Journal of Environmental Management 182 (2016) 134–140

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

# Waste recycling by vermicomposting: Maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators

Hossein Alidadi [a], Ahmad Hosseinzadeh [b, *], Ali Asghar Najafpoor [a], Habibollah Esmaili [c], Jafar Zanganeh [d], Maryam Dolatabadi Takabi [b], Fardin Ghasemy Piranloo [e]

[a] Department of Environmental Health Engineering, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Student Research Committee, Department of Environmental Health Engineering, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Department of Biostatistics, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Newcastle Institute of Energy and Resources, The University of Newcastle, Australia
[e] Biospher Technology Company, Environmental Laboratory, Abhar, Iran

A R T I C L E I N F O

*Article history:*
Received 19 February 2016
Received in revised form
10 July 2016
Accepted 11 July 2016

*Keywords:*
Vermicompost
Compost
Maturation
Stability

A B S T R A C T

Present study aims to examine the dynamics of maturation and qualification indicators in various vermicompost treatments and selection of the best treatment along with best maturation time in this regard. In this empirical study, dynamics of chemical (pH, electrical conductivity (EC), total nitrogen (TN), phosphorous, lignin, water soluble carbon (WSC), C/N, $NH_4/NO_3$) and biological (dehydrogenase enzyme (DEH) and DEH/WSC) properties were investigated in four various treatments, including various ratios of compost produced from municipal solid waste (MSW) and carbonaceous materials (50:50, 70:30, 85:15 and 100:0) over 100 days.

Results showed a significant fluctuation in EC, DEH and DEH/WSC proportions over the process. In addition, a noticeable increase was observed for the dynamics of TN, phosphorous and lignin. In contrast, the C/N, $NH_4/NO_3$ and WSC values gradually decreased during the process. Moreover, it was observed that the length of 75 days for the process is an appropriate time for maturation of all treatments. However, the first and second treatments resulted in better outcomes compared with the other types of treatments. From the point of view of quality obtained vermicompost was nitrogen enriched product in all treatments. Whereas, for the phosphorous elements this method is appropriate for the first treatment only.



## 1. Introduction

At present, owing to ever increasing population and anthropogenic activities, Municipal Solid Waste (MSW) generation shows a noticeable increase with varying characteristics. MSW management therefore has become an important issue, especially when it comes to safe disposal. In most cities and towns, however, there is an unscientific and nonsystematic MSW management involved in dumping of solid waste in the outskirts of cities. Landfill and incineration are the most widely used types of waste management options, which in comparison to stabilization by composting and vermicomposting, have minimum utilization. One of the most important MSW recycling technologies is their bioconversion to organic fertilizers that is referred to as composting and vermicomposting. MSW compost and vermicompost are increasingly applied in agriculture as a soil conditioner and fertilizer (Paul et al., 2011). Whereas, in contrast to vermicompost, two of the most important drawbacks of MSW compost are less macro and micronutrients and much higher levels of electrical conductivity (EC) than agricultural soils, which in its application in agriculture, can potentially cause the prevention of seed germination (Hargreaves et al., 2008; Iqbal et al., 2010). Additionally, according to the properties of MSW compost produced and valid guidelines, the produced MSW compost does not meet the appropriate conditions for the amendment of soils. MSW compost, therefore, is

* Corresponding author. Department of Environmental Health Engineering, School of Health, Mashhad University of Medical science, Danashgah Ave., Mashhad, Iran.
*E-mail address:* Hosseinzadeh26@gmail.com (A. Hosseinzadeh).

http://dx.doi.org/10.1016/j.jenvman.2016.07.025
0301-4797/

considered more as a soil conditioner than a soil fertilizer and it is even introduced as a secondary waste by some scientists (Gomez, 1998; Gray and Biddlestone, 2013; Hargreaves et al., 2008; Stonehouse, 2013). So for successful use of MSW compost as a fertilizer, all the existent problems should be solved. Vermicomposting is a thought–provoking process in this regard. Vermicomposting is able to not only enrich the final product from the aspect of both macro and micro nutrients and improve the other important parameters such as Carbon to Nitrogen (C/N), $NO_4/NO_3$, EC and proportion of its cellulose component decomposition, but also, augment the diversity of soil microbial communities after permanent applications (Aira et al., 2006; Alidadi et al., 2007; Alidadi et al., 2005; Doan et al., 2014; Fornes et al., 2012). On the other hand, the greater the diversity of soil microbial community structure, the more recycling of soil nutrients and other chemical and physical processes of the soil will occur. Therefore, for maintaining soil fertility and also providing an appropriate and healthy web of soil food, existence of different microbial species in soil is necessary (Wang et al., 2014). Furthermore, the produced vermicompost will be perfectly organic, important to provide soil health and therefore the product health (Lohani et al., 2011). Additionally, the degree of maturity and stability of compost and vermicompost are key parameters affecting the successful use for agricultural practices. Utilization of immature and unstable vermicompost may create an anaerobic condition for soil microorganisms. This is due to microorganisms taking advantage of available $O_2$ for breaking down materials and production of organic acids during the early stages of this process. Phytotoxicity may also be another drawback for using immature and unstable vermicompost. Although there are some conceptual differences amongst maturity and stability, these two terms are both frequently used in order to determine the organic matter decomposition degree over the vermicomposting process. Stability of vermicompost is defined as the microbial biomass activity level which can be determined with the rate of $CO_2$ production and $O_2$ uptake or with the released heat proportion resulting from microbial activity. Maturity of vermicompost is the level of phytotoxic organic substance degradation created over the active stage of this process (Benito et al., 2003). In describing maturity, a single criterion is not sufficient; thus, it is best evaluated with the measurement of two or more properties of vermicompost. There are some criteria and parameters for evaluating the vermicompost maturity which are based on different properties. These include physical (colour, odour, temperature, particle size and inert materials etc), chemical (C/N, Cation Exchange Capacity (CEC), pH, EC, $NH_4/NO_3$, Water Soluble Carbon (WSC), lignin, complex carbohydrates, humification index etc) and biological (microbial activity, enzyme activity etc) (Bernal et al., 2009). Chemical components of vermicompost feedstocks along with their decomposition stage are effective on maturity, stability and quality of vermicompost (Benitez et al., 1999; Bernal et al., 2009). So in the present study, conversions of MSW compost and agricultural wastes (carbonaceous materials), a main part of MSW into vermicompost, as an organic fertilizer have been studied. The specific objective of the present study was to investigate the effect of various materials (MSW compost and carbonaceous materials) on chemical (pH, EC, Total Nitrogen (TN), C/N, $NH_4/NO_3$, lignin, phosphorous and WSC) and biological (dehydrogenase enzyme activity (DEH) and DEH/WSC) properties during the vermicomposting process over 100 days and determine the quality, stabilization degree and maturation time of the produced vermicompost coupled with selection of the best treatment in this regard.

## 2. Materials and methods

### 2.1. Experimental design

Feed materials used in this empirical study were composed of two main parts. This includes the compost produced from MSW in Mashhad's compost factory, where the length of composting process has been 45 days and carbonaceous organic materials (COM) include sawdust, boxwood leaves and cardboard. Firstly, the compost used was washed to reduce its electrical conductivity (EC). Four treatments with three replicates were prepared from compost and COM with varying proportions including A) 50%:50%, B) 70%:30%, C) 85%:15% and D) 100%:0% to provide appropriate conditions. In each replicate 1 kg of dry weight and 100 g of Eisenia Fetida earthworms was used so that, the mean weight of each earthworm was 2 g and the total number of earthworms used were fifty in each treatment (Ndegwa et al., 2000). The duration of the present study was 100 days and moisture content of the treatments was maintained at ~50–80% by spraying water on the surface. The experimental bins were kept in the laboratory at room temperature and covered with a mosquito net to prevent any intrusion of pests. During this process, sampling was conducted five times. These took place on the 0th, 25th, 50th, 75th and 100th days.

### 2.2. Analytical methods

Upon completion of mixing, the process samples were taken and analyzed. The moisture content was determined by drying the samples in an oven at 105 °C for 24 h until constant weight was obtained. The pH of samples was determined potentiometrically in a 1/10 suspension placed on a mechanical shaker at 230 rpm for 30 min. EC was measured in a 1:10 (w/v) water extract. Measurement of total carbon content was performed via combustion in ovens at 750 °C for 2 h (Tim Haug, 1980). Total Kjeldahl Nitrogen digestion method was used for determination of total nitrogen (Theroux et al., 1936). C/N ratio of samples was calculated by dividing the weight of total carbon by the total weight of nitrogen. The proportion of $NH_4^+$and $NO_3^-$ was measured using the KCl extraction method (Eldridge et al., 1943). Dehydrogenase activity measurement was conducted by the methods described by Tabatabai. Based on this method, 0.5 g of vermicompost sample was completely mixed with 0.1 g of $CaCO_3$. Then, 1 mL of 3% aqueous solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) along with 2.5 mL of distilled water were added. After incubation at 37 °C for 24 h, 10 mL of methanol was added. The obtained suspension was filtered and the proportion of triphenyl formazan (TPF) in the filtrate was measured using a spectrophotometer at 485 nm (UV/VIS T80/T80+, England). A control without the addition of TTC was included for each sample (Page, 1982). Water Soluble Carbon (WSC) of samples was extracted with distilled water (1/5 w/v), and the extracted carbon with pyrophosphate was measured by oxidation with potassium dichromate and measurement of absorbance at 590 nm (Sims and Haby, 1971). Ascorbic acid method was used for determination of total phosphorous. Firstly, samples were digested and then read at 880 nm on a Spectrophotometer (UV/VIS T80/T80+, England) (Way, 2012). The lignin samples were dried at 50 °C for 24 h prior to weighing into glass culture tubes. The weighed sample was placed in a vacuum desiccator over $P_2O_5$ with the caps off for at least 18 h before analyzing. The standard procedure involved eliminating the sample from the desiccator, adding 2.5 ml of fresh prepared acetyl bromide reagent, capping immediately, and heating at 70 °C. Then 100 μL of perchloric acid was added to samples. Heating time duration was 30 min. After heating, the samples were quantitatively transferred with the aid of acetic acid to 50 mL volumetric flasks that contained 10 mL of 2 M NaOH and

12 mL of acetic acid. Hydroxylamine (350 μL of 0.5 M) was added to each flask and samples were diluted to 50 mL with acetic acid. In each sample, absorption spectra were determined using a Spectrophotometer (UV/VIS T80/T80+, England). The maximum absorption was at 280 nm (Hatfield et al., 1996). Data processing and analysis was carried out by using statistical package (SPSS Ver. 16).

## 3. Results

### 3.1. Raw material analysis

Table 1 shows the results of first stage analysis on the 0th day of processes (beginning day of processes).

### 3.2. Chemical parameter analysis

The experimental results of pH, EC, C/N and WSC as four of the measured chemical indicators in first and second treatments are presented in Table 2. Table 3 demonstrates the results of pH, EC, C/N and WSC, measured during vermicomposting process in third and fourth treatments. The obtained experimental results of phosphorous, TN, $NH_4/NO_3$ and lignin are depicted in Figs. 1–4.

### 3.3. Biological parameters analysis

The attained results of DEH enzyme and DEH/WSC ratio as two of the most important biological indicators are illustrated via Figs. 5 and 6.

## 4. Discussion

### 4.1. Dynamics of pH

Optimum pH for earthworm growth is neutral to rarely alkaline (Canti and Piearce, 2003). At the end of processes in all treatments, proportions of pH demonstrated an increase from the initial values. Eventually, the pH values in all treatments were 8, 7.93, 8 and 8.12 respectively. Therefore, pH of all treatments was in an appropriate range. Humic acids and ammonium ions ($NH_4^+$) are products of organic matter degradation being able to raise the amount of pH. Also, $NH_4$ production might be another cause of pH increase. On the other hand, carboxylic and phenolic groups of humic substances are two of the most important materials for plummeting of pH (Pramanik et al., 2007).

### 4.2. Dynamics of EC

Available excess salts in the structure of soils have adverse effects on the plants. EC of the soils are associated with the content of dissolved soil solutes content dissolved. In a survey in United States, it has been reported that the EC of the MSW compost was significantly higher than agricultural soils EC. Since, their application is able to potentially prevent seed germination and decline in the biological activity of soils, the lower EC of the compost or vermicompost will be more useful in the fertility of the soils (Hargreaves et al., 2008). By virtue of aforementioned reasons, the MSW compost used was firstly washed. The produced effluent can be used for providing the moisture of compost piles. According to Table 2, the largest amount of EC was observed in the second treatment (1368 μS/cm). Fourth, third and first treatments occupied the next ranks with 936, 864 and 720 μS/cm, correspondingly. The results of repeated measures from the ANOVA (RMANOVA) test demonstrated that there is a significant difference among them ($P < 0.001$). These results are well agreed with the findings reported by Khalil et al. (Khalil and Sanaa, 2009). The reason of EC rise in these treatments may be attributed to the release of nutrient ions through the organic matter mineralization process; and decrease of this parameter after the fourth stage (75th day) might be owing to stabilization of the ions released during the process (Afifi et al., 2012). So, according to this index, stabilization and maturation of treatments occurred after the 75th day of the process.

### 4.3. Dynamics of phosphorous

For phosphorous, a remarkable upward trend was experienced by all treatments (Fig. 1). At the end of process, their values were 2132, 3428, 4986 and 4200 mg phosphate/L, in that order. The result of RMANOVA test revealed that there is a significant difference among them, (P value $< 0.001$). The cause of their augmentation has been attributed directly to earthworm's gastrointestinal enzymes and indirectly to micro flora stimulation (Kizilkaya et al., 2011). On the other hand, increase of phosphorous during the process might be by virtue of phosphorous mineralization by bacterial and enzymatic activities, especially the phosphatase enzyme activity in an earthworm's gastrointestine (Dominguez et al., 2000). Raphael and Velmourougane reported similar results (Raphael and Velmourougane, 2011). Phosphorous, like nitrogen, is an essential nutrient for plant growth (Pang et al., 2007). Obtained findings of phosphorous in only the first treatments have been found in the range of 'end use test values recommended for compost' by USA (800–2500 mg/l) (Brinton, 2000).

### 4.4. Dynamics of C/N ratio

The values of C/N ratios in all treatments showed a remarkable reduction during this process, particularly over the first 50 days (Table 3). At the end of processes, the measured proportions of this ratio were 14.01, 12.93, 16.61 and 28.16 in vermicompost products

**Table 1**
The results of raw materials analysis in each treatment with three replicates (Mean ± SE).

| Parameters | First treatment | Second treatment | Third treatment | Forth treatment |
|---|---|---|---|---|
| Moisture (percent) | 78.04 ± 1.85 | 75.04 ± 2.71 | 64.26 ± 3.05 | 54.29 ± 1.83 |
| pH | 7.32 ± 0.07 | 7.27 ± 0.06 | 7.56 ± 0.08 | 7.50 ± 0.07 |
| EC (μS/cm) | 712.8 ± 5.19 | 821.4 ± 10.4 | 916.13 ± 6.49 | 1051 ± 48.49 |
| TN (mg/kg) | 0.35 ± 0.005 | 0.43 ± 0.003 | 0.45 ± 0.008 | 0.63 ± 0.009 |
| C/N | 102.63 ± 1.69 | 77.45 ± 1.95 | 71.63 ± 2.00 | 56.5 ± 2.07 |
| $NH_4/NO_3$ | 15 ± 0.04 | 16 ± 0.27 | 10 ± 0.08 | 9.09 ± 0.01 |
| Lignin (percent) | 0.007 ± 0.002 | 0.004 ± 0.000 | 0.003 ± 0.001 | 0.001 ± 0.000 |
| WSC (mg/l) | 319.77 ± 2.73 | 286.64 ± 3.97 | 267.82 ± 7.84 | 228.48 ± 8.55 |
| Phosphorous (mg phosphate/l) | 12.46 ± 0.27 | 18.86 ± 0.91 | 24.32 ± 1.07 | 29.94 ± 1.03 |
| DEH (μg/gr.24 h) | 29.64 ± 0.41 | 20.86 ± 1.32 | 11.39 ± 0.64 | 0.00 |
| DEH/WSC | 0.090 ± 0.003 | 0.077 ± 0.010 | 0.040 ± 0.005 | 0.00 |

EC: Electrical Conductivity; TN: Total Nitrogen; C/N: Carbon to Nitrogen; $NH_4/NO_3$: Ammonium to Nitrate; WSC: Water Soluble Carbon; DEH: Dehydrogenase Enzyme.

**Table 2**
The experimental results of pH, EC, C/N and WSC during the vermicomposting process of first and second treatments with three replicate (Mean ± SE).

| Parameters | First treatment | | | | Second treatment | | | |
|---|---|---|---|---|---|---|---|---|
| | 25th(d) | 50th (d) | 75th (d) | 100th (d) | 25th (d) | 50th (d) | 75th (d) | 100th (d) |
| pH | 8.56 ± 0.07 | 8.85 ± 0.02 | 8.11 ± 0.01 | 8.01 ± 0.00 | 8.58 ± 0.03 | 8.69 ± 0.02 | 8.24 ± 0.02 | 7.93 ± 0.01 |
| EC (μS/cm) | 648.64 ± 28.52 | 655.20 ± 12.58 | 1569.60 ± 17.32 | 720.00 ± 23.09 | 655.20 ± 23.09 | 1044.0 ± 6.00 | 1404.13 ± 17.20 | 1368.0 ± 0.57 |
| C/N | 87.11 ± 3.55 | 36.19 ± 0.43 | 25.31 ± 0.69 | 14.01 ± 0.09 | 68.86 ± 0.23 | 36.18 ± 0.81 | 19.35 ± 0.32 | 12.93 ± 0.15 |
| WSC (mg/l) | 256.86 ± 2.91 | 197.76 ± 6.33 | 180.22 ± 3.14 | 151.02 ± 2.71 | 231.37 ± 2.16 | 209.43 ± 5.76 | 162.76 ± 6.84 | 149.22 ± 1.12 |

EC: Electrical Conductivity; C/N: Carbon to Nitrogen; WSC: Water Soluble Carbon; d: day.

**Table 3**
The experimental results of pH, EC, C/N and WSC during the vermicomposting process of third and fourth treatments with three replicate (Mean ± SE).

| Parameters | Third treatment | | | | Fourth treatment | | | |
|---|---|---|---|---|---|---|---|---|
| | 25th(d) | 50th (d) | 75th (d) | 100th (d) | 25th (d) | 50th (d) | 75th (d) | 100th (d) |
| pH | 8.49 ± 0.08 | 8.61 ± 0.05 | 8.26 ± 0.02 | 8.01 ± 0.03 | 8.22 ± 0.07 | 7.97 ± 0.03 | 8.21 ± 0.05 | 8.12 ± 0.01 |
| EC (μS/cm) | 886.45 ± 2.70 | 900.00 ± 5.77 | 1296.70 ± 2.30 | 864.00 ± 11.54 | 994.73 ± 20.97 | 828.00 ± 17.32 | 1296.00 ± 0.40 | 922.66 ± 24.03 |
| C/N | 53.57 ± 1.4 | 34.73 ± 0.82 | 19.54 ± 0.03 | 16.6 ± 0.01 | 55.50 ± 0.59 | 48.57 ± 1.28 | 42.49 ± 1.26 | 28.16 ± 0.97 |
| WSC (mg/l) | 227.16 ± 1.44 | 223.60 ± 5.85 | 151.75 ± 4.08 | 128.77 ± 2.47 | 228.00 ± 0.87 | 180.30 ± 4.41 | 159.19 ± 4.59 | 124.37 ± 1.73 |

EC: Electrical Conductivity; C/N: Carbon to Nitrogen; WSC: Water Soluble Carbon; d: day.

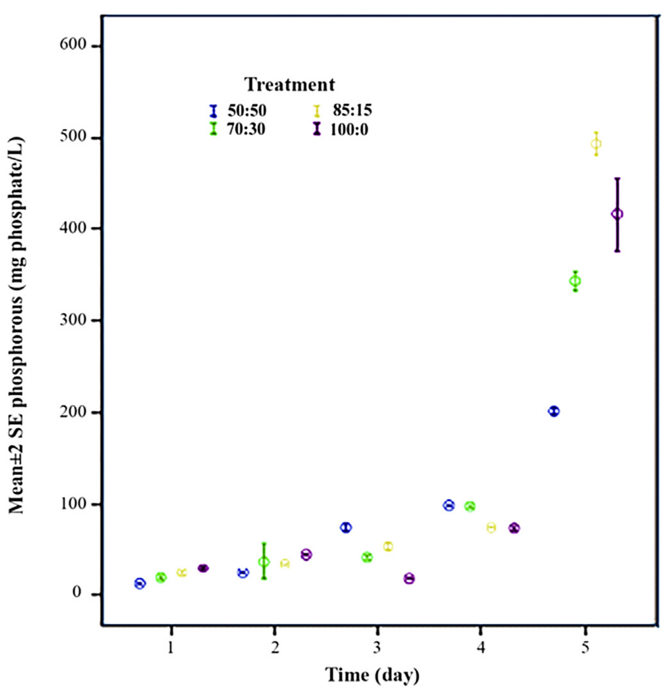


**Fig. 1.** Changes in phosphorous during the vermicomposting process of different treatments.

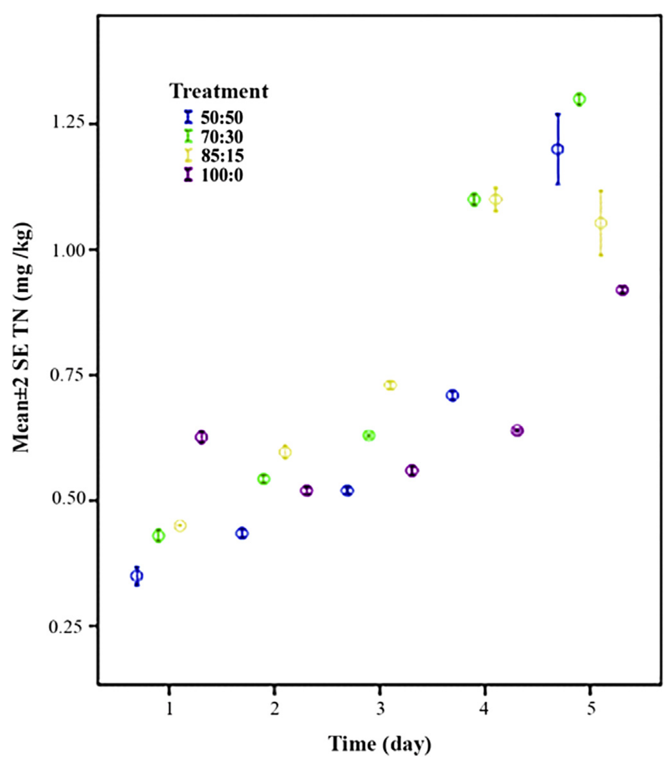


**Fig. 2.** Changes in Total Nitrogen (TN) during the vermicomposting process of different treatments.

of all treatments, in that order. RMANOVA test showed significant difference between treatments as well (P value < 0.001). In the same studies by Afifi et al., similar results were obtained (Afifi et al., 2012). C/N ratio decline was expected due to production and emission of $CO_2$ over this process and also production and disposal of nitrogenous substances. This ratio is a very important factor in vermicompost quality assessment as microorganisms will make benefit from available nitrogen in soil for degradation of organic carbon which leads to lack of nitrogen in the soil (Fuchs et al., 2001). A C/N ratio below 20 is mentioned by many authors as a maturation vermicompost index (Jimenez and Garcia, 1991). Thus after 75 days, second and third treatments can be considered as mature vermicompost with the proportions of 19.29 and 19.55, respectively. However, this ratio reached to the aforementioned range in the first treatment after 100 days, which may be owing to high initial proportion of this ratio in it. For this parameter, the second treatment had the best condition.

### 4.5. Dynamics of TN

The trend of Total Nitrogen (TN) proportion was increasing in all treatments (Fig. 2). At the end of the process, TN treatments were 1.2, 1.3, 1.05 and 0.92%, respectively. All of these proportions are higher than the recommended amount of TN by Nova Scotia Department Of Environment (NSDOE) (0.6%); that is, from the point

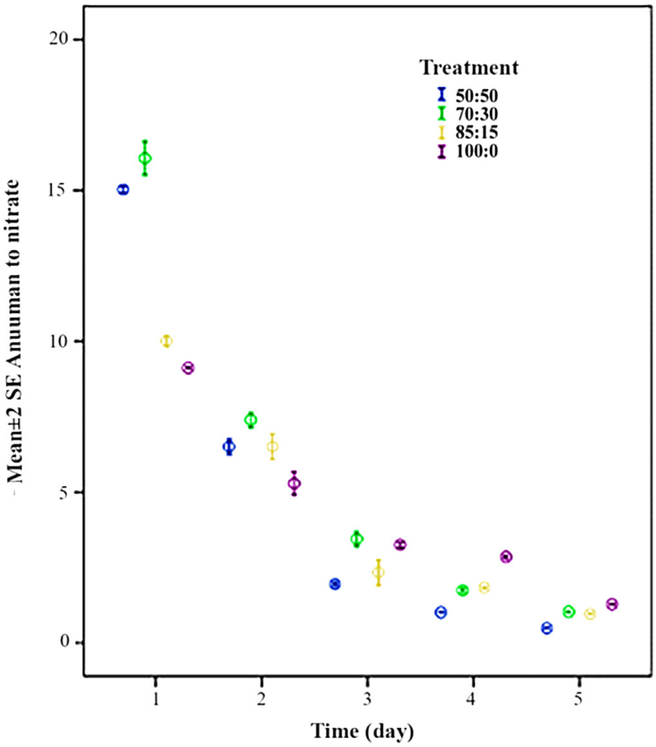


**Fig. 3.** Changes in $NH_4/NO_3$ during the vermicomposting process of different treatments.

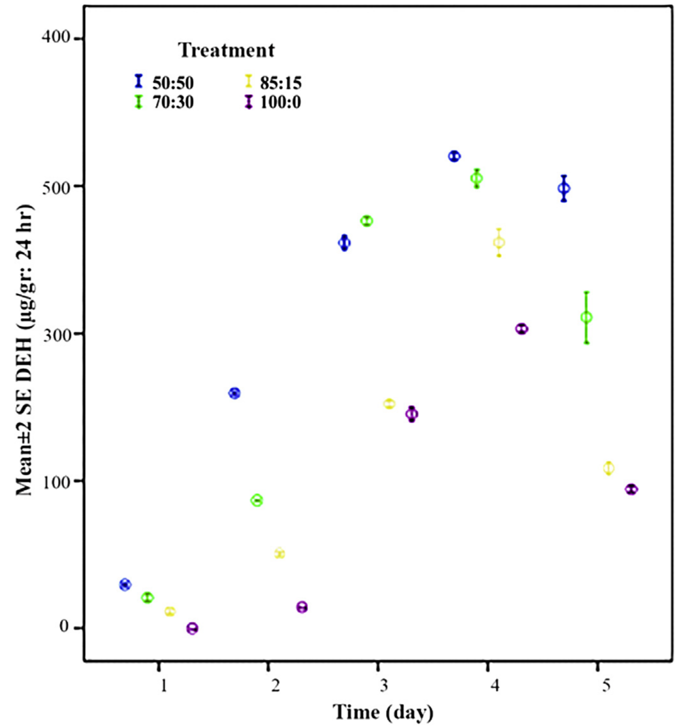


**Fig. 5.** Changes in dehydrogenase enzyme activity (DEH) during the vermicomposting process of different treatments.

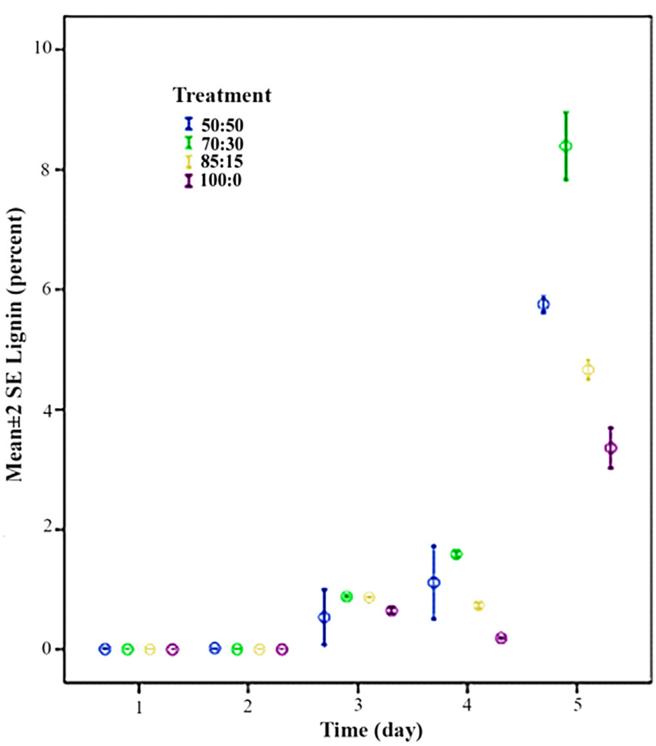


**Fig. 4.** Changes in lignin during the vermicomposting process of different treatments.

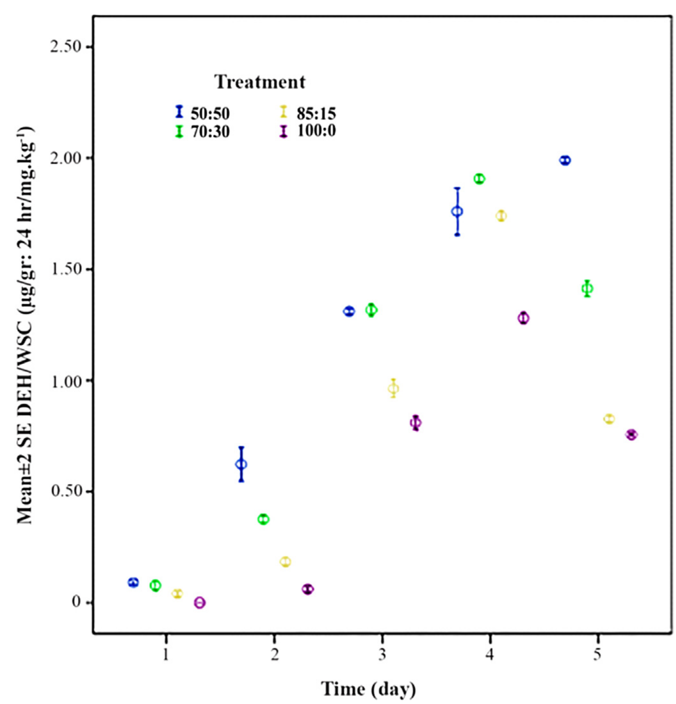


**Fig. 6.** Changes in DEH/WSC during the vermicomposting process of different treatments.

of view of nitrogen, all treatments have an appropriate condition (NSOGA, 1995). RMANOVA test demonstrated significant difference among treatments as well (P value < 0.001).

### 4.6. Dynamics of $NH_4/NO_3$ ratio

$NH_4/NO_3$ ratio of these treatments displayed a downturn trend during the process (Fig. 3). The results of RMANOVA test showed a significant difference amongst the treatments ($p_{value} < 0.001$) for this ratio as well. Other authors have observed similar trends during organic amendments processes (Ko et al., 2008). Ammonium amount in all treatments experienced a downturn trend. The reason for ammonium decline might be due to its oxidation to nitrate by nitrifying bacteria and also its volatilization because of pH decline (Afifi et al., 2012; Benito et al., 2003). Deamination of amino acids oxidative of proteins has also been reported as another reason (Morisaki et al., 1989). Conversely, variation proportion of nitrate showed with absolute certainty an upward trend. Its reason may be because of the nitrification process (Bernal et al., 2009). At the end of processes, a considerable proportion of nitrate – nitrogen, which is readily available for plants to uptake, can be observed in all treatments. According to (California Compost Quality Council 2001), if the amount of this ratio is < 0.5 in compost, this amendment will be very mature, but if it is in the range of (0.5–3) or > 3, it will be mature or immature, correspondingly (Morisaki et al., 1989). Therefore, first and third treatments were mature vermicompost at the end of first fifty days with 1.94 and 2.3, correspondingly. The other treatments could be considered as mature vermicompost after 75th day with the amounts of 1.74 and 2.84, in that order. In the end, the first treatment with a ratio of 0.483 was the best treatment (very mature vermicompost) and the proportions of this parameter in the rest improved. The maturation time of the first and third treatments was 50 days of process and the time for the second and fourth treatments was 75 days.

### 4.7. Dynamics of WSC

As Table 3 shows the WSC concentrations in all treatments had a downturn trend so that the slope of this trend plummeted in all treatments after the 75thday. RMANOVA test demonstrated a significant difference between all of the treatments (P value < 0.001). Similar results have been reported by Benitez et al. (Benitez et al., 1999). Since water soluble carbon of immature vermicompost is composed of carbohydrates, hemicelluloses, phenolic components, organic acids, amino acids, peptides and other components it is easily biodegradable. In presence of such matters the pace of degradation will be augmented during vermicompost process (Zhang et al., 2000). Alternatively, because WSC of mature vermicompost has been composed of humic substances which are more resilient to biodegradation, its biodegradation will be decreased during the process. Therefore, it can be considered that their maturation time was after the 75th day. Generally, WSC decline is used as a vermicompost maturation index (Garcia et al., 1991).

### 4.8. Dynamics of lignin

Lignin percentage of all treatments had approximately same trend until the 50th day. After this stage, a noticeable increase was observed in all of them (Fig. 4). RMANOVA test showed a significant difference among all treatments (P value < 0.001). Results obtained by Tang et al. were concordant with the dynamics trend of lignin in the present study (Tang et al., 2006). This increase may be due to the bioconversion of cellulose substances to lignin by cellulase and lignocellulase enzymes produced by earthworm's gastrointestinal micro flora (Benitez et al., 1999). Thus, the trend of the process demonstrated that after the middle of vermicomposting process (50th day); easily biodegradable materials have plummeted. This can show that after this stage, vermicompost maturation in all treatments has occurred as one of the factors being able to indicate organic matter humification is formation of aromatic materials and lignin is as an aromatic constituent (Senesi, 1989).

### 4.9. Dynamics of dehydrogenase enzyme activity

In general, biological oxidation of organic matters is a dehydrogenation process. The dehydrogenase enzyme systems play an important role in the oxidation of organic compounds as they transfer hydrogen from substrates to acceptors (Page, 1982). In the present study, the up and down trend of DEH enzyme proportion was observed similarly for all treatments and its decrease began after the 75th day (Fig. 5). Also, a significant difference amongst all treatments has been shown by RMANOVA test. Obtained results by Lazcano showed an up and down trend for this parameter during process as well (Lazcano et al., 2008). Its augmentation is because the high activity of microorganisms originated from the existence of high amount of WSC in their initial substrate. Dehydrogenase activity stabilization can be attributed to the complete degradation of available organic matters (Benitez et al., 1999). So, this point demonstrated the maturation time of vermicompost.

### 4.10. Dynamics of DEH/WSC ratio

The DEH/WSC ratio is introduced as a ‘potential metabolic index’; that is, this index is applied to distinguish the hydrolytic and maturation phases in the vermicomposting process (Masciandaro et al., 1998). According to Fig. 6, first twenty days of process has been an initial adaptation period for earthworms in various treatments and after this stage, its trend was absolutely ascending until the 75th day of the process. This time interval can be considered as a hydrolytic phase. After that in all treatments except the first, the decreasing trend of this parameter started the maturation phase of the process. Its augmentation might be owing to the decomposition of ready available biodegradable organic matters by present microorganisms in substrate or in gastrointestinal systems of earthworms. Maturation phases happen most likely due to decline of easily biodegradable organic matters. The amount of this ratio was absolutely rising in first treatment during the entire process. This showed that WSC was not a limiting substance in the entire process. Benitez et al. has also reported a similar result (Benitez et al., 1999). For this variable, RMANOVA test results showed that there was a significant difference among all treatments (P value < 0.001).

## 5. Conclusions

The vermicomposting process is considered as an easy practical method in converting waste materials to organic fertilizers. This study aimed to investigate the effect of various ratios of COM and compost on improving the maturity and quality of final products. Results of EC, WSC, DEH and C/N to this study indicated that the length of 75 days for the process is an appropriate time for maturation of all treatments. The maturation results were found more pronounced after 75 days of process. Based on $NH_4/NO_3$, the maturation time of first and third treatments was on the 50th day and for second and fourth treatments the maturation time was determined to be the 75th day. According to lignin indicator, maturation phase had started since the 50th day in all treatments. Eventually, concerning DEH/WSC ratio, the maturation phase was started on the 75th day in all treatments except the first one. Unlike phosphorous, which was at a good level in the first treatment only, while nitrogen level was appropriate in all treatments. So according to the obtained results, first treatment is selected as best treatment.

## Acknowledgments

The authors would like to express their gratitude to the authorities of environmental chemistry lab of health school for their cooperation in this study. This study was financially supported by

Mashhad University of Medical Sciences (Project No. 911119).

## References


Afifi, M.M.I., Estefanous, A.N., El-Akshar, Y.S., 2012. Biological, chemical and physical properties of organic wastes as indicators maturation of compost. J. Appl. Sci. Res. 4, 1857–1869.

Aira, M., Monroy, F., Domínguez, J., 2006. Eisenia fetida (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb. Ecol. 4, 738–747.

Alidadi, H., Parvaresh, A.R., Shamansouri, M.R., 2005. Combined compost and vermicomposting process in the treatment and bioconversion of sludge. Iran. J. Environ. Health Sci. Eng. 4, 251–254.

Alidadi, H., Najafpoor, A.A., Parvaresh, A., 2007. Determination of carbon/nitrogen ratio and heavy metals in bulking agents used for sewage composting. Pak. J. Biol. Sci. 22, 4180–4182.

Benitez, E., Nogales, R., Elvira, C., Masciandaro, G., Ceccanti, B., 1999. Enzyme activities as indicators of the stabilization of sewage sludges composting with Eisenia foetida. Bioresour. Technol. 3, 297–303.

Benito, M., Masaguer, A., Moliner, A., Arrigo, N., Palma, R.M., 2003. Chemical and microbiological parameters for the characterisation of the stability and maturity of pruning waste compost. Biol. Fert. Soils 3, 184–189.

Bernal, M.P., Alburquerque, J., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour. Technol. 22, 5444–5453.

Brinton, W.F., 2000. Compost Quality Standards and Guidelines: an International View. Final Report to New York State Association of Recyclers. Wood End Research Laboratory.

California Compost Quality Council (CCQC), 2001. Compost Maturity Index, technical report.

Canti, M.G., Piearce, T.G., 2003. Morphology and dynamics of calcium carbonate granules produced by different earthworm species. Pedobiologia 5–6, 511–521.

Doan, T.T., Bouvier, C., Bettarel, Y., Bouvier, T., Henry-des-Tureaux, T., Janeau, J.L., Lamballe, P., Nguyen, B.V., Jouquet, P., 2014. Influence of buffalo manure, compost, vermicompost and biochar amendments on bacterial and viral communities in soil and adjacent aquatic systems. Appl. Soil. Ecol. 73, 78–86.

Dominguez, J., Edwards, C.A., Webster, M., 2000. Vermicomposting of sewage sludge: effect of bulking materials on the growth and reproduction of the earthworm Eisenia andrei. Pedobiologia 1, 24–32.

Eldridge, E.F., Therouz, F.R., Mallmann, W., 1943. Laboratory Manual for Chemical and Bacterial Analysis of Water and Sewage, third ed. McGraw Hill Book Co., Inc., New York City.

Fornes, F., Mendoza-Hernández, D., García-de-la-Fuente, R., Abad, M., Belda, R.M., 2012. Composting versus vermicomposting: a comparative study of organic matter evolution through straight and combined processes. Bioresour. Technol. 118, 296–305.

Fuchs, J., Galli, U., Schleiss, K., Wellinger, A., 2001. ASCP Guidelines 2001: Quality Criteria for Composts and Digestates from Biodegradable Waste Management. Association of Swiss Compost Plants (ASCP) in collaboration with the Swiss Biogas Forum.

Garcia, C., Hernandez, T., Costa, F., 1991. Changes in carbon fractions during composting and maturation of organic wastes. Environ. Manage 3, 433–439.

Gomez, A., 1998. The evaluation of compost quality. Trac-Trend. Anal. Chem. 5, 310–314.

Gray, K., Biddlestone, A., 2013. Biological Husbandry: a Scientific Approach to Organic Farming. Elsevier.

Hargreaves, J.C., Adl, M.S., Warman, P.R., 2008. A review of the use of composted municipal solid waste in agriculture. Agr. Ecosyst. Environ. 1, 1–14.

Hatfield, R., Brei, K., Grabber, J., 1996. Revising the Acetyl Bromide Assay to Optimize Lignin Determinations in Forage Plants. U.S. Dairy Forage Research Center: Research Summaries, pp. 63–65.

Iqbal, M.K., Shafiq, T., Hussain, A., Ahmed, K., 2010. Effect of enrichment on chemical properties of MSW compost. Bioresour. Technol. 15, 5969–5977.

Jimenez, E.I., Garcia, V.P., 1991. Composting of domestic refuse and sewage sludge. 1. Evolution of temperature, pH, C/N ratio and cation-exchange capacity. Resour. Conserv. Recy 1, 45–60.

Khalil, H.M., Sanaa, S., 2009. Applcation of sewage sludge in composting technology for eradication of pathogenic bacteria. Aust. J. Basic & Appl. Sci. 4, 4591–4600.

Kizilkaya, R., Karaca, A., Turgay, O.C., Cetin, S.C., 2011. Earthworm interactions with soil enzymes. In: Karaca, A. (Ed.), Biology of Earthworms. Springer Berlin Heidelberg, Berlin, pp. 141–158.

Ko, H.J., Kim, K.Y., Kim, H.T., Kim, C.N., Umeda, M., 2008. Evaluation of maturity parameters and heavy metal contents in composts made from animal manure. Waste Manage 5, 813–820.

Lazcano, C., Gomez-Brandon, M., Dominguez, J., 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 7, 1013–1019.

Lohani, N., Kumar, R., Tewari, L.M., Joshi, G.C., 2011. Effect of different organic treatments on ex situ conservation of Polygonatum cirrhifolium Royle. Intl. J. Biodivers. Sci. Eco. Serv. Mgt 2, 134–140.

Masciandaro, G., Ceccanti, B., Gallardo-Lancho, J.F., 1998. Organic matter properties in cultivated versus set-aside arable soils. Agr. Ecosyst. Environ. 2, 267–274.

Morisaki, N., Phae, C.G., Nakasaki, K., Shoda, M., Kubota, H., 1989. Nitrogen transformation during thermophilic composting. J. Ferment. Bioeng. 1, 57–61.

Ndegwa, P.M., Thompson, S.A., Das, K.C., 2000. Effects of stocking density and feeding rate on vermicomposting of biosolids. Bioresour. Technol. 1, 5–12.

NSOGA, 1995. Directory of organic growers in Nova Scotia. Org. Times 1, 12–13.

Page, A.L., 1982. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America.

Pang, J., Cuin, T., Shabala, L., Zhou, M., Mendham, N., Shabala, S., 2007. Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. Plant Physiol. 1, 266–276.

Paul, J.A.J., Karmegam, N., Daniel, T., 2011. Municipal solid waste (MSW) vermicomposting with an epigeic earthworm, Perionyx ceylanensis Mich. Bioresour. Technol. 12, 6769–6773.

Pramanik, P., Ghosh, G.K., Ghosal, P.K., Banik, P., 2007. Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresour. Technol. 13, 2485–2494.

Raphael, K., Velmourougane, K., 2011. Chemical and microbiological changes during vermicomposting of coffee pulp using exotic (Eudrilus eugeniae) and native earthworm (Perionyx ceylanesis) species. Biodegradation 3, 497–507.

Senesi, N., 1989. Composted materials as organic fertilizers. Sci. Total Environ. 81, 521–542.

Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. Soil Sci. 2, 137–141.

Stonehouse, B., 2013. Biological Husbandry: a Scientific Approach to Organic Farming. Elsevier.

Tang, J.C., Maie, N., Tada, Y., Katayama, A., 2006. Characterization of the maturing process of cattle manure compost. Process Biochem. 2, 380–389.

Theroux, F.R., Eldridge, E.F., Mallmann, W.L., 1936. Laboratory manual for chemical and bacterial analysis of water and sewage. In: Laboratory Manual for Chemical and Bacterial Analysis of Water and Sewage. McGraw-Hill.

Tim Haug, R., 1980. Compost Engineering; Principles and Practice. Technomic Publishing CO, Inc.

Wang, K.-H., Radovich, T., Pant, A., Cheng, Z., 2014. Integration of cover crops and vermicompost tea for soil and plant health management in a short-term vegetable cropping system. Appl. Soil. Ecol. 82, 26–37.

Way, C., 2012. Standard Methods for the Examination of Water and Wastewater, twenty-seco$^{nd}$ ed. Water Environment Federation, Secaucus, NJ, USA.

Zhang, B.-G., Li, G.-T., Shen, T.-S., Wang, J.-K., Sun, Z., 2000. Changes in microbial biomass C, N, and P and enzyme activities in soil incubated with the earthworms Metaphire guillelmi or Eisenia fetida. Soil Biol. Biochem. 14, 2055–2062.