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ORIGINAL RESEARCH

Bioconversion of agro-industrial wastes: Combined compost and vermicompost processes using *Eisenia fetida* for stabilization of poultry litter

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Abstract

Purpose A combined treatment of composting and vermicomposting of poultry litter (PL), rice hulls (RH) and/or eucalyptus sawdust (ES) was carried out in order to obtain a high-quality organic fertilizer and avoid the environmental problems associated with the use of raw poultry manure and fresh agricultural waste materials.

Methods Three composting mixtures were made at volume proportions: 1:3 RH/PL (M1); 1:2 ES/PL (M2) and 0.5:1:2 RH/ES/PL (M3). Composting (120 days) followed by vermicomposting (90 days) using earthworms (*Eisenia fetida*) were conducted during the autumn-winter season, outdoors and sheltered. Moisture content was kept at 60-70%. Physicochemical (pH, electrical conductivity, lignin, total carbon and nitrogen, organic matter, ammonium and specific cations), total microbial activity (fluorescein diacetate hydrolysis) and microbiological parameters were recorded throughout both processes. Phytotoxicity tests were lastly performed by means of *Lactuca sativa*, L. seed germination, to compare the quality of the organic fertilizers obtained.

Results A sequential compost-vermicompost process enhanced the properties of the final products. Particularly, electrical conductivity was markedly lower in all vermicomposts (1.81-2.28 mS cm^{-1}) and within the values recommended for the growth of sensitive plants; microbial activity reached 187.1-203.8 µg FDA g_{soil}^{-1} h^{-1}. Germination Index values in vermicomposts were greater than 60%, indicating the high quality of the products obtained, being the mixture 0.5RH:1ES:2PL, the one which showed better quality and higher maturation degree.

Conclusion The system that combines both processes (composting + vermicomposting) was effective to produce a stabilized organic fertilizer from poultry litter with other waste organic materials.

Keywords Organic wastes, Earthworms, Cold season vermicomposting, Physicochemical parameters, Microbial activity, Germination index

Introduction

Animal manures have been used as natural crop fertilizers for centuries since they are a source of essential plant nutrients such as nitrogen, phosphorus, potassium, and organic matter (Saleem et al. 2018). Because of poultry manure's high nitrogen content, it has long been recognized as one of the most desirable manures which helps improve the soil's moisture and nutrient retention (Davis et al. 2017).

Poultry litter (PL) is a mixture of fecal and urinary excretions of poultry, including feathers, wood shavings, sawdust, rice hulls, straw and other bedding material for the disposition of manure. In Argentina, the poultry litter generated could be estimated in 1.3-1.6 million tons/year (Global broiler meat production 2019).

Land application of PL returns nutrients and organic matter to the soil, building soil fertility and quality; however, excessive application of PL to soils in the long term often increases the P transfer to nearby water bodies and causes eutrophication. In most cases, eutrophication restricts water use for fisheries,

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recreation, drinking, and industry due to the increased growth of undesirable algae, oxygen shortages, and formation of carcinogens during water chlorination (Sharpley et al. 2004).

Horticulture in Argentina is an important activity of great social and economic value that represents a valuable complement to the traditional primary productions (Castagnino et al. 2011). The province of Santa Fe participates with 8% of the cultivated area of vegetables in Argentina (Lozano 2012). A common practice to fertilize the horticultural crops is using fresh PL, which otherwise accumulates in the same area without previous treatment. The environmental problems associated with the use of fresh poultry manure could be mitigated by stabilizing its nutrient and organic matter contents by composting with agricultural wastes before its application to soils (Saleem et al. 2018).

Composting and vermicomposting are procedures to remove toxic substances and stabilize organic wastes. The rendered products, composts and vermicomposts, can be used as substrate or substrate constituents for soilless culture or to amend soils with organic matter (Mendoza-Hernandez et al. 2014). Composting is the aerobic process in which indigenous microorganisms (thermophile and mesophile) sanitize, decompose and stabilize the organic material. Vermicomposting is the process by which earthworms are used to convert organic matter (usually wastes) into a humus-like matter (Gupta and Garg 2009). The vermicomposts obtained are finely divided peat-like materials with the high porosity, good aeration, drainage, water-holding capacity, and very high microbial activity, which make them excellent as soil amendments or conditioners and as plant growth media (Tejada and González 2009).

Valuable results have been obtained by Leconte et al. (2009) regarding composting efficiency of different proportions of rice hulls, sawdust and poultry manure. Rice hulls and sawdust are wastes generated from timber and rice agro-industries with the following properties: high C/N ratio (106-109), high cellulose (57.4-59%) and lignin (20.9-24.1%) content, and very low nutrient concentrations. Thus, this kind of wastes is appropriate for composting in mixtures with materials rich in N and easily degradable C as animal manures (Leconte et al. 2009), although no study includes the combination of composting followed by vermicomposting for these wastes.

Lazcano et al. (2008) showed that the combined treatment (composting + vermicomposting) was the most effective in terms of stabilization of the cattle manure. Moreover, earthworms promoted the retention of nitrogen and gradual release of P, as well as a reduction in electrical conductivity, thereby producing improved substrates for agricultural use. Also, some authors suggest that vermicompost stimulate higher soil microbial activity than composts (Tognetti et al. 2005; Fornes et al. 2012) and that the combination of the processes shortens stabilization time (Ndegwa and Thompson 2001).

The aim of this work is to introduce a vermicomposting stage for mixtures of poultry litter, rice hulls and/or sawdust following the conventional composting, in order to obtain a higher quality organic fertilizer and avoid the environmental problems associated with the use of raw poultry manure and fresh agricultural waste materials. The evaluation of the success of these processes in the least frequently reported cold season is also of major interest. This strategy would allow sustainable waste management of local raw materials and the recycle of the nutrients in agricultural field, which in turn maintain soil health.

Materials and methods

Waste organic materials and earthworms

Three compost mixtures were prepared from *Eucalyptus sp*. sawdust (ES), rice hulls (RH) and poultry litter (PL). These waste materials were locally supplied from different sites within the province of Santa Fe (Argentina), allowing their reutilization. ES was obtained from a commercial sawmill located in Helvecia, PL was kindly provided by a poultry farm located in San Agustín and RH from a rice mill nearby in Franck. Adult clitellated earthworms (*Eisenia fetida*) were employed as test model organisms, for the vermicompost stage. They were provided by the bioterium belonging to INTEC (Instituto de Desarrollo Tecnológico para la Industria Química).

Compost and vermicompost experiments

The experimental study was designed in two stages: (1) compost and (2) vermicompost. They were carried out within a pilot plant at INTEC installations, located in Santa Fe, Argentina (31° 38' 23.2" S, 60° 39' 59.8" W). The three compost mixtures were obtained mixing the individual waste organic materials in the following volume proportions: 1:3 RH/PL (M1); 1:2 ES/PL (M2) and 0.5:1:2 RH/ES/PL (M3).

These proportions were chosen taking into account the study of Leconte et al. (2009). Each of the mixtures

was placed within a plastic recipient of 45 L (24×35×54 cm) and located outdoors, sheltered, to avoid excess of moisture due to rainfall. Composting was carried out for 120 days, from April to July 2018 (Autumn-Winter). Moisture was surveyed following the method described by Rynk et al. (1992) and kept constant at 60-70% by manual watering. Weekly, the substrates were manually turned to improve aeration and homogenization of the mixtures and within the same time frame (1-2 PM), temperature and pH were registered in four fixed spots within the top 10 cm of the mixtures. Samples were also taken at 0 and 120 days for the rest of the physicochemical and biological characterization.

For the vermicompost stage, each composted mixture (after the 120 days) was combined with fresh soil (S) (30%), given three different vermicompost treatments: T1= M1+S, T2= M2+S and T3= M3+S. This incorporation was done for the sake of improving the performance of the earthworms. Amounts of 1 Kg of T1, T2 and T3 were placed within glass boxes (10×20×30 cm) and 20 clitellated *E. fetida* earthworms (mean body weight 0.26 ± 0.02 g) were added. The vermicompost was carried out for 90 days at room temperature (24 ± 3 ºC) and constant light. Moisture was also kept constant at 60-70%. This experiment was run in triplicate. Periodic monitoring (at 0, 15, 30, 45, 60, 75 and 90 days) of the survival (as number of living adults per total adults) and biomass (as wet weight, g ind^{-1}) of the earthworms was carried out. Samples were also taken at 0 and 90 days for the rest of the physicochemical and biological characterization.

Characterization of the individual materials and the mixtures

The following variables were investigated in the individual composting materials and monitored in the mixtures at the beginning and at the end of the composting and vermicomposting times: pH, electrical conductivity (EC), ashes, acid-insoluble lignin, total carbon (TC), total nitrogen (TN), organic matter (OM), fluorescein diacetate (FDA) hydrolysis, ammonium (NH^+_4-N) and Na, K, Mg and Ca cations. For all the assays, all chemicals used were of analytical grade and used as received.

Electrical conductivity (EC) and pH were determined from suspensions of the mixtures in distilled water in a proportion 1:10 w/v (Leconte et al. 2011). They were agitated mechanically at 200 rpm in an orbital shaker for 2 h, then centrifuged at 2860 g for 10 min and left undisturbed for 30 min. EC and pH were measured with pH and EC meters (HACH®HQd Field Case). The content of N-NH_4^+ was determined in the same water extract, filtered through a 0.45 µm pore diameter membrane, by the indophenol-blue method using an Uraemia kit (Wiener Laboratory) (Laos et al. 2002).

Ashes were determined on dried samples by calcination at 550 °C for 2 h and OM was obtained gravimetrically as the difference between the original dry weight and the ashes (Sharma and Garg 2018). Total C and N were obtained from elemental analysis of the samples (LECO CHN628 Series Elemental Determinators), while total P, Ca, Mg, Na, K elements were obtained on acid-extracted samples according to USEPA method 200.9 (USEPA 1994). P was measured by the molybdenum blue method (Murphy and Riley 1962) and the other elements by flame atomic absorption spectroscopy using a Pekin Elmer AAnalyst 800 atomic absorption spectrometer (AA) equipped with hollow cathode lamps (HCLs) for each element.

The content of acid-insoluble lignin (Klason) was assessed in the individual materials and during the compost stage. Samples were first dried at an appropriate temperature to avoid lignin decomposition, then ground and screen-sieved. The fraction of particle <500 µm was selected. The lignin content was determined in accordance to TAPPI methods (TAPPI 2002) on extractives-free samples. These were previously obtained with a 24-cycles extraction in a Soxhlet apparatus, using a mixture of chloroform-ethanol as solvent (Antczak et al. 2006).

FDA hydrolysis, as a measure of the total microbial activity, was determined according to Adam and Duncan (2001) with some modifications. To 1 g of sample and 6 mL of phosphate buffer pH 7.6, an amount of 0.1 mL of FDA stock solution was added to start the reaction. The tubes were then incubated at 25 °C for 1 h; afterwards, 6 mL acetone was added to stop the reaction. After centrifugation at 450 g, the supernatant was filtered and FDA concentration was calculated from the absorbance measure at 490 nm (Perkin-Elmer Lambda 35 UV-Vis spectrophotometer) and a fluorescein calibration curve.

Presence of total and fecal coliforms, *Salmonella sp*. and *Escherichia coli* was also investigated in the mixtures at the beginning and end of the composting time to identify health risk. Total and fecal coliforms were determined from the broth macro dilution test according to methods 9221B and 9221E, respectively (APHA et al. 1998), while presence of *Salmonella sp*. and *E. coli* was evaluated by plate counts in agar.

Phytotoxicity tests

The phytotoxicity and maturity of the final compost and vermicompost products (M1, M2, M3, T1, T2 and T3) were assessed by means of seed germination bioassays. They were performed according to USEPA (1996) after an aqueous extraction of the samples. An amount of 4 g of each compost or vermicompost sample was placed into a 50 mL Falcon tube, 40 mL of distilled water was added and then mechanically shaken at 200 rpm for 60 min at room temperature. Then, the samples were left to settle at room temperature for 1 h and the aqueous extracts were subsequently used. Two different dilutions of each sample were evaluated: 25% and 50%, as it is widely known that pure extracts are not used for this kind of assays. Twenty *Lactuca sativa*, L. seeds were sown over germination paper that was placed at the bottom of a Petri dish (9.1 cm diameter) embedded with 4 mL of each dilution. In addition, a control employing only distilled water was used. The samples were run in quintuplicate (n=100). Petri dishes were incubated at 23 °C in the dark for 5 days. After this period, the root length of the germination seeds was measured and the percentage of Germinated seeds relative to the control (G) and the Germination Index (GI) were evaluated. These parameters were analysed according to Zucconi et al. (1985) and calculated employing the following equations:

$$G = (G_s - G_c) \times 100 \quad (1)$$

$$IG = (G_s/G_c) \times (L_R/L_{RC}) \times 100 \quad (2)$$

where G_s is the number of germinated seeds in the sample; G_c ,the number of germinated seed in the control; L_R, the average root length in the sample and L_{RC},the average root length in the control.

Statistical analyses

Physicochemical parameters are presented as mean values of at least two independent measurements, and their standard deviations (SD) are calculated and reported. The data obtained were subjected to analysis of variance (ANOVA, with a 95% confidence level) and a means separation was conducted using Tukey Test ($P \leq 0.05$). Statistical analyses were conducted using IBM SPSS Statistics version 24.

Results and Discussion

Characterization of the individual materials

Table 1 shows the physicochemical characteristics of the individual composting materials. Hygroscopic behavior of wood led to the highest humidity percentage for ES. Moreover, the measured chloroform-ethanol extractives gave values of 1.04 ± 0.07 % (RH), 0.35 ± 0.07 % (ES) and 2.8 ± 0.5 % (PL) The lowest content of these extractives was associated with the highest moisture content, in accordance with the findings of Jankowska et al. (2017). Alkaline pH obtained for PL was in direct relation to the higher amount of all cations measured, especially Ca. PL showed higher nutrient concentrations regarding the content of nitrogen, P and K and a slightly lower content of TC. Similar results were obtained by Leconte et al. (2009) for the same materials.

Table 1 Physicochemical characteristics of individual materials

	RH	ES	PL
Moisture (%)	7.4 (0.2)	41 (0.5)	18.5 (0.5)
pH	6.61 (0.02)	5.21 (0.03)	8.96 (0.02)
EC (mS cm^{-1})	0.68 (0.03)	0.09 (0.02)	5.71 (0.03)
Ashes (%)	17.9 (0.3)	0.7 (0.3)	18.7 (0.5)
Lignin* (%)	41.2 (0.1)	32.4 (0.6)	17.3 (1.4)
TC (%)	36.59(0.01)	35.6(0.3)	34.1(0.2)
TN (%)	0.45(0.01)	0.27(0.02)	2.33(0.12)
OM (%)	74.7 (0.5)	58.3(0.4)	62.8(0.6)
P (g Kg^{-1})	1.1(0.2)	0.8(0.2)	6.0(0.2)
Ca^{++} (%)	0.12(0.03)	0.21(0.05)	2.6(0.04)
Mg^{++} (%)	0.07(0.02)	0.02(0.02)	0.59(0.03)
Na^{+} (%)	0.04(0.02)	0.06(0.05)	0.52(0.05)
K^{+} (%)	0.27(0.06)	0.06(0.02)	2.03(0.05)

EC= electrical conductivity; TC= total carbon; TN= total nitrogen; OM= organic matter *Extractives-free basis, (. . .) Standard deviations

Regarding the lignin content, similar values to those obtained in this work for ES have been reported for *Eucalyptus oblique* (Trevorah et al. 2018) and clones of *Eucalyptus spp*. (Corradi Pereyra et al. 2013). Those obtained for PL were also found in accordance with the literature (Perondi et al. 2017). However, high values were obtained for RH in comparison to those generally reported by other authors (Chin-Pampillo et al. 2015; Leconte et al. 2009). This could be related to the different methods used in literature for its determination or an incomplete acid digestion due to the high resistance of the material, leading to an overestimation of this value. A dependency of the lignin content with the average particle size has also been reported (Leconte et al. 2011).

Evolution of composting mixtures

Determining how the main phases in composting take place is a key factor to follow the process. As observed in Fig. 1, at the beginning of the composting time (24-72 h), the temperature of the three mixtures varied

between 53-56 °C. These values were clearly higher than the maximum ambient temperature (28 °C); therefore, the systems were going through the thermophilic phase. After 7 days, temperature decreased and M2 and M3 began the mesophilic phase, with temperatures around 32 °C; while for M1, the beginning of this phase was delayed for another week. This behavior could be related to the higher proportion of PL in the mixture M1, thus this material would introduce microorganisms that extend the thermophilic phase. The brief duration of the thermophilic phase of the mixtures was possibly related to the typical ambient temperatures of the season and also to the amount of substrate used in the systems (less than 1 m^3). This phase is critical for the reduction of pathogens of the material because the elevated temperatures increase the population of thermophilic microorganisms and the rate of the biochemical reactions, thus contributing to the elimination of undesirable organisms (Lazcano et al. 2008). From the second week to the end of the composting time (week 17), temperatures oscillated between 13-28 °C, defining a prolonged mesophilic phase, where mesophilic microorganisms prevailed again. Possibly, in the last weeks (14-17) of composting, the mixtures began the maturation phase, with values closer to (maximum) ambient temperature (Fig. 1).

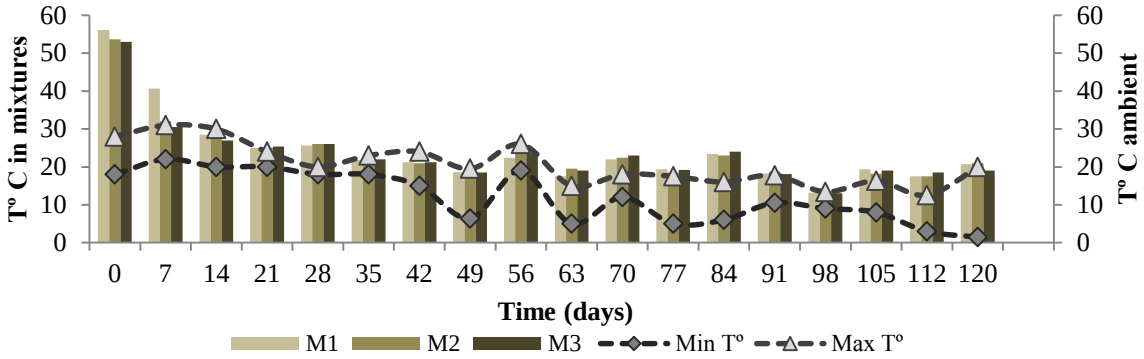

Fig. 1 Temperature profile of the mixtures M1, M2 and M3 during compost stage

The variations in the physicochemical characteristics of the three mixtures during the compost are presented in Table 2. The values of pH at the beginning were all similar and within the alkaline range. This suggests that the contribution of PL to this parameter is strong. There was no significant variation after 120 days ($P \leq 0.05$) although a small increment in M1 and M2 was experimentally observed. Initial electrical conductivity was similar in all mixtures, in the range of 3.26-3.70 mS cm^{-1}, and increased at the end of compost stage between 13 to 43% depending on the sample ($p \leq 0.05$). The highest values of EC and pH registered for M2 could be related to nitrogen compounds present in the PL, i.e. ammonia and ammonium ion (Rynk et al. 1992; Paterlini et al. 2017). The final EC values of the three composted mixtures allowed them to be classified as high-saline substrates (Şevik et al. 2018). This characteristic can, in turn, interfere with the growth of sensitive crops (Fornes et al. 2012).

Table 2 Physicochemical characteristics of mixtures at the beginning and end of the compost stage

	M1(RH/PL)		**M2 (ES/PL)**		**M3 (RH/ES/PL)**	
	Day 0	Day 120	Day 0	Day 120	Day 0	Day 120
pH	8.41 (0.03)	8.54 (0.02)	8.70(0.01)	9.01 (0.02)	8.29 (0.01)	8.17 (0.02)
EC (mS cm^{-1})	3.70(0.02)	4.18 (0.03)	3.59 (0.02)	5.14 (0.03)	3.26 (0.03)	4.37 (0.02)
Lignin* (%)	19.0 (0.8)	32.7(1.2)	19.1(1.6)	29.2(1.6)	27.4(0.5)	32.4(0.6)
Extractives (%)	2.5(0.3)	0.7 (0.2)	1.8 (0.2)	0.6 (0.0)	2.6 (0.3)	0.6 (0.3)
Ashes (%)	18.6(0.5)	28.6(0.3)	16.1(0.5)	28.9 (0.3)	17.6(0.6)	30.2(0.5)
TC (%)	34.04 (0.07)	30.2(0.4)	32.78 (0.02)	32.3(0.4)	33.9 (0.8)	28.67(0.06)
TN (%)	2.1 (0.04)	2.07 (0.07)	2.06 (0.09)	2.20 (0.15)	2.22 (0.12)	1.84 (0.05)
C/N	16.2	14.6	15.9	14.7	15.3	15.6
N-NH_4^+ (mg Kg^{-1})	1381(40)	165(10)	1663(45)	131(13)	997(15)	47(5)
P (g Kg^{-1})	4.7(0.2)	7.8(0.2)	5.1(0.2)	10.5 (0.2)	3.1(0.2)	7.2(0.2)
Ca^{++} (%)	1.62(0.05)	1.17(0.03)	1.73(0.08)	2.25(0.05)	1.35(0.05)	3.35(0.08)
Mg^{++}(%)	0.38(0.06)	0.99(0.07)	0.38(0.05)	0.92(0.03)	0.29(0.04)	0.95(0.05)
Na^+ (%)	0.50(0.05)	0.28(0.03)	0.43(0.07)	0.69(0.05)	0.36(0.03)	0.43(0.06)
K^+ (%)	1.61(0.07)	0.46(0.05)	1.51(0.04)	0.93(0.05)	1.15(0.05)	0.43(0.08)

*Extractives-free basis, (. . .) Standard deviations

Lignin contributes to biomass recalcitrance, which is a crucial factor to define biodegradability and digestibility of the fibers. Changes in lignin content during composting are also presented in Table 2. As expected, initial values of M3 were experimentally higher than the rest of the mixtures due to a higher proportion of the individual materials with higher content of lignin. After 120 days, all composted mixtures exhibited increased percentages of lignin, ranging similar final values between 29.2-32.4% ($p \leq 0.05$). Higher final values have also been obtained by other authors (Amorim Orico et al. 2012; Domínguez et al. 2018). It is reasonable to think that this increase is a consequence of the reduction of other organic components of the mixtures which were more easily biodegraded, and that the lignin was less susceptible to this degradation, therefore leading to a proportional "enrichment" of the content of the later. Moreover, microorganisms responsible for the degradation of lignin may have been inhibited due to the low temperatures of the season. An interesting result arose when determining extractives at the beginning and at the end of the composting time. To the extent of our knowledge, this is the first time this parameter is informed for the composting process. A significant reduction was observed (see Table 2), pointing to an almost complete degradation of these fractions during the compost process ($p \leq 0.05$). Some of the lipophilic extractives usually present in *Eucalyptus* sawdust are fatty acids and alcohols, sterols and their esters, triglycerides, hydrocarbons, steroid hydrocarbons and ketones (Kilulya et al. 2014); while phenolic acids derived from hydroxybenzoic and hydroxycinamic acids as well as some flavonoids have been reported in rice hulls (Wanyo et al. 2014).

Ashes significantly increased (see Table 2) ($p \leq 0.05$) due to the degradation of the organic matter during the composting process (Ogunwande et al. 2008). The reduction in TC values after 120 days ($p \leq 0.05$) is a consequence of the biochemical processes that lead to the mineralization and gasification of carbon and nitrogen (Paterlini et al. 2017). However, this variation was only experimentally noticeable for M1 and M3. TN showed a decrease only for sample M3 and little variation was observed for the other two mixtures. This is due to the little volatilization of NH_3 and to that NH_4^+ stayed dissolved in the compost pile. When the active composting period ends, most of the available N was in the form of the later (Rynk et al. 1992). This result suggests a small loss of N during composting, which in turn is beneficial for keeping the value of the mixtures as fertilizers. Nevertheless, no significant variation is shown when ANOVA test was applied. C/N ratios decreased for samples M1 and M2, which is considered a good indicator of compost stability, while almost no variation was observed for M3. On the other hand, the amount of $N\text{-}NH_4^+$ in the three mixtures was greatly reduced at the end of the compost stage (also Table 2) ($p \leq 0.05$), owing to the conversion to NO_3^- and, to a lesser extent, to the loss of NH_3 through processes of deamination, volatilization and nitrification (Milinković et al. 2019; Tiquia et al. 2002). Moreover, this is an indicator of a good quality compost and the values obtained after 120 days are within the recommended limits (<400 mg $N\text{-}NH_4^+$ Kg^{-1}) for matured composts (Şevik et al. 2018). Total P increased significantly by the end of the composting period for all the samples ($p \leq 0.05$), since P is not lost by volatilization nor leaching during composting (Toumpeli et al. 2013). Regarding the cations surveyed, there was an experimentally increase in the amount of Mg and a decrease in the amount of K for all the samples at the end of the compost stage. Unlike P, the K in plant material is not usually bound to organic compounds, thus existing in its free ionic form and usually declining due to leaching (Ultra et al. 2005) (Table 2). Amounts of Ca and Na only increased for M2 and M3, the samples with the highest increase in ashes content.

Microbial activity, measured as FDA hydrolysis, is illustrated in Fig. 2a. It was significantly increased during composting of the three mixtures in 39% (M1), 44% (M2) and 49% (M3) ($p \leq 0.05$). The higher FDA values for M1(that was statistically proved) is related to the higher proportion of PL in the mixture; while for M2 and M3, the lower microbial activity is linked to the antibacterial and antifungal properties of some structural and non-structural components of wood sawdust which can inhibit the enzymatic action of microorganisms (Bamidele et al. 2014). Results regarding to the presence of pathogens are presented in Table 3. As observed, all three mixtures before and after the composting time had relatively low amount of these microorganisms and the values obtained were below the limits for pathogens as defined by (USEPA 1993) in spite of the minor increase in coliforms and *E.coli* registered mainly for M2.

Table 3 Pathogens present in the three mixtures at the beginning and end of the compost stage

Pathogen	M1 (RH/PL)		M2 (ES/PL)		M3 (RH/ES/PL)	
	Day 0	Day 120	Day 0	Day 120	Day 0	Day 120
Total coliforms (NMP/g)	< 3	< 3	1100	1100	1100	< 3
Fecal coliforms (NMP/g)	< 3	< 3	< 3	27	< 3	< 3
Salmonella sp.	Absence	Absence	Absence	Absence	Absence	Absence
E. coli (UFC/g)	320	280	24	700	293	360

Evolution of vermicomposting mixtures

The variations in the physicochemical characteristics of the three mixtures before and after the vermicompost stage are presented in Table 4. As observed, pH values further increased (M2 >M3 >M1) after 90 days of vermicomposting, becoming more alkaline ($p \leq 0.05$). Singh et al. (2013) attributed that increase in pH to the activity of carbonic anhydrase, located within the calciferous glands of earthworms, which catalyzes the fixation of CO_2 as $CaCO_3$. EC reflects the salinity of the organic substratum and it is a good indicator of the quality of the compost/ vermicompost for agricultural applications (Yadav and Garg 2011). The three vermicomposted mixtures showed a decrease in the EC after the 90 days (Table 4) ($p \leq 0.05$). According to Domínguez et al. (2018), this could be related to the reduction of soluble ions by immobilization attributable to microorganisms and earthworms or precipitation as non-soluble salts. These values are within the reference range recommended for the growth of sensitive plants (1-3 mS cm^{-1}) (Edwards et al. 2011). This is an important result because one of the main drawbacks to the use of composts as substrate constituent relates to the high salinity of composts obtained from cattle manure or agricultural wastes (Mendoza-Hernández et al. 2014).

T1 and T2 showed further decrease in the TC values, which could be a consequence of earthworms and microorganisms using a large portion of carbon as sources of energy (Bhat et al. 2013). The content of TN was also reduced, leading to an increase in C/N ratio after the vermicomposting stage. Although, T3 presented a C/N value slightly higher than 20, the three mixtures can be considered acceptable as stable and mature substrates (Mazzarino et al. 2012). Following the same tendency observed during the compost stage, the amount of $N\text{-}NH_4^+$ in the three vermicomposted mixtures continued to decrease, falling within the acceptable values for mature vermicomposts (Şevik et al. 2018). Phosphorus and K showed an upward trend in all the samples ($p \leq 0.05$). This increase may be attributed to the reduction in weight and degradation of organic compounds through the release of CO_2 (Sharma and Garg 2018). Ghosh et al. (1999) have reported that vermicomposting also helps in the transformation of unavailable forms of phosphorus to easily available forms for plants. Moreover, the C/P ratios obtained for the three treatments (2.5-3.0) were even lower than those for composted mixtures (3.1-4.0), indicating enhanced rate of decomposition (Parthasarathi et al. 2016). The quality of the final vermicomposts were high in terms of the most limiting nutrients for plant growth.

Table 4 Physicochemical characteristics of the three treatments at the beginning and end of the vermicompost stage

	T1(M1+S)		T2(M2+S)		T3(M3+S)	
	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
pH	8.94 (0.02)	9.50 (0.01)	8.72 (0.03)	9.66 (0.01)	8.08 (0.02)	9.60 (0.01)
EC (mS cm^{-1})	2.27 (0.03)	1.81 (0.02)	2.40(0.02)	2.28(0.03)	2.42 (0.02)	1.83 (0.02)
Ashes (%)	65.1(0.5)	62.7 (0.4)	68.6 (0.5)	67.2 (0.4)	67.2 (0.5)	68.8(0.5)
TC (%)	19.8(0.3)	15.6(0.3)	15.07(0.5)	13.4 (1.3)	13.2 (0.4)	14.59(0.06)
TN (%)	1.08 (0.03)	0.79(0.00)	1.01 (0.03)	0.80(0.01)	0.81 (0.01)	0.70(0.05)
C/N	18.3	19.8	14.9	16.7	16.3	20.8
$N\text{-}NH_4^+$ (mg kg^{-1})	185(20)	7(5)	60(8)	7(4)	31(9)	16(9)
P (g kg^{-1})	5.32(0.19)	5.80(0.20)	4.88(0.25)	5.40(0.18)	4.43(0.23)	4.90(0.25)
Ca^{++} (%)	2.33(0.06)	0.92(0.05)	1.79(0.03)	1.07(0.02)	1.89(0.05)	1.12(0.07)
Mg^{++} (%)	0.80(0.05)	0.26(0.07)	0.83(0.05)	0.23(0.03)	1.01(0.04)	0.17(0.07)
Na^+ (%)	0.28(0.07)	0.78(0.05)	0.73(0.03)	0.81(0.02)	0.29(0.05)	0.82(0.06)
K^+ (%)	0.16(0.03)	0.72(0.04)	0.44(0.05)	1.04(0.06)	0.31(0.07)	0.74(0.04)

Microbial activity for the vermicompost stage is illustrated in Fig. 2b. This stage began with less FDA values than those registered at the end of the compost stage ($p \leq 0.05$). (Fig. 2a), due to the addition of fresh soil. After the 90 days, FDA levels significantly increased in 25% (T1), 22% (T2) and 31% (T3) ($p \leq$

0.05), reaching the same (M1) of higher (M2 and M3) values than during composting ($p \leq 0.05$). This is most likely associated to the action of the earthworms which modify and enhance the microbial population and the enzymatic activities due to their gut-associated processes and nutrient enrichment of the substratum (Parthasarathi et al. 2016). In accordance with the compost stage, the three vermicomposted treatments presented values below the limits required for reduction of pathogens (USEPA 1993) (data are given in Online Resource 1).

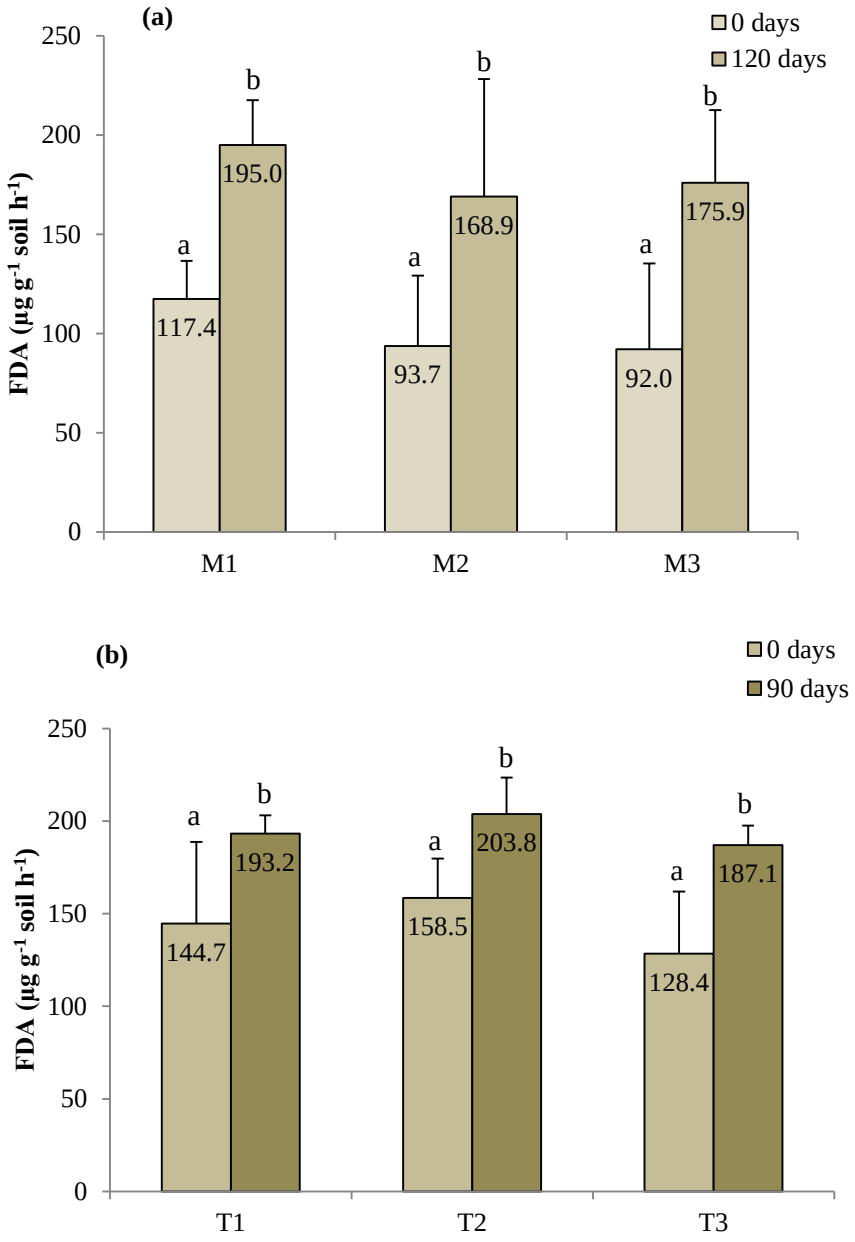

Fig. 2 FDA content during the **(a)** compost and **(b)** vermicompost stages of the three mixtures. Different lowercase letter indicates significant differences ($p < 0.05$) between treatments

Dynamics of earthworms in different vermicomposted organic substrates

The evaluation of the vermicomposting process through survival and biomass production of the earthworms is essential (Bhat et al. 2013). Earthworms grew well in all the treatments (T1, T2, T3), however, growth performance varied among substrates. No mortality was observed in any of the treatments; all earthworms survived until the termination of the

experiment. It was found that both T1 and T3 showed population increase at the end of the vermicompost stage with values of 305 and 55%, respectively. Although T2 did not increase its population, it was able to maintain the initial number of earthworms (20 individuals 0.03 m^{-2}, representing 667 individuals m^{-2}). Monitoring of the mean biomass of *E. fetida* individuals during the vermicompost stage is displayed in Fig. 3. The biomass significantly increased ($p < 0.05$) in the three treatments throughout the 90 days of the assay. T1 showed the highest increase at day 45 (0.59 ± 0.07 g ind^{-1}), while T2 and T3 showed their highest values at day 75 (T2: 0.54 ± 0.09 g ind^{-1}; T3: 0.52 ± 0.1 g ind^{-1}). After these maximums, the mean biomass weights exhibited a slight decreased, up to the end of the vermicompost stage, however the final values (after 90 days) remained higher than the initial ones in a range of 60-92 %. This would indicate that there was enough easily metabolized organic matter in the three substrates that provided the necessary nutrients to stimulate the growth of the earthworms (Yadav et al. 2013). These changes in the biomass may reflect the availability of food at the start of the experience and the exhaustion of food with time. Similar behavior was reported by other authors in the growth of *E. fetida* during the vermicomposting of different wastes (Gong et al. 2018; Sharma and Garg 2018).

Quality of the compost and vermicompost for different mixtures

Characteristic parameters obtained for this phytotoxicity test are presented in Table 5.

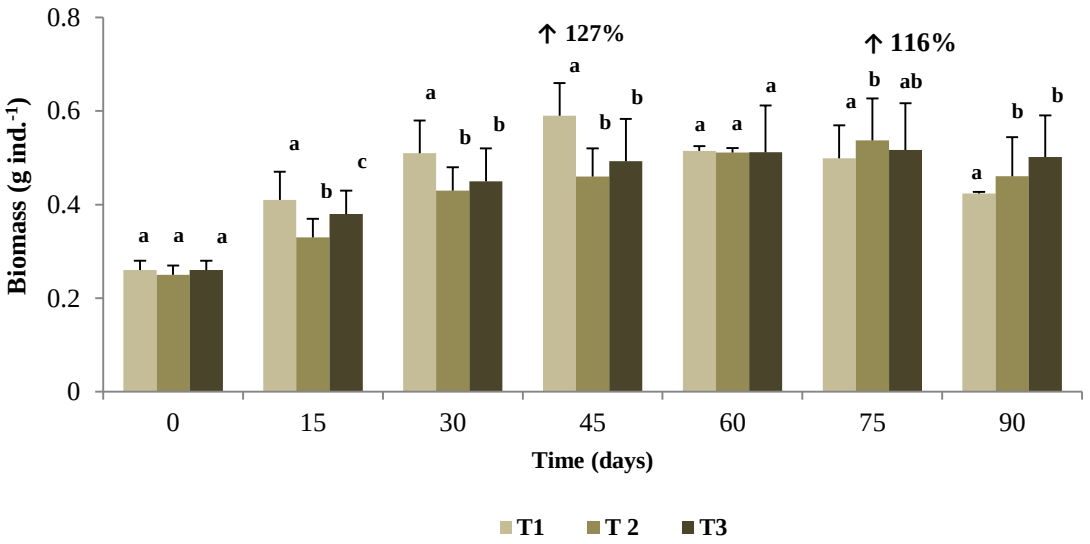

Fig. 3 Mean biomass of *E. fetida* during the 90 days of vermicomposting for the three treatments (T1, T2 and T3). Different lowercase letter indicates significant differences ($p < 0.05$) between treatments

Table 5 Phytotoxicity assay parameters for compost and vermicompost products

Parameters	Composting						Vermicomposting					
	M1		M2		M3		T1		T2		T3	
	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%
L_R (mm)	13.32	12.31	16.72	11.26	14.97	11.49	13.41	12.14	16.4	15.23	17.3	15.2
GI (%)	59.8	64.1	82.3	56.2	79.1	58.2	75.7	65.0	85.4	73.8	102.6	86.9

*LR for control was 13.96 mm

According to Zucconi et al. (1985), composts and substrates that present GI values greater than 60% are considered not phytotoxic and greater than 80% are considered mature. It is observed that for both dilutions in vermicompost products, GI values were experimentally greater than 60% indicating the high quality of the product obtained. On the other hand, only three values of GI were greater than 60% for composting products (M1 50%, M2 25% and M3 25%) but they were minor in comparison with T1, T2

and T3 at the same dilutions. These results confirm the hypotheses that vermicomposting process enhances the properties and quality of the final product after the composting process. Within the different substrates employed, it is inferred that the combination of eucalyptus sawdust and poultry litter (M2/T2) and eucalyptus sawdust, poultry litter and rice hulls (M3/T3) rendered higher values of GI than rice hulls and poultry litter (M1/T1) after the composting and vermicomposting processes, being M3/T3 the mixture that showed better quality and higher maturation degree. Finally, it is also worth noting the success of the combined-processes products despite the fact that the experiments were run during the cold season, when metabolic rates of earthworms and microorganism are slowed down.

Conclusion

The system that combines both processes (composting + vermicomposting) was effective for the stabilization of poultry litter with rice hulls and sawdust. Despite the fact that assays were conducted during the autumn-winter seasons, the processes combination allowed to achieve stable products. Furthermore, electrical conductivity was markedly lower in all vermicomposts in comparison with composts, reducing its detrimental effect in plant growth and wellness. Phytotoxicity tests, based on the germination index of a sensitive species, indicated that vermicomposting process enhanced the properties and quality of the final product. Particularly, the mixture 0.5RH: 1ES: 2PL had better quality and higher maturation degree.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

References

Adam G, Duncan H (2001) Development of a sensitive and rapid method for the measurement of total microbial activity using fluoresce in diacetate (FDA) in a range of soils. Soil Biol Biochem 33: 943-951. https://doi:10.1016/S0038-0717(00)00244-3

Amorim Orrico AC, Ribeiro Centurion S, Marques de Farias R, Previdelli Orrico Junior MA, Garófallo Garcia R (2012) Effect of different substrates on composting of poultry litter. R Bras Zootec 41 (7): 1764-1768. https://doi:10.1590/S1516-35982012000700028

Antczak A, Radomski A, Zawadzki J (2006) Benzene substitution in wood analysis. In: Annals of Warsaw Agricultural University SGGW-AR - Forestry and wood technology 58. Warsaw Agricultural University Press, Warsaw, pp15-19

APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Environment Federation) (1998) Part 9221: Multiple-tube fermentation technique for the members of the coliform group. Standard Methods for the examination of water and wastewater, 20th edn. APHA, Washington DC, 1325 pp

Bamidele, JA, Idowu AB, Ademolu KO, Atayese AO (2014) Microbial diversity and digestive enzyme activities in the gut of earthworms found in sawmill industries in Abeokuta, Nigeria. Rev Biol Trop 62 (3): 1241-1249

Bhat SA, Singh J, Vig AP (2013) Vermiremediation of dyeing sludge from textile mill with the help of exotic earthworm *Eisenia fetida* Savigny. Environ Sci Pollut R 20: 5975-5982. https://doi:10.1007/s11356-013-1612-2

Castagnino AM, Rosini MB, Benson S (2011) Horticulture in Argentina: A productive alternative with great potential. Ital J Agron 6 (37): 39-41. https://doi:10.4081/ija.2011.e37

Chin-Pampillo S, Ruiz-Hidalgo K, Masís-Mora M, Carazo-Rojas E, Rodríguez-Rodríguez CE (2015) Adaptation of biomixtures for carbofuran degradation in on-farm biopurification systems in tropical regions. Environ Sci Pollut Res 22(13): 9839-9848. https://doi:10.1007/s11356-015-4130-6

Corradi Pereira BL, Carneiro ACO, Carvalho AMML, Colodette JL, Costa Oliveira A, Fontes MPF (2013) Influence of chemical composition of Eucalyptus wood on gravimetric yield and charcoal properties. BioResource 8 (3): 4574-4592. https://doi:10.15376/ biores.8.3.4574-4592

Davis MA, Sloan DR, Kidder G, Jacobs RD (2017) Poultry Manure as a Fertilizer. UF/IFAS. https://edis.ifas.ufl.edu/aa205. Accessed: 20 August 2019

Domínguez J, Gómez-Brandón M, Martínez-Cordeiro H, Lores M (2018) Bioconversion of Scotch broom into a high-quality organic fertilizer: Vermicomposting as a sustainable option. Waste Manage Res 36: 1092-1099. https://doi:10.1177/0734242X18797176

Edwards CA, Subler S, Arancon N (2011) Quality criteria for vermicomposts. In: Edwards CA, Arancon N, Sherman R (eds) VermicultureTechnology. Earthworms, Organic Wastes and Environmental Management. Taylor &

Francis Group, US, pp 287-302. https://doi:10.1080/00207233.2012.624327

Fornes SF, Mendoza-Hernández D, García De La Fuente R, Abad Berjón M, Belda Navarro RM (2012) Composting versus vermicomposting: A comparative study of organic matter evolution through straight and combined processes. Bioresour Technol 118: 296-305. https://doi:10.1016/j.biortech.2012.05.028

Ghosh M, Chattopadhyay GN, Baral K (1999) Transformation of phosphorus during vermicomposting. Bioresour Technol 69 149-154. https://doi.org/10.1016/S0960-8524(99)80001-7

Global broiler meat production (2019) by selected country. Accessed: 20 August 2019. https://www.statista.com/statistics/237597/leading-10-countries-worldwide-in-poultry-meat-production-in-2007/

Gong X, Cai L, Li S, Chang SX, Sun X, An Z (2018) Bamboo biochar amendment improves the growth and reproduction of *Eisenia fetida* and the quality of green waste vermicompost. Ecotox Environ Safe 156: 197–204. https://doi:10.1016/j.ecoenv.2018.03.023

Gupta R, Garg VK (2009) Vermiremediation and nutrient recovery of non-recyclable paper waste employing *Eisenia fetida*. J Hazard Mater 162: 430-439. https://doi:10.1016/j.jhazmat.2008.05.055

Jankowska A, Drożdżek M, Sarnowski P, Horodeński J (2017) Effect of extractives on the equilibrium moisture content and shrinkage of selected tropical wood species. Bio Resource 12 (1): 597-607. https://doi:10.15376/biores.12.1.597-607

Kilulya K, Msagati TAM, Mamba BB, Ngila JC, Brush T (2014) Effect of site, species and tree size on the quantitative variation of lipophilic extractives in Eucalyptus woods used for pulping in South Africa. Ind Crop Prod 56: 166-174. https://doi:10.1016/j. indcrop.2014.02.017

Laos F, Mazzarino MJ, Walter I, Roselli L, Satti P, Moyano S (2002) Composting of fish offal and biosolids in northwestern Patagonia. Bioresour Technol 81: 179-186. https://doi:10.1016/S0960-8524(01)00150-X

Lazcano C, Gómez-Brandón M, Domínguez J (2008) Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 72: 1013-1019. https://doi:10.1016/j.chemosphere.2008.04.016

Leconte MC, Mazzarino MJ, Satti P, Iglesias MC, Laos F (2009) Co-composting rice hulls and/or sawdust with poultry manure in NE Argentina. Waste Manage. 29: 2446–2453. https://doi:10.1016/j.wasman.2009.04.006

Leconte MC, Mazzarino MJ, Satti P, Crego MP (2011) Nitrogen and phosphorus release from poultry manure composts: the role of carbonaceous bulking agents and compost particle sizes. Biol Fertil Soils 47: 897-906. https://doi:10.1007/s00374-011-0591-z

Lozano JF (2012) La producción de hortalizas en Argentina. Secretaría de Comercio Interior, Corporación del Mercado Central de Buenos Aires. 29 pp. http://www.mercadocentral.gob.ar/ziptecnicas/la_produccion_de_hortalizas_en_argentina.pdf. Available: 29 August 2015

Mazzarino MJ, Satti P, Roselli L (2012) Indicadores de estabilidad, madurez y calidad de compost. In: MJ Mazzarino and P Satti (eds) Compostaje en la Argentina: Experiencias de producción, calidad y uso. UNRN. Orientación Gráfica Editora, Buenos Aires, pp 13-28

Mendoza-Hernández D, Fornes F, Belda R (2014) Compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary. Sci Hortic-Amsterdam 178: 192-202. https://doi:10.1016/j.scienta.2014.08.024

Milinković M, Lalević B, Jovičić-Petrović J, Golubović-Ćurguz V, Kljujev I, Raičević V (2019) Biopotential of compost and compost products derived from horticultural waste - Effect on plant growth and plant pathogens'suppression. Process Saf Environ 121: 299-306. https://doi:10.1016/j.psep.2018.09.024

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27: 31-36. https://doi:10.1016/S0003-2670(00)88444-5

Ndegwa PM, Thompson SA (2001) Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. Bioresour Technol 76: 107-112. https://doi:10.1016/S0960-8524(00)00104-8

Ogunwande GA, Osunade JA, Adekalu KO, Ogunjimi LAO (2008) Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency. Bioresour Technol 99: 7495-7503. https://doi:10.1016/j.biortech.2008.02.020

Parthasarathi K, Balamurugan M, Prashija KV, Jayanthi L, Basha SA (2016) Potential of Perionyxexcavatus (Perrier) in lignocellulosic solid waste management and quality vermifertilizer production for soil health. Int J Recycl Org Waste Agricult 5: 65-86. https://doi:10.1007/s40093-016-0118-6

Paterlini H, González MV, Picone MI (2017) Calidad de la cama de pollo fresca y compostada. Ciencia del Suelo (Argentina) 35 (1): 69-78

Perondi D, Poletto P, Restelatto D, Manera C, Silva JP, Junges J, Collazzo GC, Dettmer A, Godinho M, Vilela ACF (2017) Steam gasification of poultry litter biochar for bio-syngas production. Process Saf Environ Protect 109: 478-488. https://doi:10.1016/j.psep.2017.04.029

Rynk R, van De Kamp M, Wilson GB, Singley ME, Richard TL, Kolega JJ, Gouin FR, Laliberty Jr. L, Kay D, Murphy DW, Hoitink HAJ, Brinton WF (1992) On-farm composting handbook. In: Rynk R (ed) Northeast Regional Agricultural Engineering Service. Ithaca, NY, 109 pp

Saleem A, Irshad M, Ping A, Haroon B (2018) Loss of phosphorus by runoff from soils after amendment with poultry litter co-composted with crop waste. Int J Recycl Org Waste Agricult 7 (3): 211–215. https://doi:10.1007/s40093-018-0207-9

Şevik F, Tosun I, Ekinci K (2018) The effect of FAS and C/N ratios on co-composting of sewage sludge, dairy manure and tomato stalks. Waste Manage 80: 450–456. https://doi:10.1016/j.wasman.2018.07.051

Sharma K, Garg VK (2018) Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm *Eisenia fetida* (Sav.). Bioresour Technol 250: 708-715. https://doi:10.1016/j.biortech.2017.11.101

Sharpley AN, McDowell RW, Kleinman PJ (2004) Amounts, forms, and solubility of phosphorus in soils receiving manure. Soil Sci Soc Am J 68: 2048-2057. https://doi: 10.2136/sssaj2004.2048

Singh R, Soni SK, Singh SP, Chauhan UK, Kalra A (2013) Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of Pogostemoncablin (patchouli) Benth. Appl Soil Ecol 70: 48-56. https://doi:10.1016/j.apsoil.2013.04.007

TAPPI T 222 om-02 (2002) Acid-insoluble lignin in wood and pulp. TAPPI Press, Atlanta, GA, USA

Tejada M, González JL (2009) Application of two vermicompost on a rice crop: Effects on soil biological properties and rice quality and yield. Agron J 101: 336-344. https://doi:10.2134/agronj2008.0211

Tiquia SM, Richard TL, Honeyman MS (2002) Carbon, nitrogen and mass loss during composting. Nutr Cycl Agroecosyst 62: 15-24. https://doi:10.1023/A: 1015137922816

Tognetti C, Laos F, Mazzarino MJ, Hernández MT (2005) Composting versus vermicomposting: a comparison of end product quality. Compost Sci Util 13 (1): 6-13. https://doi:10.1080/1065657X.2005.10702212

Toumpeli A, Pavlatou-Ve AK, Kostopoulou SK, Mamolos AP, Siomos AS, Kalburtji KL (2013) Composting Phragmitesaustralis Cav. plant material and compost effects on soil and tomato (Lycopersiconesculentum Mill.) growth. J Environ Manag 128: 243-251. https://doi:10.1016/j.jenvman.2013.04.061

Trevorah RM, Huynh T, Vancov T, Othman MZ (2018) Bioethanol potential of *Eucalyptus obliqua* sawdust using gammavalerolactone fractionation. Bioresour Technol 250: 673–682. https://doi:10.1016/j.biortech. 2017.11.084

Ultra Jr. VU, Mendoza DM, Briones AM (2005) Chemical changes under aerobic composting and nutrient supplying potential of banana residue compost. Renew Agr Food Syst 20: 113-125. https://doi:10.1079/ RAF2005104

USEPA (1993) Standards for the use or disposal of sewage sludge. U.S. Gov. Print. Office, Federal Register 58: 9248-9415. Washington DC

USEPA (1994) Method 200.9. Determination of trace elements by stabilized temperature graphite furnace atomic absorption. 2nd revision: JT Creed, TD Martin, JW O'Dell. Cincinati, Ohio, 42 pp

USEPA (1996) Ecological effects test guidelines. Seed germination/ root elongation toxicity test. OPPTS 850.4200. https:// nepis.epa.gov/Exe/ZyPDF.cgi/P100RF5I.PDF? Dockey= P100RF5I.PDF Accessed: 27 January 2019

Wanyo P, Meeso N, Siriamornpun S (2014) Effects of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk. Food Chem 157: 457–463. https://doi:10.1016/j.foodchem.2014.02. 061

Yadav A, Garg VK (2011) Recycling of organic wastes by employing *Eisenia fetida*. Bioresour Technol 102: 2874-2880. https://doi:10.1016/j.biortech.2010.10.083

Yadav A, Gupta R, Garg VK (2013) Organic manure production from cow dung and biogas plant slurry by vermicomposting under field conditions. Int J Recycl Org Waste Agricult 2 (21): 1-7. https://doi:10.1186/ 2251-7715-2-21

Zucconi F, Monaco A, Forte M, Bertoldi MD (1985) Phytotoxins during the stabilization of organic matter. In: Composting of agricultural and other wastes, Gasser JKR (ed) Elsevier Applied Science Publishers, England, pp 73-86