in both compost and vermicompost, opens an exciting line of work in the field of vermicomposting for environmental protection. Many of the microorganisms identified in compost and vermicompost have been characterized as potential degraders of synthetic polymers (Shah et al. 2008, Sivan 2011, Bhardwaj et al. 2013, Lambert et al. 2014, Ojha et al. 2017, Osman et al. 2018). Moreover, the levels of carboxylesterase activity, which seems to play an important role in biodegrading polyester polymers (Bhardwaj et al. 2013, Zumstein et al. 2017), are particularly high in vermicompost (Sanchez-Hernandez and Domínguez 2017, Domínguez et al. 2017).

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