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Evaluation of Different Types of Vermicompost by the Assessment of Microbial Enzymatic Activities

Diploma Thesis

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DECLARATION

I declare that the Diploma Thesis "Evaluation of Different Types of Vermicompost by the Assessment of Microbial Enzymatic Activities" is my own work and all the sources I cited in it are listed in Bibliography.

Prague, 9.4.2015 _____

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SUMMARY

The intensive agricultural and industrial activities produce a large amount of organic wastes which represent a serious environmental problem. Vermicomposting has been described as a suitable technology for the valorisation and stabilization of different types of organic waste. In this study was evaluated the feasibility of vermicomposting of horse manure (HM), apple pomace (AP), wine pomace (WP), digestate (D) and kitchen waste (KW) in a continuous-feeding system for 6 months. Five vermicomposters, one per each initial organic waste (HM, AP, WP, D and KW) were set up. The experiment was conducted in triplicate. Earthworms were inoculated in cow manure and fed every 2 weeks. Earthworm biomass and number were monitored every 2 months and variations in chemical parameters and microbial activities were evaluated as well.

The earthworm biomass was enhanced after 4 and 2 months in the case of HM and AP treatments, respectively. The pH was generally alkaline in all types of treatments, except of HM and D, in which was neutral. The EC ranged in values from 0.4 to 1.7 mS cm^{-1}. The transformation of organic matter result in a decrease in the TOC content in HM and AP treatments. On the contrary, total soluble C increased after 4 months in all vermicomposting treatments, meanwhile, the enhancement in soluble N content was recorded only in the case of KW. Increased values in N-NO_3^- was found in AP, D and KW treatments, whereas HM and D increased the content in N-NH_4^+. However, the content in both inorganic N forms were reduced in WP treatment. The assessment of dehydrogenase showed a low activity at the end of the process indicating the stabilization of the organic matter contained in each initial material. On the other hand, β-glucosidase activity was enhanced only in the case of WP vermicomposting treatment. Phosphatase was increased during the vermicomposting of HM, and it was found to be sensitive to changes in pH. Likewise, protease and *o*-diphenol oxidase showed higher activity during the vermicomposting of HM and WP, respectively.

Generally, the initial chemical composition found in different types of organic wastes determined its subsequent transformation, enzymatic activities as well as the chemical composition of final product (vermicompost). However, the vermicomposting of HM and AP in a continuous-feeding system were more efficient compared with other organic wastes. Nevertheless, new set of experiments is need in order to evaluate more specific parameters related to mortality of earthworms, toxicity of final vermicompost and its agronomical use.

Key words: earthworms, enzymatic activity, continuous-feeding vermicomposting system, organic wastes, vermicompost.

SOUHRN

Intenzivní zemědělské a průmyslové aktivity produkují velké množství organických odpadů, které představují vážný environmentální problém. Vhodnou technologií pro zhodnocení a stabilizaci různých druhů organických odpadů se jeví vermikompostování. V této studii byla zhodnocena proveditelnost vermikompostování koňského hnoje (KH), jablečných výlisků (JV), matoliny (M), digestátu (D) a kuchyňského odpadu (KO) v kontinuálním systému krmení po dobu šesti měsíců. Bylo založeno pět vermikompostérů, každý pro jednotlivý druh organického odpadu (KH, JV, M, D a KO). Experiment byl proveden ve třech opakováních. Do kravského hnoje byly naočkovány žížaly, jež byly krmeny každé dva týdny. Každé dva měsíce byla monitorována biomasa a počet žížal spolu se změnami chemických parametrů a mikrobiálních aktivit.

V případě varianty KH se zvýšila biomasa žížal po čtyřech měsících a u varianty JV po dvou měsících. Hodnota pH ve všech variantách byla obecně zásaditá, s výjimkou KH a D, ve kterých bylo pH neutrální. Elektrická vodivost se pohybovala v rozmezí od 0,4 do 1,7 mS cm^{-1}. Přeměna organické hmoty vedla k poklesu v obsahu celkového organického uhlíku ve variantách KH a JV. Ve všech variantách došlo naopak po čtyřech měsících ke zvýšení celkového rozpustného uhlíku, zatímco nárůst obsahu rozpustného dusíku byl zaznamenán jen v případě KO. Vyšší hodnoty $N\text{-}NO_3^-$ se vyskytovaly ve variantách JV, D a KO, kdežto vyšší obsah $N\text{-}NH_4^+$ ve variantách KH a D. Obsah obou anorganických forem dusíku byl zredukován ve variantě M. Stanovením dehydrogenázy byla zjištěna slabá aktivita na konci procesu, která poukazovala na stabilizaci organické hmoty obsažené v každém výchozím materiálu. Aktivita β-glukosidázy vykazovala zvýšení pouze v případě varianty M. U fosfatázy došlo ke zvýšení během vermikompostování KH a bylo zjištěno, že je citlivá ke změnám pH. Během vermikompostování KH prokázala vyšší aktivitu rovněž proteáza a *o*-difenol oxidáza při vermikompostování M.

Obecně lze konstatovat, že počáteční chemické složení zjištěné v různých druzích organického odpadu určovalo jejich následnou přeměnu, enzymatické aktivity stejně tak jako chemické složení výsledného produktu (vermikompostu). V porovnání s ostatními organickými odpady bylo v kontinuálním systému krmení efektivnější vermikompostování KH a JV. Ke zhodnocení specifičtějších parametrů souvisejících s úmrtností žížal, toxicitou výsledného vermikompostu a jeho zemědělského využití je nicméně nutná další série experimentů.

Klíčová slova: žížaly, enzymatická aktivita, vermikompostování s kontinuálním systémem krmení, organické odpady, vermikompost.

CONTENT

1. INTRODUCTION

Waste production from agro-industrial and urban activities represents a serious worldwide environmental problem. Million tons of organic wastes are generated every year in the European Union (EU). The Czech Republic as a member of the EU produces large amount of waste from agro-industrial practices. Some examples are those derived from traditional extraction of wine and apple juices production which generate a waste usually known as pomace. On the other hand, the production of biogas has become as one of the most important resource of energy production in Czech Republic, but this activity also produce a large quantity of a waste known as digestate. The excessive production of horse manure has largely used as a soil fertilizer. However, it is necessary its previous stabilization since its direct application causes detrimental impact on soil properties. Some urban activities generate a large amount of kitchen waste which is being increasing during last years. The disposal of this residue in landfills represents a serious environmental problem due to water contamination and methane production. This huge quantity of wastes could be converted into a nutrient enriched bio-fertilizer and used for agricultural purposes or land restoration.

One of the most promising techniques for valorisation of residues is a vermicomposting. It is biological process involving the bio-stabilization of organic wastes by the joint action of earthworms and microorganisms. There are different techniques to perform vermicompost by a non-continuous and continuous feeding system. In the traditional non-continuous system earthworms are inoculated to organic waste and the process is carried out until all the material is transformed into the vermicompost. Meanwhile, the continuous feeding system differs respect to traditional system in the partially transformation of the material by adding new fresh feedstock to earthworms weekly. To this date many reports has analysed the feasibility of vermicomposting by a non-continuous feeding system, but not many in a continuous feeding system.

Enzyme activities have been used as indicators of the time course of organic matter in vermicomposting systems. These activities have been widely used in non-continuous vermicomposting systems. According to these statements we proposed as main objective of this master thesis to study the feasibility of a continuous feeding vermicomposting system to stabilize the organic matter in different kind of wastes (apple pomace, wine pomace, digestate, horse manure and kitchen wastes). To achieve this objective we will evaluate the quality of these wastes by the assessment of microbial enzymatic activities during the vermicomposting process.

2. SCIENTIFIC HYPOTHESIS AND OBJECTIVES OF WORK

The objective of the work is the stabilization different organic wastes from agro-industrial and urban activities in continuous-feeding vermicomposting systems by the assessment of enzymatic microbial activities and other chemical parameters as suitable tools for monitoring this processes.

The main hypotheses of the work are:

- The continuous-feeding vermicomposting system may be a suitable technology to valorise different organic wastes from agro-industrial and urban activities.
- Changes on chemical variables such as pH, EC, total organic carbon (TOC), total soluble carbon (C), total soluble nitrogen (N), and inorganic N forms ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) will be expected and will be different according to the type of organic waste used and at different time of collection during the vermicomposting processes.
- The quantification of enzymatic activities may be considered as good, sensitive and reliable bio-indicators of metabolic activity of microbial communities present in organic wastes. Alterations on their activity during vermicomposting process will be useful indicators of organic matter stabilization in each waste. Differences in the microbial activity will be also expected according to the type of organic waste used and at different time of collection during the vermicomposting processes

3. LITERATURE OVERVIEW

3.1. Waste

The EU defines a waste as "any substance or object which the holder discards or intends or is required to discard" according to the Waste Framework Directive (Commission, 2008).

In the Basel convention, wastes were defined as "substances or objects which are disposed of or are intended to be disposed of or are required to be disposed of by the provisions of national law" (UNEP, 2014).

The United Nations Statistics Division defines waste in Glossary of Environment Statistics as "materials that are not prime products (that is, products produced for the market) for which the generator has no further use in terms of his/her own purposes of production, transformation or consumption, and of which he/she wants to dispose. Wastes may be generated during the extraction of raw materials, the processing of raw materials into intermediate and final products, the consumption of final products, and other human activities. Residuals recycled or reused at the place of generation are excluded" (United Nations, 1997). The economic value use of wastes usually is relatively low. It is not produced for any particular use or it cannot be use anymore for original reason it was created. The material can contain hazardous and/or pollutants substances originated from production process. The management of the wastes can represent a serious risk for the environment due to the complexity and problematic of their disposal. In general the wastes can be classified as: agricultural, forestry, industrial and urban residues (GOV UK, 2012). In this bibliographic overview, we will focus on agro-industrial and urban wastes.

3.1.1. Agro-industrial waste

Agro-industrial waste is mainly produced from processing of agricultural or animal products. Those derived from agricultural activities include material such as straw, stem, stalk, leaves, husk, shell, peel, lint, pulp, seeds/stones, stubble from fruits, legumes or cereals (rice, wheat, corn, sorghum, barley…), bagasses generated from sugarcane or sweet sorghum milling, spent coffee grounds, brewer's spent grains, winery and juices production, and many others (Vandamme, 2009).

Their composition varies widely, thus they can be divided primarily by moisture content on dry and wet wastes. Dry wastes or residues can be consider those which derived from fields activities and seed crops production such as rice, beans, wheat, corn; fruit and nut crops

production like apples, grapes, lemons; vegetable crops production like tomatoes, cucumbers, melons and nursery crops production, in this case, there are many species of flowers and indoor plants i.e. orchids but also fruit trees. The waste generates mainly derived from their elaboration and preparation for market sales and/or during prunnings. Contrary, the wet wastes or residues are those which contains animal slurry, farmyard manure and grass silage (Nigam et al., 2009).

The production of agro-industrial wastes represents a serious environmental problem which management causes economically problems to companies. These wastes are generated in a large quantity throughout the year, in total accounting for over 250 million tonnes. The safe disposal and /or re-use of them has become in a high-cost and long-time consuming process for companies. The inappropriate waste management leads to pollution of soil, water, air and loss of valuable compounds and materials (Panesar et al., 2015). The residues are usually rich in compounds such as sugars, fibres, proteins, and minerals, which are compounds of industrial interest. Especially processes of fermentation can convert these wastes into a widely variety of valuable chemical products, including biofuels (bioethanol) and organic solvents such butanol. Nevertheless, some of these organic wastes due to its high content in organic matter are suitable to be transformed by the joint action of earthworms and microorganism in a process known as vermicomposting in order to obtain product with an agronomical value (organic amendments) (Nigam et al., 2009).

For the experiment, different types of agro-industrial wastes were chosen, such as horse manure, apple pomace, wine pomace and digestate. They are briefly described below.

3.1.1.1. Horse manure

The animal manure is an organic material consists of faeces, bedding and food remains which are removed from animal stalls. The composition of manure depends on many factors such as food composition, type of stabling, material of bedding, animal species and its age. The maturity of the manure depends on the duration and quality conditions during its storage. The storage quality can be also influenced by the amount of organic substances and nutrients, especially nitrogen contained in animal manure. The highest intensity of decomposition is greatly stimulated by air contact during the storage process, but sometimes, it can cause a great loss of organic substances present in the manure between 50 – 60 %. Likewise, the content in N can be decreased during maturation processes in a range of 30 – 40 % (Vaněk et al., 2012).

In the case of horse manure, it is generated up to 25 kg of wet waste per day (faeces and urine), since the horse usually defecate between 4 - 13 times per day , especially in those cases in which horses are kept in stall because the bedding material is up to 10 kg per day

(Westendorf and Krogmann, 2004). Therefore, the main predominant component in the composition of horse manure is bedding (Mönch-Tegeder et al., 2014). On the other hand, horse manure is characterized to be a solid material and it mainly composed by 60% and 40% of solids and liquids, respectively (Wheeler and Zajaczkowski, 2002). In addition, it has been described that horse manure is rich in nutrients such as nitrogen (0.65%), phosphorus (0.13%) and potassium (0.52%), and it also contains a high amount in organic matter (20%) (Vaněk et al., 2012). Therefore, the chemical composition of horse manure determines its suitable application as organic fertilizer. However, if horse manure is applied in higher doses can have a detrimental impact on soil reducing the availability of nutrients and water to be taken up by plants (Westendorf and Krogmann, 2004).

3.1.1.2. Apple pomace

The solid waste generated from apple juice production is usually known as apple pomace. Most of the apple production which not have suitable characteristic for human consumption is usually used for juice production, cider, pulp and jelly. The pomace represents approximately 30 % of the original fruit and it is a mixture of core, seed, peel, calyx, stem and soft tissue. The composition can vary according to the type of apple and its processing but in general, the major part is represented by water, carbohydrates such as glucose, fructose and sucrose, minerals, vitamins, some polyphenols compounds and low content of protein (Vendruscolo et al., 2008). Apple pomace is often used to produce pectin because wet residues from apples contains up to 15 % of pectin on the dry weight basis (Endreß, 2000). It has been described that the presence of pectin at this concentration has higher gelling properties compared with pectin from citrus (Schieber et al., 2003).

In last years, the growing demand for the consumption of healthy products, like fresh juices has generated a large amount of pomace which disposal represent a problem, primarily due to tendency to microbial spoilage reducing its further use. Moreover, apple pomace contains a 80 % of moisture, approximately, which determinate high-cost processes for its drying, storage and shipment (Robinson and Nigam, 2003). Nevertheless, apple pomace provides a good source of digestible fibre, and thus is commonly used as a feed for livestock (Mamma et al., 2009).

3.1.1.3. Wine pomace

Wine pomace is a solid residue derived from wine production, wine juice, raisins and jams. The major part of grapes production, around 80 %, approximately, is used for wine production.

The wine pomace is mainly composed by 50% skins, 25% seeds and 25% stalks and it is a lignocellulose residue composed by different polymers at different proportion as follow: cellulose (50 %), hemicellulose (35 %) and lignin (15 %). However, in its chemicals composition can be found other compounds such as alcohols, acids, aldehydes, esters, pectins, polyphenols, mineral substances and sugars (Ruberto et al., 2008).

The main problem associated to wine production is the generation of large amount of waste during the harvest period (August - October), in which 10 million of grape pomace tons, approximately, are produced in a few weeks during the harvest (Bustamante et al., 2008).

Wine pomace is normally prepared as a feed for livestock, but due to its low content in nutrient up to 30 % from total amount of food, it cannot be used for ruminants. In addition, a great range of products with a high industrial value are obtained from grape pomace such as ethanol, tartrates, citric acid, grape seed oil, hydrocolloids, and dietary fibre (Schieber et al., 2001).

3.1.1.4. Digestate

The biogas production is an emergency renewable energy source. The biogas production is being increasing during last decade by the implementation of plant production (Yadvikaa et al., 2004). This process is carried out by the anaerobically transformation of organic matter to produce biogas (methane and carbon dioxide) and a residue commonly known as digestate which can be used as a fertilizer (Lukehurst et al., 2010). The properties of digestate are influenced by the anaerobic process and also by the substrate used for the process (Koblenz et al., 2015). The waste of anaerobic digestion (digestate) has high content in organic matter and nutrients such as nitrogen which gives digestate a great agronomic value as a fertiliser.

However, it has been identified some pathogenic microorganism in the chemical composition of digestate as consequence of inappropriate thermophilic conditions which represent a serious risk (Bustamante et al., 2012). Moreover, digestate can contain phytotoxic volatile fatty acids and heavy metals (Zhu et al., 2014). The presence of pathogenic microorganism and/or toxic compounds after anaerobic digestion determinate a previous process of stabilization of this waste before of its application with agronomical purposes. The most suitable method is to separate the solid from liquid fraction and subsequently the solid fraction will be composted. The content of dry matter in solid fraction commonly is between 25 - 35 % (Holm-Nielsen et al., 2009).

3.1.2. Urban solid waste

Urban solid waste is usually generated from human, commercial, municipal activities, small industries, public areas waste water treatment plants (see **Table 1**) (Zhou et al., 2015). The physico-chemical characteristics of urban waste are modified by lifestyle, cultural, traditional conditions, economical status, literacy rates, dietary habits, climate and geographic location (Singha et al., 2011). Generally, urban solid waste contains recyclables materials such as paper, plastic, glass, and metals; toxic substances like paints, pesticides, used batteries; and compostable organic matter such as rest of fruit and vegetable skins and pulps (Singha et al., 2011).

Nowadays, the high increase in the amount of urban waste produced is directly proportional to the population growth, thus the techniques available to manage these wastes , such as landfilling, thermal conversions (incineration, pyrolysis, gasification), bio-chemical conversions (composting, vermicomposting, anaerobic digestion) and chemical conversions (trans-esterification and other processes to convert plant and vegetable oils to biodiesel) are increasing in a proportional ratio (Singh et al., 2011). Currently, most of the urban wastes are disposed in landfills and/or open dumps, especially in the developed countries. The landfilling requires a huge portion of land mass and usually has a negative impact on the environment. In addition, the presence of toxic substances and heavy metals in this urban waste limits its further re-use and/or disposal. On the other hand, urban wastes have high content in organic matter and significant amount of recyclable plant nutrients. Therefore they can be applied to soil, but only with a previous stabilization in order to eliminate the toxic substances. Vermicomposting may be suitable for processing of urban wastes as well as a good option for the waste management (Singha et al., 2011).

For our work, kitchen waste was chosen as a representative of urban wastes. Detailed information can be found bellow.

	Locations and activities in which wastes are generated	Types of waste
Residential	Apartments, single-family and multi-family homes	Food wastes, rubbish, paper waste, ashes, special wastes
Commercial, institutional	Restaurants, markets, office buildings, hotels, shopping malls, schools, print shops, auto repair shops, prisons	Food wastes, rubbish, ashes, demolition and construction wastes, special wastes, hazardous wastes
Public areas	Parks, streets, alleys, vacant lots, playgrounds, beaches, highways, recreational areas	Street sweepings, roadside litter, rubbish, etc.
Treatment plant sizes	Water, sewage and industrial waste water treatment processes	Treatment plant sludges

Table 1 General classification of urban solid waste, Singha et al. (2011).

3.1.2.1. Kitchen waste

Kitchen waste is an organic waste mainly generates from urban activities. Food is usually discarded because it has expired date, spoiled, oversupplied and/or due to individual eating habits (FAO, 2013). The composition of kitchen waste can significantly vary according to different eating habits and feedstock used. Generally, it consists of carbohydrate polymers (starch, cellulose, and hemicellulose), lignin, other organics compound such as proteins, lipids, and acids and a small inorganic portion know as ashes. The main characteristic is its high content in water, up to 80 %, as previously has been reported by Zhang et al. (2014). Due to its high moisture, kitchen waste can be easily degraded. In addition, the kitchen waste is known to contain hazardous persistent pollutants such as heavy metals which are detrimental for the sustainability of the ecosystems (Pattnaik and Reddy, 2011).

Traditionally kitchen waste is managed by environmentally and non-eco-friendly techniques such as landfilling and incineration. Decomposition of kitchen waste on landfills produces a high emission of methane and carbon dioxide, which has a negative impact on the environment increasing the climate change. In addition, during the degradation also leachates are generated, which may be toxic as previously has been shown by Wang et al. (1997). The disposal by incineration is a high-cost technique as consequence of high moisture content and discharge of harmful gas and toxic ashes (Lee et al., 2004). The stabilization of kitchen waste using aerobic composting systems represent a suitable technique for transformation of organic matter and reduce the heavy metals contained in kitchen waste (Zhang et al., 2014).

3.2. Vermicomposting

Vermicomposting is defined as a bioxidative process in which epigenic species of earthworms interacts with microorganisms and other fauna within decomposer community accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties to convert into a valuable material, vermicompost, which can be used as a soil organic amendment (Edwards, 2011). Vermicomposting is reported to be a low-cost technology and its end product is characterized by high level of microorganisms, enzymes and humus-like substances with low C:N ratio (Fernández-Gómez et al., 2012). The biochemical decomposition of the organic matter during vermicomposting process is primary accomplished by microorganisms, but earthworms are crucial drivers of the process since they may affect the microbial decomposer activity by grazing directly on microorganisms. Moreover, the presence of earthworms increases highly the surface area available for microbial attack after the comminution of organic matter by earthworms (Gómez-Brandón et al., 2011). All these activities enhance the turnover rate and the productivity of microbial communities, thereby increasing the rate of decomposition. Vermicompost, the end product of vermicomposting, is characterized by its high content in nutrients such as NH_4^+, NO_3^-, P, K, Ca, and Mg as well as plant growth hormones and regulators. This effect is as consequence of higher rates of mineralization which occurs during vermicomposting process in the organic matter rich in earthworms cast. Moreover, vermicompost present higher porosity and holding water capacity as consequence of the material finely divided which determinate the easily availability of nutrients to be taken up by plants. In recent years, vermicomposting has progress considerably due to the large amount of wastes generated by different activities. It was reported a huge variety of residues susceptible to be vermicomposted such as sewage sludge, paper industry waste, urban residues, food and animal waste as well as horticultural waste from plants (Domínguez and Gómez-Brandón, 2012).

3.3. Earthworms

3.3.1. Body structure

Earthworms are macroscopic clitellate oligochaete annelids that live in soil (Gajalakshmi and Abbasi, 2004). They are segmented worms, bilaterally symmetrical, with an external gland commonly known as clitellum for producing the egg case - cocoon (see **Fig. 1**). The clitellum secretes the fibrous cocoon and the clitellar gland cells produce a nutritive albuminous fluid that fills the cocoon. The clitellum and its position can differ widely among different species (Domínguez and Edwards, 2011a).

On every segment, except of the first segment, earthworms have bristles - setae - which are used to anchor parts of the animal body during the movement and allow earthworms to fix in the burrows when they are attacked by a predator. The body wall consist of a thin outer cuticle, epidermis, it prevents to loss water from earthworm body (Edwards and Bohlen, 1996). Goblet cells are located in this layer, which produce mucus that covers earthworm body. Under epidermis, layer of nervous tissue including great amount of sensory cells can be found, the cells are responsible for stimuli such as heat, touch and light. The epidermis and nervous tissue are connected by basal membrane. In addition, inside of the membrane are two muscle layer: the first is circular and the second longitudinal. Finally, a layer of coelomic epithelial cells known as - peritoneum - divides the body wall from the body cavity (Nancarrow and Taylor, 1998).

Moreover, earthworms do not have lungs (except of some aquatic species), they bring the oxygen to their body by dissolving through the body surface, the moisture is kept by cover from mucus (Nancarrow and Taylor, 1998).

Earthworms are hermaphrodites animals and reproduction normally occurs through copulation and cross-fertilization, flowing which each of the mated individuals produces cocoons which containing 1-20 fertilized ova. The resistant cocoons are usually deposited near the soil surface and hatch after incubation period according to environmental conditions. Under favourable conditions earthworms will reach sexual maturity within several weeks after emergence (Gajalakshmi and Abbasi, 2004; Domínguez and Edwards, 2011a).

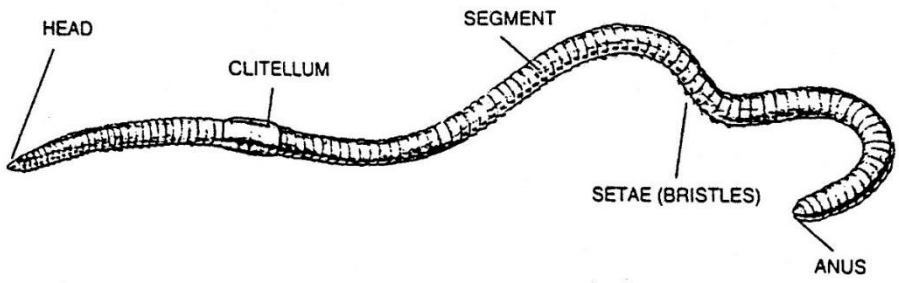

Fig. 1 Structure of earthworm body, available online from: http://g3animals.wikispaces.com/Worm%20Facts

3.3.2. Classification

Earthworms are invertebrates which belong to the phylum of *Annelida* and class *Oligochaeta* (Gajalakshmi and Abbasi, 2004). Earthworms represent the major animal biomass in most terrestrial ecosystems (Domínguez and Gómez-Brandón, 2012). In fact, more than 8,300 species have been described and classified on the basis of their feeding and burrowing strategies into three categories: epigeic, endogeic and anecic (Pižl, 2002; Domínguez and Gómez-Brandón, 2012). Epigeic species, litter dwellers and transformers, live in organic horizon and ingest primarily fresh organic substrate. Endogeic species live (soil feeders) in the upper layer of soil, feed on high amounts of the mineral soil and create horizontal burrows. Finally, anecic species (burrowers) live in greater depth, ingest medium amounts of soil and built vertical burrows (Domínguez and Gómez-Brandón, 2012).

3.3.3. Species suitable for vermicomposting

The main characteristic for selecting suitable earthworms for vermicomposting process are based on their ability to colonize organic wastes, high rates of consumption, digestion and assimilation of organic matter, short life-cycles, resistance to a wide range of environmental factors, high-reproductive rates and endurance and tolerance of handling (Gajalakshmi and Abbasi, 2004). Epigeic species has been described to show potential for vermicomposting. It has been only found few earthworms species which display all these characteristics and only five have been extensively used in vermicomposting such as: *Eisenia andrei, Eisenia fetida, Dendrobaena veneta,* and in a lesser extend *Perionyx excavatus, Eudrilus eugeniae* (Domínguez and Edwards, 2011a).

E. andrei and *E. fetida* species are common earthworms used for vermicomposting because they are ubiquitous with a worldwide distribution and colonize organic substrates, their life cycles are short, they have a wide temperature and moisture tolerance range and they can be

readily handled. *E. fetida* corresponds to the tripped or banded morph with the area round the intersegmental groove having no pigmentation and appearing pale or yellow, thereby its common name of “tiger”. Meanwhile, *E. andrei* the common "red" worm corresponds to the uniformly reddish morph. Apart from their differences in pigmentation, the two species are morphologically similar and requirements the same. However, *E. andrei* shows higher growth rate and reproduction compared with *E. fetida*. The similarities found between *E. andrei* and *E. fetida* have provoked that their taxonomical classification is still unresolved, and moreover in much of the current literature both species are termed indiscriminately as *E. andrei* or *E. fetida*. Nevertheless, studies performed by Domínguez et al. (2005), Pérez-Losada et al. (2005) have confirmed that both species are two different phylogenetic species (Domínguez and Edwards, 2011b).

The optimal range of temperature and moisture for both species is 25°C and 85 %, respectively. The viability of the cocoons and viable individuals produced by *E. andrei* and *E. fetida* is up to 82 % and 3.8 %, respectively. Earthworm life cycles are comprised from 45 to 51 days (from cocoon to adult earthworm) and the length of their life is maximally 5 years, but under natural conditions may be short (Domínguez and Edwards, 2011a).

3.4. Vermicomposting food web

During the vermicomposting process a large number of microorganisms and invertebrates can be found as results of the recycling of organic matter and release of nutrients, thus vermicomposting systems sustain a complex microbial and invertebrate food web. The decomposers microorganisms like bacteria and fungi interact with the soil fauna in the vermicomposting by interactions which include competition, predation and mutualism (Sampedro and Domínguez, 2008). The most numerous and diverse member of this food web are microorganisms, especially bacteria, fungi and ciliates. However there also abundant protozoa and many invertebrates of varying sizes, including nematodes, micro-arthropods and large populations of earthworms (Sampedro and Domínguez, 2008). Microorganisms are the primary consumers of the vermicomposting food web, which are able to degrade and transform the organic material. Soil invertebrates are considered as the secondary consumers including earthworms, which exist with microorganism feeding on and dispersing them throughout the organic matter. The organic material passes through the gizzard of the earthworm it is finely ground prior to digestion. The enzymatic extracellular activity of the endo-symbiotic microbes degrades the material. These enzymes are able to degrade cellulose and phenolic compounds, thus increasing the degradation of the ingested material which is released from earthworm's body in form of casts. During the degradation of organic wastes increase the burrowing and tunnelling activities by earthworms which contribute to aerate the substrate and enable water nutrients, and thus, stimulate the activity of microorganisms. After earthworm dies, new food is available to be degraded by decomposers microorganisms. Earthworms are crucial drivers in vermicomposting system due to accelerate the decomposition processes, but it is unknown how they obtain their input energy whether from decaying organic matter or microorganisms, micro-fauna or from combination of all. Earthworms are able to use a huge range of substrate for feeding to grazing and moreover, they have the ability to shift between living and non-living carbon sources (Domínguez, 2011).

3.5. The progress of vermicomposting process

In general the vermicomposting process can involve two main phases: (i) an active phase in which earthworms are transforming and modifying the organic substrate, thereby changing the state and microbial composition of the initial material (Lores et al., 2006); and (ii) a maturation phase which is characterized by the displacement of earthworms towards to the layer of fresh material (undigested material), in which microorganisms take over the decomposition of processed substrate by earthworms. The duration of maturation phase is not fixed, and it is usually determined by the species and density of earthworms as well as the portion in which the residue is applied (Domínguez and Gómez-Brandón, 2012).

In the active phase, the impact of earthworms on the decomposition of the organic matter during the vermicomposting process is mainly due to *gut associated processes* (GAPs) such as ingestion, digestion and assimilation of the organic matter and microorganisms in the earthworms gut, and then casting (**Fig. 2**). Specific microbial communities respond diversely to the gut environment, and selective effects on the presence and abundance of microorganisms during the passage of organic material through the gut of these earthworm species have been observed (Monroy et al., 2009). For instance, some microorganisms can be digested in the intestinal tract; some can be remained unaffected, whereas others can be activated during the passage through the intestinal tract. Such selective effects on microorganisms as a result of gut transit can modify the decomposition pathways during the vermicomposting process. It may be due to modifications in the composition of microbial communities involved in the decomposition processes, as microorganisms from gut which are released with the faecal material and then continue the decomposition of egested organic matter. Consequently, earthworm cats contain different microorganisms in comparison with those presented in initial material (Domínguez and Edwards, 2011b). It is well-known that the inoculum of these microorganism communities in fresh organic matter promotes alterations similar to those detected when earthworms are present modifying microbial community levels of activity and altering the functional diversity of microbial populations in vermicomposting process (Domínguez and Gómez-Brandón, 2012). GAPs contain all changes that microorganisms and organic matter undergo when passing through earthworm intestinal tract, such as modification of the microbial diversity and activity, modification of the microfaunal populations, homogenization, the intrinsic processes of digestion, assimilation, production of mucus and excretory substances such as sugars, urea, and NH_4^+, which are readily available nutrients sources for microorganisms (Domínguez and Edwards, 2011b).

Casts are the result and final product of GAPs. With production of casts is GAPs completed and casts undergo *cast-associated processes* (CAPs). CAPs are more related with ageing of process, modification of excreted material, the impact of earthworms are primarily indirect and associated with previous GAPs (Domínguez and Edwards, 2011b). During the maturation process vermicompost is expected to reach its optimum represented promoting of plant growth and suppression of plant diseases. Currently, there is not enough knowledge to determine when the vermicompost achieves optimum and how it can be determined (Domínguez, 2011).

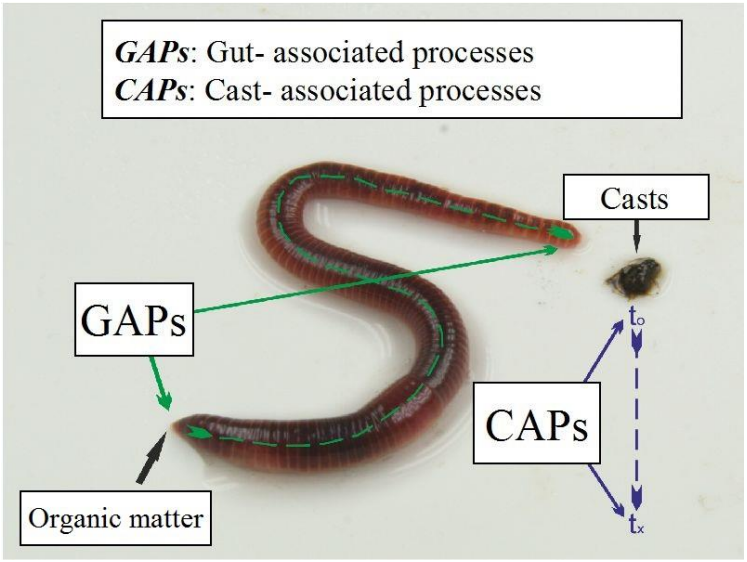

Fig. 2 Earthworms influence the degradation of organic matter within vermicomposting process through ingestion, digestion and casting - gut associated processes. Cast associated processes are related with ageing processes (Domínguez and Gómez-Brandón, 2012), available online: http://www.intechopen.com/books/biomass-now-cultivation-and-utilization/animal-manures-recycling-and-management-technologies

3.6. Microbial communities during vermicomposting

Microorganisms are the main agents of biochemical decomposition, whereby earthworms are involved in the indirect stimulation of microbial populations through organic matter fragmentation increasing the surface area available for microorganism. Earthworms also modify the microbial populations through digestion, stimulation, and dispersion in cast. Therefore, it is necessary to establish the effects of earthworms on microorganism, because whether the earthworms stimulate or depress the microbiota, or modify the structure and function of microbial communities, they can have different effects on the rates and form of organic matter decomposition. The first studies performed on analysis of microbial communities in vermicompost have observed an enhancement in the microbial biomass immediately after the waste pass through the earthworm intestinal tract. However, it was found a decrease in the microbial biomass present in the earthworm cats (Domínguez, 2011). The activity of epigeic earthworm drastically reduces the viable microbial biomass during the vermicomposting process, and this reduction is proportionally higher for fungi than for bacteria, possibly because earthworms may use fungi for food selectively. Likewise, the presence of earthworms not only affects to the microbial biomass since the microbial structure and diversity can be seriously altered. According to studies performed by Lores et al. (2006) the initial composition of microbial communities present in the organic waste can be modified by the earthworm action during vermicomposting process, but it can be associated to autochthonous microbial communities in the organic waste and it depend on the earthworm specie used for vermicomposting. Definitely, earthworm activity helps microbial communities use the available energy more efficiently and plays a key role in shaping the structure of the microbial communities in organic wastes during vermicomposting processes (Domínguez, 2011).

3.7. Factors influencing vermicomposting process

Epigeic earthworms are tolerant to wide range of environmental factors, but they also have specific requirements on the basis of, moisture and temperature range. If the conditions are optimal, earthworms are able to decompose satisfactory the organic waste and reproduce rapidly. On the contrary, if the conditions are non-well-appropriate, earthworms usually migrate to those parts of the organic waste in which the conditions are more suitable, and even they can die, in this case vermicomposting process is carried out very slowly (Domínguez and Edwards, 2011a). The factors are summarized in **Table 2**.

3.7.1. Temperature

Temperature represents one of the most important parameters for the life of earthworms, because influences their reproduction, growth and metabolism. Most of the earthworm species have a range of temperature between 10-35°C. According to a study performed by Edwards (1998) the optimal range of temperature for *E. fetida* was observed at 25°C. However, if this range reach values up to 35°C, the metabolic activity decrease and the earthworm death can occur (Munroe, 2007). Under extreme temperature conditions, earthworms are moved to deeper layers and a hibernation process is started. Lower ranges of temperature between 4 – 10 °C reduces feeding and reproduction activities. During colder seasons such as autumn or winter, they can acclimate but they cannot survive for a long period of time under freezing range of temperature (Domínguez and Edwards, 2011a). The maintenance of a constant range of temperature during vermicomposting system play a key role for successful process. It can be archieved by adding thin layer of fresh material on the surface, thus the material does not overheating (Garg and Gupta, 2009).

3.7.2. Moisture

Moisture content is the second most important characteristic for survival of earthworms. The most rapid growth of *E. fetida* and *E. andrei* was reported in range between 80 - 90% of water content in organic wastes (Edwads, 1988). The moisture content depends on many factors such as vermicomposting system used, waste physical condition and porosity. Higher moisture conditions in organic waste can become in an anaerobic situation responsible of unpleasant smells. Meanwhile, lower content in water can produce the earthworm death. To create optimal conditions of moisture a periodic sprinkling of water is need during the vermicomposting process (Garg and Gupta, 2009).

3.7.3. Aeration

Earthworms are highly sensitive to low oxygen conditions, in fact it has been observed that anaerobic conditions by high level of water content or fatty substances can endanger the life of earthworms. The regularly aeration by manually mixing and turning of the organic waste contribute to create better oxygen conditions (Garg and Gupta, 2009; Domínguez and Edwards, 2011a).

3.7.4. pH

The optimal range of pH for earthworms is found between 7.5 – 8, but in some cases they are able to survive in ranges of pH between 5 – 9 (Edwards, 1998). The range of pH can considerably change during vermicomposting process; at the beginning is lower due to production of CO_2 and organic acids, but during the process, these values increase as consequence of proteins decomposition and NH_4^+ production (Garg and Gupta, 2009).

3.7.5. C:N ratio

The C:N ratio influences the decomposition of organic waste during the vermicomposting process. The optimal range for C:N ratio is found between 25 - 30 which determinate the easily stabilization of organic matter according to Pramanik et al. (2007). However, excessive content in nitrogen can produce the loss of N as consequence of volatilization processes by the quickly decomposition of the organic waste. Meanwhile, higher C concentration reduces the microbial activity quickly (Edwards et al., 2011). Organic wastes with a C:N ratio higher than 40:1 indicate slowly processes of organic matter decomposition, thus an extra-exogenous N source need to be added. The ratio C:N change during vermicomposting process since loss of C in the form of CO_2 are expected as consequence of metabolism of microorganism and earthworms which determinate an enhancement in C:N ratio (Garg and Gupta, 2009).

3.7.6. Initial feed material

The organic waste used for vermicomposting may have some specific requirements. In the first place, the physical characteristics are important in order to favour the movement of earthworms as well as supplies O_2 in the material. Therefore, organic wastes with high porosity are optimal material to be vermicomposted. Moreover, it is necessary to avoid excessive compaction of substrate and maintain an equilibrate balance between the surface area and volume. The organic waste with poor structure or high water content (i.e. sludge, food waste)

may be mixed with bulking agents (i.e. wood chips, dry manure) to enhance porosity and provide favourable conditions of moisture (Garg and Gupta, 2009). On the second place, the composition of organic wastes influences significantly the quality of the end product, vermicompost. The initial material may contains toxic or non-degradable substances (i.e. glass, metal, plastic, detergents) which represents a risk for earthworms, especially whether the purpose of the vermicomposting is the production of soil organic amendments for agricultural purposes (Garg and Gupta, 2009). In the third place, earthworms are not able to tolerate high levels of salts or NH_4^+ in organic wastes. The optimal range in NH_4^+ and salts is up to 1 $mg.g^{-1}$ and 0.5%, respectively. The high levels in salts can be reduced by adding water and/or by a pre-composting process (Domínguez and Edwards, 2011a).

3.7.7. Light

Earthworms are photo-sensible; the light is detected through photoreceptor cells distributed on their surface (Gajalakshmi and Abbasi, 2004). One hour of exposure to ultraviolet rays from sunlight cause paralysis and after few hours can be lethal. The worm breathes through its skin and if the skin is dryness can produce the earthworm death (Singh and Singh, 2014).

3.7.8. Predators, parasites and pathogens

Many species of mammals and birds are responsible of earthworms predation as well as other fauna such as ants, centipedes, carabid and staphylinid bettles and their larvae. Internal parasites, represented by Protozoa (the most common genus belong to Gregarina), Nematoda, Rotatoria and Platyhelminthes genera are able to infest the earthworms body. Some Bacterial species such as *Spirochaeta* sp., *Bacillus* sp. and fungal pathogens have been described as potential pathogens of earthworms (Domínguez and Edwards, 2011a).

Parameters of substrate	Values
C:N ratio	25:1 to 30:1
Initial particle size	10 - 20 mm
Moisture content	80% - 90%
Oxygen	aerobic conditions
Temperature	15°C - 25°C
pH	5 - 9
Ammonia content	<0.5 $mg.g^{-1}$
Salt content	<0.5%

Table 2 Optimal parameters for vermicomposting processes. Adapted from Domínguez and Edwards (2011b).

3.8. Different types of vermicomposting systems

Singh and Singh (2014) have defined two basic systems of vermicomposting the basic small-scale and large-scale system (see **Fig. 4**) which can be performed in bins or in compost pile. Usually the large-scale system requires high amount of wastes and it is mainly used for commercial purposes. The structure is described in **Fig. 3**.

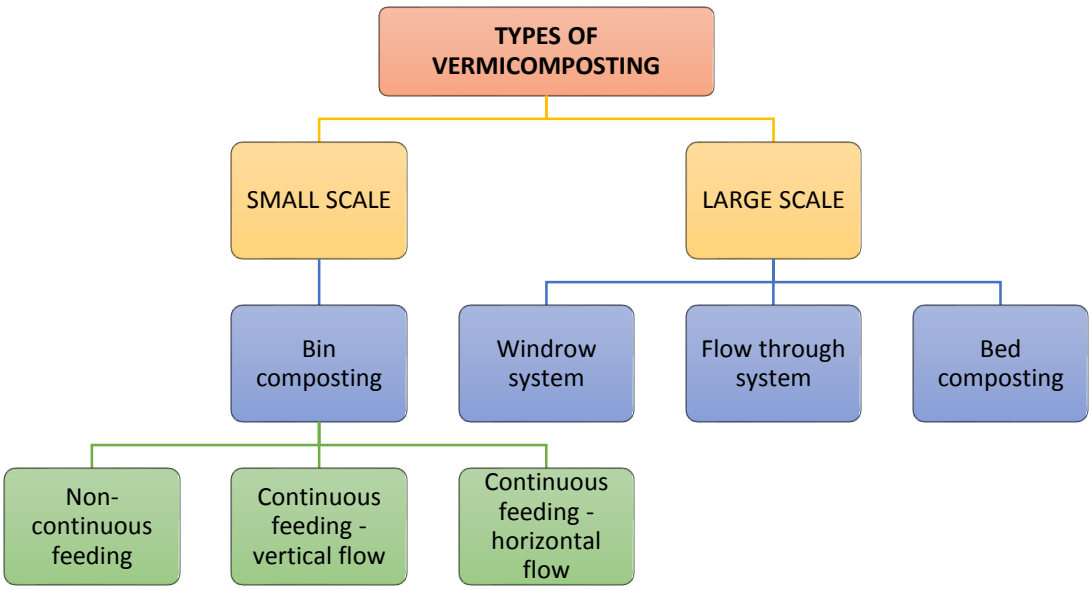

Fig. 3 Diagram showing the division of different types of vermicomposting systems, Singh and Singh (2014).

3.8.1. The small-scale system

The small scale vermicomposting systems are suitable for households and offices, since it usually takes place in bins. There are various types of bins commercially available which are made up of wood, plastic or metal (Hanč a Plíva, 2013b). The size of bin depends on the amount of material to be vermicomposted, number of earthworms and area of the storage (Singh and Singh, 2014). The bins used usually have holes on the wall sides for better air circulation and also at the bottom of the bins for leachate draining. The small scale bins are divided into three categories: non-continuous, continuous vertical flow and continuous horizontal flow (Fulekar, 2013)

(i) **Non-continuous feeding system** is carried out in non-divided bins. The bedding is added on the bin bottom and earthworms are inoculated. On the layer of bedding is

added feedstock and the earthworms start to decompose both materials. This system is often used due to its small size and easily maintenance. However, the problem lies in the removing of final vermicompost since the entire volume have to be emptied (Fulekar, 2013).

(ii) **Continuous-feeding in vertical flow system** is performed on series of trays stacked up vertically. In the last tray situated in the bottom of vermicomposters, the bedding is placed and earthworms are inoculated, then the organic waste is added. When the bottom tray is full, new fresh organic waste is added to the tray situated above. Earthworms decompose the substrate in the bottom tray and then move towards to the above tray. The final product is easier to collect using this system, since the bottom tray should be relatively free of earthworms at the end of the vermicomposting process (Fulekar, 2013).

(iii) **Continuous-feeding in horizontal flow system** is performed using containers similar to in a non-continuous system. The bins are placed horizontally and divided in two parts, which are separated often with gauge screen of chicken wire. At the beginning of the process only one part is filled with bedding and organic waste whereas the second part is filled up immediately after the first is full of vermicompost. The vermicompost will be collected from the first tray after the earthworm movement to the new fresh material contained in the second tray (Fulekar, 2013).

3.8.2. The large-scale system

The basic process of vermicomposting in a large-scale is the same as in small-scale. However, in the large-scale system is used for processing of a large amount of organic waste and the vermicompost produced are mostly used for industrial purposes. This technology is widely used in Canada, Italy, Japan, the United States of America, Malaysia and the Philippines. There are three main methods of the large-scale system which are classified as: windrow system, flow through system and beds composting (Singh and Singh, 2014).

(i) **Windrow system** use linear long piles on the ground containing organic material. They can be open or under cover which consist in a bedding material for earthworms and organic matter to prevent the predation of earthworms. Even though the windrow has no physical borders, the earthworms do not leave from piles due to large amount of organic material available. The fresh organic matter is added from

the front side of pile and the back side when the decomposition process finish can be removed (Singh and Singh, 2014).

(ii) **Flow through system** is performed using huge vermireactors. The organic material is added from the top of this vermireactors and when the organic waste is stabilized, the vermicompost is collected from the bottom of the vermireactor (Hanč a Plíva, 2013b). The name "flow through" means that earthworms are never disturbed in their beds. The waste comes from the top, flow through the reactor and after degradation of organic matter, it falls down on the ground (Munroe, 2007).

The flow through reactor was designed by Dr. Clive Edwards in the 1980s and it was reported that the vermireactor surface must be around 93 m^2 to be able to process between 2 - 3 tonnes per day of organic waste (Munroe, 2007).

(iii) **Beds composting** can be conducted in many different ways. The first type is usually known as top-fed bed in which the organic waste is placed in a safe area surrounded by four walls with floor. However, this bed composting can be performed in stacked bin in order to reduce the place need for vermicomposting. It commonly contains mixed material with earthworms added (Singh and Singh, 2014).

Fig. 4 The small-scale system (A) versus the large-scale system (B). (A) continuous-feeding in vertical flow system vermicomposter, similar type was used for the experiment; (B) flow through system vermireactor. Available online: (A) http://wormcompostingbin.net/gusanito-worm-wrangler-review/;
(B) https://sonomavalleywormfarm.wordpress.com/2013/01/28/a-great-start-for-the-new-year/

3.9. Characteristics of vermicompost

Vermicompost is a value-added product generated from the biological degradation of organic matter by interactions between earthworms and microorganisms. Earthworms ingest organic material passing through their gizzard in which the material is fragmented into finer particles. The microorganisms living in organic matter are a source of nourishment for earthworms. This process leads the acceleration of microbiological degradation of the organic matter and thus the microbial population increase. In addition, vermicomposting modifies chemical and physical structure of the initial material resulting in the enhancement of the humification process in which the non-stable organic material is oxidized and stabilized (Arancon et al., 2011).

However, there are many factors affecting considerable the quality of the end product. The most important factors for vermicompost characterization are: earthworm specie used, processing time, type of initial feedstock, type of vermicomposting system used, pre-processing of vermicompost (leaching, pre-composting) or amendments application (Edwards et al., 2011).

3.9.1. Physical characteristics

Vermicompost is characterized by dark colour, homogeneity, increased surface area, strong capacity of adsorption and nutrients retention (Arancon et al., 2011). In the high-quality vermicompost, the organic matter content may be between 20 - 50 % compared with inert materials in which this amount is less than 1 % of the total weight. The size of the particles present in vermicompost is less than 0.2 mm due to the grinding of organic material in the earthworm gut. Other important factor is the humidity of vermicompost, although the recommended water content during vermicomposting process must be in a range between 80 - 90%, the final moisture content can widely vary depending physical characteristic of organic waste. However, a level in humidity can represent a problem because the management of vermicompost is more expensive. Contrary, if the content in water is relatively low in the vermicompost, it can seriously reduce its properties for conferring plant disease resistance. In general the optimal range is accepted to be between 30 - 50% (Edwards et al., 2011).

3.9.2. Chemical characteristics

The vermicompost acidity has great importance for plant growth and fertility. The final pH value depends on the type of organic waste used for producing vermicompost. The optimal pH range for vermicompost produced from pig manure and sheep manures is of 5.3 – 8.6,

respectively. In any case, it has been described cases in which a pH range of 6 – 7 in vermicomposts favours the plant growth (Edwards et al., 2011).

The content in nutrients is other important chemical variable in vermicompost. (Arancon et al., 2011) It has been observed that vermicompost made up from manure waste has a great amount of mineral such as NO_3^-, soluble form of P, N, Ca and Mg elements compared with commercial fertilizers since they are easily uptake by plants (see **Table 3**). The content in total organic carbon (TOC) in vermicompost is associated to the organic matter present in the waste and thus it can be used as a good indicator of the degree of stabilization during vermicomposting process (Pramanik et al., 2007). Likewise, the total nitrogen found in vermicompost can vary between 0.1 - 4% (Atiyeh et al., 2002). The C:N ratio can provides information about stability of the organic matter. The optimal range for C:N ratio is stablished in a range of 20 - 22. The concentration in NO_3^- must be relatively high respect to NH_4^+ in vermicompost. Optimal NO_3^- content is in range of 100-200 mg^{-1} whereas NH_4^- levels should not be higher than 300 mg^{-1} (Edwards et al., 2011). The total P, K, Ca, Mg, S and B should be specified whether the nutrient value in vermicompost is important (see **Table 3**). However, some vermicompost produced from urban waste, paper waste, animal manures can contain heavy metal such as lead, mercury, cadmium, chromium and zinc, which can has an adverse effect on the environment (Morgan, 2011)

	Plant growth medium	**Vermicompost from pig manure**
Conductivity (mmhos/cm)	1.35	11.76
pH	5.90	5.30
Organic C (%)	31.78	27.38
Total N (%)	0.43	2.36
P (%)	0.15	4.50
K (%)	1.59	0.40
Ca (%)	1.03	8.60
Fe (%)	2.58	0.80
Mg (%)	3.52	0.50
Cu (µg/g)	34.09	378.8
Mn (µg/g)	526.04	1170.0
Zn (µg/g)	115.26	824.7
B (µg/g)	41.85	45.3

Table 3 Chemical characteristics of vermicompost from pig manure in comparison with soilless plant growth medium (MM360), Atiyeh et al. (2002).

3.9.3. Biological characteristics

The biological characteristics present in vermicomposts have been described to be higher compared with soil amendment (Edwards et al., 2011). Vermicompost contains large number of bacteria, actinobacterial and fungi. In addition it possesses plant growth hormones such as auxin, gibberellin, cytokines and plant growth regulators as fulvic and humic acids. According to Eastmen et al. (2001) there is evidence that some human pathogens can be reduced during vermicomposting process.

On the other hand, enzymatic activities can be considered as another biological characteristic of vermicompost (Fernández-Gómez et al., 2012). Their activity is positively influenced by earthworms. Enzymes are associated to microbial activity, soil fertility plant growth, and plant disease resistance. The determination of their activity could indicate the overall microbial activity of the vermicompost (Edwards et al., 2011).

3.10. Monitoring of vermicomposting process

The effectiveness of a vermicomposting process is crucial for the stabilization of organic wastes and it is intimately conditioned to the optimal growth and development of earthworms. The monitoring of changes in parameters during vermicomposting process is highly needed and it has been described in previous studies performed on the recycle of different organic wastes (Garg and Gupta, 2009; Fernández-Gómez et al., 2010a; Hanc and Chadimova, 2014). Chemical parameters such as pH, content in TOC, N, NO_3^- and NH_4^+ and microbial activities can be considered as good, reliable and sensitive indicators for monitoring the organic matter stabilization in the vermicomposting process.

3.10.1. Chemical parameters

During the vermicomposting processes many chemical parameters such as temperature, humidity, aeration and pH must be monitored since earthworms require optimal values on these variables for their growth and development and thus, the organic waste will be processes and stabilized in a short period of time (Pramanik et al., 2007).

At the beginning of vermicomposting process, pH value is lower due to production of CO_2 and organic acids during the decomposition of organic matter leading the formation of NH_4^+, which increase the pH of the system. The effect of organic acids and NH_4^+ regulates the vermicompost pH and as results these shifts run towards the neutrality (Pramanik et al., 2007).

TOC and inorganic N forms (NO_3^- and NH_4^+) play an essential role for cell synthesis, growth and metabolism in all living organisms. To ensure a proper nutrition for earthworms during vermicomposting, the presence of C and N must be in the substrate in an optimal range. It is also necessary for the optimal earthworm digestion. Likewise, it has been described that the C:N ratio for the optimal organic matter stabilization is 25:1 (Ndegwaa and Thompson, 2000).

The optimal humidity in the organic waste is usually constant during the vermicomposting process, but sometimes the application of water is need depending physical structure of organic waste as well as its ability for retaining water (Edwards and Bohlen, 1996). The temperature is highly important since the metabolic activities of earthworm communities depend on this variable. Therefore, temperature must be in specifics ranges according to the specie of earthworm used. Even though earthworms are highly photo-sensitive, therefore vermicomposting process has to be conducted in darkness conditions in order to avoid an earthworm paralysed under light exposure (Gershuny, 2011).

3.10.2. Microbial activities

Enzyme activities have been used widely as an index of soil fertility or ecosystem status because they are involved in the biological transformations of native and foreign compounds in soils (Tate, 2000). Several enzymatic activities have been measured to describe organic matter decomposition in two microbial-driven processes, composting and vermicomposting processes in a large and small scale (Benitez et al., 2002; 2005). Vermicomposting involves the bio-oxidation and stabilization of organic matter through the joint action of earthworms and microorganisms.

Microbial activity is achieved through the action of the enzymes that are responsible for the hydrolysis of complex macromolecules that constitute the organic wastes. As a consequence of this activity, simple water-soluble compounds which support microbial growth are released favoring the continuity of the process. Moreover, the extent to which this enzymatic activities are produced gives information on the rate of decomposition of organic matter and, therefore, on the product stability (Mondini et al., 2004). The complete transformation of organic matter during the vermicomposting process requires the joint action of many different enzymes as consequence of the high complexity organic wastes. The analysis of some of the most significant enzymes provides information for a good estimation of the events that take place through the vermicomposting process:

(i) **Dehydrogenases** are intracellular enzymes which is commonly used as good bioindicator of microbial activity on account of its role on the oxidative phosphorylation process, and therefore in the respiratory metabolism of microorganism (Delgado et al., 2004). The increase of dehydrogenase activity during the vermicomposting process indicates an enhancement on microbial biomass. Meanwhile, a decrease in the activity of dehydrogenase may be associated to a low content in the easily organic matter, thus dehydrogenase activity is directly depending the substrate available (Garg et al., 2008)

(ii) **β-Glucosidases** are involved in the carbon cycle through the hydrolysis of glucosides, which can be found in decomposing plant residues. Consequently, β-glucosidase is associated with the carbon cycles and organic matter breakdown, thus during vermicomposting process shows the bioavailability of different carbon sources (García et al., 1995).

(iii) **Phosphatases** are an enzyme implicate in phosphorus cycles, catalysing hydrolysis of esters and anhydrides of phosphoric acid to different inorganic forms which can

be metabolized by plants (Gupta et al., 2014). The active phosphatase has two basic forms: alkaline and acid (Bakshi and Varma, 2010). These enzymes are responsible of the release of inorganic phosphorous compounds through organic substances during the vermicomposting processes. In this sense, this enzyme is a good indicator of the mineralization rates of organophosphorus compounds during and/or after a vermicomposting process (Gupta et al., 2014).

(iv) **Proteases** are enzymes are involved in the hydrolysis of the proteins and during vermicomposting processes are good indicator of mineralization of nitrogen organic compounds (Bakshi and Varma, 2010).

(v) ***O*-diphenol oxidases** participate in the oxidation of phenolic compounds into quinones. These enzymes are directly involved in humus substances formation and thus, they are implicated in the humification process. The o-diphenol oxidase may be reliable indicators of the organic matter stabilization during vermicomposting processes (Ke et al., 2010).

Most of these enzymatic activities have been previously monitored in a wide range of organic wastes during vermicomposting processes (Benitez et al., 2005; Pramanik et al., 2007; Fernández-Gómez et al., 2012). In general, it is well-known that enzymatic activities increase during the first stages of organic waste transformation in vermicomposting systems. Subsequently their activity decrease according to the biodegradation of the waste till values significantly reduced compared with initials. Thus, the vermicomposts produced may have lower activity compared with the fresh organic wastes. On the other hand, the microbial activity analysed in the stabilized material (vermicompost) can be used as indicator of the metabolic functionality of the microbial communities presents in the organic wastes (Vivas et al., 2009).

4. MATERIALS AND METHODS

4.1. Organic waste collection

The vermicomposting processes were performed using the initial organic waste from horse manure (HM), apple pomace (AP), wine pomace (WP), digestate (D) and kitchen waste (KW). HM was collected from horse farm placed in town near to Prague (Kladno, Czech Republic), which contained a mixture of solid and liquid horse faeces and bedding. AP was provided by the company Severofrukt (Terezín, Czech Republic, 50° 30′ N, 14° 9′ E). This waste was mainly obtained from apple juice production. WP was originated from Winery Company located in the South Moravia (Czech Republic). The waste was composted mainly by rest of pulp and stones of grapes after wine production. D was obtained from the biogas plant from an agricultural cooperative (Krásná Hora nad Vltavou, Czech Republic). The composition of D was approximately 50% manure slurry, 40% corn silage and 10% haylage. KW was collected from employees of faculty of Agrobiology Food and Natural Resources from the Czech University of Life Sciences (Prague, Czech Republic). Chemical characteristic were analysed in each organic waste used and are provided in the **Table 4**.

Initial organic waste	pH	EC (mS cm^{-1})	TOC (%)	N_{tot} (%)
HM	7.83	1.36	40	2.7
AP	3.85	1.26	40	2.5
WP	3.73	3.21	42	2.1
D	8.54	3.51	36	2.2
KW	-	-	-	-

Table 4 The chemical composition of initial organic waste used for vermicomposting process. Electrical conductivity (EC), total organic carbon (TOC), total nitrogen (N_{tot}).

4.2. Earthworms collection

Non-cllitelated earthworms (*E. andrei*) were obtained from the company Jakub Filip located in Lužice u Hodonína (Czech Republic). The earthworms provided by this company were contained in cow manure (CM).

4.3. Experimental design

The experiment was conducted in a special treated room from the research station of Faculty of Agrobiology, Food and Natural Resources located in Červený Újezd (Prague, Czech Republic). The vermicomposting process was carried out in darkness, at room temperature (22 ºC) with a relative humidity 80% and aeration every 12 hours for 15 min.

The vermicomposters used in this experiment were type VermiHut Worm Bin. Fifteen vermicomposters were set up, three per each organic waste tested (HM, AP, WP, D, KW). Vermicomposters were composed of five trapezoidal trays (0.4 x 0.4 x 0.18 m) with perforated bottom (for allowing movement of earthworms from one tray to another). Only two trays from the top of the vermicomposters were selected for performing vermicomposting process. The last three trays placed on the bottom were already filled with composted material, which have not been used for our analysis. In the first tray, 5 L of organic waste above mentioned was added and subsequently, the earthworms were placed in the second tray. The earthworms were obtained from 20 L from the original cow manure substrate. The cow manure was applied in order to provide appropriate habitat for worms and reduce their initial shock due to the environmental changes as previously was observed by Hanc and Chadimova (2014). For each treatment, 1 L of cow manure with earthworms was collected from the stock (20 L) and was placed in the second tray. The earthworm number and biomass were also determined in 1 L of substrate in which the earthworm number was 167 with a total biomass of 7.1 g. Each treatment was conducted in triplicate.

The earthworms were fed every two weeks by adding 5 L of new fresh material. Samples were collected after 0, 2, 4 and 6 months and were kept at 4 ºC for chemicals and enzymatic assays. Earthworms were picked by hand, counted, weighed and removed in each time of collection. Due to the low degradation and homogenization in the KW treatment was not possible not analyse all parameters at initial time.

4.4. Chemical analysis

The **pH** and **electrical conductivity (EC)** of in each treatment were measured by pH meter (CRISON micro pH) in 1 g of material diluted with 10 ml of distilled water, in a ratio 1:10 (w:v). **Total organic carbon (TOC)** was determined using the dichromate oxidation by Mingorance et al. (2007). 0.2 g of sample was mixed with 3 mL 0.16 M $K_2Cr_2O_7$, 6 mL H_2SO_4 and 11 mL distilled water. The mixture was incubated for 24 h and the Cr^{3+} resulting from organic C oxidation were determined using spectrophotometry at 590 nm. The g of TOC was calculated using a calibration curve of saccharose (0-10 mg mL^{-1}). For determination of **total soluble carbon (C)** and **nitrogen (N)**, 1 g of sample was mixed with 10 mL $CaCl_2$ and was shaking for two hours at room temperature. 60 ml of supernatant was centrifuged at 3000 rpm. The SKALAR SANPLUS SYSTEM ® (Skalar, Netherlands) was used for the quantification of total soluble C and N. The assessment of C was performed as follows: samples were acidified with 0.5 M H_2SO_4 solution and subsequently bubbled with N gas. Then, it was added a reagent persulfate/ tetra borate and the solution flowed through an UV digestion coil, which caused oxidation from organic carbon (C_{org}) to carbon dioxide (CO_2). The amount of CO_2 was measured by infra-red detection. The determination of N was carried out by mixing sample with a borax buffer and then with potassium persulphate solution. The solution run through the UV digestion and NO_3^- were reduced to NO_2^-. The NO_2^- were assessed by the Griess reaction and measured spectrophotometrically at 540 nm. For the analysis of **NO_3^-** and **NH_4^+** samples were mixed with 0.01 M $CaCl_2$ solution in ratio 1:10 (w:v). The solution was shaked for 2 hours at room temperature. 60 mL of supernatant were centrifuged at 3000 rpm. The determination was realized on the automated SKALAR SANPLUS SYSTEM ® (Skalar, Netherlands). The NO_3^- quantification was done by the Cd reduction method. This reaction consists in the reduction of NO_3^- to NO_2^- by bringing into granulated Cu/Cd column. Then, the NO_2^- was diazoted with sulphanilamida and coupled with α – naphthylethylenediamine dihydrochloride. This reaction was measured at 540 nm spectrophotometrically. NH_4^+ was chlorinated to monochloramine, which reacted with salicylate to aminosalicylate. The green complexes were formed after its oxidation and it was measured at 660 nm.

4.5. Enzymatic activity analysis

Hydrolytic (β–glucosidase, phosphatase, and protease) and redox enzymatic activities (dehydrogenase, and *o*-diphenol oxidase) were measurement in triplicate and quantify spectrophotometrically.

The determination of **dehydrogenase** (EC 1.1) activity was assayed as previously has been described by Camiña et al. (1998). 1 g of sample was placed into test tube and mixed with 1.5 mL of 1 M Tris buffer (pH 7.6) and 2 mL of 0.5% iodotetrazolium violet (INT). The reaction was incubated for 1 h at 40 ºC. The iodonitrotetrazolium formazan INTF produced was extracted by adding 10 mL of mixture 1:1 (v:v) ethanol:dimethylformamide and the absorbance was measured at 490 nm spectrophotometrically. **β–glucosidase** (EC 3.2.1.21) and **phosphatase** (EC 3.1.3.1) activity were determined using the methods described by Eivazi and Tabatabai (1977; 1988), respectively. 0.5 g and 0.2 g of samples were mixed with 0.5 mL of 0.05 M p-nitrophenyl-D-glucopyranoside and 0.115 M p-nitrophenyl-phosphate for β-glucosidase and phosphatase, respectively in 0.1 M Maleate buffer pH 6. Samples were incubated at 37°C for 2 h in darkness. The quantification of p-nitrophenol originated was measured by adding 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH at 400 nm spectrophotometrically. The concentrations were extrapolated using a standard curve of p-nitrophenol (0-50 μg mL^{-1}). **Protease** (EC 3.4.2.21-24) activity was measured following the method described by Ladd and Butler (1972). 1 g of sample was mixed with 5 mL of 0.05 M Tris-HCl buffer (pH 8.1) and 5 mL of 2% casein. Samples were shaking and incubated for 2 h at 50°C. Then, the reaction was stopped by adding 5 mL of 15 % TCA. The extract was mixed with 7.5 mL a mixture of 0.06 M Na OH, 5 % Na_2CO_3, 0.5 % $CuSO_4 \times 5H_2O$, 1 % potassium sodium tartrate, and 5 mL of 33 % Folin-Ciocalteu reagent. The colour was quantified at 700 nm spectrophotometrically and the concentrations were extrapolated using a standard curve of L-Tyrosine (0-25 mg mL^{-1}). The determination of ***o*-diphenol oxidase activity** (EC 1.10.3.1.) was done following the method described by Perucci et al. (2000). 1 g of sample was incubated with 2 mL of 0.1 M phosphatase buffer (pH 6.5), 1.5 mL of 0.2 M proline and 0.2 M pyrocatechol for 10 min at 30°C. The reaction was stopped by adding 5 mL of ethanol and all samples were cooled in freezer for 10 min. Then, the suspension was filtrated and absorbance was measured at 525 nm. It was used as standard pyrocatechol (0-70 μg mL^{-1}).

4.6. Statistical analysis

All data presented are the mean of three replicates. The data analysis was performed using software SPSS® Windows Version 17.0 (Chicago, Illinois, USA). One-way ANOVA was used to analyse statistically significant differences in each treatment at different time of collections and between different treatments in each time of collection. Both analyses were measured in each parameter of the vermicomposted material with mean separation based on Tukey's test. Statistical analysis were carried out on confidence level >95% ($P \leq 0.05$).

5. RESULTS

5.1. Earthworm biomass

5.1.1. Biomass

Changes on earthworm biomass were monitored during the vermicomposting process in each treatment at different time of collection (2, 4, and 6 months) and significant variations were related to initial values of earthworm biomass added for performing vermicomposting process (**Fig. 5**). In the case of HM vermicomposting treatment, it was observed an enhancement in the earthworm biomass at 2, 4 and 6 months, but the maximum biomass was recorded after 4 months. Similarly, AP samples increased the earthworm biomass during vermicomposting processes, but the maximum was reached at 2 months. WP and D increased the earthworm biomass at all times of collection except to after 2 months (**Fig. 5**). On the other hand, in the case of KW vermicomposting sample was the enhancement in the earthworm biomass was only observed after 6 months of vermicomposting (**Fig. 5**).

Differences in earthworm biomass according to type of organic waste used for vermicomposting were also found (**Fig. 5**). After 2 months, the earthworm biomass was higher in AP vermicomposting treatment respect to HM, WP, D and KW. However, HM vermicomposting treatment showed maximum values in earthworm biomass after 4 months respect to all vermicomposting treatments, whereas KW vermicomposting treatment reduced greatly the earthworm biomass compared with AP, WP and D vermicomposting treatments. Finally, the earthworm biomass increased significantly in KW after 6 months respect to HM, AP, WP, and D vermicomposting treatments (**Fig. 5**).

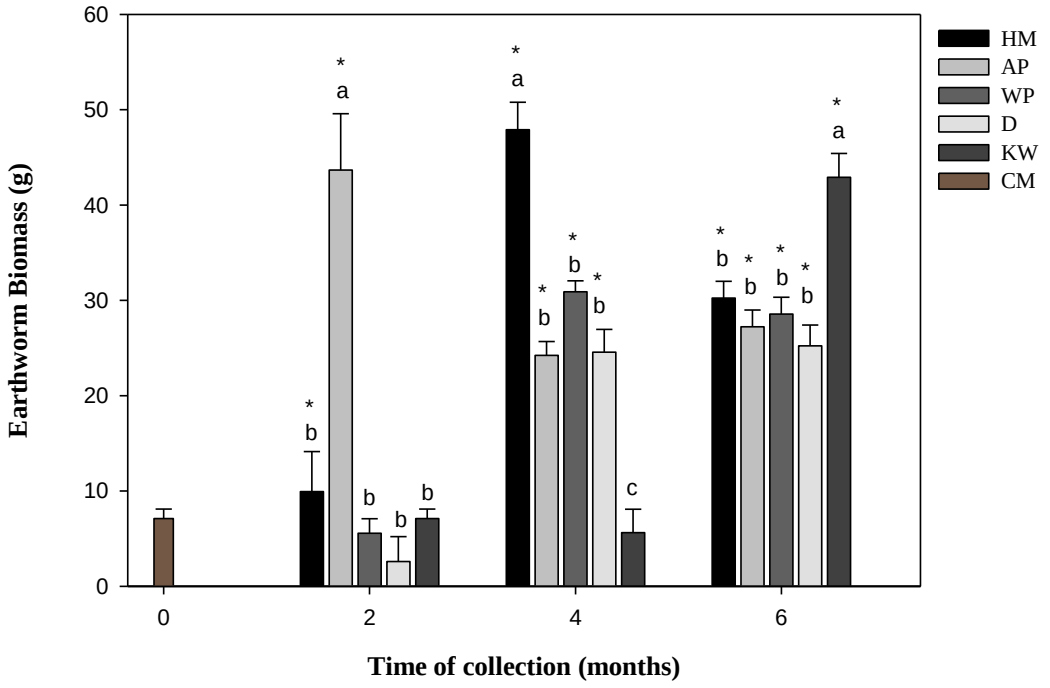

Fig. 5 Earthworm biomass during the vermicomposting process of organic wastes. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect initial earthworm biomass at different time of collection. Bars represent standards errors.

5.1.2. Earthworm mortality and reproduction

Earthworms were counted in each time of collection (2, 4, and 6 months) by hand in order to evaluate the mortality and reproduction during the vermicomposting process. The significant differences observed in each treatment at all times of collection were related to the initial earthworm number added for performing vermicomposting (**Fig. 6**). In the case of HM vermicomposting sample, it was only observed an enhancement in the earthworm number at 4 months, and this parameter was significantly reduced after 6 months. Contrary, AP vermicomposting treatment showed a greatly increase in the earthworm number at 2 and 6 months, whereas after 4 months no significant differences were observed respect to the initial time. However, WP vermicomposting sample did not modify the earthworm number at 2 months, and even it was found a decline in this parameter after 4 and 6 months. D samples reduced dramatically the earthworm number at 4 and 6 months, whereas in the case of KW vermicomposting sample it was observed only after 8 months.

Changes in the earthworm number between different vermicomposting treatments in each time of collection were also found (**Fig. 6**). Thus, AP vermicomposting sample showed higher values in the earthworm number respect to HM, D and KW vermicomposting treatments after 2 months. Meanwhile, it was found an enhancement in the earthworm number present in HM vermicomposting sample respect to others vermicomposting treatments after 4 months. The earthworm number was significantly higher in AP compared with WP, D and KW vermicomposting samples. Meanwhile, D showed a decline in the earthworm number respect to KW vermicomposting treatments whereas no differences were found between WP and KW vermicomposting samples (**Fig. 6**). After 6 months, AP was found to increase the earthworm number respect to other vermicomposting treatments, whereas HM and KW showed significant enhancement compared with WP and D vermicomposting treatments. The earthworm number in D was slightly reduced respect to WP vermicomposting treatment (**Fig. 6**).

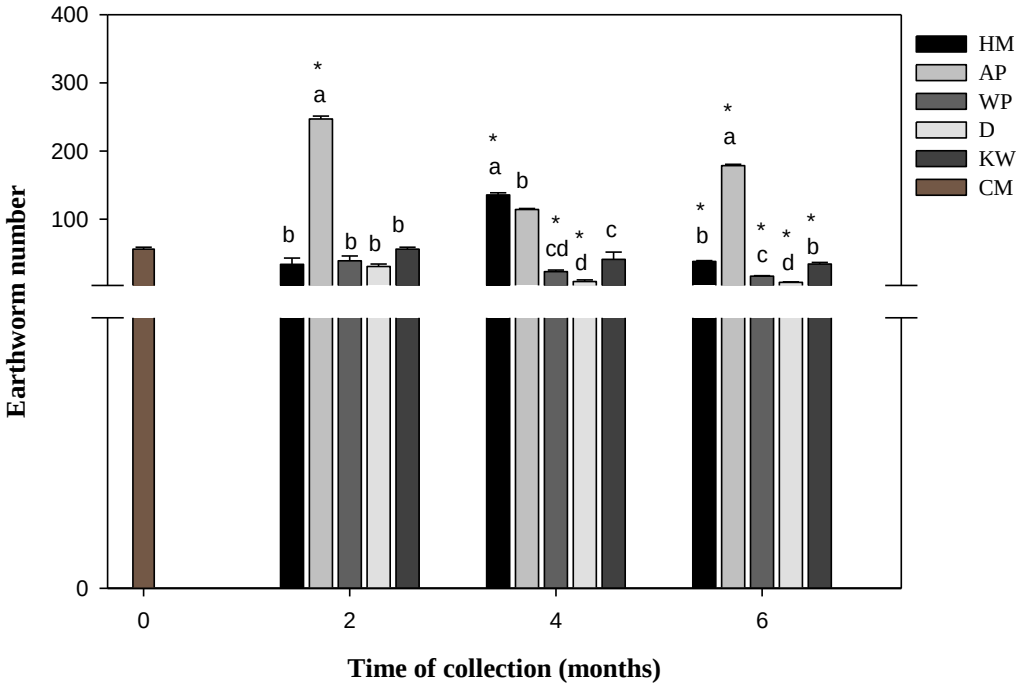

Fig. 6 Earthworm number during the vermicomposting process of organic wastes. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect initial earthworm number at different time of collection. Bars represent standards errors.

5.2. Chemical parameters

5.2.1. pH

The **Table 5** shows changes in pH values during the vermicomposting process. HM vermicomposting sample revealed a significant decrease after 2, 4 and 6 months respect to initial time. Meanwhile, in AP and WP vermicomposting treatment were found an enhancement in the pH values after 2, 4 and 6 months. Likewise, D vermicomposting sample also showed a decrease after 2, 4 and 6 months. On the other hand, it was observed and enhancement in the pH values in KW vermicomposting sample after 6 months respect to initial time.

The pH values quantified in HM, AP, WP, D and KW vermicomposting treatments showed differences among themselves after 0, 2, 4 and 6 months (**Table 5**). The pH in AP and WP was lower respect to HM, AP and D vermicomposting treatments at initial time. Likewise, HM showed a reduction in the pH value respect to D vermicomposting treatments (**Table 5**). KW vermicomposting treatment showed the highest pH value at 2 and 4 months. Meanwhile, significant differences in this parameter were found in the order WP>HM>AP>D (**Table 5**). Similar trend was observed after 4 months of vermicomposting process, but in this case the significant differences between vermicomposting treatments were found in the order WP>AP>HM>D. On the other hand, WP showed maximum values in pH respect to other vermicomposting treatments after 6 months, whereas the lowest pH was observed in HM vermicomposting treatment. The pH in AP and D were significantly reduced respect to KW vermicomposting treatment (**Table 5**).

Time (months)	pH				
	HM	**AP**	**WP**	**D**	**KW**
0	7.8 ± 0.04^{b}	3.9 ± 0.04^{c}	3.8 ± 0.02^{c}	8.5 ± 0.04^{a}	-
2	7.3 ± 0.04^{c} *	7.0 ± 0.05^{d} *	7.9 ± 0.03^{b} *	6.5 ± 0.02^{e} *	8.6 ± 0.01^{a}
4	6.9 ± 0.02^{d} *	7.2 ± 0.04^{c} *	8.1 ± 0.02^{b} *	6.4 ± 0.02^{e} *	8.6 ± 0.03^{a}
6	7.1 ± 0.01^{d} *	8.0 ± 0.01^{c} *	9.2 ± 0.03^{a} *	8.1 ± 0.03^{c} *	8.9 ± 0.04^{b} *

Table 5 The pH values measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.2. Electrical conductivity (EC)

The changes in EC during vermicomposting process are shown in the **Table 6**. The EC values in HM, AP, WP, and D vermicomposting treatments decreased significantly after 2, 4 and 6 months respect to initial time. Meanwhile, it was observed a significant decrease in the EC values in KW vermicomposting treatment at 4 months, whereas a slight enhancement was detected after 6 months (**Table 6**).

Significant differences in the EC values were found between HM, AP, WP, D and KW vermicomposting treatments at different time of collection (**Table 6**). The EC analysed in WP and D samples was higher respect to HM and AP vermicomposting treatments at initial time (**Table 6**). Likewise, D showed the highest values in EC at 2 months whereas significant differences in this parameter between vermicomposting treatments were found in this order KW>HM>WP>AP. Similar trend was observed at 4 months (**Table 6**). Finally, the EC values in D and KW were highly increased compared to HM, AP and WP vermicomposting treatments after 6 months. AP showed the lowest values in EC, whereas in the case of HM, this parameter was significantly enhanced respect to WP vermicomposting treatment. (**Table 6**).

Time (months)	EC (mS cm^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	1.3 ± 0.05^{b}	1.3 ± 0.13^{b}	3.2 ± 0.07^{a}	3.5 ± 0.03^{a}	-
2	1.0 ± 0^{c} *	0.4 ± 0.01^{e} *	0.8 ± 0.03^{d} *	2.0 ± 0.03^{a} *	1.6 ± 0.03^{b}
4	1.0 ± 0.01^{c} *	0.4 ± 0.01^{e} *	0.7 ± 0.01^{d} *	1.8 ± 0.01^{a} *	1.2 ± 0.03^{b} *
6	1.1 ± 0.02^{b} *	0.4 ± 0^{d} *	1.0 ± 0.03^{c} *	1.6 ± 0.03^{a} *	1.7 ± 0.02^{a} *

Table 6 EC measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.3. Total organic carbon (TOC)

The quantification of the TOC content in all vermicomposting treatments (HM, AP, WP, D and KW) during the vermicomposting process revealed that HM and AP vermicomposting treatments decreased the content this parameter after 2, 4 and 6 months respect to the initial time. Meanwhile, WP and D increased the TOC at 6 months. No changes were detected in KW vermicomposting treatment at all times of collection (**Table 7**).

Significant differences in the TOC content between HM, AP, WP, D, and KW vermicomposting treatments at each times of collection were found (**Table 7**). The content in TOC was higher in HM and WP samples respect to AP and D at initial time. (**Table 7**). After 2 month, the TOC content in WP vermicomposting treatment was greatly increased compared with AP, but no significant changes were observed respect to other vermicomposting treatments. WP increased significantly the TOC content respect to other vermicomposting treatments after 4 months, whereas no changes were detected between HM, AP, D and KW vermicomposting treatments (**Table 7**). At 6 months, WP showed the highest values in TOC content respect to other treatments, whereas in this case AP was found the lowest TOC content. D increased significantly this parameter respect to HM and KW vermicomposting treatments (**Table 7**).

Time (months)	TOC (g kg^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	40.0 ± 1.12 ^{a}	40.0 ± 0.03 ^{b}	42.1 ± 1.57 ^{a}	35.9 ± 0.99 ^{b}	-
2	35.6 ± 2.76 ^{ab} *	31.0 ± 2.18 ^{b} *	39.5 ± 0.23 ^{a}	33.9 ± 1.24 ^{ab}	36.3 ± 1.20 ^{ab}
4	33.2 ± 0.51 ^{b} *	34.0 ± 3.97 ^{b} *	42.1 ± 0.82 ^{a}	32.9 ± 0.83 ^{b}	35.3 ± 0.54 ^{b}
6	35.1 ± 0.09 ^{c} *	31.5 ± 1.88 ^{d} *	45.2 ± 0.18 ^{a} *	40.2 ± 1.44 ^{b} *	35.5 ± 0.76 ^{c}

Table 7 TOC measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.4. Total soluble C

The changes in the total soluble C content in each vermicomposting treatment at different time of collection during vermicomposting process are shown in **Table 8**. The total soluble C content in HM, AP, WP, and KW samples was significantly enhanced after 4 and 6 months respect to initial time. Meanwhile, D samples increased the content in total soluble C at all times of collection (**Table 8**).

Significant differences were found in the total soluble C between vermicomposting treatments in each time of collection (**Table 8**). The content in total soluble C analysed at initial time was greatly increased in HM respect to AP, WP, and D vermicomposting treatments (**Table 8**). Likewise, HM vermicomposting sample enhanced significantly the total soluble C content respect to AP, WP, D and KW vermicomposting treatments after 2 months (**Table 8**). However, WP showed the lowest total soluble C content, whereas no significant differences were observed between AP, D and KW vermicomposting treatments. The quantification of total soluble C in AP revealed an increase in this parameter respect to WP, D and KW vermicomposting treatments at 4 months, but no differences were detected compared with HM. No significant differences were observed between HM and D vermicomposting samples, but this parameter in HM and D was higher compared with WP and KW vermicomposting treatments. Meanwhile, WP showed an enhancement in the total soluble C content respect to KW vermicomposting treatment (**Table 8**). The total soluble C quantified at 6 months of vermicomposting process revealed that HM and WP increased these values significantly respect to others vermicomposting treatments, whereas no significant differences were detected between AP, D and KW (**Table 8**).

Time (months)	C (g kg^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	2.00 ± 0.074^{a}	1.77 ± 0.039^{b}	1.74 ± 0.022^{b}	1.74 ± 0.021^{b}	-
2	2.02 ± 0.025^{a}	1.85 ± 0.044^{b}	1.70 ± 0.020^{c}	1.88 ± 0.032^{b} *	1.83 ± 0.027^{b}
4	2.92 ± 0.024^{ab} *	2.99 ± 0.037^{a} *	2.66 ± 0.025^{c} *	2.85 ± 0.028^{b} *	2.43 ± 0.042^{d} *
6	2.46 ± 0.055^{a} *	2.10 ± 0.015^{b} *	2.42 ± 0.059^{a} *	2.16 ± 0.031^{b} *	2.18 ± 0.037^{b} *

Table 8 Total soluble C content measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.5. Total soluble N

The changes in total soluble N content in each vermicomposting treatment at different time of collection during vermicomposting process are shown in the **Table 9**. The content in total soluble N determined in HM and AP vermicomposting treatments was significantly decreased at 2 and 4 months respect to initial time, but no differences were found after 6 months. Contrary, WP vermicomposting treatment did not show any differences in total soluble N content at all time of collection. However, a high increase in this parameter was observed in D vermicomposting treatment after 2 and 4 months, whereas no changes were detected at 6 months. In the case of KW, it was only observed an enhancement after 6 months (**Table 9**).

Significant differences in the total soluble N between vermicomposting treatments in each time of collection during the vermicomposting process were found (**Table 9**). Total soluble N quantified in D increased significantly respect to HM, AP and WP vermicomposting treatments at initial time. Meanwhile, HM showed higher values respect to AP and WP vermicomposting treatments (**Table 9**). Likewise, it was observed that the highest values in total soluble N were found in D respect to other vermicomposting treatments after 2 months, whereas in AP, WP and KW vermicomposting treatments showed a reduction in this parameter respect to HM (**Table 9**). Similarly, the quantification of total soluble N revealed that D had maximum values respect to others vermicomposting treatments at 4 months. In this case, AP showed negatively differences in this parameter compared with HM, WP and KW vermicomposting treatments, whereas the N content observed in WP and KW vermicomposting treatments was significantly reduced respect to HM (**Table 9**). Finally, D showed higher values in total soluble N respect to other vermicomposting treatments at 6 months. HM increased this parameters significantly compared with AP, WP and KW vermicomposting treatments, whereas in the case of AP and WP were reduced respect to KW vermicomposting treatment (**Table 9**).

Time (months)	N (g kg^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	0.87 ± 0.015^{b}	0.34 ± 0.036^{c}	0.37 ± 0.003^{c}	1.26 ± 0.051^{a}	-
2	0.71 ± 0.004^{b} *	0.24 ± 0.006^{c} *	0.33 ± 0.032^{c}	1.89 ± 0.122^{a} *	0.39 ± 0.012^{c}
4	0.78 ± 0.006^{b} *	0.19 ± 0.004^{d} *	0.41 ± 0.007^{c}	1.79 ± 0.040^{a} *	0.33 ± 0.030^{c}
6	0.82 ± 0.032^{b}	0.26 ± 0.019^{d}	0.36 ± 0.024^{d}	1.46 ± 0.022^{a}	0.68 ± 0.035^{c} *

Table 9 Total soluble N measured content during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each vermicomposting treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.6. $N\text{-}NO_3^-$ content

The significant differences in the $N\text{-}NO_3^-$ content during vermicomposting process were found in all vermicomposting treatments at different time of collection (**Table 10**). Statistically significant changes were detected in HM after 2 and 6 months compare to initial time. In the case of AP and KW vermicomposting treatments, it was found higher $N\text{-}NO_3^-$ content after 6 months, whereas it was observed an enhancement in the $N\text{-}NO_3^-$ content in WP vermicomposting sample at 2 months, and after 2 and 4 months in the case of D vermicomposting treatment (**Table 10**).

The quantification of $N\text{-}NO_3^-$ content in all vermicomposting treatments at initial time revealed that D increased this parameter respect to HM, AP, and WP, whereas in AP and WP vermicomposting treatments was significant reduced compared with HM (**Table 10**). Similarly, D vermicomposting treatment showed an increase in $N\text{-}NO_3^-$ content after 2, 4 and 6 months respect to others treatments. Likewise, AP, WP, and KW were not significantly different among themselves, but these vermicomposting treatments showed a decrease in the $N\text{-}NO_3^-$ content respect to HM after 2 and 4 months. Meanwhile, it was found no differences in the $N\text{-}NO_3^-$ content between HM and KW vermicomposting treatments at 6 months, but it was found an increase in this parameters respect to AP and WP. No significant differences were detected between AP and WP vermicomposting treatments after 6 months (**Table 10**).

Time (months)	$N\text{-}NO_3^-$ (g kg^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	0.68 ± 0.024^{b}	0.02 ± 0.005^{c}	0.06 ± 0.011^{c}	1.11 ± 0.043^{a}	-
2	0.49 ± 0.014^{b} *	0.04 ± 0.005^{c}	0.15 ± 0.025^{c} *	1.67 ± 0.123^{a} *	0.03 ± 0.001^{c}
4	0.60 ± 0.005^{b}	0.02 ± 0.005^{c}	0.06 ± 0.015^{c}	1.51 ± 0.058^{a} *	0.06 ± 0.007^{c}
6	0.55 ± 0.045^{b} *	0.05 ± 0.003^{c} *	0.04 ± 0.001^{c}	1.19 ± 0.027^{a}	0.48 ± 0.034^{b} *

Table 10 $N\text{-}NO_3^-$ content measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.2.7. N-NH_4^+ content

The quantification of N-NH4$^+$ content in each vermicomposting treatment (HM, AP, WP, D and KW) at all time of collection revealed significant differences during vermicomposting process (**Table 11**). N-NH_4^+ content in HM and D vermicomposting treatments enhanced at 4 and 6 months, whereas in AP vermicomposting sample decreased considerably after 2, 4 and 6 months respect to initial time. Likewise, N-NH_4^+ content in WP vermicomposting treatment was significantly decreased at 2 and 6 months whereas in the case of KW vermicomposting treatment was only detected an enhancement in this parameter after 6 months.

Significant differences were found between different vermicomposting treatments in each time of collection (**Table 11**). Thus, N-NH_4^+ content quantified in WP was higher compared with HM, AP and D vermicomposting treatments at initial time. Likewise, an enhancement was detected in AP respect to HM and D vermicomposting treatments (**Table 11**). KW was significantly different respect to other vermicomposting treatments at 2 months, whereas no changes were detected between HM, AP, WP and D vermicomposting treatments (**Table 11**). Similarly, WP showed an enhancement in this parameter respect to others vermicomposting treatments at 4 months, but it was not also found differences between HM, AP, D and KW vermicomposting treatments (**Table 11**). Meanwhile, no significant changes were detected in N-NH_4^+ content between vermicomposting treatments after 6 months (**Table 11**).

Time (months)	N-NH_4^+ (g kg^{-1})				
	HM	**AP**	**WP**	**D**	**KW**
0	0.12 ± 0.008 ^{c}	0.27 ± 0.029 ^{b}	0.34 ±0.006 ^{a}	0.11 ± 0.001 ^{c}	-
2	0.11 ± 0.003 ^{b}	0.11 ± 0.020 ^{b} *	0.12 ± 0.029 ^{b} *	0.09 ±0.010 ^{b}	0.26 ± 0.017 ^{a}
4	0.15 ± 0.007 ^{b} *	0.15 ± 0.010 ^{b} *	0.36 ± 0.020 ^{a}	0.15 ± 0.011 ^{b} *	0.21 ± 0.027 ^{b}
6	0.16 ± 0.008 ^{a} *	0.17 ± 0.002 ^{a} *	0.17 ± 0.009 ^{a} *	0.16 ± 0.005 ^{a} *	0.15 ± 0.004 ^{a} *

Table 11 N-NH_4^+ content measured during vermicomposting process in different types of organic wastes. All values are the mean and standard error of three replicates. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection. Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.3. Enzymes activities

5.3.1. Dehydrogenase

Significant changes were found in the dehydrogenase activity in each treatment at different time of collection during the vermicomposting process (**Fig. 7**). HM vermicomposting treatment showed a slight increase in the dehydrogenase activity at 2 months respect to initial time of collection. Meanwhile, after 4 months no differences were found. However, the dehydrogenase activity was dramatically reduced reaching values up to 0 μg INTF g^{-1} h^{-1} at 6 months. On the other hand, dehydrogenase activity in AP was drastically reduced at 4 and 6 months respect to initial time, whereas in the case of WP and D vermicomposting treatments, it was observed at 2, 4 and 6 months. In the case of KW vermicomposting treatment the dehydrogenase activity quantification showed a progressive reduction respect to initial time at 4 and 6 months (**Fig. 7).**

The quantification of dehydrogenase activity between different vermicomposting treatments in each time of collection revealed significant differences (**Fig. 7**). AP increased the dehydrogenase activity respect to HM, WP and D vermicomposting treatments at the initial time. Meanwhile, the activity in WP was significantly higher respect to HM, but not compared to D and no differences were observed between HM and D vermicomposting treatments (**Fig. 7**). After 2 month, the dehydrogenase activity reached values strongly higher in KW respect to HM, AP, WP and D vermicomposting treatments. The activity found in AP only showed differences compared with D vermicomposting treatment. No significant differences were found in the dehydrogenase activity between HM, AP and WP vermicomposting treatments (**Fig. 7**). On the other hand, the quantification of the dehydrogenase activity did not show any significant difference between vermicomposting treatments after 4 month, except in the case of KW, in which it was greatly enhanced (**Fig. 7**). Finally, only the dehydrogenase activity in WP and KW showed an increase compared to HM, AP and D vermicomposting treatments at 6 months, whereas no changes were detected between HM, AP and D vermicomposting treatments (**Fig. 7**).

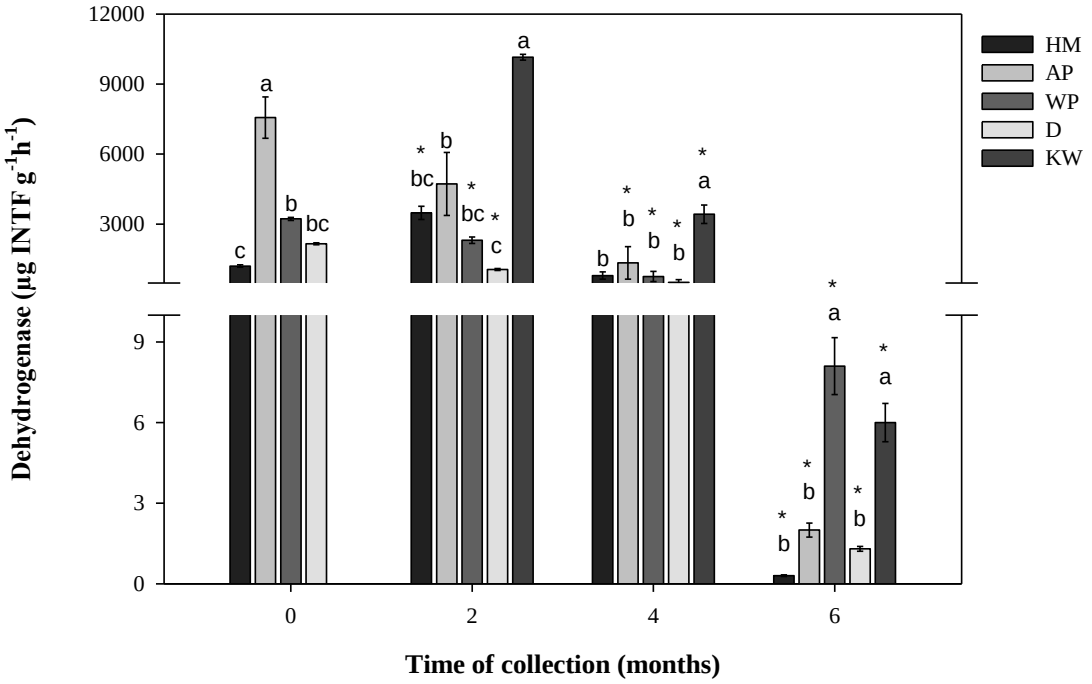

Fig. 7 Dehydrogenase activity during vermicomposting process in different types of organic wastes. Data with the same letter are not significantly different between treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each vermicomposting treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.3.2. β-Glucosidase

The quantification of β-glucosidase activity revealed significant changes in each vermicomposting treatment at different time of collection during the vermicomposting process (**Fig. 8**). In the case of HM vermicomposting treatment, it was found a decrease after 2 and 4 months in the β-glucosidase activity respect to initial time. Meanwhile, AP did not show any modification in the β-glucosidase activity at all time of collection. However, WP vermicomposting treatment increased β-glucosidase activity at 2 and 4 months respect to initial time, whereas no significant changes were found after 6 months. In the case of D and KW vermicomposting treatments, the quantification of β-glucosidase did not reveal any significant change during the vermicomposting process (**Fig. 8**).

Significant changes were detected in the β-glucosidase activity between different vermicomposting treatments at all time of collection (**Fig. 8**). At initial time, HM and WP showed a higher activity compared with AP and D vermicomposting treatments. However, no significant changes were observed after 2 months, except in the case of WP in which the activity was found significantly increased compared to HM, AP, D and KW vermicomposting treatments (**Fig. 8**). Likewise, at 4 months WP showed an enhancement in the β-glucosidase activity compared with HM, AP, D and KW vermicomposting treatments. However, in HM this activity was greatly increased respect to AP, D and KW vermicomposting treatments, whereas no significant differences were found between AP, D and KW vermicomposting treatments (**Fig. 8**). In the last sampling time (6 months), the higher β-glucosidase activity was found in HM vermicomposting treatment. Meanwhile, WP was significantly different respect to D vermicomposting treatment. No changes in the β-glucosidase activity were detected between AP, D and KW vermicomposting treatments (**Fig. 8**).

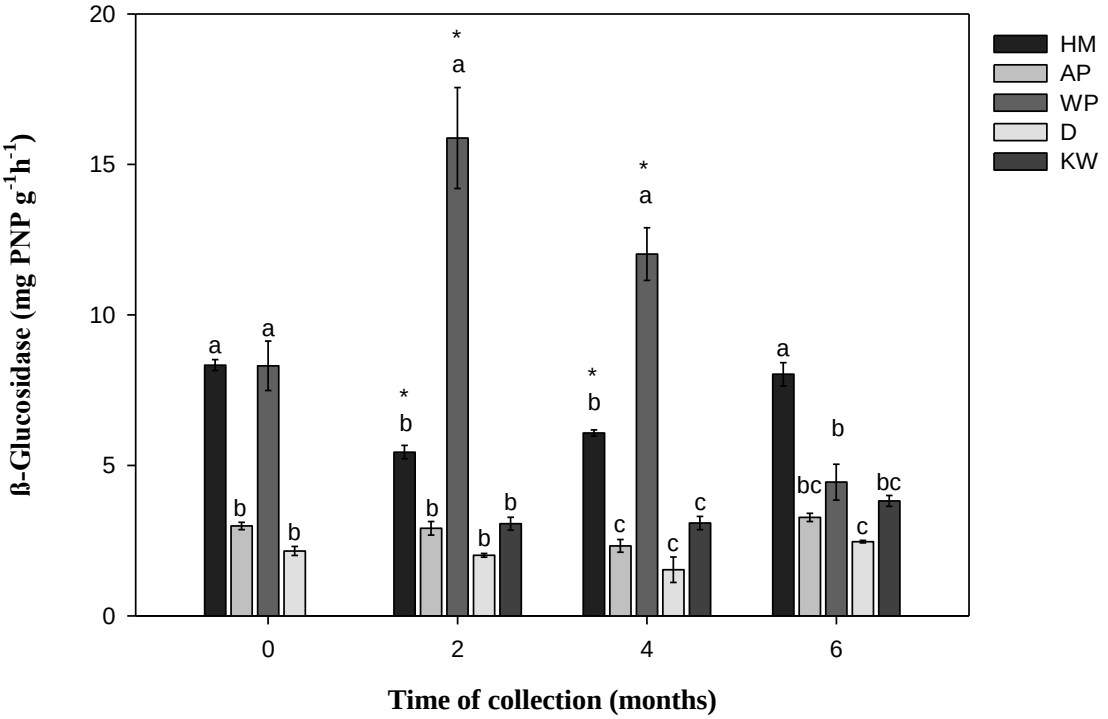

Fig. 8 β-Glucosidase activity during vermicomposting process in different types of organic wastes. Data with the same letter are not significantly different between treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each vermicomposting treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.3.3. Phosphatase

Changes in the phosphatase activity were found in each vermicomposting treatment at different time of collection during the vermicompost process (**Fig. 9**). This activity was significantly increased in HM after 4 and 6 months respect to initial time, meanwhile in AP and WP vermicomposting treatments was not found any significant change at all time of collection. However, in D and KW vermicomposting treatments the phosphatase activity increased significantly in the last time of collection (6 months) (**Fig. 9**).

The phosphatase activity quantified in HM, AP, WP, D and KW vermicomposting treatments did not show any statistical difference among themselves at initial time (**Fig. 9**). However, a significant increase was observed in AP and WP after 2 months respect to HM, D and KW vermicomposting treatments (**Fig. 9**). Likewise, after 4 months the phosphatase activity showed an enhancement in HM, AP and WP compared with D and KW vermicomposting treatments (**Fig. 9**). Finally, the phosphatase activity was strongly increased after 6 months in HM respect to AP, WP, D and KW vermicomposting treatments. Contrary, the lowest activity was found in WP, whereas AP and KW showed a great enhancement of phosphatase activity respect to D and no differences were observed between AP and KW vermicomposting treatments (**Fig. 9**).

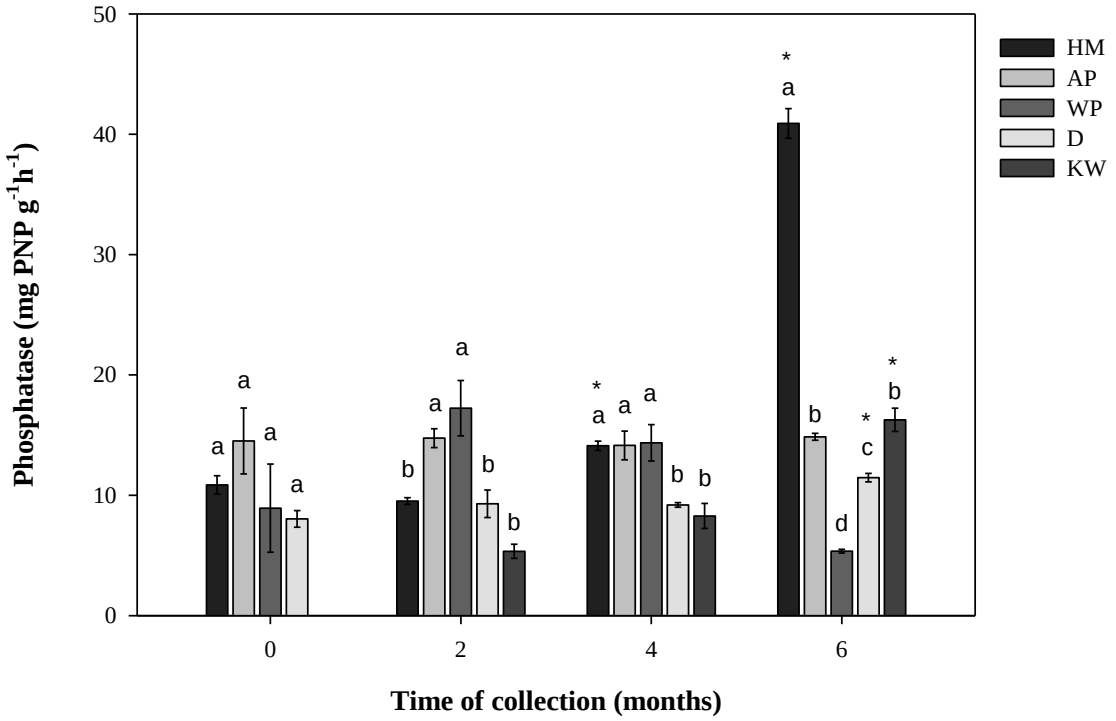

Fig. 9 Phosphatase activity during vermicomposting process in different types of organic wastes. Data with the same letter are not significantly different between treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.3.4. Protease

The protease activity quantified in all vermicomposting treatments (HM, AP, WP, D, and KW) revealed changes at different time of collection during the vermicomposting process (**Fig. 10**). HM were significantly reduced after 2 months of vermicomposting, whereas an enhancement in this activity was found after 6 months. Meanwhile, protease activity observed in AP vermicomposting treatment did not change at any time of collection. In the case of WP, it was observed the increase in the protease activity at 2 and, on the contrary a decrease in activity at 6 months respect to the initial time. The quantification of protease activity in D showed a reduction after 6 months, whereas after 4 and 6 months it was observed an enhancement in KW vermicomposting samples (**Fig. 10**).

Significant differences were found between vermicomposting treatments in each time of collection (**Fig. 10**). The quantification of the protease activity revealed that the higher activity was detected in D compared with HM, AP and WP vermicomposting treatments at initial time. Likewise, it was observed a great enhance in the protease activity in HM and WP vermicomposting treatments respect to AP, but no significant differences were found between HM and WP vermicomposting treatments (**Fig. 10**). Protease activity reached higher values in WP and D vermicomposting samples at 2 months, whereas KW vermicomposting treatment showed the lowest value for this activity. The protease activity observed in HM vermicomposting sample was higher compared with AP vermicomposting treatment (**Fig. 10**). After 4 months of vermicomposting, the highest protease activity was detected in HM and D vermicomposting samples, whereas no significant changes were detected in the protease activity in AP, WP and KW vermicomposting treatments (**Fig.10**). It was found that HM vermicomposting samples showed higher values in the protease activity respect to all vermicomposting treatments at 6 months. The lowest activity was found in WP vermicomposting sample, whereas it was observed similar values in the protease activity in D and KW vermicomposting treatments which were significantly different respect to AP vermicomposting sample (**Fig.10**).

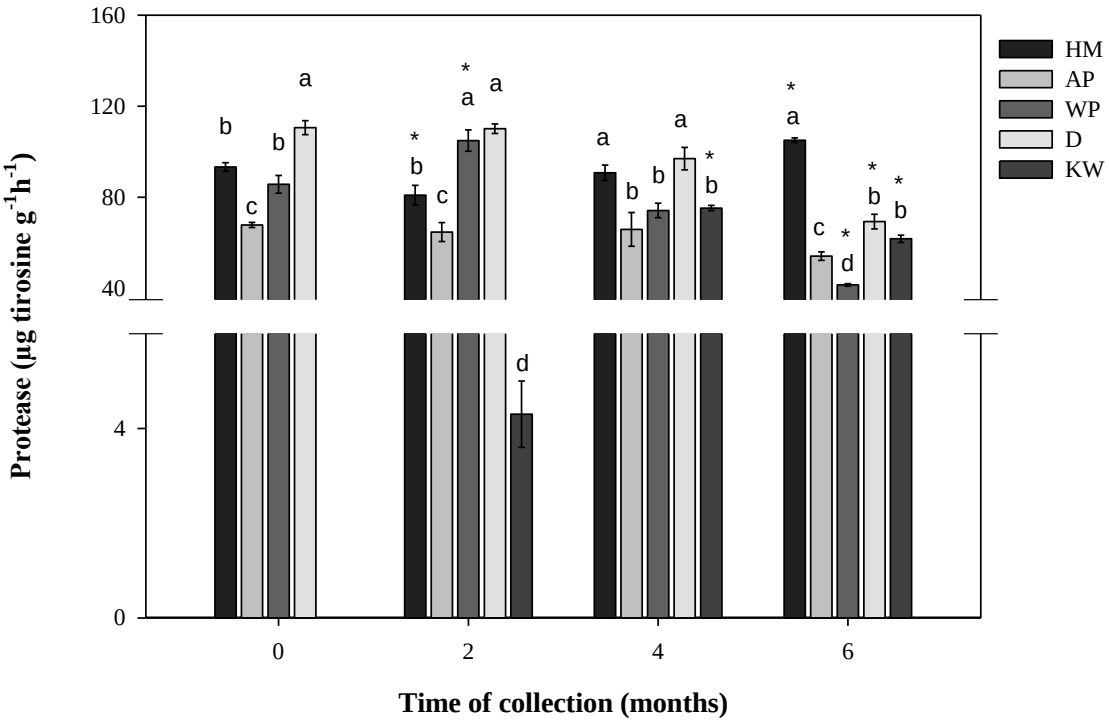

Fig. 10 Protease activity during vermicomposting process in different types of organic wastes. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

5.3.5. *O*-diphenol oxidase

Changes in the *o*-diphenol oxidase activity were found in each vermicomposting treatments at different time of collection during the vermicomposting process (**Fig. 11**). The activity detected in HM and AP vermicomposting samples were only increased after 2 months respect to initial time. Contrary, an increase in this activity was found in WP vermicomposting sample after 6 months. Meanwhile, in the case of D vermicomposting treatment, it was observed a decrease after 4 months, but this activity was significantly enhanced after 6months respect to initial time. No significant changes were found in the *o*-diphenol oxidase activity in KW vermicomposting sample at all time of collection (**Fig. 11**).

The *o*-diphenol oxidase activity analysed in D vermicomposting sample were highly increased compare with AP and WP vermicomposting treatments at initial time, but not respect to HM. However, this activity showed similar values to those found in AP and WP vermicomposting treatments (**Fig. 11**). On the other hand, it was not found significant changes in the *o*-diphenol oxidase activity after 2 and 4 months between vermicomposting samples (**Fig. 11**). Nevertheless, a significant increase in this activity was detected in WP vermicomposting sample compared with HM, AP, D and KW at 6 months. AP vermicomposting sample was negatively different compared with KW, whereas no differences were observed between HM, D and KW vermicomposting treatments. (**Fig. 11**).

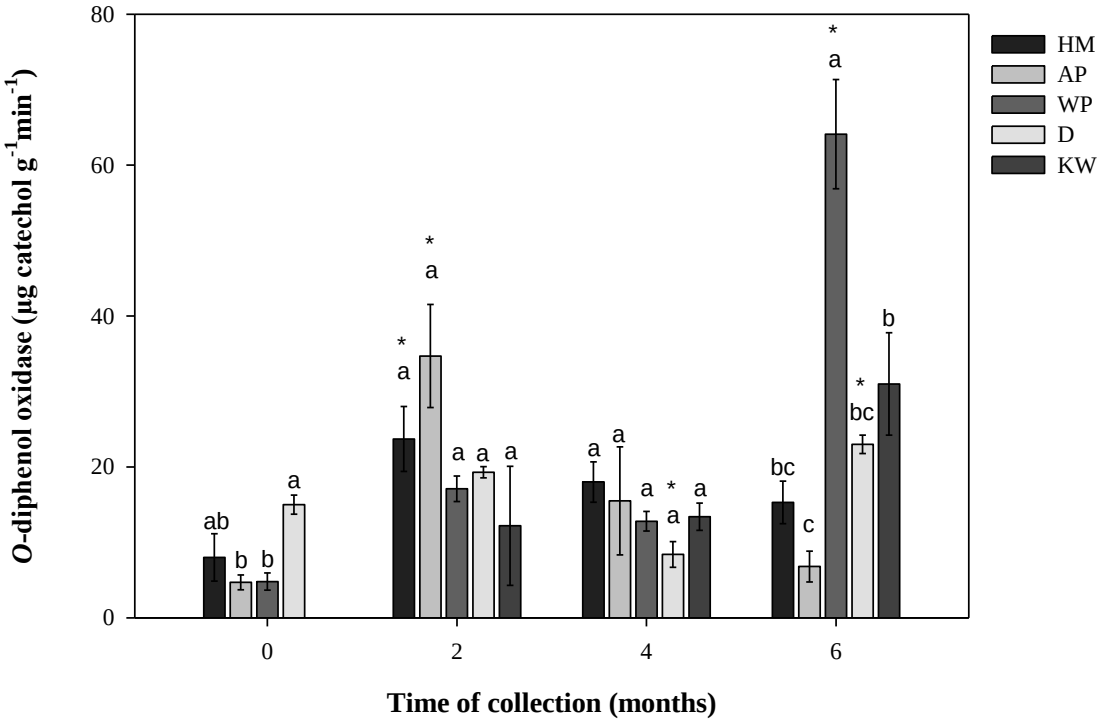

Fig. 11 *O*-diphenol oxidase activity during vermicomposting process in different types of organic wastes. Data with the same letter are not significantly different between vermicomposting treatments in each time of collection ($P < 0.05$). Asterisks indicate significant differences in each treatment respect to initial time (2 months in the case of KW) at different time of collection. Bars represent standards errors.

6. DISCUSSION

6.1. Earthworm development

In this experiment was observed that the application of fresh organic waste every two weeks provoked the maximum values in **earthworm biomass** in HM, WP and D vermicomposting treatments after 4 months. This result is in agreement with previous finding observed by Garg, et al. (2005), Nogales et al. (2005), Hanc and Vasak (2015). However, different trend in our experiment was observed in the case of AP and KW vermicomposting treatments, in which, the maximum were reached after 2 and 6 months, respectively. In a study performed by Hanc and Chadimova (2014) was found an enhancement in earthworm biomass after 4 months but in a non-continuous vermicomposting system. This result suggests that the conditions for earthworm growing are more optimal under a continuous feeding system compared with a non-continuous feeding system. In the case of KW vermicomposting treatment, the low earthworm biomass observed after 2 and 4 months of the process could be explained by high pH 8.6 detected in this waste. Higher values in the pH has been observed to have a negative influence on earthworm growing as previously was reported by Fernández-Gómez et al. (2010a). **Earthworm number** reached maximum values in HM vermicomposting treatment after 4 months indicating that at this time was found the optimal conditions for earthworm development. Similarly, earthworm number showed maximum values in AP vermicomposting treatments at 2 and 6 months following the same trend found in earthworm biomass. This result suggests that the chemical changes induced during vermicomposting process at this time of collection favouring the earthworm growth. However, some contradictory results in the earthworm number were found in WP, D and KW vermicomposting treatments since this parameter was dramatically reduced, especially after 6 months. This finding may be associated with changes in some chemical parameter such as pH, EC and NH_4^+ content, which has been found lethal for earthworms (Edwads, 1988). The increase in NH_4^+ content can be connected with higher ratio of organic waste mineralization at the end of vermicomposting process. It is important to highlight, that under alkaline pH, the NH_4^+ can be converted to NH_3 increasing its toxicity (Fernández-Gómez et al., 2010a). Nevertheless, a further study analysing specific parameter of earthworm growth and reproduction is need in order to unravel in which way changes in the chemical composition of vermicomposting samples can interfere in the earthworm development during the vermicomposting of these organic wastes (WP, D and KW).

6.2. Chemical parameters during vermicomposting

The **pH** value, as has been previously mentioned, is connected with processes of organic matter transformation. In general, the pH found in all organic waste tested was alkaline, but it was observed differences according to the organic waste and time of collection were found during the vermicomposting process. Thus, the minimum values in this parameter were detected in AP and WP at initial time, probably due to the presence of many phenolic compounds in their chemical composition. Generally, HM and D showed a decline in the pH value during the vermicomposting process, whereas AP, WP and KW treatment increased this parameter. Changes in the pH values during vermicomposting may be associated to the mineralisation of nitrogen and phosphorus into nitrites/nitrates and orthophosphates as well as to the bioconversion of the organic material into intermediate species of organic acids, as previously was reported by Garg et al. (2006) However, the enhancement in the pH values at the end of the vermicomposting process was observed in all types of organic wastes and it was probably associated to the formation of NH_4^+ as the results of the proteins mineralization at the end of vermicomposting process (Pramanik et al., 2007). These results are in line with previous findings in which the pH value increased up to 10.4 after 210 days of tomato fruit waste vermicomposting in a continuous-feeding vermicomposting system (Fernández-Gómez et al., 2010a).

The **EC** reflects the releasing of free ions and minerals that are generated through the ingestion and excretion processes by the earthworms during the decomposition of organic substances in a vermicomposting process (Garg et al., 2006). In our experiment, an enhancement in the EC parameter was only observed in the case of KW vermicomposting treatment. A similar finding has been observed during the vermicomposting of tomato fruit wastes in a continuous feeding system in which were observed values up to 4.4 mS cm^{-1} (Fernández-Gómez et al., 2010a). However, in the case of HM, AP, WP, and D vermicomposting treatments were observed a reduction in the EC values at the end of the vermicomposting process. The content in organic salts is also very crucial for the survival of earthworms but levels lower than 0.5 % are considered acceptable for vermicomposting systems (Edwads, 1988; Domínguez and Edwards, 2011a). Moreover, in these vermicomposting treatments were found the maximum levels in earthworm biomass and number showing that probably EC values between 0.4 – 1.7 mS cm^{-1} may be optimal for earthworm development.

Epigeic earthworms are known to accelerate the rate of organic matter decomposition during vermicomposting, thereby leading important losses of **TOC** throughout this

biotransformation process (Garg et al., 2006; Domínguez et al., 2010). In our study a decline in the TOC content was observed in HM and AP vermicomposting treatments after 2 months. Similar result was found during vermicomposting of vegetable greenhouses residues (Fernández-Gómez et al., 2010b). However, WP and D vermicomposting treatments increased the content in TOC at the end of process, and KW treatment did not modify this parameter. This result can be explained by the fact that new fresh material was added during continuous-feeding vermicomposting. Also, this result is in line with previous finding found by Fernández-Gómez et al. (2010a). It was also determined the **total soluble C** in order to analyse differences in some soluble organic compounds. Soluble acids C are one of the most readily biologically active parameters able to define the stability of vermicompost since it represents the easily metabolized organic matter (Campitelli and Ceppi, 2008). In our study, the quantification of total soluble C in all types of vermicomposting treatments was highly increased after 4 and 6 months, which suggests that probably during the process of organic matter transformation are being produced some organic C compounds such as acid fulvic and/or humic which could be involved in organic matter stabilization. Similar results has been previously observed by Li et al. (2011) and Romero et al. (2007) in different types of organic wastes. However, in this experiment it is need to perform a deeply study for the assessment of changes in fulvic and humic acids production during vermicomposting process.

Earthworms have a great impact on N transformations in organic wastes, moreover, their activity enriches the N levels of vermicompost through addition of mucus and secretion into the material (Atiyeh et al., 2000; Yadav and Garg, 2009). At the end of vermicomposting process, **total soluble N** did not considerable change in all types of organic waste, except in the case of KW vermicomposting treatment in which an enhancement was found. In general the final content of N in vermicomposting is dependent on initial nitrogen present in the waste and the extent of decomposition. Earthworm activity enriches the N profile of vermicompost through microbial mediated nitrogen transformation, through addition of mucus and nitrogenous wastes secreted by earthworms. Changes in pH may be an important factor in N retention as N_2 is lost as volatile ammonia at high pH values (Khwairakpam and Bhargava, 2009). Nevertheless, during the vermicomposting process of HM and AP was observed a decline in total soluble N. In this two treatments were found higher earthworm biomass and number which suggest this reduction in N content may be associated to processes of transformation conducted by the earthworm and microbial activity. Meanwhile, in the case of D treatment was found an enhancement, probably caused by the release of excretory products,

mucus and enzymes by earthworms which can contribute an increase the level in N (Yadav and Garg, 2009).

Vermicompost mainly present higher content in **N-NO_3^-** than **N-NH_4^+** (Edwards et al., 2011). In our study, HM, D, and KW vermicomposting treatments was found higher values in N-NO_3^- respect to N-NH_4^+, whereas AP and WP treatments followed the opposite trend. The content in N-NO_3^- was increased in AP and KW vermicomposting treatments probably due to an enhancement in the nitrification process as consequence of earthworm decomposition after 6 months. Meanwhile, the decrease found in this parameter in the case of HM and D vermicomposting treatments may be associated to denitrification process and/or immobilization of N in microbial biomass (Atiyeh et al., 2000). The overall highest values in N-NO_3^- content found in D vermicomposting treatment respect to other treatments may be related to higher levels in N, especially in NH_4^+, found in the initial composition of this organic waste (Tambone et al., 2015). In the case of N-NH_4^+, it was found that HM and D vermicomposting treatments showed higher values in this parameter at the end of the process probably as consequence of a higher mineralization of N organic. A study conducted in a continuous-feeding vermicomposting system reported an increase in NH_4^+ content after 210 days in tomato-fruit waste (Fernández-Gómez et al., 2010a). Meanwhile, lower values in N-NH_4^+ content can be related to higher pH values found in AP, WP and KW vermicomposting treatments at the end of the process which could contribute to a loss of NH_4^+ by volatilization.

6.3. Enzymatic activities during vermicomposting

Some enzymatic activities are correlated with overall microbial activity, soil fertility, plant growth and plant disease resistance (Edwards et al., 2011). During vermicomposting, earthworms selectively enhance some enzymatic activities (Pramanik et al., 2007). In this study it was evaluated the activity of dehydrogenase, β-glucosidase, phosphatase, protease and *o*-diphenol oxidase. The quantification of these enzymatic activities may be one of the key analyses for determining the overall microbial activity in vermicompost (Edwards et al., 2011).

Dehydrogenase is considered an indicator of overall microbial activity because it occurs intracellularly in all living microbial cells and is involved in microbial respiratory process (Masciandaro et al., 200). In our experiment, all tested organic wastes showed higher values in dehydrogenase activity at initial time. However, this activity was continuously decreased at 4 and 6 months of vermicomposting, reaching values up to 8.1 - 0.3 μg INTF $g^{-1}h^{-1}$ at the end of the vermicomposting process. Similarly, Fernández-Gómez et al. (2010a) detected the highest activity in tomato-fruit wastes after 120 days which decline after 210 days in a continuous-feeding vermicomposting system. These results suggest that the dehydrogenase activity may be responsible of higher values found in total soluble C, which could be related to the formation of soluble C substances during the last stages of vermicomposting process. On the other hand, it was found a positively relation between the earthworm biomass and the dehydrogenase activity, and it was demonstrated that fresh earthworm castings released into the vermicompost substrate have higher microbial activity and viable cell numbers (Parhasarathi and Ranganathan, 1999). Thus, the dehydrogenase activity could be good indicator of microbial activity enhanced by earthworms. The extremely lowest values in the dehydrogenase activity detected after 6 months of vermicomposting process may indicate the organic matter stabilization in each organic wasted used and as consequence a decrease in the microbial activity.

β-Glucosidase catalyses the hydrolysis of cellobiose and other disaccharides and play a key role in the decomposition of organic C compounds (Benitez et al., 1999). Levels of this activity are determined by presence of readily metabolizable substrates (Vargas-García et al., 2010). In our study, it was found differences in the β-glucosidase according to organic waste tested. Generally, it was observed a reduction in the activity of this enzyme in HM vermicomposting treatment during the vermicomposting process (2 and 4 months), but at the last stage it was recovered. This finding can be related to a decrease in available organic substrates as previously has been shown by (Benitez et al., 1999). In the case of AP, D, and KW vermicomposting

treatments any change was observed in the activity of this enzyme. Levels of this activity are determined by the presence of readily metabolizable substrates. Thus, during the vermicomposting of these organic wastes the presence of such compounds may be relatively low. Nevertheless, the high content in soluble organic compounds were observed in these treatments may be due to the positively relation found between dehydrogenase and total soluble C. This finding suggests that probably the dehydrogenase could be involved in the processes of degradation of organic matter, instead of β-glucosidase, as previously has been observed by Benitez et al. (2005). On the other hand, WP vermicomposting treatment increased the β-glucosidase activity, but at the end of the vermicomposting it was found a decline. This finding could suggest that the enhancement in the β-glucosidases activity may be probably attributed to high content in carbohydrates and sugars contained in winery wastes. In addition, β-glucosidase could be presumably also associated to the high earthworm biomass found in this treatment. It was observed higher carbohydrase activities in earthworms casts grown in organic wastes, as previously was reported by Parhasarathi and Ranganathan (1999). Therefore, β-glucosidase activity, which is easy and inexpensive to analyze, appears to be useful to monitor the earthworm population while avoiding the laborious earthworm biomass determination (Fernández-Gómez et al., 2010a).

Phosphatase catalyses the hydrolysis of organic phosphorus compounds to an inorganic phosphate form, which can be taken up by plants (Vargas-García et al., 2010). The phosphatase activity was higher in HM vermicomposting treatments at 4 and 6 months, and only after 6 months in the case of D and KW. Similar results were found in vermicomposting of tomato-fruit waste in a continuous feeding system (Fernández-Gómez et al., 2010a). This result could suggest the presence of residual amounts of organic-phosphate compounds, which may act as inductor of phosphatase activity. On the other hand, WP vermicomposting treatment showed a reduction in this activity after 6 months. In this case, it was found that WP vermicomposting treatment showed higher values in pH compared with other treatments after 6 months. In a previous study performed by Fernández-Gómez et al. (2010a) it was observed a decline in the phosphatase activity at higher pH levels close up to 9, which is not considerably an optimal pH for this enzyme (4.5 – 6). In this case, the quantification of phosphatase activity may not be a good indicator of quality in organic wastes derived from winery industry. In the case of AP vermicomposting treatment no changes were detected in the phosphatase activity during vermicomposting process. This result suggests that probably during the transit of materials through the earthworm gut during the vermicomposting of AP produced the higher degradation organophosphorus compounds as previously has been observed by Garg et al. (2012).

Protease activity is closely related to the N cycle and catalyses the hydrolysis of pectidics bound in proteins. It is considered to be good indicator of organic matter decomposition (Gupta et al., 2014). In our study, the protease activity was decreased at 2 months, but after 4 months it was found a slight enhancement in HM vermicomposting treatment. In this case, the protease activity could be involved in the hydrolysis of N organic compounds which may determine the enhancement found in the $N\text{-}NH_4^+$ content after 6 months. AP vermicomposting treatment did not alter the protease activity during the vermicomposting process. However, in WP vermicomposting treatment the activity increased at 2 months, but it was observed a progressive decline along the vermicomposting process. This finding may be associated with the low content in $N\text{-}NH_4^+$ observed at this time of collection which could indicate that $N\text{-}NH_4^+$ after the hydrolysis of organic compound must be transform into $N\text{-}NO_3^-$ by mineralization. In the case of D vermicomposting treatment was showed a reduction in protease activity after 6 months. Protease was reported as a very good indicator of the level of maturity of a substrate, thus the significant decrease at the last stage of vermicomposting may indicate high degree of decomposition (Aira et al., 2007). However, KW vermicomposting treatment increased the protease activity after 4 and 6 months. In this case, it was found a negative relation between the content in $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ which may suggest that probably a nitrification processes is involved in the transformation of $N\text{-}NH_4^+$ to $N\text{-}NO_3^-$ Moreover, it has been observed that the earthworms death could release peptides stimulating protease activity which could potentially influence the protease patterns (Fernández-Gómez et al., 2010a)

The ***o*-diphenol oxidase** catalyses the oxidation of a phenolic compound and is directly involved in the humus substances formation. Therefore this enzymatic activity is implicated in the humification process (Perucci et al., 2000; Benitez et al., 2004). HM and AP vermicomposting treatments increased in *o*-diphenol oxidase activity at 2 months but a progressive decline was detected at the end of the vermicomposting process. As mention above, this enzyme participate in humification process and hence, higher levels of activity could reflect an increase in humification of these types of vermicompost at this time. Other trend was observed in the case of WP and D in which the *o*-diphenol oxidase activity increased at the last stage of vermicomposting. This was probably due to presence of phenolic compounds in organic waste which could increase the activity of this enzyme activity. Grapes and wine contain high amounts of phenolic compounds, mainly flavonoids. Likewise, residues of wine production are also characterised by high contents of phenolic compounds due to an incomplete extraction during wine production (Rockenbach et al., 2011). Similarly, Levén et al. (2006) reported that the phenol content in D is mainly dependent on the feedstock composition in the

anaerobic digestion process. KW vermicomposting treatment did not show significant changes in this activity during the process. However, KW is mainly composed by fruits, vegetables and beverages which are rich in phenolic compounds, thus the stimulation in the *o*-diphenol oxidase activity could be expected (Balasundram et al., 2006). In addition, the decline in the earthworm numbers after 6 months in these organic wastes could also cause an increase in *o*-diphenol oxidase activity. According to Castillo et al. (2013) *E. fetida* has the capability to bio-accumulate high amounts of harmful chemicals (including phenol) in their tissues and either biodegraded or biotransformed by *o*-diphenol oxidase enzymes, which are involved in the formation of melanin, a key compound in cellular pathogen defence. This finding could affect the activity of this enzyme during the vermicomposting process. On the other hand, the passage of organic compounds through the earthworm gut may influence process of phenolic compounds stabilization by the stimulation of the microbial activity in castings (Castillo et al., 2013).

7. CONCLUSION

- The vermicomposting of organic wastes such as HM and AP in a continuous-feeding system favoured the growth and development of earthworm whereas in the case of WP, D and KW were seriously affected.
- The continuous-feeding system favoured optimal changes in pH value only for HM and D, whereas in the rest of the vermicomposting treatments (AP, WP and KW) was relatively high.
- The EC values were positively influenced by vermicomposting in a continuous- feeding system in all types of organic wastes.
- The transformation of organic matter presented was highly favoured during the vermicomposting of HM and AP in a continuous-feeding system compared with WP, D and KW. Moreover, it was found that this system of vermicomposting stimulated the enhancement in the total soluble C in all vermicomposting treatment.
- Total soluble N was positively affected during the vermicomposting of KW in a continuous-feeding system. Inorganic N forms were favoured in HM, D and KW treatments, whereas in AP and WP vermicomposting treatments nitrification processes were predominant.
- The dehydrogenase activity showed to be good and sensitive indicator of organic matter stabilization in all tested organic wastes, especially in the in the case of AP and KW.
- β-Glucosidase activity was not an useful microbial activity indicator in all organic wastes tested except in the case of WP.
- Phosphatase activity showed a high sensibility to high pH in the tested material, therefore it was not confirmed to be precise detector for monitoring vermicomposting process in organic wastes such as WP.
- In a continuous-feeding system, the protease activity indicated the stabilization of different organic wastes such as WP, D and KW, but not in the case of HM and AP.
- The *o*-diphenol oxidase activity can be considered a good indicator for monitoring vermicomposting processes of organic wastes rich in phenolic compounds since in our study it was found that this activity was influenced in materials such as AP, D and WP which have a high content in phenolic compound.

8. LIST OF ABBREVIATIONS

AP	apple pomace
C	total soluble carbon
CAPs	*cast-associated processes*
CM	cow manure
C:N	carbon-to-nitrogen ratio
CO_2	carbon dioxide
C_{org}	organic carbon
D	digestate
E. andrei	*Eisenia andrei*
E. fetida	*Eisenia fetida*
EC	electrical conductivity
EU	European Union
Fig.	figure
GAPs	*gut associated processes*
HM	horse manure
INT	iodotetrazolium violet
INTF	iodonitrotetrazolium formazan
KW	kitchen waste
N	total soluble nitrogen
NH_4^+	ammonium
N-NH_4^+	ammonium nitrogen
N-NO_3^-	nitrate nitrogen
NO_2^-	nitrite
NO_3^-	nitrate
N_{tot}	total nitrogen
sp.	**s**pecies
TOC	total organic carbon
v:v	volume to volume
w:v	weight to wolume
WP	wine pomace

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