

Research Article

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Biodynamic preparations on static pile composting from prickly pear cactus and moringa crop wastes

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Abstract: Biodynamic agriculture, which considered biodynamic preparations (BP) and compost as essential to farms sustainability, surged as an alternative almost a century ago. Composting is a way to obtain either biofertilizers or soil amendments, whereas the static piles method reduces energy and cost because no turnings are needed. The present study aims to evaluate the BP effect on physical, chemical and biological properties of static piles compost from prickly pear cactus and moringa crop wastes (regional principal substrates) over 100 days of composting. The experiment was carried out in an organic farm (Nuevo León, Mexico) considering four treatments: T1, Prickly pear cactus+BP; T2, Moringa+BP; T3, Prickly pear cactus and T4, Moringa. Results showed significantly higher bacterial activity ($p < 0.05$) in T1 (until 1.38×10^{10} CFU), therefore it had the highest temperatures and mineralization. Treatments with prickly pear cactus attained the highest temperatures, compared with those with moringa (significantly in 71% of total sampling days, $p < 0.05$). An aerobic environment was maintained by the passive aeration system (holed PVC pipes placed at the bottom layer). The final material was considered to be sanitized, according to Enterobacteriaceae, *Escherichia coli* and *Salmonella/Shigella* analysis for quality control. Results indicate the BP efficiency on regional substrates decomposition, by using the static piles method.

Keywords: microorganisms, mineralization, *Moringa oleifera*, *Opuntia ficus-indica*, principal component analysis

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1 Introduction

The biodynamic agriculture (BA) surged during the first half of 1920's as an alternative way to produce in a sustainable and self-sufficient way (Pfeiffer 2011). The fundamentals of the biodynamic movement were presented by the Austrian philosopher Rudolf Steiner (in Koberwitz, currently Poland), who accepted farmers requesting an answer to progressive soil fertility, seed viability and animal health decrease (Steiner 2009). BA has common characteristics with organic production, such as crop rotation, use of biofertilizers, pests and diseases biocontrol, but the holistic biodynamic approach considers the inclusion of farm animals and plants. Moreover, the cornerstone of biodynamic farming are the biodynamic preparations (BP), sprayed on the soil and crops and applied in compost. There are six BP used on compost, which are exposed to a fermentation period from 6 months to 1 year and buried in the topsoil. After the BP harvest, those are applied at rates between 1 and 5 g approximately (von Wistinghausen et al. 2000; Reeve et al. 2010).

Although the BA has been practiced for almost a century, reports and information in peer reviewed journals regarding the discernible effect of BP on the final compost product are still scarce (Carpenter-Boggs et al. 2000; Reeve et al. 2010). In most countries, such as Mexico, where there are currently more than 83,000 organic farms, BA is not well known. There are only 5 identified biodynamic farms in Mexico (from <https://www.demeter.net/>). Nevertheless, attention has been increasing in different continents, such as Asia, America, Africa, but especially in Europe, and area under BA and members of BA organizations are growing (Villanueva-Rey et al. 2014).

Despite the low interest in alternative ways of production, neither the techniques to produce soil improvement promoters, biofertilizers, disease and pest biocontrols nor the social or environmental impact of the agriculture have received appropriate scientific attention. During the last decades, organic wastes coming from the industrial or agricultural sectors are increasing. These

deserve more attention in order to develop and improve the optimal management of those wastes. Within these methods, the composting process is a way to reduce odors coming from the uncontrolled decomposition of organic matter, environmental contamination, and the potential for human pathogenic microorganisms to spread. Composting waste also produces a high value sub-product, which improves physical, chemical and biological characteristics in the soil (Stoffella and Kahn 2006). However, the conditions for an optimal process must be considered. These include moisture content, aeration rate, bulk density, carbon:nitrogen ratio (C:N) and energy cost. Compost maturity is a key parameter to evaluate its quality and positive effect through its application (Sen et al. 2007).

Static pile composting promotes an efficient process, mainly through energy and costs reduction, because no turning is needed (Hubbe et al. 2010). Nasini et al. (2016) reported the use of static piles composting and its efficiency to convert olive oil extraction and grape transformation by-products into high quality soil amendment, considering the stability, maturity and environmental impact.

The design and management of biodynamic composting processes for farmers from areas of under-developed countries need to be addressed in terms of energy and cost reduction. Therefore, the purpose of this study was to evaluate the influence of one of the biodynamic agriculture fundamentals, the BP. The composting process was carried out in static piles, using the crop wastes of prickly pear cactus (*Opuntia ficus indica* L. Mill.) and moringa (*Moringa oleifera* Lam.) as principal substrates. This is the first study on evaluating the BP effect, focusing on the complete removal of energy that is typically used for turning piles or aeration of the system. Furthermore, the whole process was carried out under local conditions and without a specific modification of the materials, making it easier to implement.

2 Materials and methods

2.1 Experiment location

The experiment was established under open field conditions in the organic farm “Zu-Nopalito” (certified by BioAgriCert), located in Zuazua, Nuevo León (25° 53' N, 100° 02' W), which is about 355 m above sea level with an annual precipitation of 520 mm.

2.2 Biological material

The principal substrates for the static pile composting were prickly pear cactus and moringa crop wastes, coming from an organic farm and greenhouse under conventional production, respectively. Dairy and chicken manure were used on each pile to adjust C:N ratio in a proportion of 1:1.

The compost BP (Table 1) were obtained from Demeter certified farm “El Equimite” (Veracruz, Mexico).

2.3 Static pile composting

The materials proportions for each pile were calculated in order to obtain an initial C:N ratio of approximately 20:1. The final volume (2 m³) and total weight (1.5 ton) of each compost pile were similar, in order to decrease the variability that may be caused by the pile structure and dimensions (for all treatments: 1.3, 0.9 and 2.5 m of width, height and length, respectively). The substrates were arranged in three layers, with each layer being about 30 cm in height. Moringa and prickly pear cactus wastes were chopped up to obtain 5-10 cm pieces and used in the bottom layer to promote aeration. In the middle and top layer plant materials were chopped up until a size lower than 5 cm was obtained to increase the mineralization.

Table 1: Biodynamic preparations identification number (IN) and components descriptions

IN	Composition of biodynamic preparations
502	Yarrow blossoms (<i>Achillea millefolium</i> L.)
503	Chamomile blossoms (<i>Matricaria recutita</i> L.)
504	Stinging nettle shoots (<i>Urtica dioica</i> L.)
505	Oak bark (<i>Quercus robur</i> L.)
506	Dandelion flowers (<i>Taraxacum officinale</i> L.)
507	Valerian flower extract (<i>Valeriana officinalis</i> L.)

In each pile, the crop wastes were first placed above the ground to retain or decrease lixivates.

Four piles were constructed, two treated with BP and the other two were analyzed as controls, without BP (T1, prickly pear cactus+BP; T2, moringa+BP; T3, prickly pear cactus; T4, moringa). The BP proportion consisted of 2 g of each solid preparation (502-506) and 5 mL of the liquid one (507). Masson and Masson (2013) mention the previous BP amounts for 10-15 m³ of raw material.

A micro-sprinkler system was installed for compost pile irrigation. The irrigation time was the same for all treatments, calculated after moisture analysis, in order to attain an adequate environment for microorganism development (approximately 60% moisture). Water was obtained from a ground water storage.

2.4 Chemical, physical and biological analyses of compost piles

Five samples per treatment were taken and analyzed on a specific frequency depending on the parameter and according the value change over time. On each sampling day, samples were taken along the 2.5 m length and at a half depth of the pile. The different parameters' values determined when the compost was "ready", but principally the microorganism activity and temperature stabilization (near ambient), were considered as the cooling stage (Hubbe et al. 2010).

2.4.1 Physical parameters

The temperature reading interval was modified depending on the temperature rising or decreasing rates. Three different layers were monitored (bottom, middle and top), with thermometers being placed at 8 locations along the length of the pile. The moisture percentage was determined by drying at a constant temperature of 105°C for 24 hours (Escudero et al. 2012). The total volume was calculated by the difference between the final and initial compost piles dimensions.

2.4.2 Chemical parameters

Electrical conductivity (EC) and pH value were measured in a 1:10 compost:water extract (Nasini et al. 2016), using a multiparameter meter (Thermo Scientific Orion 4-Star).

The compost samples were ashed using a muffle furnace set to 400°C over 24 h, where the organic matter

(OM) content was calculated from the initial and final weights (Solano et al. 2001). Compost total nitrogen was quantified using a Kjeldahl digestion procedure (Etheridge et al. 1998). The P content was determined using the vanadate-molybdate method with a spectral photometer (Krey et al. 2013). The K, Ca, Mg, Cu, Zn, Fe and Mn content were estimated by atomic absorption spectroscopy (UNICAM Solar®, model 9626).

2.4.3 Biological parameters

All samples were prepared in a 1:10 dilution (sample:potassium phosphate buffer) and in consecutive dilutions until the optimal counts of the target microorganisms were reached, ranging from 30-300 CFU (Wang et al. 2012). Dilutions of 1:10⁶ and 1:10⁷ were used to assess yeast/molds and bacteria growth, respectively.

The inoculation of 1 mL per sample was done in 3MTM PetrifilmTM plates. For yeast and molds, 3MTM PetrifilmTM RYM plates were incubated at 28±2°C for 5 days. For bacteria, 3MTM PetrifilmTM AC Plates were incubated at 35±2°C for 48 h. Final enumeration was done with the aid of a SOL-BAT Q-14 colony counter.

After a 100 days period, samples were taken from different points, considering the whole depth and length of the piles, and analyzed for detection of Enterobacteriaceae, *Escherichia coli*/Coliform and *Salmonella/Shigella*, using respective count plates and incubation periods: 3MTM PetrifilmTM Enterobacteriaceae/ambient temperature for 24 h; 3MTM PetrifilmTM *E. coli*/Coliform and *Salmonella-Shigella* Agar BBL for 48 h at 35±2°C.

2.5 Statistic design and analysis

The values of the physical, chemical and biological parameters measured over the time (temperature, pH, EC, moisture percentage, C:N ratio, OM, nitrogen and microorganisms CFU) were analyzed by the selection of nonlinear regression models with criteria of the highest determination coefficient (R²) and minimum mean squared error. Sigma-plot software was used for regression analysis.

The data obtained for each variable in the different sampling points were subjected to an analysis of variance (ANOVA). Mean comparison was performed using Tukey honestly significance difference test (p<0.05). At the end of the experiment, 18 variables were analyzed and subjected to a principal component analysis (PCA). The

statistical software package SPSS was used for ANOVA, means comparison and PCA.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Physical parameters

During the first 24 hours, compost piles temperatures increased until a maximum of $55.8 \pm 1.3^\circ\text{C}$. Because of the high increase/decrease temperature rates during the first month, it was necessary to record thrice per week. Approximately 35 days from the material mixing, the temperature showed a lower rate of change, accordingly, recording was then done on a weekly basis until it reached nearly ambient temperature (from day 50).

By comparing layers within treatments, it was observed that in piles with prickly pear cactus as the principle substrate (T1 and T3), the mean temperature of the top and middle layer during the first 55 days were statistically higher than those of the bottom layer for 92% of the sampling days. By using moringa as principle substrate (T2 and T4), such differences were statistically different in 81% of the cases, until day 52. After those periods, temperatures were statistically similar between layers (Figure 1).

By comparing treatments per layer, Figure 1 depicts that the mean temperatures of treatments with prickly pear cactus (T1 and T3) were statistically higher than those using moringa (T2 and T4) in 71% of sampling days during first 100 days of composting (except for the first 2 days in T1, upper layer).

Significant temperature differences were found between treatments with and without BP. Regarding the piles with prickly pear cactus (T1 and T3) the mean temperatures of T1 were statistically higher than T3 in 64, 64 and 42% of the sampling days for the top, middle and bottom layers, respectively, during 100 days of composting. In case of piles with moringa (T2 and T4), mean temperatures of T2 were statistically higher than T4 for the top, middle (except first day) and bottom layers in 38, 38 and 31% of sampling days, respectively, during 100 days of analysis. Just 4% of the all sampling days showed a higher mean temperature in a pile without BP, compared with the same principal substrate with BP.

The moisture content of piles showed a significant difference at different sampling days. During the first eight days, the moisture of T2 was statistically lower than

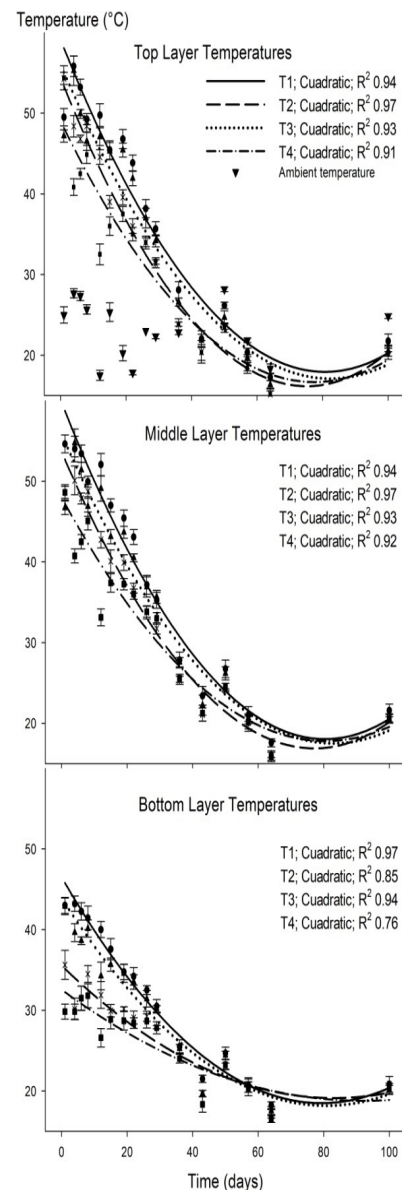


Figure 1: Mean temperature of static piles (T1 ● prickly pear cactus+BP; T2 × moringa+BP; T3 ▲ prickly pear cactus; T4 ■ moringa) per layer over the most active phases (100 days). Means (\pm SD) were calculated from five replicates for each treatment and sampling day. Statistically different (Tukey test, $p < 0.05$)

the other treatments. At day 64 the lowest moistures were recorded for T1 and T3. And at the end of the analyzed period, the lowest values were found in T1 and T4 (Figure 2).

3.2 Chemical parameters

The maximum and minimum pH values, over the analyzed period (100 days), ranged between 8.2 and 9.3 (Figure 3).

Significant differences of pH values were recorded for the following treatments and sampling days (time of composting): lowest value for T3, first day; maximum values for T2 and T4 on days 8, 22, 36 and 100.

EC varied depending on the sample point and piles' material. The statistically maximum values, over the time, were attained for the following treatments and sampling days: T2-T3 (first); T2-T4 (22) and T4 (100). The maximum value was achieved at the end of the analysis period (T4, $3.99 \pm 0.44 \text{ dS m}^{-1}$) (Figure 3).

OM, nitrogen and C:N ratio values decreased over the evaluated period and according to the different treatments. Significant differences of OM values were recorded for the following treatments and sampling days (time of composting): the lowest value for T1, day 64; the maximum values for T2 and T3, day 100. Nitrogen values showed the following significant differences for the respective treatments and sampling days: the maximum values for T4 and minimum values for T1, day 36, 64 and 100. C:N ratio was significantly affected over the time. T2 and T4 showed the minimum values at day 36 and 100 of composting (Figure 4).

By analyzing macro- and microelements in compost samples after 100 days of composting, interactions between evaluated factors (BP use and compost substrate) were found for N, K, Zn and Mn values. BP inclusion showed a highly statistical difference ($p < 0.01$) in K and Mn concentrations. Statistically higher values ($p < 0.01$) of Ca/Mg and Cu were found in compost from prickly pear cactus and moringa substrates, respectively (Figure 5).

3.3 Biological parameters

Total microorganism population (CFU g^{-1} compost) in the biological material showed the highest values in biodynamic preparations compared with the crop and animal organic wastes (Table 2).

Total bacteria, molds and yeast populations (CFU g^{-1}) contained in compost piles showed significant differences over time. Bacteria population values showed significant differences for the following treatments and sampling days: maximum values for T2 and T4, day 8; T1, day 22; T1, T2 and T3, day 36; T3 and T4, day 64. The maximum value for bacteria was attained for T1 at day 22 ($1.38 \times 10^{10} \pm 3.04 \times 10^9$). Molds and yeast population values were significantly higher for the following treatments and sampling days: T2 and T4, day 8; and T3, day 64. The maximum yeast and molds population was found in T3 at day 64 ($7.20 \times 10^7 \pm 0.00$) (Figure 6).

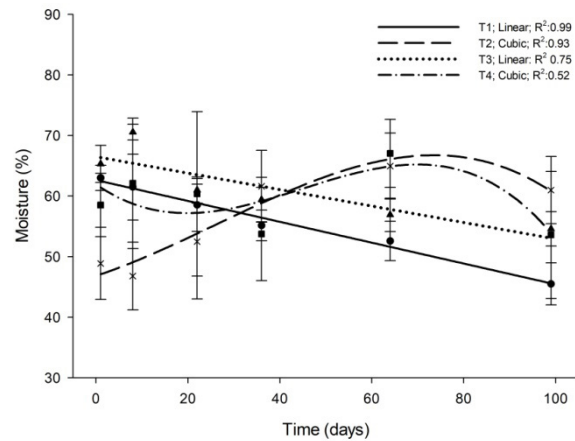


Figure 2: Moisture of static piles (T1 ● prickly pear cactus+BP; T2 × moringa+BP; T3 ▲ prickly pear cactus; T4 ■ moringa) over 100 days of analysis. Means (\pm SD) were calculated from five replicates for each treatment and sampling day. Statistically different (Tukey test, $p < 0.05$)

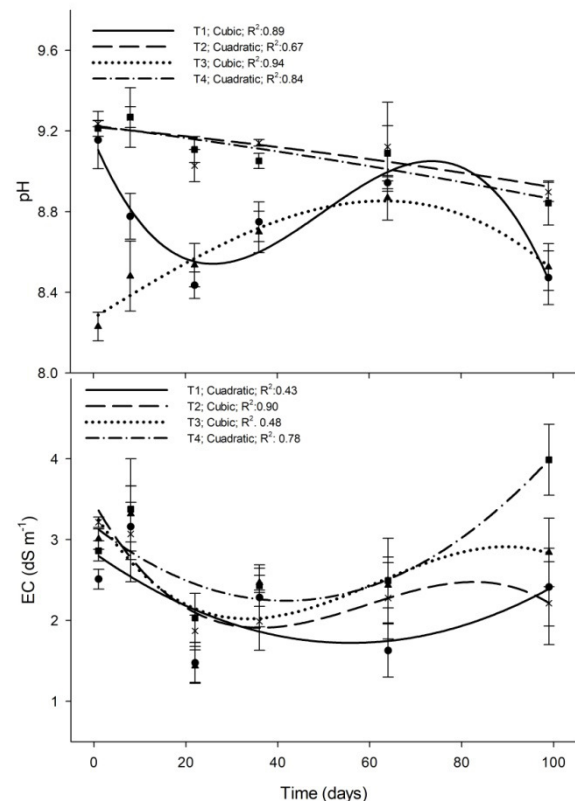


Figure 3: pH and Electrical conductivity (EC) of static piles (T1 ● prickly pear cactus+BP; T2 × moringa+BP; T3 ▲ prickly pear cactus; T4 ■ moringa) over 100 days of analysis. Means (\pm SD) were calculated from five replicates for each treatment and sampling day. Statistically different (Tukey test, $p < 0.05$)

Table 2: Total molds, yeast and bacteria (CFU g⁻¹ compost) in principal substrates and biodynamic preparations used in static piles

Substrate and Biodynamic preparations	Molds and yeast (CFU g ⁻¹)	Bacteria (CFU g ⁻¹)
Prickly pear cactus	4.20x10 ⁶	7.35x10 ⁴
Moringa	1.03x10 ⁶	3.20x10 ⁵
Cow manure	2.00x10 ⁶	2.49x10 ⁵
Chicken manure	2.50x10 ⁶	5.52x10 ⁵
Biodynamic preparations		
502	3.75x10 ⁷	9.80x10 ⁶
503	1.66x10 ⁷	3.20x10 ⁷
504	6.00x10 ⁶	4.40x10 ⁶
505	3.50x10 ⁶	4.80x10 ⁶
506	7.50x10 ⁶	3.18x10 ⁷
507	1.28x10 ⁷	2.38x10 ⁷

Regarding the Enterobacteriaceae, *Escherichia coli*/Coliform and *Salmonella*/*Shigella* analysis, a concentration of 62 CFU of *Escherichia coli*/Coliform per gram of compost (dry weight basis) was detected in T3.

3.4 Physical, chemical and biological variables interaction through a PCA

The first and second principal components were selected, which explain approximately 89% of the total variance. The first component showed correlation between N, P, K, Cu, Zn, Fe, Mn, pH and volume, associated to T4. Moreover, in the same component, a correlation between calcium, magnesium and C:N ratio was associated to T1. The second component showed a correlation between bacteria and temperature, associated to T1 and T2. In addition a correlation between EC, OM, molds and yeast, was associated with T3 (Figure 7).

4 Discussion

4.1 Physical parameters

By analyzing layer temperatures per treatment, increasing values over time (suggesting microbial activity) showed the passive aeration system efficacy on maintaining an aerobic environment, without pile turning. Maximum differences between upper (top and middle) and bottom layers temperatures were 12.6, 19.9, 15.6 and 24.5°C in T1, T2, T3 and T4, respectively (achieved during the first four days), because of the holed PVC pipes placed above the

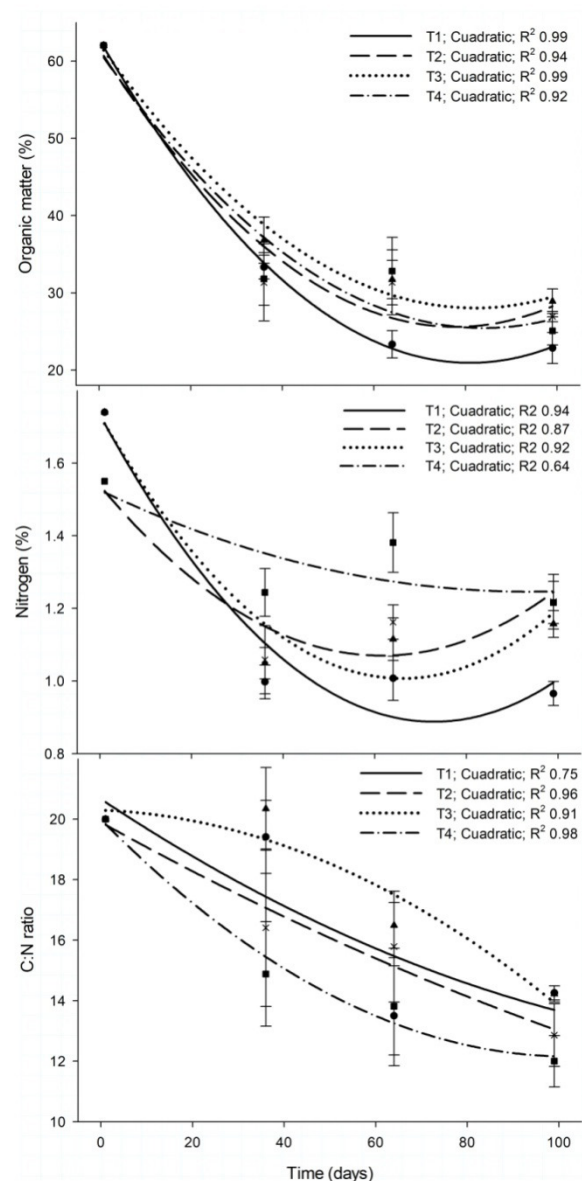


Figure 4: Organic matter, nitrogen (N) and C:N ratio of static piles (T1 ● prickly pear cactus+BP; T2 × moringa+BP; T3 ▲ prickly pear cactus; T4 ■ moringa) over 100 days of analysis. Means (±SD) were calculated from five replicates for each treatment and sampling day. Statistically different (Tukey test, p<0.05)

ground. The lowest increase in temperature of the bottom layers in the piles was with moringa (T2 and 4), which contributed the maximum differences (Figure 1).

Temperature values were not the same for all piles. The middle and top layer from each treatment achieved values above 50°C, being optimal for the composting process (Isobaev et al. 2014). On the other hand, the piles dimensional drawback was the temperature attained in bottom layers (under 45°C). However, it could be modified in order to reach the desirable value by increasing the piles

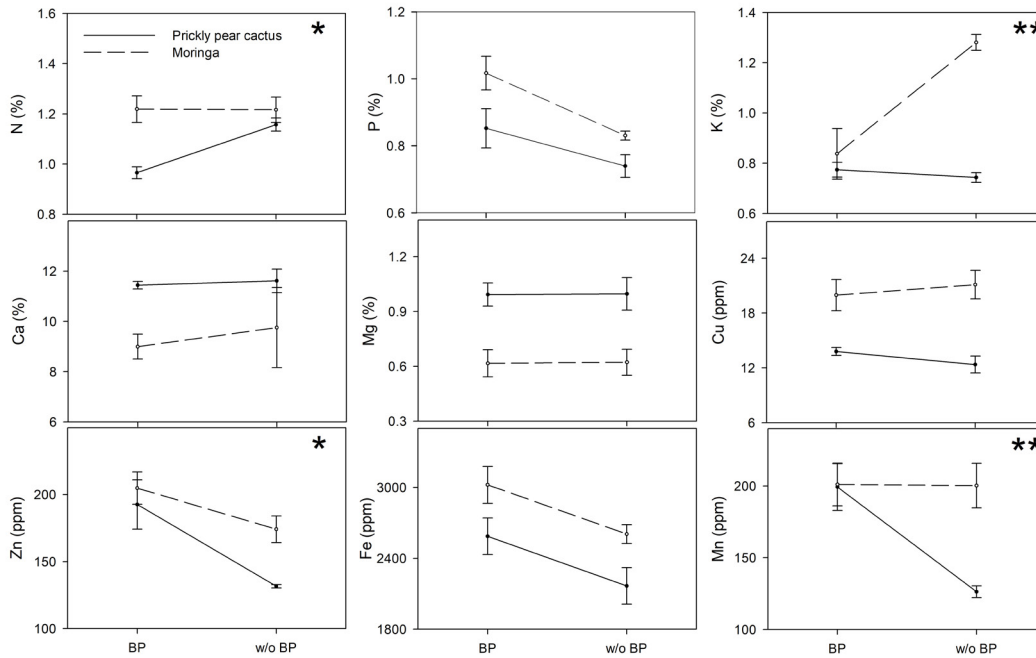


Figure 5: Compost macro- and microelements after 100 days composting and its significant (* $p < 0.05$) and highly significant (** $p < 0.01$) interaction between evaluated factors (BP: Biopreparations and compost substrate). Means (\pm SD) were calculated from five replicates for each treatment and sampling day

height. Under traditional turned windrow composting, the bottom layer temperature increases at the same or higher rates than upper layers (Hubbe et al. 2010; Luo et al. 2008).

In Figure 1, the comparison between principal substrates (T1 and T3 vs T2 and T4) shows the highest mean temperatures in static piles with prickly pear cactus, because of the optimal growth conditions for the microorganisms, promoted by mucilage. This polymeric compound of carbohydrate substances is considered as an optimum microorganisms' growth medium, containing L-arabinose, D-Galactose, L-rhamnose, D-xylose, galacturonic acid in various proportions, low acidity and high soluble solids content (Gebresamuel and Gebre-Mariam 2012). It is structured by pectin and mucilage, with Ca^{2+} /gelling and no gelling properties, respectively (Matsuhiro et al. 2006; Sepúlveda et al. 2007).

By comparing treatments' temperatures per layers, the mean values of piles with BP were higher than those with the same substrate and without BP. The significant differences during the 100 days of composting showed the effect of BP inclusion on the temperature increase, which is a consequence of the microorganism activity on organic matter decomposition, producing heat as energy is released (Yang et al. 2016). Researchers report the acceleration on decomposition processes by using

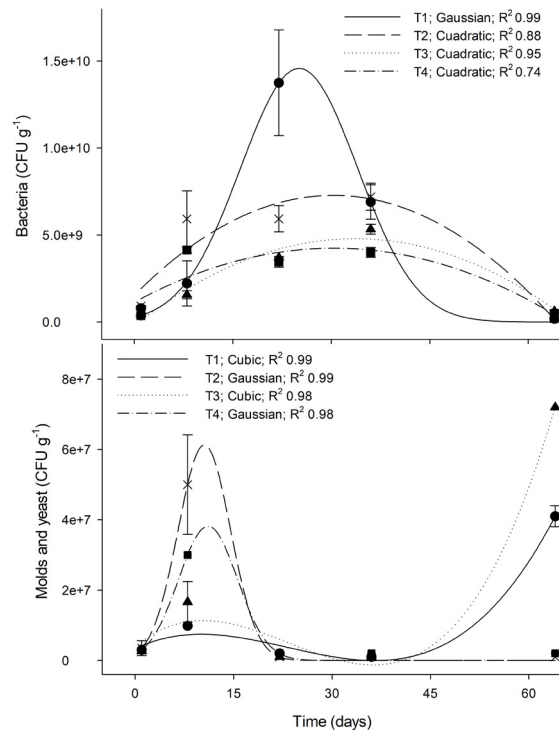


Figure 6: Total CFU g^{-1} compost of bacteria, molds and yeast in static piles (T1 ● prickly pear cactus+BP; T2 × moringa+BP; T3 ▲ prickly pear cactus; T4 ■ moringa) over 65 days of analysis. Means (\pm SD) were calculated from five replicates for each treatment and sampling day. Statistically different (Tukey test, $p < 0.05$)

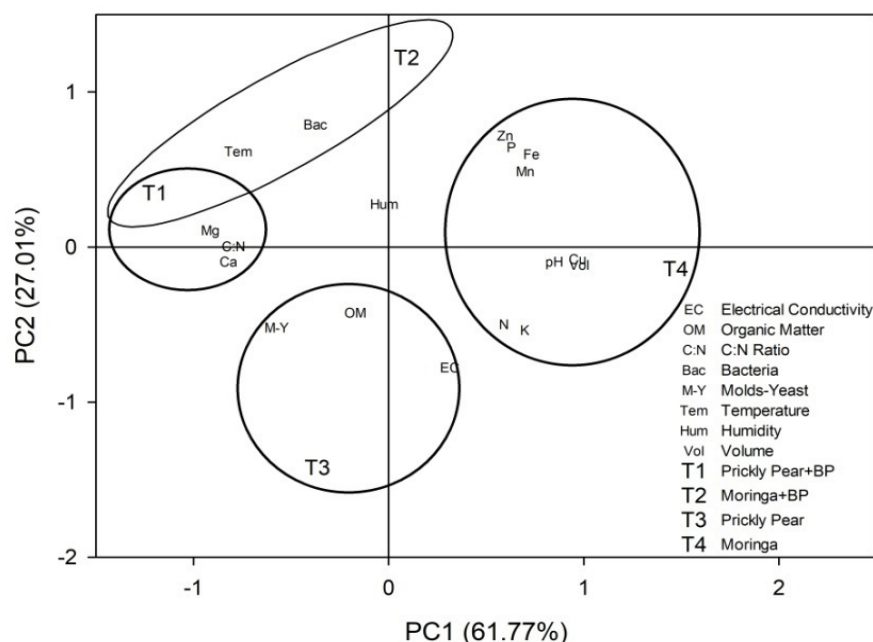


Figure 7: Principal component analysis of physical, chemical and biological variables of static piles. BP: biodynamic preparations, N: nitrogen; P: phosphorus; K: potassium, Ca: calcium, Mg: magnesium, Cu: copper, Zn: zinc, Fe: iron, Mn: manganese

BP in compost. It showed higher temperatures, nitrogen content, dehydrogenase enzyme activity (suggesting higher microbial activity), nutrient holding capacity, and microorganism population, in contrast with the untreated compost (Carpenter-Boggs et al. 2000; Mäder et al. 2002; Sradnick et al. 2013).

The maintenance of the mentioned temperature guarantees the sanitization and hygienization of the composting product, referring to weed seeds, and potential human and plant disease development (Barberi 2002; Cayuela et al. 2008; Deportes et al. 1998). The United States Environmental Protection Agency (EPA) specify about 55°C for at least 3 and 15 days in aerated static pile or in-vessels and in turned windrow composting (5 turns), respectively, as the temperature requirement for biosolids composting. The EPA usually refers to the marketable end product as Class A, which provides physical-chemical characteristics to the soil as an organic fertilizer and without detectable pathogen levels (US EPA 2002).

The moisture behavior followed a temperature increase function. The lowest values were observed in treatments with BP, because of the higher temperature increase. However, the moisture over the analyzed period ranged between an optimum value for microorganism population development (Figure 2). Moisture has been cited as a critical parameter for systems optimization (Luo et al. 2008). It depends on the correct materials selection

and mixing proportion.

4.2 Chemical parameters

Masson and Masson (2013) consider an optimal initial C:N ratio of 30:1. However, the principal substrates used in the present study contained a low C:N ratio (prickly pear cactus 24.88, moringa 36.67, chicken manure 8.02, cow manure 13.77) to attain the optimal value after mixing. The management of low values of C:N ratio in the piles can increase the use of manures; nevertheless, it also increases the loss of nitrogen in the form of NH_3 or NH_4^+ , depending on the pH (Hubbe et al. 2010).

In Figure 3, by comparing pH behavior between piles with the same substrate, the difference between T1 and T3 can be observed during the first days. This is because of the maximum microbial activity in T1, releasing NH_3 to the medium and increasing the pH value (Singh et al. 2016). Differences between treatments with prickly pear and moringa were observed over the time because of the nature of the material.

EC values evolution over time depended on multiple variable interactions. The progress in the composting process was reflected through the increasing/decreasing rates of change. EC attained values between 1.3 ± 0.3 to $4.0 \pm 0.44 \text{ dS m}^{-1}$ over the 100 day composting (Figure 3).

Such behavior is generally expected, as supported by results from Tatàno et al. (2015), who reported increasing values in different experimental composters, with values up to 5.5 dS m^{-1} .

Although at day 100 of composting there was no statistical difference in C:N ratio between piles with the same principal substrate, there needs to be a study of the individual factors where it comes from (Figure 4). By analyzing OM and N between piles with prickly pear cactus, T1 showed statistically lower values than T3, which suggests a higher material decomposition in the first one. The highest OM reduction was promoted by the BP inclusion and substrate. Similar results during composting processes were reported by Gigliotti et al. (2012), where the OM loss reached values of around 50% and Jiang et al. (2011) found a total organic carbon loss between 33.8% and 54.0%. In piles with moringa, OM values were statistically similar with and without BP inclusion, but N concentration at day 36 and 64 of composting were significantly lower in those with BP due to element volatilization or lixiviation, promoted by microorganism activity, temperature, moisture and their interaction (Singh et al. 2016).

Nitrogen concentration of different treatments decreased over the time, however during the coldest period (between day 65-80, average 15°C) the lowest nitrogen levels were observed (Figure 1 and 4). After the mentioned period, ambient temperature started increasing, along with the nitrogen concentration for all treatments. We can assume that microorganisms increased together with temperature, which may be responsible for nitrogen fixation. As Singh et al. (2016) mentioned, free living bacteria play an important role in this process. Similar results were obtained by Gigliotti et al. (2012), who reported a nitrogen increase from day 47 to 126, with values from 0.89 to 1.76%, respectively. Further studies are suggested to analyze the behavior of nitrogen increase related to microorganisms.

4.3 Biological parameters

Total bacteria CFU were statistically higher in piles with BP (T1 and T2) than those without BP (T3 and T4), as observed in Figure 6. Such results agree with Reeve et al. (2010), who reported that BP (as fermented substances) stimulate organisms present in the raw material, which increases microorganism activity followed by decomposition increases. Masson and Masson (2013) suggest that under biodynamic pile composting, temperatures above 55°C are not required because the microorganism population can be negatively affected. It is known that between the whole

microorganism population in the soil (about 1.5 billion CFU per gram), 2-5% belongs to the beneficial microorganisms, improving processes such as the nitrogen fixation, phosphorus solubilization, increasing iron availability to the plant, etc. (Stoffella and Kahn 2006). Molds and yeast were the first microorganisms to colonize different treatments. As Stoffella and Kahn (2006) report, these are the first kind of microorganism that breakdown the material, which continues as the bacteria population increases.

Enterobacteriaceae, *Salmonella* and *Shigella* were not detected through the compost piles length and depth, but in T3 *Escherichia coli*/Coliforms were below detectable levels, which is to be considered as a sanitized material (Gantzer et al. 2001). These results showed that the sanitization or hygienization not only depends on the temperature, but on the substrate material source. Turner (2002) demonstrated that at 55°C *E. coli* inactivation occurs rapidly, but at 50°C it may depend on moisture and nature of the material. Moreover, it could be explained that the bacterial pathogens destruction could depend not only on temperature, but on free ammonia concentration, heat duration and microorganisms biocontrol and competition (Singh et al. 2016). Other authors report the no detection of *Escherichia coli* 0157:H7 (food-borne infectious pathogen of public health importance that causes diarrhea and hemorrhagic colitis) and *Salmonella enteritidis* by lower temperatures during periods of 72 and 48 h, respectively, or considering degree days of about 180 and 300 (Lung et al. 2001; Hess et al. 2004). Droffner and Brinton (1995) examined *Salmonella* and *Escherichia coli* during aerobic composting of municipal wastes and biowastes, suggesting that the microorganisms' removal is complex and not merely depends on the thermal physical environment. In the same study, both bacteria survived at about 60°C , but became undetectable during the cooling and curing stage.

The microbial activity is strongly correlated with the increase and decrease of temperature, hence its importance to be monitored in order to evaluate the composting process efficiency and stabilization (Bustamante et al. 2008).

4.4 Physical, chemical and biological variables interaction through a PCA

The PCA agreed with the results described above, regarding the association between compost piles and variables behavior over time. Macro- and microelements were correlated (except Ca and Mg) and associated with T4, because of the lowest decomposition rate and

elements were still found in the raw material. In addition, the correlation of variables related to microbial activity (temperature and bacteria) associated with T1 and T2 is confirmed, promoted by the BP inclusion. The highest C:N ratio associated to T1 was promoted by the microbial activity (mainly bacteria) and nitrogen lost (Figure 7).

5 Conclusions

BP promoted the microorganisms' reproduction (principally bacteria), temperature increases and decomposition of the raw material, in comparison with those piles without BP. The use of prickly pear cactus as a principal substrate positively affected the composting process. The combination of both factors, previously described, attained the best quality final compost in the present study (T1, prickly pear cactus+BP).

The passive aeration system provided enough air flux to the system, maintaining an aerobic environment without need of turning. Further analyses confirmed the sanitization of the final compost.

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