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Investigating the efficiency of co-composting and vermicomposting of vinasse with the mixture of cow manure wastes, bagasse, and natural zeolite

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ABSTRACT

Fermentation of ethanol as a product of sugarcane agro-industry causes the discharge of large amounts of a liquid waste called vinasse into the environment. In this study, co-composting followed by vermicomposting process of the mixtures of vinasse, cow manure, and chopped bagasse was performed for 60 days using earthworms of *Eisenia fetida* species. The results showed that the trend of changes in C/N was decreasing. The pH of the final fertilizer was in alkaline range (8.1–8.4). The total potassium decreased during the process, ranging from 0.062 to 0.15%, while the total phosphorus increased and its values ranged from 0.06 to 0.10%. The germination index (GI) for all samples was 100%, while the cellular respiration maturity index was < 2 mg C-CO_2 g^{-1} organic carbon day^{-1}, confirming a very stable compost. The results of this study indicate that the compost obtained from the co-composting-vermicomposting process could be used as a sound soil amendment.

1. Introduction

Food and agricultural industries produce large amounts of solid and liquid wastes; if not properly managed, they create numerous problems to the environment. The residues of agro-industries are considered as the contaminants, influencing the environment significantly (Diaz et al., 2002; Siles et al., 2011). Ethanol as one of the products of sugarcane agro-industries is produced from alcohol producing factories through mesophilic fermentation of agricultural crops including sugarcane, corn, wheat, sugar beet etc. (Mota et al., 2013). Production of ethanol through fermentation results in the production of large amounts of a liquid waste called vinasse (9–14 L of vinasse is produced per one liter of ethanol) (Diaz et al., 2002; Siles et al., 2011). It contains considerable amount of biodegradable organic matter (BOD = 25,000–45,000 mg/L and COD = 70,000–120,000 mg/L) and nutrients (N = 30 g/kg, K = 30 g/kg). However, due to low pH (3–4.5), high temperature, brown color, high ash content, and electrical conductivity (250–300 dS/m), it is considered as one of the most contaminated wastewaters (Campos et al., 2014; Carvajal-Zarrabal et al., 2012; Diaz et al., 2002; Vaccarino et al., 1993; Zayas et al., 2007).

The discharge of this wastewater into the environment causes serious problems and changes in natural ecosystem including eutrophication or decreased diffusion of sunlight into aqueous environments. In addition, increasing environmental awareness coupled with more stringent regulation standards has triggered various industries to challenge themselves in seeking appropriate wastewater treatment technologies (Teh et al., 2016). In the past years, vinasse produced by Razi Alcohol Production Factory of Ahvaz city was disposed off in evaporation ponds. This treatment method generated many problems including contamination of ground and surface waters, nuisance, malodor, appearance and aggregation of insects and other disturbances to the surrounding environment (Madejón et al., 2001; Siles et al., 2011). There are different methods for the treatment of vinasse, including chemical treatment (coagulation and flocculation, chemical precipitation,

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chemical oxidation) and biological treatment (aerobic or anaerobic, trickling filter, lagoons, landfilling etc.). The majority of these methods are not very efficient due to relatively high investment and maintenance cost, the production of other hazardous compounds, and very high concentration of minerals and salts in the wastewater. Therefore, the use of advanced wastewater treatment systems is not reasonable (Eykelbosh et al., 2015; Prado et al., 2013; Siles et al., 2011). One of the suitable methods for converting agricultural wastes is composting process. Composting is regarded as a method in which aerobic mesophilic and thermophilic microorganisms consume organic matter as a substrate under controlled conditions, producing a stabilized, mature, deodorized, hygienic material, free of pathogens and plant seeds, and rich in humic substances that can be used as soil conditioner. If earthworms are applied in this process, it can be termed as an integrated composting-vermicomposting process. Earthworms can convert organic fraction of solid wastes to a nutrient rich fertilizer under aerobic conditions. *Eisenia fetida* is a species of earthworms, which under suitable environmental conditions (suitable pH, temperature, and moisture), has the potential to convert organic waste into products with a high nutritious value that can be used as conditioner for improving physical, nutritional, and biological characteristics of soil (Amouei et al., 2009; Lim and Wu, 2016; Lim et al., 2015b; Meunchang et al., 2005; Negro et al., 1999; Prado et al., 2013; Wu et al., 2014). In various studies, agricultural wastes, wastewater sludge, and industrial and municipal organic solid wastes have been used as the raw materials in composting and vermicomposting processes (Entry et al., 2005; Gigliotti et al., 1996; Molina et al., 2013; Moreno et al., 1996; Murillo et al., 1995; Yadav and Garg, 2011). Since vinasse possesses carbon and some salts including potassium and calcium, which are required for the growth of microorganisms, it may be applied in aerobic degradation processes (Mota et al., 2013). However, limited studies were conducted in this regard. For example, Molina et al. (2013) have investigated the stabilization of sewage sludge, vinasse biowastes, and rabbit manure by vermicomposting using *E. fetida*. In another study, co-composting of vinasse/grape marc has been investigated and optimized (Diaz et al., 2002). Madejón et al. (2001) also have evaluated the effect of three vinasse composts as a deep fertilizer on crops (corn and sugar-beet) and on some chemical properties of a soil by applying two successive compost applications. One of the most important requirements that must be taken into consideration for composting and vermicomposting processes is proper aeration. For this purpose, bulking materials are used to facilitate the aeration of the compost mixture. The use of agricultural and industrial wastes not only provides the required carbon but also serves as bulking agents and improves the aeration of composting mixtures. Bagasse is an agricultural waste, which over several million tons of it is annually produced in agro-industrial units in Khuzestan province; of which 100 thousand tons is used for production of animal feeds and chipboard sheet and 300–350 thousand tons is used in paper production units (Taghizadeh, 2011). The remaining amount of the produced bagasse is dumped as waste materials, which causes air pollution through frequent natural self-burning. Therefore, it is necessary to find an efficient way for managing the huge amounts of the produced bagasse in our area in an ecofriendly manner. Hence, this waste was used as bulking agent during combined composting followed by vermicomposting of vinasse. Usage of vinasse compost for increasing soil fertility is one of the economical and suitable methods. However, the high salinity caused by vinasse has significantly limited its usage as a fertilizer in agriculture. One of the solutions to decrease the salinity of final compost is the application of adsorbent materials such as natural zeolites during vermicomposting. Zeolites are porous structured compounds with an aluminosilicate framework (AlO_4 and SiO_4) that can accommodate a wide variety of cations, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and others. Some studies have used zeolites for reducing the salinity as well as for removing heavy metals from fertilizers obtained from composting of poultry manure and leachate. However, no study has been reported for reducing salinity in the fertilizer obtained from different mixtures with vinasse (Armağan et al., 2004; Turan, 2008). The present study was aimed to investigate the applicability of an integrated co-composting-vermicomposting process to produce a fertilizer from vinasse, cow manure, and bagasse. Furthermore, during the process a natural calcium zeolite was used as adsorbent to improve the quality and decrease the salinity of the final product. The physical, chemical, and biological parameters affecting the maturity and quality of the final product were also evaluated for different mixtures of the raw materials.

2. Materials and methods

2.1. Feeding materials and composting system setup

In this study, the required vinasse and bagasse were obtained from Razi Alcohol Production Factory and Dabal Khazaei Agro-industry Company, Iran, respectively. The co-composting process was carried out for 3 weeks in wooden boxes ($50 \times 15 \times 25$ cm) with internal polyethylene coating. The feeding materials consisted of cow manure, vinasse ($EC_{1:10}$ = 6.44 mS/cm; pH = 4.7; moisture = 93%), and crushed bagasse (1–1.5 cm) as the bulking agent. The wet weight ratios of bagasse to cow manure during composting were 10, 25, and 50%. Thereafter, vinasse was added to the mixture of cow manure and bagasse at a ratio of 10 (V_1), 20 (V_2), and 40% (V_3) (wet weight basis) (Diaz et al., 2002; Madejón et al., 2001; Molina et al., 2013). During the composting process, aeration of the mixtures was performed through manual mixing in a periodic fashion of twice per week (Hawrot et al., 2005). The moisture content of the mixtures was measured after mixing and then it was adjusted to the optimum range (45–55%) by sprinkling required water, and the moisture content was kept in this range during the composting process (Fountoulakis et al., 2009).

2.2. Vermicomposting by the earthworm

After composting for 3 weeks, calcium zeolite was added to the mixtures at two ratios of 10% (Z_1) and 20% (Z_2). Then, 15–18 earthworms, *E. fetida*, with a mean size of 5.5 cm, were added to each kilogram of the compost to begin the vermicomposting process. A mixture without the presence of the worms was used as the control (Molina et al., 2013). The moisture and temperature of the treatments during vermicomposting were monitored and kept constant to be in the optimal range (Molina et al., 2013).

2.3. Physical and chemical analyses

On the 1st, 10th, 20th, 40th and 60th days, after complete mixing of the mixture, about 20 g homogenized wet samples (free of earthworms and cocoons) were drawn from 5 different parts of each treatment and then dried for further chemical analyses. The parameters of electrical conductivity (EC), pH, organic carbon (OC), total nitrogen content (TKN), total phosphorus (TP), and total potassium (TK) were analyzed. Monitoring of *E. fetida* worms was performed across all stages of the process as well as at the end of the process in terms of the number of the worms and the weight of the worm mass (Gandolfi et al., 2010; Molina et al., 2013). The indices of germination, cellular respiration, population of fungi and heterotrophic bacteria were measured in the final product to determine the maturity degree.

The moisture content was measured by drying the sample at 105 °C for 24 h using an oven (Memmert, Germany) and until no further change in the dry weight was detected.

Organic carbon (OC) was measured by oxidation method of potassium dichromate (Eaton et al., 2005; Gupta, 2012). The volatile fraction was determined by burning the dried samples at 550 °C in a muffle furnace (Memmert, Germany). pH (WTW inolab pH meter, Germany) and EC (Hach conductivity meter, USA) were measured by using a glass electrode with a dilution ratio of 1:10 of the sample to water (Leege, 1998). The total nitrogen (TKN) and total phosphorus (TP) were measured by Kjeldahl analysis system (FOSS, Denmark) (Gupta, 2012) and Olsen, using a spectrophotometer, (Hach, Model DR 5000, USA) (Diaz et al., 2002) methods, respectively. The total potassium (TK) was measured by flame photometry method (M410, Sherwood, England) (Leege, 1998). The germination index (GI) for the number of the seeds germinated after three days (Kim et al., 2008) and the respiration index (CO_2) were reported according to measurement of CO_2 absorbed in the solution of 1 N NaOH and 2 ml of barium chloride 10%. The number of the bacteria and fungi was counted and reported as CFU after incubating at 37 °C for 3 days (INE 400, Memmert, Germany) (Khalil et al., 2008).

2.4. Statistical analysis

Repeated measures analysis of variance (rm-ANOVA) was used to analyze the physico-chemical characteristics of the feeds and the products. In this analysis, the investigated treatments were considered as the subjects, vinasse and zeolite contents as the between-subject factor, and the sampling time was fixed as a within-subject factor. The sphericity condition of all the variables was tested with Mauchly's test, and when they did not meet the condition, the Geisser Greenhouse (G–G) procedure was used to correct the sphericity violation (Fernández-Delgado Juárez et al., 2015). Significant differences in the main effects were analyzed by paired comparisons with the Bonferroni test. The normality and the variance homogeneity of the data were tested prior to ANOVA. Simple descriptive analysis was applied to determine the average value of the quantitative variables. Statistical analyses were performed using the SPSS software (version 22.0; SPSS Inc., Chicago, IL). P values <0.05 were considered significant as the probability levels.

3. Results and discussion

3.1. Temperature

As can be observed from Fig. 1, at the beginning of the composting process, the mixture had a temperature equal to the ambient temperature (27–29 °C). After 10 days, along with the onset of microbial activities and degradation of organic compounds, the temperature increased to over 48–55 °C and remained within this range for 1 week. The greatest peak of temperature elevation (55 °C) across all of the three vinasse ratios was observed in the treatments with the maximum content of cow manure. The increase in temperature may be due to aerobic metabolism activity of microorganisms, which produces heat. Additionally, the presence of suitable ventilation in the mixture, which provides sufficient oxygen for stimulation of biological activity and maintaining aerobic conditions, is another reason for the increased temperature (Liang et al., 2003). After this, a reduction in the temperature occurred in the treatments. At the end of the vermicomposting period (day 60), the temperature reached around 29–31 due to decreased microbial activity and organic substrate.

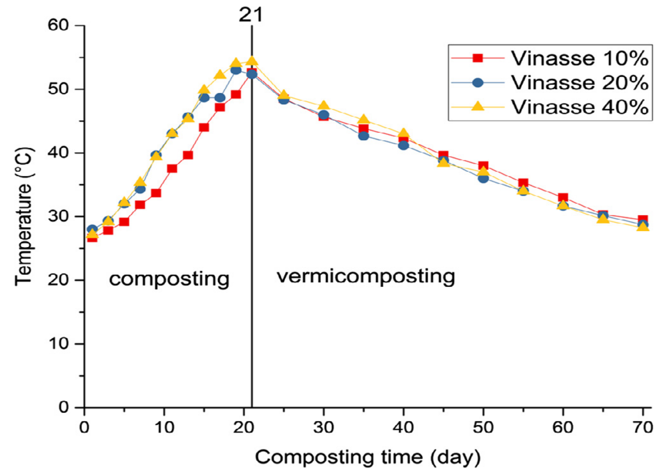

Fig. 1. Changes in the temperature in composting and vermicomposting processes for 10, 20, and 40% of vinasse treatments.

3.2. The characteristics of the composted, vermicomposted cow manure, and its mixture with vinasse and bagasse

Changes in some parameters such as pH, EC, C/N, TKN, TP, and TK during the study are shown in Table 1. pH is one of the most important factors influencing microorganisms' activities as well as chemical reactions in composting and vermicomposting. In this study, the mean value of pH in the ratios of 10 and 20% vinasse was toward alkaline ($pH \geq 7$), while for the ratio of 40% vinasse, the environment was a little acidic to neutral ($pH \geq 6.6$), which may be due to higher amounts of vinasse used in these treatments. Overall, at preliminary stages and onset of the trend, the pH range was neutral and shifted to relatively alkaline and continued until the end with minor fluctuations (near 8). pH across all ratios of the control (without vinasse and calcium zeolite) had a tendency to be alkaline, and this trend continued until the end with minor fluctuations. Microorganisms and earthworms are able to change the pH of soil, and they could degrade organic and nitrogen compound to ammonium (NH_3) ions and humic acid (Komilis and Ham, 2006). These compounds have different influence on pH. The presence of phenolic and carboxylic groups in humic acid results in a reduction of the pH, while ammonium ions results in an increase in pH. The interaction effects of these compounds could result in a reduction of the pH of vermicompost and its shift towards neutral pH (Pramanik et al., 2007). During all the mentioned treatments with vinasse with different ratios, the pH was a little higher, being at the alkaline range when compared to the control samples (see Table 1). The extreme microbial activity and degradation of organic compounds in the early weeks has resulted in the formation of the ammonium ion and elevation of pH in the compost mass. Development of pH of the matrix's compounds represents the activity of microorganisms and earthworms during the aerobic metabolism process in the presence of sufficient moisture and thus production of basic compounds (such as ammonia resulting from degradation of nitrogen-containing organic compounds) (Garg et al., 2006; Singh and Kalamdhad, 2014). ANOVA repeated measures with Greenhouse-Geisser correction showed that the mean of pH differed significantly between composting time points [$F(1.134, 13.606) = 20$, $p < 0.001$]. However, no significant difference was observed with regard to the three ratios of vinasse and two ratios of zeolite ($p > 0.05$). The Bonferroni pairwise comparison test showed a significant difference between the pH of the first day and the pH of 21th day (end of composting) and vermicomposting (day 60) ($p < 0.001$), confirming the formation of basic compound such as ammonium ion during composting process. However, no

Table 1
The values of TK, TP, C/N, EC, and pH for co-composting followed by vermicomposting of vinasse, bagasse, and cow manure with different ratios at different sampling days (1, 21, and 60 day).

Parameters	Treatments								
	$V_1 + B_1$	$V_1 + B_2$	$V_1 + B_3$	$V_2 + B_1$	$V_2 + B_2$	$V_2 + B_3$	$V_3 + B_1$	$V_3 + B_2$	$V_3 + B_3$
Day 1 (beginning of composting)									
$pH_{1:10}$	8.16	7.00	8.2	7.58	7.66	8.15	7.65	7.88	6.66
$EC_{1:10}$ (mS/cm)	0.76	0.73	0.7	0.96	0.9	0.88	1.37	1.22	1.11
C:N	37.49	52.41	40.12	38.24	35.0	44.79	36.5	40.64	43.96
TP (%)	0.045	0.02	0.04	0.03	0.04	0.04	0.05	0.035	0.025
TK (%)	0.084	0.11	0.11	0.11	0.13	0.213	0.076	0.085	0.20
Day 21 (end of composting or beginning of vermicomposting)									
$pH_{1:10}$	8.2	7.9	8.25	8.25	8.26	8.33	8.20	8.28	8.23
$EC_{1:10}$ (mS/cm)	1.90	1.73	1.69	2.40	2.25	1.95	2.42	2.40	2.30
C:N	11.06	26.53	23.84	17.40	15.21	17.68	16.73	13.76	29.20
TP (%)	0.065	0.06	0.05	0.05	0.07	0.05	0.05	0.05	0.05
TK (%)	0.125	0.2	0.26	0.161	0.146	0.24	0.10	0.105	0.52
Day 60 (end of vermicomposting)									
Zeolite (10 percent)									
$pH_{1:10}$	8.20	8.20	8.30	8.30	8.55	8.30	8.30	8.20	8.40
$EC_{1:10}$ (mS/cm)	1.70	1.55	1.4	1.94	1.75	1.67	1.83	1.70	1.63
C:N	9.37	10.67	21.62	18.66	12.05	15.35	14.32	13.5	20.72
TP (%)	0.1	0.095	0.09	0.11	0.09	0.1	0 0.06	0.08	0.06
TK (%)	0.07	0.11	0.2	0.07	0.12	0.13	0.070	0.06	0.184
Zeolite (20 percent)									
$pH_{1:10}$	8.30	8.05	7.93	8.30	8.30	8.20	8.30	8.40	8.0
$EC_{1:10}$ (mS/cm)	1.40	1.21	1.04	1.53	1.41	1.23	1.66	1.55	1.36
C:N	9.70	30.77	21.10	16.15	16.37	17.52	16.10	10.36	35.38
TP (%)	0.10	0.10	0.09	0.10	0.10	0.07	0.06	0.08	0.07
TK (%)	0.055	0.083	0.160	0.065	0.083	0.133	0.07	0.076	0.117
Control Treatment	Parameters								
	$pH_{1:10}$		$EC_{1:10}$ (mS/cm)		C:N		TP (%)		TK (%)
Day 1 (beginning of composting)									
$V_0 + B_1$	7.4		0.7		45.00		0.041		0.09
$V_0 + B_2$	7.2		0.55		56.62		0.035		0.26
$V_0 + B_3$	7.5		0.6		41.30		0.04		0.05
Day 21 (end of composting or beginning of vermicomposting)									
$V_0 + B_1$	8.2		1.18		14.72		0.042		0.05
$V_0 + B_2$	8.2		0.96		19.95		0.044		0.068
$V_0 + B_3$	8.2		1.68		13.17		0.038		0.05
Day 60 (end of vermicomposting)									
$V_0 + B_1$	8.1		1.20		14.93		0.05		0.062
$V_0 + B_2$	8.2		1.56		14.14		0.06		0.068
$V_0 + B_3$	8.2		1.12		10.26		0.05		0.078

$EC_{1:10}$; TP as %, TK as %; V_0 means compost or vermicompost without vinasse; V_1, V_2 and V_3 each respectively means mixture with 10%, 20% and 40% vinasse; B_1, B_2 and B_3 each respectively means mixture containing 10%, 25% and 50% bagasse.

significant difference was observed between composting and vermicomposting period ($p > 0.05$).

C/N ratio is an indicator for determining the maturity of the compost (Huang et al., 2006). In this study, C/N ratio on the first day of composting process was in the range of 35–52.41. It decreased during vermicomposting and reached to 9.37–35.38 at the end of the sixtieth day of vermicomposting [$F(2, 24) = 182.3$, $p < 0.001$]. The greatest C/N ratio in the final product was observed in the treatment of 40% vinasse and 50% bagasse. This can be due to the presence of high organic carbon content in vinasse and the decreased degradation of the organic materials by the microorganisms. In the control sample, C/N ratio at the beginning and at the end of the period (day 60) was 41.3–56.62 and 10.26–14.93, respectively (Table 1). The reduction in C/N ratio can be ascribed to the increase in the earthworms' population, resulting in higher degradation of organic compound along the composting. The worms and microorganisms converted a part of organic carbon to carbon dioxide, releasing from the matrix (Lim et al., 2015a). On the other hand, nitrogen in the mixtures increases due to the degradation of organic compounds as well as due to the addition of glaze, mucous, growth stimulating hormones, and the enzymes produced by earthworms to the matrix compounds, which are enriched of nitrogen. As a result, with the decrease in the organic carbon content and the increase in the total nitrogen in the materials of the matrix, the C/N ratio reduces in the final vermicompost product (Suthar, 2007). Khwairakpam and Bhargava (2009) and Suthar (2007) have attributed the reason of reduction in C/N ratio to the release of some parts of the organic carbon as carbon dioxide gas as well as nitrogen mineralization because of microbiological degradation.

Potassium (TK) is an essential nutrient for the growth and activity of the microorganisms in composting or vermicomposting process; in addition, it is another indicator for determining the maturity of the final product (Saha et al., 2008). TK varied significantly with the composting time [$F(1.282, 15.39) = 7.8$, $p < 0.05$]. The results obtained from this study indicated that the potassium content at the beginning of composting process varied from 0.08 to 0.24%. It then showed an increasing trend, so that during the composting, its values ranged from 0.08-0.52%. This phenomenon can be attributed to the production of acids during degradation of organic compounds by microorganisms, which in turn converts the insoluble potassium content of the mixtures to soluble form. Therefore, the amounts of potassium increase during composting (Kaviraj and Sharma, 2003; Pramanik et al., 2007; Saha et al., 2008). Thereafter, until 60th day (vermicomposting), the trend of potassium was decreasing and its content varied from 0.06 to 0.2% (Table 1). The lowest value of potassium was measured for 10% vinasse and 10% bagasse treatment with corresponding value of 0.06, which is due to the lower concentration of this element in 10% vinasse and bagasse. The potassium content is directly associated with the enzymes such as phosphatase present in the digestive system of earthworms and bacteria activities (Kaviraj and Sharma, 2003; Pramanik et al., 2007). Further, the use of calcium zeolite in 21th day of composting reduced the potassium content in the treatments because of its ion exchange properties and the entrapment of potassium in its pores (Turan, 2008).

The total phosphorus (TP) content increased over time for all the treatments and the control samples, resulting in significant differences in the TP content between treatments concerning time [$F(2, 24) = 112$, $p < 0.001$]. Its contents at the beginning and the end of the process were in the range of 0.02–0.05 and 0.06–0.11%, respectively (Table 1). This increasing trend in the total phosphorus content was greater and more significant in the treatments with vinasse than in the control treatments ($p < 0.05$). The highest phosphorus content at the end of the vermicomposting was observed in the treatments containing the minimum level of the bulking agent (bagasse) and relatively the lowest vinasse content. While, at the end of the process the lowest phosphorus content was observed for the treatments containing 40% vinasse. Therefore, it can be concluded that the treatments with the lower vinasse had more suitable conditions for performing the vermicomposting process and worms activities. The evolution in phosphorus content, especially during the vermicomposting process, can be attributed to the enzymes, such as phosphatase, present in the digestive tract of the earthworms. In addition, the degradation of phosphorous organic matter to soluble phosphate (mineralization) during vermicomposting process can increase the content of phosphorous in the final product (Ghosh et al., 1999; Kostecka and Kaniuczak, 2008). Furthermore, the increase in the total phosphorus content in the samples containing calcium zeolite can be attributed to this fact that when zeolite is mixed with water, it can release some nutrients including phosphorous into the composting materials. According to the previous studies, zeolites have a higher capacity for adsorbing and entrapping of potassium than phosphorus and NH_4^+. Therefore, the release of potassium from the zeolite is less than that of phosphorus; as a result, the content of phosphorus in the final product of vermicomposting increases (Ndegwa et al., 2000; Zorpas and Loizidou, 2008).

The changes in electrical conductivity after adding the zeolite with the ratio of 10% and 20% into different treatments of vinasse, bagasse, and cow manure are shown in Figs. 2 and 3, respectively. In this study, after completing the composting process (21 days), different ratios (10% and 20%) of the zeolite were added to the mixtures to investigate its effect on the salinity and electrical conductivity of the mixtures.

For all the treatment, the amount of electrical conductivity during composting period (without calcium zeolite) showed an increasing trend from the beginning (1th day) until 21th day of composting, and its values were in the range of 1.69–2.42 mS/cm on 21th day (Table 1). After the addition of 10% zeolite (21th day of composting) to the treatments, the electrical conductivity decreased and its values were in the range of 1.4–1.94 mS/cm by the end of 60th day (Table 1). The highest reduction in the EC after using 10% zeolite was obtained for $V_3 + B_2$ and $V_3 + B_3$ treatments with the value of 29%. Following the addition of 20% zeolite to the investigated treatments, the EC showed a decreasing trend and its values on 60th day (end of vermicomposting) ranged from 1.04 to 1.66 mS/cm (Fig. 3).

The amount of reduction in the EC, after using 20% zeolite, was in the range of 26–41%. The highest reduction of the EC was observed for $V_3 + B_3$ treatment. It can be concluded from the results that the application of zeolite reduced the salinity of the treatments during the integrated composting vermicomposting process. The EC content of different treatments showed a significant difference concerning composting time. A repeated measures analysis of variance (rm-ANOVA) showed that the mean of EC differed significantly between composting time points [$F(2, 24) = 1060$, $p < 0.001$]. Additionally, significant difference was observed with regard to the three ratios of vinasse and two ratios of the zeolite ($p < 0.001$). The Bonferroni pairwise comparison test showed a significant difference between EC of the first day and EC content of composting (day 21) and vermicomposting (day 60) ($p < 0.001$) as well as between EC of composting and vermicomposting period ($p < 0.001$). Post hoc tests using the Bonferroni correction revealed that the EC increased by an average of 1.157 mS/cm after 21 days (end of composting) ($p < 0.001$) and then reduced by an average of 0.584 mS/cm between composting and vermicomposting ($p < 0.001$).

EC is a measure of the amount of soluble salts in an organic amendment, and it is used as an indicator to evaluate the applicability of vermicomposting for agricultural purposes (Lim et al., 2016; Lim and Wu, 2016). The ideal value of EC for final compost product depends on its final application. The ideal compost should not have an electrical conductivity above 2 mS/cm. When the EC exceeds this value, the compost will be called "salty" (Turan, 2008). At the end of process and after the addition of calcium zeolite, the EC decreased. For the other treatments (without calcium zeolite), its increasing trend also decreased. The increased amount of EC at the beginning of the process can be due to degradation of organic matter, which in turn releases different mineral salts in available forms such as phosphate, ammonium, and potassium to the mixtures (Dao, 1999; Turan, 2008). Moreover, because of the activities of microorganisms during pre-composting as well as because of mutual activities of the earthworms and microorganisms during vermicomposting process, most of elements present in the treatments are converted to their available forms, releasing into the mixtures. As a result, their concentration and the concentration of organic acids increase gradually throughout the process, and consequently the EC increases (Zorpas and Loizidou, 2008). In some treatments, the electrical conductivity of the compost was near 2.5 mS/cm before adding calcium zeolite. However, by the addition of the zeolite, the quality of the compost increased. For instance, after the addition of the zeolite with the ratio of 10 and 20%, the EC of the final product decreased by 29% and 41%, respectively. The best usage of the zeolite with the ratio of 10% was obtained for the treatments with 40% vinasse. The highest reduction in the EC content was observed for the treatments containing zeolite with the ratio of 20% and 40% vinasse (removal efficiency = 41%); the EC content of the treatments with this ratio showed a decreasing trend, confirming that the zeolite with this ratio is able to maintain a larger amount of Na^+ and Cl^- within its structure. Garg et al. (2006) studied the production of vermicomposting from a wide variety of organic wastes by *E. fetida*, and reported that the EC increased during the process for all the treatments. They attributed the reason to the release of available ions and minerals, which had been produced along the digestion process and excrete of organic compounds from the guts of the earthworms. Yang et al. (2015) also studied the production of vermicompost from pig manure and concluded that the EC value increased significantly along the vermicomposting process. Garg et al. (2006) also confirmed this result in their study.

3.3. The biological parameters of E. fetida at the beginning and end of vermicomposting process

Table 2 provides the results related to the number of *E. fetida* worms at the beginning and end of vermicomposting process for

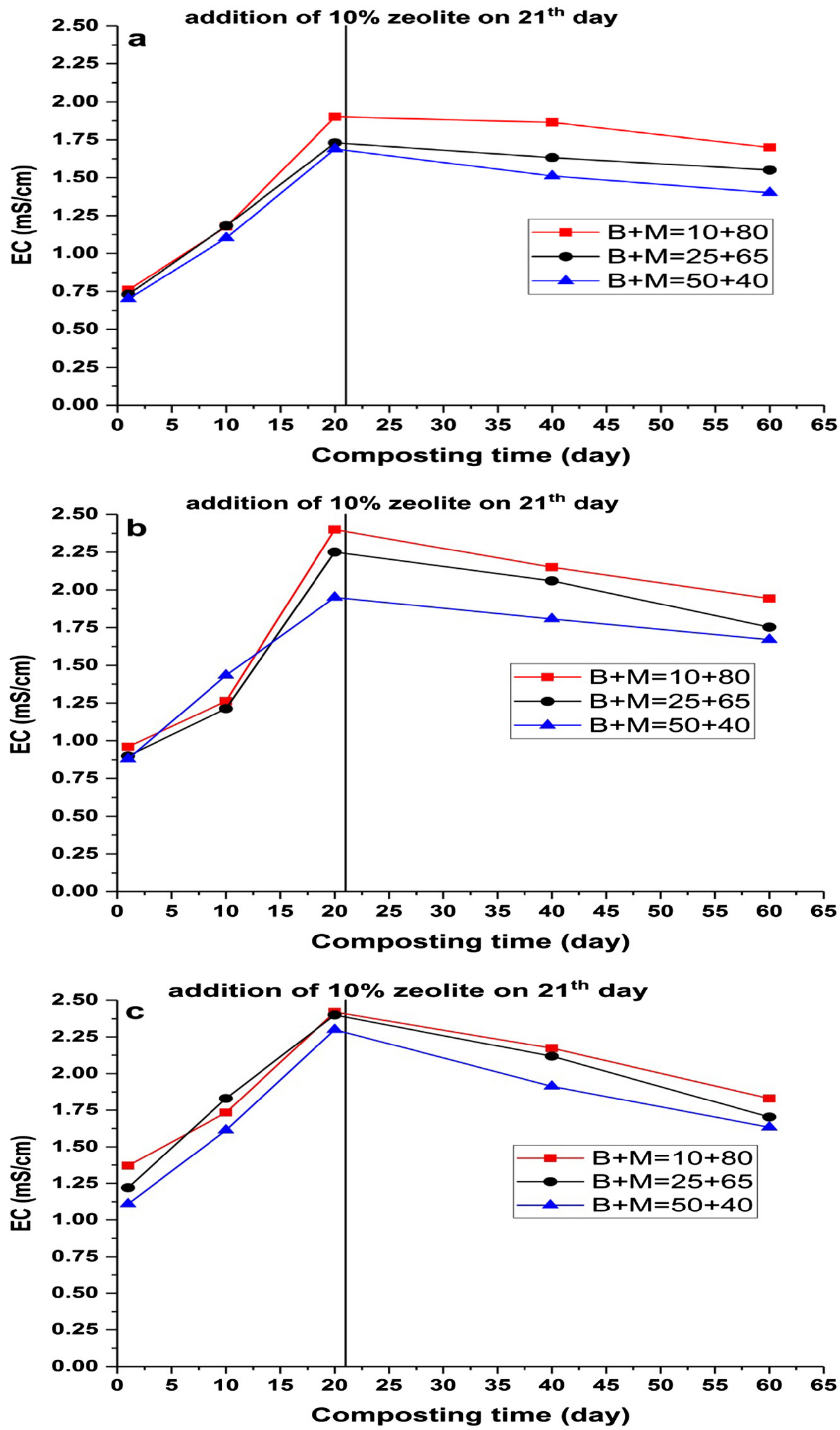

Fig. 2. Changes in the electrical conductivity by using 10% zeolite: (a) vinasse content 10%; (b) vinasse content 20%, and (c) vinasse content 40%; B and M represent weight percent of bagasse and cow manure respectively.

mixed treatments with different ratios of vinasse, bagasse, and cow manure compared to the control treatments. The initial weight and the size of the worms on the first day of vermicomposting process (or 21th day of the integrated process) were 369 mg/worm and 5.7–6.5 cm, respectively. The final weight of the worms for the treatments containing vinasse with the ratio of 10, 20, and 40% was 581, 518, and 438 mg/worm, respectively. The corresponding value for the control treatments (without vinasse) was in the range of 594–673 mg/worm. An increase in the weight and number of worms was observed for all the treatments. The increase in the number and weight of *E. fetida* worms was lower in comparison with other similar studies, which produced vermicompost from cow manure, wastewater sludge, and nutrients (Edwards and Bohlen, 1996; Fernández-Bayo et al., 2007; Suthar and Sharma, 2013). The difference in the number and weight of the earthworms among different vinasse treatments can be due to different compositions of the vermicomposting mixtures assayed, so that with increasing the dosage of vinasse added, the number and weight of the worms decreased. Our results are in consistent with the results found by other researchers (Fernández-Bayo et al., 2007;

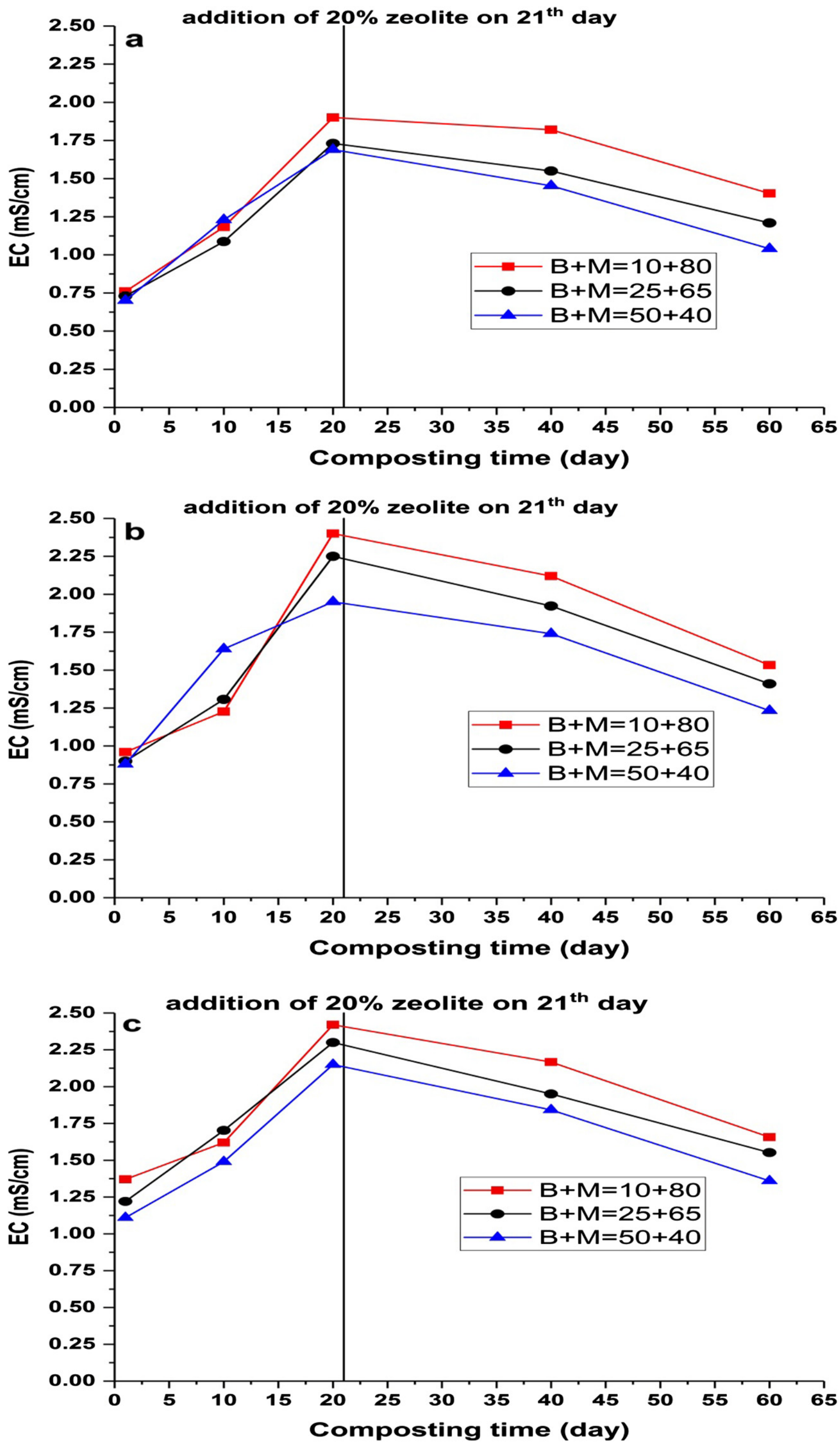

Fig. 3. Changes in the electrical conductivity by using 20% zeolite: (a) vinasse content 10%; (b) vinasse content 20%, and (c) vinasse content 40%; B and M represent weight percent of bagasse and cow manure respectively.

Table 2
The number of worms at the beginning and the end of the vermicomposting process for different treatments of mixed vinasse, bagasse, and cow manure with different ratios as well as for control treatments.

Worms number	Treatments*											
	$V_0 + B_1$	$V_0 + B_2$	$V_0 + B_3$	$V_1 + B_1$	$V_1 + B_2$	$V_1 + B_3$	$V_2 + B_1$	$V_2 + B_2$	$V_2 + B_3$	$V_3 + B_1$	$V_3 + B_2$	$\mathbf{V_3 + B_3}$
Initial number (21th day)	68	68	68	52	52	52	52	52	52	52	52	52
Final number (60th day)	108	113	102	78	73	72	73	71	69	67	65	64

* V_0 means compost or vermicompost without vinasse; V_1, V_2 and V_3 each respectively means mixture with 10%, 20% and 40% vinasse; B_1, B_2 and B_3 each respectively means mixture containing 10%, 25% and 50% bagasse.

Molina et al., 2013). The best results for the growth and reproduction of worms were observed for the treatments consisting of the lowest ratio of vinasse (10%) (581 mg/worm and 78 worms). The results revealed that by increasing the content of vinasse from 10 to 40%, the weight and number of *E. fetida* decreased. The worms of the control treatments (without vinasse) had the highest weight and number of *E. fetida* at the end of the vermicomposting process, when compared with treatments with vinasse. According to Owojori et al. (2008), *E. fetida* growth is influenced by EC and salt content. Furthermore, the obtained results showed that the total concentration of soluble salts not only affected the growth and reproduction of the worms but also influenced cationic and anionic composition, for example, phosphate or organic forms negatively influenced the worms' reproduction (Murillo et al., 1995; Ndegwa et al., 2000; Owojori et al., 2008).

3.4. The maturity indices and maturity degree of the vermicompost

The parameters determining the degree of maturity of the resulting compost were measured using germination index (GI), respiration index (CO_2), and the population of bacteria and fungi (Table 3). The GI above 80–90% is an indicator showing phytotoxic free composts. Zucconi et al. (1981) proposed that if the GI of a compost be less than 80% (GI < 80%), it is considered as an immature compost, for mature ones the GI index is > 80% and 90%. The results of this study showed that the GI of all samples was very good and stable (GI = 100%). Oviedo-Ocaña et al. (2015) reported a consistent GI for their treatments. The aim of determining the oxygen respiration or carbon dioxide evolution level is to estimate the degree of maturity of the compost and biological activity in final compost (Margesin et al., 2000). The maturity index for cellular respiration was 0.16–1.5 (mg C-CO_2 g^{-1} organic carbon day^{-1}) for the experimental treatments and 0.71–0.85 for the control samples. Epstein (1996) proposed the following limitations as compost stability index: if respiration rate (mg C-CO_2 g^{-1} organic carbon day^{-1}) is <2, the compost is very stable, 2–5 stable, 5–10 moderately stable, 10–20 unstable, and >20 extremely unstable. In the study carried out by California Compost Quality Council (CCQC) (2001), based on carbon dioxide evolution rate (mg C-CO_2 g^{-1} organic carbon day^{-1}), the compost stability is defined as follows: <2 very stable, 2–8 stable, and >8 less stable. The results of this research on the 60th day showed that in all the treatments, the cellular respiration index was less than 2 (<2), indicating that the final compost is very stable. Our results are in good agreement with the results obtained by Oviedo-Ocaña et al. (2015) and Gómez et al. (2006). The microorganisms present in the treatments can provide a suitable, mature, and stable compost product in terms of cellular respiration index if we can provide them a proper carbon source, their required organic matter, and suitable ventilation and oxygenation conditions in interior parts of the assayed mixtures (Oviedo-Ocaña et al., 2015). The highest value for cellular respiration index was reported in the treatments with 40% vinasse, ranging from 0.85 to 1.5. It might be attributed to the reduction in the activity of microorganisms owing to the higher percentage of vinasse when compared with other treatments. According to the previous studies, since the resistance of bacteria at higher temperatures is greater than that of fungi, typically their population is higher than that of fungi in the final product (Rojas-Avelizapa et al., 2005). The concentration or population of bacteria and fungi in the final compost product is another key factor that determines the degree of maturity of the product. The concentrations of bacteria and fungi for the experimental treatments were in the range of 9.5–15.21 and 9.25–9.96 log colony forming units per gram dry weight of compost (CFU/gdw), respectively. The corresponding values for the control treatments varied from 12.61 to 14.24 and 9.37 to 10.12 log (CFU/gdw), respectively. Various reports have stated that the minimum microbial population required for bioremediation is at least 6 log (CFU/gdw) (Rojas-Avelizapa et al., 2005). The results of this study showed that the microbial population was developed for all treatments by the end of the vermicomposting, and the highest bacterial population was detected for the treatments containing 40% vinasse and 50% bagasse. The level of access to the carbon source can result in changes in the microbial population. Therefore, since the microorganisms of this treatment possessed the largest sources rich of vinasse and bagasse, as well as suitable aerobic conditions and sufficient ventilation, this treatment had the largest microbial population.

4. Conclusions

In the present study, an integrated composting-vermicomposting process was used to investigate its capability in degradation of different mixtures of vinasse, bagasse, and cow manure into a mature and stable end product. In some treatments, a natural calcium zeolite was also applied as adsorbent to reduce the salinity of the final product. The results showed that despite the high level of potassium in vinasse, at the end of the process the total potassium content decreased in some treatments because of the application of calcium zeolite and adsorption of this element by the zeolite. The maturity and stability indices of the resulting

Table 3
Maturity indices of the fertilizer obtained from the composting and vermicomposting at different ratios on day 60.

Treatments*	Germination %	Respirometric ** mgC-CO_2 g^{-1} day^{-1}	Bacteria $\frac{\log CFU}{gdw}$	Fungi $\frac{\log CFU}{gdw}$
$V_1 + B_1$	100	1	11.61	9.25
$V_1 + B_2$	100	0.3	9.5	9.41
$V_1 + B_3$	100	0.69	11.46	9.4
$V_2 + B_1$	100	0.48	11.49	9.96
$V_2 + B_2$	100	0.3	11.87	9.45
$V_2 + B_3$	100	0.16	12.61	9.63
$V_3 + B_1$	100	1.5	11.51	9.3
$V_3 + B_2$	100	0.85	11.6	9.62
$V_3 + B_3$	100	0.92	15.21	9.77
Control				
$V_0 + B_1$	100	0.71	13.24	9.37
$V_0 + B_2$	100	0.73	14.24	10.12
$V_0 + B_3$	100	0.85	12.61	9.55

* V_0 means compost or vermicompost without vinasse; V_1, V_2 and V_3 each respectively means mixture with 10%, 20% and 40% vinasse; B_1, B_2 and B_3 each respectively means mixture containing 10%, 25% and 50% bagasse.
** All values are based on dry weight.

fertilizer were in an acceptable range. The number and weight of the earthworms increased at the end of the vermicomposting process for all the treatments. The higher the vinasse dosage, the lower the weight and number of the worms. The treatments consisting of the higher zeolite content and the lower vinasse content provided a final product with the best quality. It can be concluded that the integrated composting-vermicomposting process yielded a useful final fertilizer that can be used as soil conditioner for agricultural purposes.

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