

Vermicompost derived from spent coffee grounds: assessing the potential for enzymatic bioremediation

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ABSTRACT

Vermicomposting provides a low-cost, rapid, and environmentally safe solution for the disposal of the large amounts of spent coffee grounds (SCGs) generated annually worldwide. In the last decade, there has been a huge increase in the number of studies concerning the revalorization of SCGs as a biodegradable material of interest in the pharmaceutical, cosmetic, food, and energy industries. However, little attention has been paid to this organic waste as a potential biostimulatory amendment for bioremediating polluted soils. This chapter provides an overview of the main current applications of SCGs and discusses the results obtained in the processing of SCGs through vermicomposting on a pilot scale. In the very short term, vermicomposting reduced substantially the residue mass, yielding a nutrient-rich and enzymatically active vermicompost. The levels and reactivity of carboxylesterases (CbEs) in the vermicompost obtained are evaluated. Although these esterases have a well-recognized function as pesticide-detoxifying enzymes in animals, their role as extra-cellular detoxifying enzymes in vermicompost is unknown. Thus, degradation and detoxification of chlorpyrifos (a model organophosphorus pesticide) was examined in the presence of both liquid and solid vermicomposts derived from SCGs. Degradation of the pesticide in liquid vermicompost followed typical first-order kinetics ($t_{1/2} = 4.74 \text{ day}^{-1}$). Furthermore, CbE activity was strongly inhibited, suggesting a bioscavenging role for these esterases in response to chlorpyrifos contamination. The high levels of CbE activity, and other enzymes of interest regarding contaminant transformation as laccases, in the vermicompost derived from SCGs compared to levels naturally occurring in soils, suggest that this revalorized product has a great potential for the bioremediation of pesticide-contaminated agricultural soils.

Keywords: esterases; organophosphorus pesticides; liquid vermicompost; enzyme fingerprinting; bioremediation; urban agriculture

12.1 INTRODUCTION

The amount of caffeine (1,3,7-trimethylxanthine) in coffee beans is in the range of 0.8%–4.0% (w/w) depending upon the variety of coffee, that is, Arabica or Robusta (Murthy and Naidu, 2012). The stimulatory properties of this purine alkaloid on brain function and behavior may explain why coffee is one the most commonly consumed beverages in the world. Coffee beans also contain a complex mixture of different substances, including sugars, lipids, proteins, vitamins, minerals, alkaloids, and phenolic compounds, some of which may help reduce the risk of several chronic diseases, such as type 2 diabetes, Parkinson's disease, cardiovascular diseases, and some types of cancer (Doepker et al., 2016; Higdon and Frei, 2006; Ludwig et al., 2014). Worldwide consumption of coffee continues to increase, and large amounts of coffee by-products are generated during the manufacture of coffee powder (instant coffee). Depending upon the method (dry or wet) used to produce coffee powder, four types of residues are generated: coffee pulp, coffee husks, silverskin, and spent coffee grounds (SCGs) (Murthy and Naidu, 2012). Globally these residues represent a serious environmental hazard, and their revalorization should be a matter of significant concern to regulatory and environmental agencies. In the last decade, efforts have been made to develop biotechnological processes that use coffee waste to produce value-added products of environmental significance and of interest to the pharmaceutical, cosmetics, and food industries.

Among the coffee by-products generated during the production and consumption of coffee, SCGs represent the most important from an environmental viewpoint. SCGs comprise a powdered organic residue with high levels of humidity (80%–85%) and acidity (pH around 5.0). The residue is obtained during the production of instant coffee powder by treatment of raw coffee powder with hot water or steam under pressure (Mussatto et al., 2011a). It is estimated that about 2-kg wet mass of SCGs are generated from each kilogram of instant coffee produced, representing an annual global production of around 6 million tons (Mussatto et al., 2011a). Surprisingly, despite the large volume of SCGs produced, this organic residue has only recently received particular attention, unlike other coffee by-products, such as coffee pulp, husks, and silverskin (Cruz et al., 2015; Murthy and Naidu, 2012). A literature survey conducted using two common bibliographic search engines (Scopus and ISI Web of Knowledge) revealed the scarce interest that SCGs have aroused in the past two decades (Fig. 12.1). However, since 2010 there has been a significant increase in the number of scientific studies concerning the use of SCGs as a feedstock to produce value-added products of great interest in many industrial sectors (Campos-Vega et al., 2015). The purpose of this chapter is, first, to review recent findings on the industrial applications of SCGs, and subsequently, to discuss the use of vermicomposting to process SCGs and highlight the potential capacity of SCG-derived vermicompost to enzymatically degrade organophosphorus (OP) pesticides.

This chapter is divided into four parts. Section 12.2 presents a brief overview of the most common applications of SCGs in the production of biofuel and

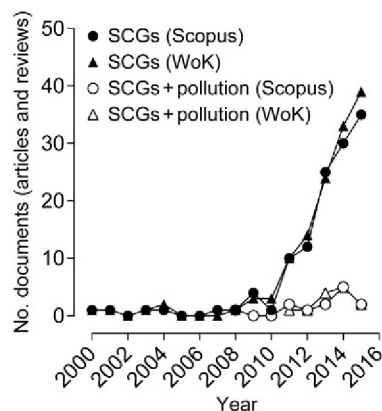


FIGURE 12.1 Global Scientific Production (Limited to Articles and Reviews) Dealing With Spent Coffee Grounds (SCGs) and Their Applications or Effects in the Environment (SCG + Pollution)

Survey was performed September 24, 2016 using two common bibliographic searching engines (ISI Web of Knowledge, or WoK, and Scopus).

other revalorized materials of interest in the pharmaceutical, food, and cosmetics industries. [Section 12.3](#) focusses on the potential agricultural applications of SCGs, whereas [Section 12.4](#) considers vermicomposting as a complementary low-cost and environmentally safe solution to the disposal of SCGs, making SCG-derived vermicompost a suitable material for alleviating pesticide pollution. The results of a case study are discussed in the context of pesticide detoxification by extracellular enzymes present in vermicompost derived from SCGs.

12.2 MULTIPLE APPLICATIONS OF SPENT COFFEE GROUNDS: AN OVERVIEW

SCGs comprise a suitable feedstock for producing liquid biofuel because of the high lipid content of the waste material; 10%–28% dry mass according to [Campos-Vega et al. \(2015\)](#). Research has mainly focused on developing new methods, or improving already-established methods, of obtaining this oil fraction with satisfactory yields. Thus, Soxhlet extraction using organic solvents, such as *n*-hexane ([Caetano et al., 2014](#)), hydrothermal liquefaction ([Yang et al., 2016](#)), catalytic hydrodeoxygenation ([Döhlert et al., 2016](#)), fast pyrolysis ([Kelkar et al., 2015](#)), and ultrasonic-assisted extraction ([Rocha et al., 2014](#)) are examples of some of the methods proposed for extracting oil from SCGs to produce liquid biofuel. Furthermore, SCGs have proved to be a viable solid fuel (pellets and biochar) for heating purposes ([Jeguirim et al., 2014](#); [Tsai et al., 2012](#); [Zuorro and Lavecchia, 2012](#)).

In the food industry, SCGs have been used in animal feed (Seo et al., 2015) and also as an ingredient in bakery products destined for human consumption (Martínez-Saez et al., 2017), as well as a source of dietary fiber with human health benefits (López-Barrera et al., 2016). SCGs have even been used in fermentative processes because of their high content of sugars (Mussatto et al., 2011b). From a pharmaceutical viewpoint, SCGs represent a source of a vast variety of chemicals with bioactive properties. Accordingly, many studies have demonstrated that SCGs contain readily extractable antioxidant substances, such as polyphenols, caffeine, and Maillard-reaction products, which may be exploited for pharmaceutical and nutritional purposes (Bravo et al., 2012). Furthermore, oils isolated from SCG by supercritical fluid extraction have been used in the formulation of topical creams, such as moisturizers and antiseborrheics (Ribeiro et al., 2013), and also as sunscreens that provide high levels of protection against UVB/A radiation (Marto et al., 2016).

The potential use of SCGs in the field of new ecofriendly materials has also been explored recently. For example, oil-free SCGs were used satisfactorily as a filler to reinforce polypropylene composite (Wu et al., 2016). Similarly, SCGs have been combined with other waste by-products derived from steel production and coal combustion to produce a geopolymer material with suitable physical features for use in pavement construction (Kua et al., 2016). By combining SCGs and pure clays, Sena da Fonseca et al. (2013) succeeded in developing a ceramic material that exhibited ideal physical and mechanical performance for construction purposes.

The presence of microplastic debris in the oceans represents, nowadays, a serious environmental hazard that must be tackled urgently (Eerkes-Medrano et al., 2015; Lambert et al., 2013). The challenge is now to develop biodegradable plastics that can be produced cheaply and that also have desirable physical and biodegradable properties. In this way, SCGs have proved suitable for elaborating biodegradable plastic composites (Baek et al., 2013; García-García et al., 2015; Wu, 2015), thus opening up various possibilities for manufacturing ecofriendly plastic materials to alleviate the environmental pressure caused by the more recalcitrant and toxic microplastics.

12.3 ENVIRONMENTAL APPLICATIONS OF SPENT COFFEE GROUNDS

12.3.1 USE OF SPENT COFFEE GROUNDS IN AGRICULTURE

Landfill disposal has traditionally been the main method used to deal with the high volume of SCGs generated yearly on a global scale. However, some studies have revealed that this practice is not free of environmental risk because of the presence of potentially toxic substances, such as caffeine, polyphenols, and tannins in fresh SCG (Mussatto et al., 2011b). However, some agricultural applications provide encouraging solutions to the disposal of SCGs. Although this organic residue is frequently

used in domestic agriculture as a soil amendment, the beneficial effects on soil quality and plant health have only recently been investigated, and there is some controversy regarding the effects of SCGs in agriculture. For example, some studies using lettuce as a plant model have demonstrated beneficial effects on plant health when SCGs are applied at doses between 5% and 30% (v/v), including enhanced photosynthetic pigments, vitamins, minerals, and antioxidant capacity (Cruz et al., 2014a,b, 2015); however, the effects were highly dependent on the state of the SCGs (fresh or composted) and the application rate. Use of composted SCGs was the preferred option because the concentrations of caffeine and polyphenols present in fresh SCGs were greatly reduced by composting. Moreover, these studies also demonstrated that the use of fresh SCGs as a soil amendment may reduce micronutrient bioavailability due to the presence of metal-chelating substances in the fresh waste (Cruz et al., 2014b). By contrast, a recent study by Hardgrove and Livesley (2016) showed that direct application of noncomposted SCGs to different types of soil significantly reduced the growth of several horticultural plants (broccoli, leeks, radishes, sunflowers, and violas) at an application rate as low as 2.5% v/v. Although bioactive compounds in the SCGs may be responsible for this toxic effect, the exact mechanism leading to growth inhibition was not elucidated. Similarly, Yamane et al. (2015) examined the effect of SCGs (application rate, 10 kg/m²) on green manure crops in a 2-year-long field study. Although SCGs caused significant inhibition of plant growth in the first cropping season, the effect decreased in successive seasons. The authors of the study suggested that caffeine, polyphenols, and tannins caused inhibition of plant growth. One interesting result of this study was the pesticide-like effect of fresh SCGs in controlling weed cover, a phenomenon also reported by Hardgrove and Livesley (2016). Despite the increasing interest in the use of SCGs in agriculture, further research is required to develop scientifically based guidelines for use of this waste material as a soil amendment.

12.3.2 BIOREMEDIATION WITH SPENT COFFEE GROUNDS

Most bioremediation studies concerning SCGs have focused on the capacity of the waste product to remove dyes and metals from water. For example, Lavecchia et al. (2016) used noncomposted and water-washed SCGs to remove Pb from water. The kinetics of adsorption of this metal to SCGs fitted a Langmuir model, with a maximum adsorption of 2.46 mg/g. Franca et al. (2009) also used fresh SCGs to remove the cationic dye methylene blue and the maximum adsorption capacity achieved was 18.7 mg/g. However, better results are achieved with activated SCGs. For example, SCG-derived carbonaceous materials have been used to remove dyes from water after a prior activation step in which the SCGs are impregnated with either H₃PO₄ (Reffas et al., 2010) or a mixture of H₃PO₄ and ZnCl₂ (Namane et al., 2005). Similarly, Jung et al. (2016) transformed SCGs into an activated carbonaceous material with excellent properties for removing dyes from aqueous media. Less laborious treatment of SCGs with solar energy (~200°C for 5 days) produced a more efficient degreased adsorbent for removal of Ni and Cd

from water than water-washed SCGs (Yen and Huang, 2015; Yen and Lin, 2015). However, scarce attention has been given to the isolation of SCGs after sorbed dyes or metals from water. In this sense, the study by Safarik et al. (2011) is an original example in which SCGs were pretreated with a methanolic ferrofluid solution (1:5, w/v) to produce magnetically modified SCGs able to remove up to 73.4 mg of acridine orange per gram of dried SCGs by simple magnetic separation techniques. Although the aforementioned studies have demonstrated the potential usefulness of fresh and activated SCGs (or the pyrolytic-derived product) in bioremediation of contaminated waters, these laboratory-scale results have not yet been corroborated at a larger scale.

The high organic matter content of SCGs causes proliferation of soil microbes when the material is applied to soil as an amendment; however, little attention has been paid to the potential use of SCGs in the bioremediation of polluted soils. In situ bioremediation is the preferred approach for recovery of contaminated soils because it is cost effective and environmentally friendly. This cleanup technology is based on the capacity of microorganisms and plants to immobilize, accumulate, and metabolize environmental contaminants in soil. However, in situ bioremediation has important drawbacks related to the biochemical, physiological, and ecological characteristics of microorganisms and plants. For example, effective microbially induced degradation of soil contaminants is often a lengthy process that is highly dependent on the microbial growth rate and bioavailability of target contaminants. Stimulation of native microbial communities by addition of organic matter, in a procedure referred to as *biostimulation*, is often the most suitable solution to long-term maintenance of in situ microorganism-assisted bioremediation. SCGs comprise an attractive material because of their high nutrient content and water-holding capacity, although prior treatment (e.g., composting) is required to reduce the phytotoxicity of some of the components. Fenoll et al. (2011, 2014a,b, 2015) have studied the sorption capacity of SCGs in response to pesticide input. To examine pesticide leaching and persistence, these researchers added degreased SCGs (washed with hot water, air dried, refluxed with *n*-hexane, and finally sieved at <3 mm) to disturbed soil columns under laboratory conditions. They reported that application of the degreased SCGs to a soil with a low organic matter content reduced the leaching of fungicides, insecticides, and herbicides, probably as a result of the presence of a greater number of organic ligands available for pesticide binding. Moreover, these studies have also shown that treatment of soil with SCGs may lead to degradation of pesticides via a biostimulatory effect (Fenoll et al., 2014b). Overall, the findings of the studies by Fenoll et al. (2011, 2014a,b, 2015) suggest that SCGs have qualities that make the product suitable as an organic amendment for bioremediation purposes, to increase pesticide adsorption, reduce the leaching capacity of pesticides, and increase biodegradation of pesticides. However, biodegradation is not always achieved because of the low bioaccessibility of some pesticides (e.g., phenylurea herbicides), which are strongly sorbed to the organic matter of SCGs (Fenoll et al., 2015).

12.4 VERMICOMPOSTED SPENT COFFEE GROUNDS: AN ENVIRONMENTALLY SAFE VALUE-ADDED PRODUCT

Vermicomposting is defined as a biooxidative process in which dead organic matter is transformed into a fine and porous peat-like material called vermicompost, which has a high water-holding capacity and nutrient burden, and well-established microbial community; physicochemical and biological properties that undoubtedly improve plant health (Domínguez and Edwards, 2011a). During vermicomposting, detritivorous earthworms cooperate with microorganisms, thus accelerating the stabilization of organic matter and significantly modifying its physical, chemical, and biological properties (Domínguez, 2004; Domínguez and Edwards, 2011a,b; Domínguez and Gómez-Brandón, 2012, 2013). Unlike composting, there is no typical thermophilic phase (45–65°C) in vermicomposting, and enzymes and other chemicals of concern for plant growth and development are therefore not destroyed by thermal denaturing (Datta et al., 2016). Biochemical degradation of organic matter during vermicomposting is mainly due to microbial enzymes, although earthworms are the key drivers of the process. Aerobic conditions are favored in organic waste as a result of the feeding, casting (deposition of feces), and tunneling activities of earthworms. Earthworms also stimulate microbial proliferation (by increasing the surface area available for microbial colonization after fragmentation and ingestion of fresh organic matter), modify microbial biomass and activity (via dispersion of microorganisms in casts), and interact closely with other biological components (e.g., protozoa and springtails) of the vermicomposting system, thereby affecting the microbiome and microfaunal community structure (Domínguez et al., 2003; Lores et al., 2006). A more detailed description of the vermicomposting process is outside the scope of this chapter, and a comprehensive description of vermicomposting (covering suitable earthworm species and raw organic feedstock, impact of vermicompost on soil quality and plant fitness, and the most common domestic- and industrial-scale vermicomposting systems) is provided in the reference book by Edwards et al. (2011).

SCGs contain significant amounts of bioactive compounds, such as polyphenols, caffeine, and tannins, which may represent an environmental hazard when the fresh waste material is applied to soils as an amendment. Vermicomposting of SCGs is a rapid solution to reduce the toxicity of these compounds, and yields revalorized SCG-derived products rich in nutrients, microorganisms, and extracellular enzymes with beneficial effects on soil quality and plant health. The excellent qualities of vermicompost as a soil amendment have been extensively investigated (Edwards et al., 2011). However, very few studies have examined the qualities of vermicompost in relation to removal of pesticide residues from soils. Nogales and coworkers have shown that vermicomposts produced from diverse types of organic waste (from the winery distillery and olive oil industries) significantly reduced the mobility of pesticides, such as imidacloprid and diuron (and their metabolites) in soils with low organic matter contents (Fernández-Bayo et al., 2007, 2008, 2009). Enhanced adsorption of pesticide in soil was suggested to be due to the high content of organic

ligands (such as lignin) in vermicompost. Apart from this adsorptive capacity, the biostimulatory effect of vermicompost on soil has scarcely been investigated. In a laboratory study, [Fernández-Gómez et al. \(2011\)](#) used the community-level physiological profiling approach to compare the microbial functional response of four different types of vermicompost to pesticide exposure. These authors found that vermicomposts with high functional diversity were most suitable for bioremediation purposes. Hence the aforementioned studies demonstrate the capacity of vermicompost to reduce the impact of pesticide residues in soil via two main mechanisms: by increasing the number of binding sites for pesticides with a moderate to high sorption coefficient, and by increasing the biodegradation capability. However, the role of certain extracellular enzymes of vermicompost as catalysts for degrading or transforming pollutants is a topic of increasing concern. In this sense, some studies have examined how a group of esterase enzymes with known function in pesticide metabolism, that is, carboxylesterases (CbEs), affects pesticide detoxification in soil bioturbated by earthworms ([Sanchez-Hernandez et al., 2014a,b, 2015](#)). These studies postulate the intriguing idea of using earthworms as biological vectors in the enzymatic bioremediation of OP-contaminated soils. On the basis of the previous results, the question that arises is whether vermicompost, and particularly vermicompost derived from SCGs, provides a rich source of these pesticide-detoxifying CbEs.

In the present study, SCGs were obtained from a local supplier (the cafeteria in the Faculty of Biological Science, University of Vigo). The SCGs were processed in pilot-scale vermireactors (3 m²) held in a greenhouse. The earthworm species *Eisenia andrei* (commonly known as redworm) was used in the vermireactors ([Fig. 12.2](#)). *E. andrei* (Oligochaeta, Lumbricidae), an epigeic earthworm with a worldwide distribution, is tolerant to a wide range of temperature and moisture conditions, and is the most common earthworm species used in vermicomposting facilities ([Domínguez and Edwards, 2011b](#)). SCGs were added to the vermireactor in successive layers, as they were processed by earthworms. Population density and biomass of earthworms were determined periodically, and samples of SCG-vermicompost were also collected to determine the chemical and biological properties.

The initial earthworm population density in the vermireactor was 3153 ± 184 mature earthworms/m² and 7987 ± 1271 juveniles/m² (mean \pm SD, $n = 5$), corresponding to an earthworm biomass of 700 ± 156 g mature earthworms/m² and 740 ± 154 g juveniles/m², respectively ([Fig. 12.2A](#)). After the addition of 60 kg of SCGs, the density and biomass of earthworms increased continuously and significantly over 14–28 days, reaching a maximum earthworm density of 13634 ± 1578 individuals/m² for an earthworm biomass of 1732 g fresh mass/m². The cocoon density reached the maximum level after 4 weeks ([Fig. 12.2A](#)). To study the effects of the SCG diet on earthworm growth, a complementary experiment was conducted with 30 newly hatched (baby earthworms) (0.055 ± 0.002 g, initial mean weight) held individually in SCGs. The results of this trial showed an exponential growth rate within the first 6 weeks ([Fig. 12.2B](#)), demonstrating the viability of vermicomposting of SCGs despite the high content of caffeine and other bioactive ingredients. This growth curve clearly indicated a phase of adaptation of juveniles to SCGs, probably marked by physiological adjustment to nutrients supplied by SCGs and energy expenditure

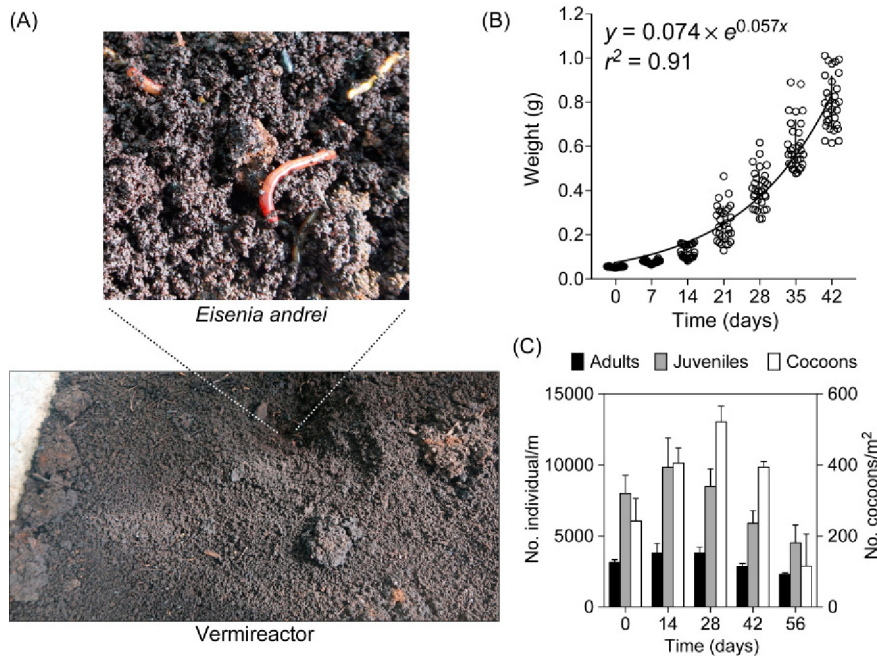


FIGURE 12.2 Vermicomposting of Spent Coffee Grounds (SCGs) Using *E. andrei*

(A) Adult individuals (*E. andrei*) in the vermireactor. (B) Growth rate of juvenile ($n = 30$ individuals) *E. andrei* individually kept in Petri dishes containing SCGs. (C) Time-course dynamics of earthworm density (adults, juveniles, and cocoons) during the first 56 days of vermicomposting.

against exposure to potential toxic compounds (e.g., caffeine). Indeed, the typical growth curves of *E. andrei* in nontoxic substrates, such as cattle manure fit a hyperbolic-like model with a rapid growth rate in the first weeks of feeding (Elvira et al., 1996). Overall, the earthworm population in the vermireactor was similar to those reached in other types of organic residues, such as cow manure (8,000 individuals/m²), and pig manure (14,600 individuals/m²) (Monroy et al., 2006). The chemical components of fresh SCGs (caffeine, polyphenols, and tannins) may be toxic to earthworms and pose a serious threat to earthworm survival, growth, and reproduction. Nevertheless, the study findings indicate that vermicomposting of SCGs is feasible in terms of waste treatment, production of earthworm biomass, and yield of vermicompost with good characteristics. The chemical and biochemical properties and the microbial activity of fresh SCGs and the vermicompost derived from SCGs are summarized in Table 12.1. Concentrations of trace elements in the SCGs used in this pilot study were within the same range of variation as those reported by other authors. As a consequence of the rapid C mineralization during vermicomposting, there was a significant increase in the concentration of micro- and macronutrients in the SCG-derived vermicompost. Furthermore, the pH of the fresh SCGs was slightly acid and became neutral in the final product, and concentrations of cellulose and

Table 12.1 Mean (\pm SD, $n = 5$) Values of Chemical Composition and Microbial Activity of Spent Coffee Grounds (SCGs) as Raw Feedstock ($t = 0$ Days) and as Vermicompost ($t = 12$ Months)

	SCG Feed-stock ($t = 0$ Months)	SCG Ver-micompost ($t = 12$ Months)	SCGs ^a
pH	6.34 \pm 0.10	6.94 \pm 0.06	—
Electrical conductivity (μ S/cm)	179 \pm 25	118 \pm 7.5	—
Moisture (%)	66.4 \pm 1.1	79.7 \pm 4.4	—
Organic matter (%)	98.7 \pm 0.07	94.4 \pm 1.13	90.5
Total carbon (%)	53.2 \pm 0.76	46.7 \pm 4.35	—
Total nitrogen (%)	2.34 \pm 0.01	4.54 \pm 0.28	2.3–2.79 \pm 0.10
C/N ratio	22.7 \pm 0.34	10.3 \pm 0.46	16.91 \pm 0.10
Phosphorus (g/kg d.w.)	1.10 \pm 0.08	3.01 \pm 0.32	1.47–1.8
Potassium (g/kg d.w.)	3.00 \pm 0.42	6.52 \pm 0.37	3.55–11.7
Calcium (g/kg d.w.)	1.23 \pm 0.03	6.00 \pm 0.52	0.77–1.2
Magnesium (g/kg d.w.)	1.40 \pm 0.05	3.51 \pm 0.14	1.29–1.9
Sulfur (g/kg d.w.)	0.43 \pm 0.02	1.57 \pm 0.67	1.6
Iron (g/kg d.w.)	0.067 \pm 0.005	1.25 \pm 0.27	0.052–0.11
Manganese (g/kg d.w.)	0.029 \pm 0.001	0.24 \pm 0.06	0.028–0.040
Zinc (g/kg d.w.)	0.022 \pm 0.010	2.60 \pm 1.43	0.008–0.015
Boron (mg/kg d.w.)	90.5 \pm 8.3	66.1 \pm 15.0	8.40 \pm 1.10
Copper (mg/kg d.w.)	21.3 \pm 0.44	60.0 \pm 4.16	18.6–32.3
Basal respiration (mg CO ₂ /g d.w./h)	0.70 \pm 0.18	0.30 \pm 0.06	—
Lignin (%)	19.5 \pm 0.96	57.7 \pm 4.66	23.9 \pm 1.7
Cellulose (%)	30.6 \pm 2.13	17.2 \pm 3.44	8.6 \pm 1.8– 12.4 \pm 0.79
Hemicellulose (%)	28.1 \pm 1.76	12.6 \pm 4.36	36.7 \pm 5.0– 39.1 \pm 1.94

^aChemical composition of SCGs taken from [Ballesteros et al. \(2014\)](#), [Mussatto et al. \(2011a\)](#), and [Murthy and Naidu \(2012\)](#).

hemicellulose were halved. All in all, values of most physicochemical properties of SCG-derived vermicompost matched with those considered to have the quality criteria of a good vermicompost ([Edwards et al., 2011](#)).

12.4.1 ENZYME ACTIVITIES IN VERMICOMPOST FROM SPENT COFFEE GROUNDS

The breakdown of macromolecules, such as cellulose, hemicellulose, lignin, and tannin during vermicomposting requires the action of a battery of extracellular enzymes, most of which are produced by microorganisms. Accordingly, changes in enzyme

activities during vermicomposting have been widely studied in an attempt to elucidate the biochemical interactions between microorganisms and earthworms during organic matter decomposition, as well as to specify a biochemical fingerprint for mature vermicompost to be used as an indicator of vermicompost quality. The fraction of organic compounds available during vermicomposting seems to be the driving force of extracellular enzymes, such as β -glucosidase, phosphatase, urease, and protease (Benitez et al., 1999). However, the increasing concentrations of humic substances that appear as vermicomposting progresses provide chemical support for binding extracellular enzymes, rendering them more stable and protecting them against proteases or adverse environmental conditions (desiccation or high temperatures). It is therefore not surprising that the activity of extracellular enzymes remains high in the mature vermicompost, marked by nutrient depletion and absence of earthworms (Aira et al., 2007; Benitez et al., 2005; Castillo et al., 2013; Domínguez, 2004). In fact, the activities of some extracellular enzymes are higher in the mature vermicompost than during the vermicomposting process (Castillo et al., 2013).

However, it is not clear whether the enzymatic burden of vermicompost has an additive effect on soil biochemical performance, thereby accelerating the nutrient decomposition in soil (Aira and Domínguez, 2011; Benitez et al., 2005). Likewise, little is known about the effects of the extracellular enzymes present in vermicompost on the persistence of environmental contaminants in soil. In an attempt to address this question, some efforts have started characterizing the enzymatic profiles of vermicomposts derived from grape marc, cattle manure, or SCGs. The levels of several extracellular enzyme activities in vermicompost produced from SCGs are presented. Particular emphasis is given to laccase and CbE because of their potential role in bioremediation of organic contaminants.

Enzyme activities in vermicompost were generally measured following established protocols in soil enzymology. Soil enzymes are diverse, as they include intracellular (living, resting, and dead microbial cells) and extracellular forms (free in soil solution or associated with organo-mineral complexes of soil) (Nannipieri et al., 2002). As vermicompost is a stabilized material with a high organic matter content and a well-developed microbial community (Edwards et al., 2011), enzyme activities are expected to be mainly intracellular and linked to organic complexes. Most methods of determining soil enzymes measure catalytic activity in soil-water suspensions, which are preferred as they integrate the activity of the multiple forms in which enzymes are located in the soil matrix (German et al., 2011). Common procedures for processing aqueous suspensions of soil generally involve continuous magnetic stirring (Deng et al., 2013; Turner, 2010), homogenization in a blender (Bell et al., 2013) or a Brinkmann Polytron (Saiya-Cork et al., 2002), or rotating mixing (Jackson et al., 2013; Sanchez-Hernandez et al., 2015), before removal of aliquots to determine enzyme activity. However, these procedures do not yield efficient (complete) homogenization of vermicompost. Thus, in a preliminary assay, CbE activity was compared in aqueous suspensions of vermicompost (3% w/v) prepared using two different procedures: by rotating mixing for 30 min, or by homogenization in a glass-PTFE Potter-Elvehjem grinder (commonly used for tissue homogenization).

The activity of the esterase was 4 times higher in the vermicompost homogenates [$142 \pm 41 \mu\text{mol/h/g}$ dry mass for the hydrolysis of 1-naphthyl butyrate (1-NB) and $473 \pm 110 \mu\text{mol/h/g}$ for the hydrolysis of 4-nitrophenyl butyrate (4-NPB)] than in the vermicompost: water suspensions obtained by rotating mixing ($31.2 \pm 4.0 \mu\text{mol/h/g}$ dry mass for 1-NB and $122 \pm 16 \mu\text{mol/h/g}$ for 4-NPB). In view of these results, homogenates of vermicompost derived from SCGs were used, to determine the activity of 11 enzymes. Seven of these are representative of the C-, N-, P-, and S-cycles; two (laccase and peroxidase) are considered to measure the oxidative potential of soil (Bach et al., 2013), and the other two enzymes (dehydrogenase and catalase) are commonly used as indicators of microbial activity because of their intracellular location (Trasar-Cepeda et al., 1999; von Mersi and Schinner, 1991). Table 12.2 summarizes the assay conditions adapted to a microplate format for each enzyme activity.

The enzyme activities measured in the vermicompost derived from SCGs are summarized in Table 12.3. The enzyme activities of vermicomposts produced from horse manure and from grape marc are also included for comparison. The chemical nature of the feedstock and the presence of different substrates in the fresh organic waste processed during vermicomposting significantly influence the levels of enzyme activities in mature vermicomposts. Thus, higher levels of urease and protease activities were found in SCG-derived vermicompost than in the other two types of vermicompost as a result of a higher content of nitrogen compounds in SCGs. Likewise, the high oil content of SCGs also explains the higher CbE activity (1-NB) in the vermicompost produced from this organic waste than in the vermicompost obtained from horse manure. Similarly, SCGs and grape marc contain high concentrations of polyphenols, tannins, cellulose, and hemicellulose, thus explaining the higher activity of laccases and peroxidases in these vermicomposts than in vermicompost made from horse manure. As laccases and CbEs are important detoxifying enzymes in polluted soils, the high levels of these enzymes in vermicomposts made from SCGs and from grape marc suggest that vermicomposts are of potential use in the bioremediation of polluted soils.

Laccases have a long history of use in bioremediation. These copper-containing oxidases, which are mainly produced by white-rot fungi, plants, and some species of bacteria, catalyze the oxidation of various aromatic compounds, such as phenols and aromatic amines at the expense of molecular oxygen (Eichlerova et al., 2012). They can also transform or degrade environmental pollutants of current concern, such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), trinitrotoluene, synthetic dyes, pesticides, and endocrine disrupters, such as nonylphenol and bisphenol A (Rao et al., 2014). Nevertheless, large amounts of crude preparations or purified laccases are needed to detoxify polluted soils, and this is one of the challenges in the biotechnological application of laccases in bioremediation. Induction of laccases in several wild fungal strains by specific chemical inducers or through genetically modified organisms has been the most common strategy used to obtain preparations of the enzyme (Gianfreda et al., 1999). However, this approach is not always the best option because of poor enzyme yields, high costs, and tedious purification procedures. Use of vermicompost may represent

Table 12.2 Enzyme Activities Measured in Vermicompost and Spectrophotometric Conditions for Microplate-Scale Assay

Enzymes (EC)	Assay Methods	Substrate (Final Concentrations)	Buffers	Wavelength (Product)	References
CbE (3.1.1.1)	Stopped assay (30 min at 20°C, orbital shaking incubator)	1-NB (2 mM); 4-NPB (2 mM)	Tris-HCl (pH = 7.4)	530 nm (1-naphthol), 405 nm (4-nitrophenol)	Sanchez-Hernandez et al. (2015)
Acid phosphatase (3.1.3.2)	Stopped assay (4 h at 20°C, orbital shaking incubator)	4-Nitrophenyl phosphate (5 mM)	Modified universal buffer 20 mM (pH = 6.5)	405 nm (4-nitrophenol)	Popova and Deng (2010)
Alkaline phosphatase (3.1.3.1)	Stopped assay (4 h at 20°C, orbital shaking incubator)	4-Nitrophenyl phosphate (5 mM)	Modified universal buffer 20 mM (pH = 11.0)	405 nm (4-nitrophenol)	Popova and Deng (2010)
β -Glucosidase (3.2.1.21)	Stopped assay (4 h at 20°C, orbital shaking incubator)	4-Nitrophenyl- β -D-glucanopyranoside (5 mM)	Modified universal buffer 20 mM (pH = 7.4)	405 nm (4-nitrophenol)	Popova and Deng (2010)
Protease (3.4.21.92)	Stopped assay (4 h at 50°C, orbital shaking incubator)	Casein (1% w/v)	Tris-HCl 0.1 M (pH = 8.1)	700 nm (tyrosine equivalents)	Schinner et al. (1996)
Urease (3.5.1.5)	Stopped assay (4 h at 20°C, rotating mixer)	Urea (40 mM)	Unbuffered method	690 nm (ammonium)	Schinner et al. (1996)
Arylsulfatase (3.1.6.1)	Stopped assay (4 h at 20°C, orbital shaking incubator)	—	Modified universal buffer 20 mM (pH = 7.4)	405 nm (4-nitrophenol)	Popova and Deng (2010)
Laccase (1.10.3.2)	Stopped assay (3 h at 20°C, orbital shaking incubator)	Pyrogallol (5 mM) L-DOPA (25 mM)	Maleic acid 25 mM (pH = 7.4)	460 nm (oxidized substrates)	Bach et al. (2013)
Peroxidase (1.11.1.7)	Stopped assay (3 h at 20°C, orbital shaking incubator)	Pyrogallol (5 mM)	Maleic acid 25 mM (pH = 7.4) + 0.3% H ₂ O ₂	460 nm (oxidized substrates)	Bach et al. (2013)
Dehydrogenase (1.1)	Stopped assay (1 h at 40°C, water bath shaking)	Iodotetrazolium chloride (0.14% w/v)	Tris-HCl 1 M (pH = 7.0)	464 nm (reduced iodotetrazolium formazan)	von Mersi and Schinner (1991)
Catalase (1.11.1.6)	Stopped assay (5 min at 20°C, rotating mixer)	H ₂ O ₂ (8.8 mM)	Unbuffered method	505 nm (colored product formed from oxidative coupling reactions between O ₂ , 4-aminoantipyrine, and phenol)	Trasar-Cepeda et al. (1999)

CbE, Carboxylesterase; L-DOPA, L-3,4-dihydroxyphenylalanine; 1-NB, 1-naphthyl butyrate; 4-NPB, 4-nitrophenyl butyrate.

Table 12.3 Mean (\pm SD, $n = 6$) Enzyme Activity in Vermicompost Obtained From Spent Coffee Grounds (SCGs), Horse Manure, and Grape Marc

Enzyme	SCGs	Horse Manure ^a	Grape Marc ^a
<i>C-cycling enzymes</i>			
CbE 4-NPB ($\mu\text{mol/h/g}$ dry mass)	527 \pm 57	172 \pm 31	1079 \pm 102
CbE 1-NA ($\mu\text{mol/h/g}$ dry mass)	351 \pm 61	155 \pm 75	1180 \pm 160
CbE 1-NB ($\mu\text{mol/h/g}$ dry mass)	263 \pm 68	35.2 \pm 14.3	63.3 \pm 30.1
β -Glucosidase ($\mu\text{mol/h/g}$ dry mass)	12.3 \pm 0.80	8.58 \pm 1.17	29.4 \pm 3.21
<i>P-cycling enzymes</i>			
Acid phosphatase ($\mu\text{mol/h/g}$ dry mass)	5.00 \pm 0.27	9.20 \pm 0.16	16.3 \pm 2.05
Alkaline phosphatase ($\mu\text{mol/h/g}$ dry mass)	28.4 \pm 1.00	8.05 \pm 0.96	33.3 \pm 3.63
<i>N-cycling enzymes</i>			
Urease ($\mu\text{mol NH}_4^+ \text{--N/h/g}$ dry mass)	387 \pm 30	159 \pm 5.30	78.5 \pm 12.2
Protease (mg tyrosine equivalent/h/g dry mass)	21.7 \pm 0.76	14.31 \pm 0.66	13.10 \pm 2.54
<i>S-cycling enzymes</i>			
Arylsulfatase ($\mu\text{mol/h/g}$ dry mass)	4.82 \pm 0.20	3.22 \pm 0.29	0.67 \pm 0.06
<i>Oxidative enzymes</i>			
Laccase ($\mu\text{mol pyrogallol/h/g}$ dry mass)	12.7 \pm 1.00	8.90 \pm 0.93	30.0 \pm 2.60
Laccase ($\mu\text{mol L-DOPA/h/g}$ dry mass)	0.60 \pm 0.13	0.35 \pm 0.14	0.78 \pm 0.24
Peroxidase ($\mu\text{mol pyrogallol/h/g}$ dry mass)	7.45 \pm 1.01	5.50 \pm 0.34	5.60 \pm 0.65
<i>Microbial activity indicators</i>			
Dehydrogenase ($\mu\text{mol INTF/h/g}$ dry mass)	1.52 \pm 0.21	0.92 \pm 0.25	0.180 \pm 0.02
Catalase (mmol H ₂ O ₂ consumed/h/g dry mass)	180 \pm 4.0	165 \pm 7.2	103 \pm 12.2

CbE, Carboxylesterase; L-DOPA, L-3,4-dihydroxyphenylalanine; INTF, iodonitrotetrazolium formazan; 1-NA, 1-naphthyl acetate; 4-NPB, 4-nitrophenyl butyrate.

^aData from Domínguez, J., Sanchez-Hernandez, J.C., Lores, M., 2017. Vermicomposting of winemaking by-products. In: Galanakis C. (Ed.), *Handbook of Grape Processing By-Products*. Academic Press, Elsevier, 55–78.

an alternative, cost-effective strategy for supplementing polluted soils with laccases. For instance, vermicomposts produced from polyphenol-rich feedstocks (SCGs or grape marc) had a higher laccase content than vermicompost made from horse manure, and even higher than in different types of soil (Bach et al., 2013). These findings encourage further research on the stability, catalytic efficiency, and reactivity of laccases after application of vermicompost (as solid or liquid preparations) to polluted soils.

Laccase-induced detoxification involves oxidation of contaminants to free radicals or quinones that subsequently undergo polymerization and may be incorporated into humic substance in formation (Riva, 2006). This detoxifying system leads to immobilization of the pollutant in naturally occurring polymeric compounds. CbE activity may provide a comparable detoxification system. The interest in these esterases for bioremediation purposes arises from their capacity to bind OP pesticides by covalent attachment between the phosphoryl moiety of the pesticide and the serine hydroxyl group of the active site of the enzyme (Jackson et al., 2011), as discussed in the next section.

In the present study, CbE activity was detected in vermicompost derived from SCGs by using three different substrates (Table 12.3). In animal tissues and soil, this enzyme is generally found as multiple isozymes with a markedly different substrate preference. In fact, our results showed that there was a significant difference between the hydrolysis rates of 1-NB and 4-NPB among the different types of vermicompost. Likewise, the enzyme activity was resistant to vermicompost desiccation and thermal denaturing (data not shown), which suggested an extracellular location linked to organic matter. To our knowledge, this is the first report of CbE activity in vermicompost derived from SCGs. The activity was one order of magnitude higher than in agricultural soils (Sanchez-Hernandez et al., 2015, 2017), measured using the same enzyme assay. The high CbE activity suggests that vermicompost derived from SCGs has an enzymatic bioremediation capacity, which would enhance its properties as revalorized product.

12.4.2 PESTICIDE DETOXIFYING ESTERASES IN VERMICOMPOST FROM SPENT COFFEE GROUNDS

This part of the study focused on the role of vermicompost-derived CbE activity in OP pesticide detoxification. The ubiquitous CbEs hydrolyze a vast range of carboxylic acid esters, thioesters, and amides (Yan, 2012). Their environmental interest has been addressed in a special issue published in the *Journal of Pesticide Science* in 2010 (Wheelock and Nakagawa, 2010). In pesticide toxicology, CbEs play a pivotal role in detoxifying anticholinesterase (organophosphate and methyl carbamate) and pyrethroid pesticides. These esterases can hydrolyze pyrethroids and methyl carbamates, as they contain a carboxylic ester in their chemical structure (Jackson et al., 2011). Detoxification of OP pesticides by CbEs, however, is achieved by an inhibition-based mechanism. The OP binds to the serine hydroxyl group of the active site of the enzyme to yield a stable “enzyme–inhibitor” complex. The enzyme cannot be reactivated in this phosphorylated state (Sogorb and Vilanova, 2002; Yan, 2012), and can undergo an “aging” process, whereby one alkyl group may leave the OP molecule in the presence of water (dealkylation), leading to permanent inactivation of the enzyme.

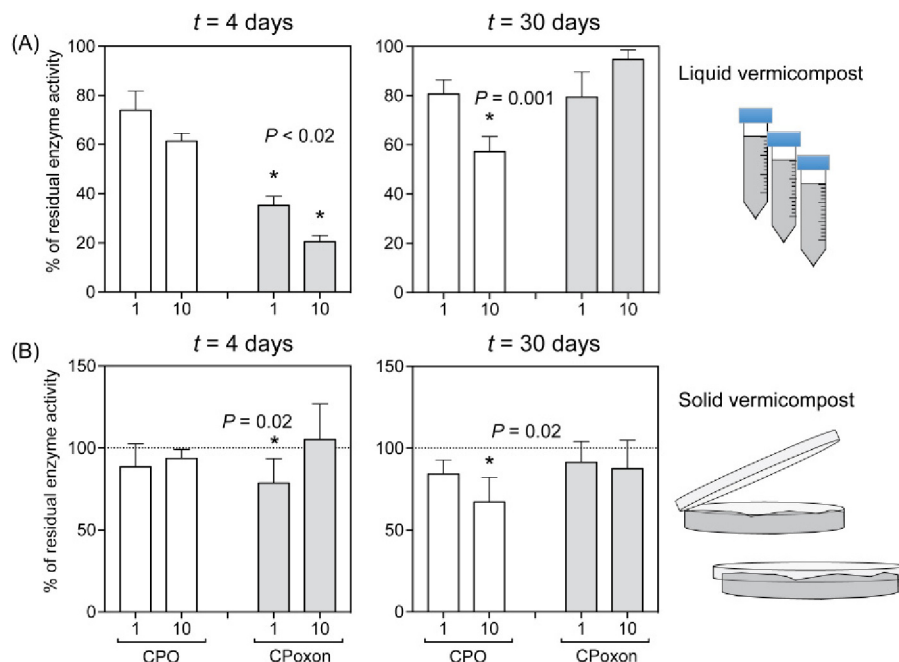
CbEs have multiple environmental applications. Thus, inhibition of CbE activity by OP pesticides is often used as a complementary indicator of the exposure of non-target to these types of agrochemicals (Wheelock et al., 2008). The esterases are not only important as simple biomarkers of pesticide exposure, but also because they contribute to developing insecticide resistance in some pest species (Farnsworth

et al., 2010; Hemingway and Ranson, 2000). They have been satisfactorily included in the Toxicity Identification Evaluation scheme, whereby purified preparations of CbE are used in a logical weight-of-evidence approach to identify the cause of toxicity in environmental matrices, such as water or sediment (Phillips et al., 2010; Wheelock et al., 2008). More recently, the potential use of CbE activity in enzymatic bioremediation has been investigated in earthworm-bioturbed soil (Sanchez-Hernandez et al., 2014a,b, 2015). Although pending further research, the results of these studies suggest that soil CbE activity, induced by earthworm activity, irreversibly binds OP pesticides, thereby acting as biomolecular scavengers. In addition, earthworm casting was postulated as a significant source of stable and active CbE activity to soil, preventing saturation of the stoichiometric detoxification mechanism by CbEs caused by an excess of pesticides (Sanchez-Hernandez et al., 2014a,b).

In a similar approach, the sensitivity of CbE in vermicompost derived from SCGs to the insecticide chlorpyrifos (CPO) and its metabolite chlorpyrifos-oxon (CPoxon) was assessed. To this end, wet solid (80% w/v, moisture) and liquid vermicomposts were individually spiked with a low (1 ppm) and a high (10 ppm) concentration of both OP pesticides ($n = 4$ replicates per treatment), and incubated for 30 days at 20°C in darkness. CbE activity was measured 4 and 30 days after pesticide treatment. In the case of liquid vermicompost, the enzyme activity was determined directly in the test tubes according to the protocol in Table 12.2. For solid vermicompost, however, a subsample (1.5 g) was removed from each treatment and homogenized (3% w/v) in distilled water before determination of CbEs.

The results of this toxicity assay show that the response of the enzyme activity to the pesticides strongly dependent on the OP type, time of exposure, and the initial state of vermicompost (solid or liquid). In the liquid vermicompost, CPoxon significantly inhibited CbE activity after 4 days of exposure ($H_{(4)} = 17.8$, $P = 0.0013$, Kruskal–Wallis test), and the response was dose dependent (Fig. 12.3A). Although the CbE activity decreased in response to the CPO treatment, it was not statistically different from control values. This was expected because oxygen-analog metabolites of phosphorothioate pesticides display a higher affinity for the active sites of CbEs than the parent compounds (Chambers et al., 2010). Surprisingly, CbE activity in the solid vermicompost was not equally affected by OP treatments in short-term exposure (Fig. 12.3B).

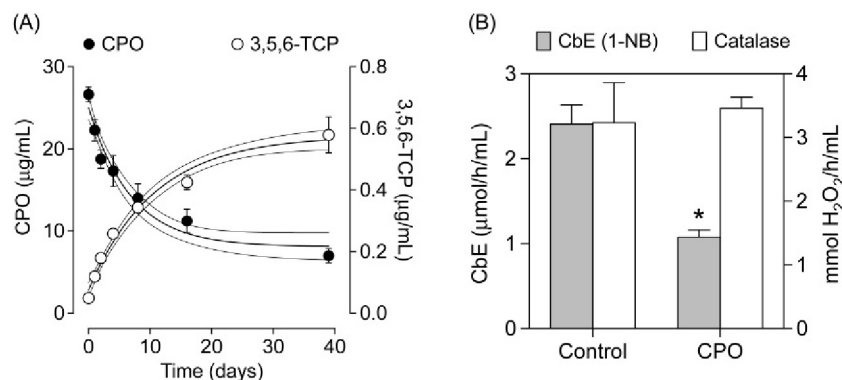
For longer exposure times ($t = 30$ days), the CbE activity recovered fully in the liquid vermicomposts treated with CPoxon. However, spontaneous reactivation of the phosphorylated enzyme activity should not be ruled out. To investigate the possible mechanisms responsible for this recovery of enzyme activity, the catalase activity was measured as an indicator of microbial activity. There was a significant increase of this intracellular enzyme activity in all treatments (controls and pesticide-spiked vermicomposts) from 1.67 ± 0.03 mmol O₂ consumed per hour per milliliter vermicompost (mean \pm standard deviation, $n = 20$) at $t = 4$ days to 4.82 ± 0.11 ($n = 20$) after 30 days of pesticide exposure. Therefore, enhanced microbial activity during the 30-day exposure time may contribute to reverse CbE activity in the liquid vermicomposts treated with CPoxon. Indeed, cometabolism of the pesticide by microorganisms may also contribute to restoring CbE activity. However, CbE activity was

**FIGURE 12.3**

Effect of chlorpyrifos (CPO) and chlorpyrifos-oxon (CPoxon) on carboxylesterase (CbE) activity in both (A) liquid and (B) solid spent coffee ground (SCG)-derived vermicomposts treated with 1 and 10 ppm of pesticides. Data are the mean and standard deviation ($n = 4$ replicates). Asterisk indicates significant differences compared with controls (Wilcoxon rank-sum test).

significantly inhibited in liquid vermicomposts treated with the highest concentration of CPO relative to the activity in control samples (Fig. 12.3A). One plausible explanation for this is that the esterase enzyme was inhibited by formation of CPoxon during degradation of CPO, as freshly secreted CbE from microbial proliferation was inhibited by CPoxon on formation. Further experiments must be conducted to test this hypothesis and to provide solid evidence for the actual role of CbE activity in liquid vermicompost as a detoxifying mechanism. Nonetheless, the results obtained so far indicate the following: (1) CbEs acted as molecular scavengers for OP pesticides, displaying higher affinity for the toxic metabolite CPoxon than for the parent compound (CPO), and (2) the liquid vermicompost provided a better substrate for this CbE-induced detoxification system, probably because of higher bioaccessibility of the pesticides to the active site of the enzyme than in solid vermicompost.

Degradation of CPO in liquid vermicompost was confirmed by incubation of liquid vermicompost made from SCGs ($n = 4$ replicates) in the presence of 25 $\mu\text{g}/\text{mL}$ CPO (20°C and dark). At this pesticide concentration, the degradation kinetics of the OP was monitored by liquid chromatography with diode-array detection

**FIGURE 12.4**

(A) Degradation kinetic of Chlorpyrifos (CPO) (25 $\mu\text{g/mL}$) in liquid vermicompost, and formation rate of the metabolite 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). (B) Carboxylesterase (CbE) and catalase activities in control (pesticide free) and CPO-treated liquid vermicomposts after 40 days of pesticide treatment. The esterase activity was measured using the substrate 1-naphthyl butyrate (1-NB) as summarized in Table 12.1. Data are the mean and standard deviation ($n = 4$ replicates). Asterisk indicates Wilcoxon rank-sum test.

(Sanchez-Hernandez et al., 2015). Degradation of CPO fitted a first-order kinetics model with a half-life ($t_{1/2}$) of 4.74 day^{-1} , which was concomitant with a progressive increase in the concentration of the metabolite 3,5,7-trichloro-2-pyridinol (Fig. 12.4A). Moreover, at the end of the degradation trial, CbE activity was more strongly inhibited (55.3% of controls) in the liquid vermicompost spiked with CPO than in the controls (pesticide-free vermicompost), whereas the catalase activity did not change. The mean ($\pm\text{SD}$) catalase activity of uncontaminated liquid vermicompost was $3.23 \pm 0.63 \text{ mmol O}_2$ consumed per hour per milliliter, whereas the mean activity of the enzyme was $3.46 \pm 0.17 \text{ mmol O}_2$ consumed per hour per milliliter in the CPO-spiked liquid vermicompost (Fig. 12.4B). The result of this assay confirmed that CbE activity in liquid vermicompost is involved in immobilizing CPoxon during degradation of CPO, reducing the potential toxicity of this metabolite to microorganisms (Singh and Walker, 2006).

Although it is still too early to draw firm conclusions, the data obtained in this study suggest that the role of CbEs as molecular scavengers of OP pesticides is highly dependent on the form of vermicompost that receives the pesticide. Homogenization of liquid vermicompost disperses extracellular enzymes and microorganisms in the aqueous medium and thus facilitates their contribution to pesticide degradation or transformation. In view of these findings, vermicompost could be potentially used for bioremediation of OP-contaminated soils following the conceptual scheme illustrated in Fig. 12.5. Binding of the pesticide to organic matter in vermicompost (step 1 in Fig. 12.5) represents the main route whereby vermicompost would reduce pesticide transport and toxicity in soil. Indeed, as discussed earlier, vermicomposts derived from SCGs and from grape marc provide a rich source of lignin that acts as an organic

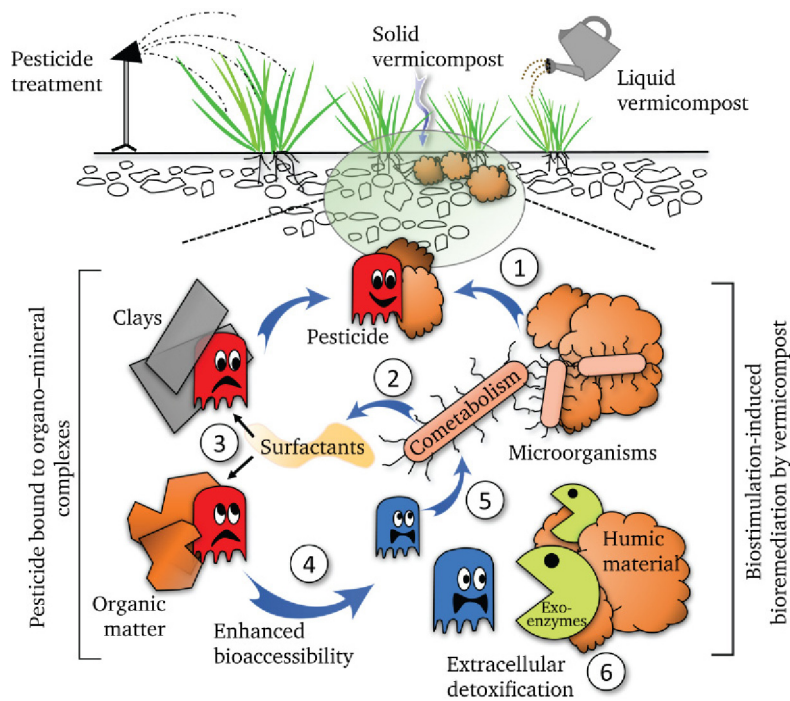


FIGURE 12.5 Conceptual Model of the Potential Role of Vermicompost (Liquid and Solid) on Pesticide Degradation and Transformation in Soil

ligand for pesticides. Furthermore, vermicompost can have a biostimulatory effect in soil when environmental conditions are favorable (step 2). The enhancement of microbial activity and biomass has two effects on bioavailability and biodegradation of pesticides. First, pesticides sorbed on clay and soil organic matter may be desorbed by the action of natural surfactants, such as humic acids and surfactants produced by microorganisms (e.g., glycolipids, phospholipids, lipopeptides, lipoproteins, and lipopolysaccharides) (Megharaj et al., 2011). As vermicompost is rich in humic substances and microorganisms, it is expected to exert a surfactant-like effect on sorbed pesticides to organo-mineral complexes of soil (step 3). Second, microbes may also directly attack sorbed pesticides through the catalytic action of exoenzymes (EE) that they release (Megharaj et al., 2011). Surfactants facilitate the bioavailability of pesticides for subsequent degradation by proliferating microorganisms (steps 4 and 5) and for transformation or breakdown by the action of extracellular detoxifying enzymes (CbEs, laccases, and peroxidases) associated with the organic matter in vermicompost (step 6). Pending further studies, the addition of liquid vermicompost to OP-contaminated soils or to agricultural soils that are periodically treated with OP will contribute to reducing the mobility and toxicity of these agrochemicals via the action of chemical (humic substances) and biochemical (e.g., CbEs) factors in the vermicompost.

12.4.3 POTENTIAL USE OF SCG-VERMICOMPOST FOR SAFE CULTIVATION IN URBAN AGRICULTURE

From an agronomic and environmental viewpoint, urban agriculture could benefit from SCG vermicompost. This kind of agriculture, defined as the production of crop and livestock within and around cities and towns, is a form of sustainable agriculture that has experienced an exponential growth worldwide in the last decade (Zezza and Tasciotti, 2010). Its popularity lies in its capacity to stimulate the local economy and social integration (Badami and Ramankutty, 2015), which is needed at a crucial time in human history: in 2007, for the first time, the world population became more urban than rural (Orsini et al., 2013). In this scenario, urban agriculture may be considered as a form of alleviating socioeconomic pressure caused mainly by two concomitant events: rural–urban migration, especially in developing countries, and the global financial crisis that initiated in 2007 (Hodson and Quaglia, 2009). Another bibliographic analysis of scientific studies involving the terms “urban agriculture” or “urban farming” shows an inflexion point in 2007 in the progress of urban agriculture studies since the 1980s (Fig. 12.6), which in fact coincides with these two aforementioned global events. These data could provide an idea of how important urban

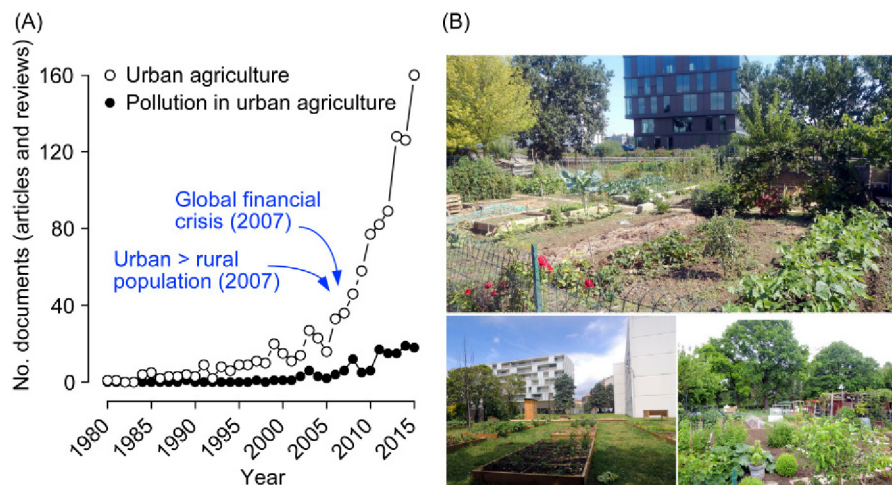


FIGURE 12.6

(A) Global scientific production (articles and reviews) dealing with urban agriculture and pollution. Arrows indicate two critical events in this century: the global financial crisis initiated in 2007 and, for the first time in human history, the urban population exceeding the rural, as of May 2007 (Orsini et al., 2013). Survey was performed using the bibliographic searching engine Scopus (October 2, 2016). (B) Photographs of several urban orchards in France.

Part B Photos by Camille Dumat, Available from: <http://reseau-agriville.com>

agriculture will be in cities around the world in the coming years, and how important the implementation of methodologies and monitoring programs for its sustainability and safety development will be.

One of the challenges of urban agriculture is the quality of urban soil to support safe crops. Urban soils are perhaps among the most contaminated soils in the world, and metals, such as Pb and Cd, as well as organic pollutants, such as PAHs and PCBs, are the most common environmental pollutants found in urban soils (Meuser, 2010). For example, a study performed in the city of Shanghai, China, that included the analysis of the 16 US-EPA priority PAHs in 57 soil samples collected in 2011, revealed concentrations of these aromatic hydrocarbons (Σ PAHs = 1970 $\mu\text{g/kg}$, mean value) that may pose a potential risk to human health. Similarly, a survey performed in east London, United Kingdom, in 2009 in which 76 soil samples were collected from an area of 19 km^2 (Vane et al., 2014) showed that total concentrations of the US-EPA PAHs were similar to other cities (Σ PAHs = 18 mg/kg , mean value), whereas concentrations of PCBs representing those congeners with a high chlorine content were higher (Σ PCBs = 120 $\mu\text{g/kg}$, mean value), which could suggest local sources for these PCBs, as opposed to a global atmospheric transport. Likewise, PAH concentrations were determined in soils collected from three European cities (Glasgow, Torino, and Ljubljana) during the spring of 2004 (Morillo et al., 2007), indicating that soils from Glasgow had the highest concentrations (1.48–51.8 mg/kg). In these latter two studies, a pyrogenic origin for PAHs was suggested, especially from motor vehicle exhaust. Moreover, climatic conditions and organic carbon content of soils were suggested as the main driving forces for PAH accumulation in soil.

Metal contamination is also very frequent in urban soils (Wortman and Lovell, 2013). High concentrations of Pb, for instance, are frequently detected in urban soils as a consequence of the legacy of Pb-based gasoline. Residues from traffic in cities (e.g., car tire and brake wear) and nearby industrial areas also contribute to increase metal concentrations in urban soils (Table 12.4). Accordingly, growing horticultural plants and fruits in urban environments has an elevated risk of metal accumulation from the soil (Antisari et al., 2015). However, metal pollution of the edible parts of vegetables occurs mainly by air deposition of metal-bound particles and simply washing the vegetables with water removes a significant fraction of metals (Nabulo et al., 2010; von Hoffen and Säumel, 2014). Nevertheless, although the high concentrations of metals in soil may pose a risk for plant uptake depending on plant species and soil physicochemical properties, it is important to consider that these pollutants may cause deterioration of soil quality, thus rendering the soil less productive from an agronomic viewpoint.

Vermicomposting provides a unique opportunity to revalorize SCGs in the cities that are, in turn, the main sources of this organic waste. Phosphate fertilization and compost amendment are two common practices in bioremediation of urban soils to reduce metal uptake by horticultural plants (Wortman and Lovell, 2013). Organic residues with a high organic matter content (>50%) and high concentrations of

Table 12.4 Mean and Range of Metal Concentrations (mg/kg) in Urban Soils From Several Cities Around the World

City/Country	Cd	Cu	Ni	Pb	Zn
New York (USA)	1.6	110	32	600	327
Bangkok (Thailand)	0.15	27	23	29	38
Hong Kong (China)	0.33	10	4	71	78
Berlin (Germany)	0.35	31	8	77	129
Oslo (Norway)	0.34	24	24	34	130
Connecticut (USA)	<0.5	40	12	176	163
Zagreb (Croatia)	0.5	18	49	23	70
Baltimore (USA)	0.56	17	2.8	100	92
Szczecin (Poland)	0.5–1.8	13.8–32.4	8.2–13.6	48–140	63–398
Sofia (Bulgaria)	0.11–0.33	32.6–65.7	—	30.9–34.3	37.9–81.0
Berger (Norway)	<0.1–1.5	4–2850	1–310	3–5780	8–998
Trondheim (Norway)	<0.1–11.3	1.7–706	6–231	9–976	7–3420
Seoul (South Korea)	1.0–4.4	11–471	—	93–1636	55–596
Beijing (China)	<0.01–0.97	2–282	2.8–169	5–117	22–400
Ljubljana (Slovenia)	—	14–135	14–45	10–387	56–581
Seville (Spain)	—	9–365	16–62	15–977	21–443
Torino (Italy)	—	15–430	77–830	14–1440	53–880

Data from Cheng, Z., Paltseva, A., Li, I., Morin, T., Huot, H., Egendorf, S., Su, Z., Yolanda, R., Singh, K., Lee, L., Grinshtein, M., Liu, Y., Green, K., Wai, W., Wazed, B., Shaw, R., 2015. Trace metal contamination in New York City garden soils. *Soil Sci.* 180, 167–174; Meuser, H., 2010. *Contaminated Urban Soils*. Springer, Dordrecht; Heidelberg; London (p. 318).

iron, phosphorus, and manganese are able to reduce Pb bioavailability (Wortman and Lovell, 2013). Hence, vermicompost derived from SCGs should be a suitable organic amendment because of the high concentrations of these elements and organic matter (Table 12.1). Additionally, vermicomposts are rich in humic substances, which favor binding of metals and organic pollutants. Another important issue of concern in urban agriculture is the high consumption of irrigation water, which can be aggravated in periods of water restriction in megacities. Vermicompost derived from SCGs has a very high water-holding capability that undoubtedly could alleviate water demand. Additionally, the innovative tendency of soilless cultures in urban agriculture to avoid the use of soil, obviously reducing the risk of plant contamination (Eigenbrod and Gruda, 2015), can benefit from liquid vermicompost, which is rich in nutrients and enzyme activities; these latter are essential for the mineralization of organic matter. Taken together, the physicochemical and biological properties of SCG-derived vermicompost make this revalorized product a promising strategic product for bioremediation (e.g., metal immobilization) and improvement (e.g., nutrient and extracellular enzyme addition) of urban agriculture soils, helping to reduce contamination of vegetable crops from these soils.

12.5 CONCLUSIONS

SCGs proved to be a suitable feedstock for vermicomposting, and a high density of earthworms was observed in the first few weeks of the decomposition process. Chemical and microbiological analyses revealed that mature and high-quality vermicomposts were obtained. Vermicomposting of SCGs is, therefore, a practical mean for the disposal of SCGs, yielding a value-added product with attractive properties for bioremediation purposes. In this way, highly polluted soils, such as those commonly used in urban agriculture could benefit from the physicochemical and biological properties of SCG-derived vermicompost. Measurement of selected extracellular enzyme activities involved in C-, N- S-, and P-cycling revealed higher activities of protease, urease, laccase, and CbE in vermicompost made from SCGs than in other types of vermicompost (e.g., vermicompost made from horse manure). The chemical composition of the primary feedstock influenced the levels of extracellular enzyme activity in vermicompost. Therefore, enzymatic fingerprinting can be considered for inclusion in the chemical and microbiological tests of vermicompost quality. Nevertheless, enzyme assays must first be standardized and accurate quality-control methods developed before biochemical fingerprinting can be used to define the quality of vermicompost.

The laccase and CbE activities were higher in the vermicompost made from SCGs than those from other vermicomposts produced from organic residues. This indicates the possibility of using this type of vermicompost in the enzymatic bioremediation of OP-contaminated soils. A survey of the scientific literature revealed that the use of vermicompost to decontaminate soil is relatively new and that further investigation must be conducted to reveal the real potential of the product in biostimulation-induced bioremediation. The CbE activity in vermicompost made from SCGs, particularly liquid vermicompost, was found to be sensitive to inhibition by CPoxon (toxic metabolite of CPO), leading to immobilization of this pesticide by irreversible inhibition of the enzyme activity. Although this detoxification mechanism is not catalytic, and is therefore susceptible to saturation due to phosphorylation of CbE activity, microbial proliferation may contribute to restoring the initial levels of extracellular CbE activity, thus contributing to the long-term maintenance of this enzymatic detoxification.

However, the toxicity assays were conducted with small sample sizes and the findings may not be transferable to field conditions. Thus, further research must be carried out to address some questions, such as the earthworm health during vermicomposting of SCGs, yields of vermicomposting of mixing feedstock rich in substrates that induce laccase and CbE activity, and the effect of application of liquid vermicompost on the biochemical performance of soil and its natural attenuation capacity.

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