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Vermicomposting of Sewage Sludge: Earthworm Population and Agronomic Advantages

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Optimal substrates for vermicompost should ensure survival and reproduction of earthworms, as well as vermicompost quality. This concerns especially waste material substrates. Therefore, the objectives of this study were to estimate the adaptation of *Eisenia foetida* to mixtures of sewage sludge and composted cow dung, and also to evaluate the quality of the vermicompost as fertilizer. Four treatments contained sewage sludge and composted cow dung in ratios of 70:30 (A), 80:20 (B), 90:10 (C) and 95:5 (D), with 60-70% moisture. The samples were incubated at 17 ± 2°C with 50 adult earthworms each. Following 50 days of composting, cocoons, juveniles and adult earthworms were counted and several physicochemical parameters were determined. *E. foetida* could not survive treatment D. Treatments A and B had the highest survival and reproduction. However, treatment C showed the highest amounts of available N and P (2189.80 and 2310.63 mg kg^{-1}, respectively) and a C:N ratio of 12.46, making it a better fertilizer. The values of bulk density (0.64 g cm^{-3}), organic matter (54.82%), organic C (31.80%), available K (1801.92 mg kg^{-1}) and total N (2.55%) for treatment C were within the ranges for vermicompost recommended in the Mexican guidelines. Significant differences were found in all physical and chemical parameters ($p<0.05$), except for P. Fractionation of P in treatment C and sewage sludge showed that the largest proportion of P is slightly soluble in water and so, available to plants. Overall, this study suggests that sewage sludge and cow dung vermicomposted with *E. foetida* can improve fertility and nutrient availability, as well as earthworm population growth, compared with the sewage sludge alone.

Introduction

Wastewater treatment plants in Mexico generated in the last decade an estimated 493,000 to 1,500,000 Mg yr^{-1} of sewage sludge due to biological processes that digest organic matter from households (Garrido *et al*. 2005a). Current disposal of sewage sludge using sludge reactors or dehydration systems is costly (Cardoso-Vigueros and Ramírez-Camperos 2002), while on the other hand, sewage sludge has been used on agricultural land for a long time to add macro and micro nutrients and organic matter to the soil (Rostagno and Sosebee 2001; Hernández-Herrera *et al*. 2005). Environmental problems, e.g. the egression of human pathogens to water, soil and air, are also associated with the management of sewage sludge (Franco-Hernández *et al*. 2003; Penn and Sims 2002; Shober and Sims 2003; Esteller *et al*. 2009). These issues could be significantly reduced by stabilizing sludge before its disposal or agricultural use (Epstein 2003). Composting and vermicomposting are two of the best-known processes for the biological stabilization of different natural and anthropogenic solid organic wastes, including sewage sludge (Short *et al*. 1999; Kaushik and Garg 2003; Capistrán *et al*. 2004; Vaca *et al*. 2005; Díaz *et al*. 2007; Frederickson *et al*. 2007). Vermicomposting involves among other processes, the biooxidation, digestion, and mineralization of organic material; in contrast to composting, it depends on the joint action of earthworms, and microorganisms; finally it does not need nor indeed must involve a thermophilic stage (Capistrán *et al*. 2004; Díaz *et al*. 2007). During vermicomposting, the important nutrients such as nitrogen, phosphorus, potassium, and calcium present in the feed material are converted through microbial action into forms that are much more soluble and available to plants than those in the parent substrate (Kaushik and Garg 2003). Microorganisms produce the enzymes that cause the biochemical decomposition of organic matter, but earthworms are crucial drivers of the process as they aerate and fragment the substrate, and mobilize the nutrients, thereby drastically altering the microbial activity (Trigo *et al*. 1999). Earthworms mechanically comminute and blend the organic matter, increase the surface area exposed to microorganisms who in turn reduce the C:N ratio – making microbial decomposition easier (Albanell *et al*. 1988; Garg *et al*. 2006; Liu *et al*. 2005). All materials digested by the earthworms' intestines show enhanced agricultural properties (fine texture, more easily attacked by bacteria, fungi and actinomycetes) and are enriched with substances commonly called growth regulators (Díaz *et al*. 2007). Earthworms also modify microbial biomass and activity through stimulation, digestion and dispersion in casts, thereby affecting the structure and function of microbial communities (Feller *et al*. 2003; Barois *et al*. 1999). The application of soil amendment products digested by earthworms enriches microbial metabolites which, in turn, stimulate vegetal crop yield (Díaz *et al*. 2007).

Recent studies have used different proportions of sludge as substrate for vermicomposting: 0-70% mixed with cow dung (Kaushik and Garg 2003); 49-70% with water hyacinth (Cardoso-Vigueros and Ramírez-Camperos 2002); and 50-85% with cow dung (Contreras-Ramos *et al*. 2005). Other experiments have used composted and fermented sewage sludge to feed *Eisenia foetida* (Kaushik and Garg 2003; Liu *et al.* 2005; Garg *et al.* 2006). However, the problems in this set of reports seem to be: a relatively wide uncertainty as regards the optimal mixtures (what to mix and in which proportions). And also, composting and fermentation imply time as well as economic costs associated to periodical homogenization of pile or vessel contents. Accordingly, this study will try to improve on previous reports by using fresh sludge (without previous composting), in higher proportions than studied up until now (70-95%).

The objectives of this study are firstly, to estimate survival and reproduction of *E. foetida* in different mixtures of sewage sludge with cow dung. And secondly, to evaluate the physicochemical characteristics and quality of the vermicompost as fertilizer.

Materials and Methods

Collection of E. foetida, Sewage Sludge and Cow Dung

Adult earthworms (*E. foetida*) were randomly collected from horse dung at the Faculty of Agricultural Science of the Mexico State Autonomous University. Fresh sewage sludge was obtained from the Toluca North wastewater treatment plant, located in Toluca, State of Mexico. Cow dung (a mixture of feces and urine composted during one year to benefit from spontaneous microbial colonization) was obtained from a farm in Lerma, State of Mexico. Composted cow dung was used, as it is more suitable than fresh manure for earthworm population growth (Gutiérrez-Vázquez *et al*. 2007), and as it has more oxygen and avoids fermentation gases which bring about earthworm mortality.

Experimental Design

Four 0.8-kg mixtures of sewage sludge and cow dung (A, B, C and D) were prepared with the following dry weight ratios: 70:30, 80:20, 90:10 and 95:5%, respectively. The use of more than 70% of fresh sewage sludge was intended to reuse the highest amount possible of sewage sludge, to reduce the problem of its disposal. The mixtures were manually homogenized and placed in plastic bottles (3 L, 12 cm diameter, 25 cm depth) perforated in the base to release leachate. Each treatment was replicated thrice. In these mixtures, 50 adult *Eisenia foetida* earthworms were incubated (Contreras-Ramos *et al*. 2005; Garg *et al*. 2006). Moisture was maintained at 60-70% by weight during 50 days (Contreras-Ramos *et al*. 2005). All the containers were kept in the dark at 17 ± 2 °C. Following 50 days of vermicomposting (Liu *et al*. 2005), the total number of adult and juvenile earthworms and cocoons was counted and the earthworms were manually removed to carry out physicochemical analyses. Three samples of sewage sludge were air dried and sieved to be included as controls. The unmixed cow dung samples were also analyzed.

Chemical Analyses

At the end of 50 days of vermicomposting, three samples of each vermicomposted mixture were air dried in the shade at room temperature, ground and sieved (2 mm diameter) according to the Mexican vermicomposting standard (NMX-FF-109-SCFI-2007). Bulk density, electrical conductivity (Porta *et al*. 2003), and pH (Jackson 1982) were determined. Organic matter was estimated by ignition at 550°C and total organic carbon was obtained assuming that organic carbon corresponds to 58% of total organic matter (Primo and Carrasco 1981). Total Kjeldahl nitrogen was determined (Cardoso-Vigueros and Ramírez-Camperos 2002). Available phosphorus and nitrogen were estimated as suggested by Olsen and Summers (1982) and by extracted KCl, respectively (Siddique and Robinson 2004; Mariani *et al*. 2007). P fractionation was carried out according to Penn and Sims (2002). Available K and Na were determined using atomic emission spectroscopy (Liu *et al*. 2005).

Statistical Analyses

One–way analysis of variance (ANOVA) was used to test treatment effects (Contreras-Ramos *et al*. 2005). Differences between treatment means were calculated using the Tukey test, at a 0.05 level of significance. Statistical analyses were performed using SAS v. 5.0.

Results and Discussion

In terms of earthworm population dynamics, using high proportions of sewage sludge (≥ 95%) provoked the death of *E. foetida*, possibly due to oxygen deficiency (Kaushik and Garg 2003) and toxicity following ammonification in fresh sewage sludge (Carbonell *et al*. 2009). At the end of the vermicomposting process, treatment A showed 55 adults earthworms, treatment B maintained the initial number (50 adults), and treatment C showed a decrease to 40 units (Table 1); however, this trend of lower adult survival related to higher sewage sludge proportions in the substrate was not statistically significant. As to juvenile earthworms, averages of 18 were found in treatments A and B as against 4 in treatment C. Treatments A and B also showed higher numbers of cocoons. The foregoing suggests that the proportion of sludge in the mixtures influenced mainly the reproduction of the earthworms.

As regards physical characteristics, the bulk densities were 0.80, 0.65 and 0.64 g cm^{-3} (treatments A, B and C, respectively). These values are within the 0.40-0.90 g cm^{-3} range (Table 2) which is appropriate for vermicompost in the Mexican standards (NMX-FF-109-SCFI-2007). Bulk density is most relevant to plant health, as an indicator of soil porosity and its resistance to root growth. Electrical conductivity in sewage sludge was 2.64 dS m^{-1}, slightly higher than 2.20 dS m^{-1} in cow dung (Table 2). Vermicompost conductivity was 4.16 dS m^{-1} for treatment A and 3.28 dS m^{-1} for B and C, similar to Carbonell *et al*. (2009). These values were generally below 4 dS m^{-1}, a threshold value for moderate but significantly higher mortality and lesser earthworm population growth, attributable to acid pH and higher amounts of salts dissolved in water (Dayananda *et al*. 2008). Electrical conductivity is dependent on freely available minerals and ions, generated during ingestion and excretion by the earthworms (Garg *et al*. 2006). The electrical conductivity in the sewage sludge, cow dung and vermicomposts were classified as low in salts according to the Mexican standard for soils (NOM 021-SEMARNAT-2000).

Regarding chemical characteristics, the sewage sludge had an acid pH (6.02) at the beginning of the experiment (Table 2) which was further lowered after 50 days of vermicomposting (5.65-5.82). Treatment A was significantly different from B and C (Table 2). Acid pH probably owed to CO_2 and organic acids produced during mixture decomposition by microbial metabolism, as well as to the presence of NO_3^- and NO_2^- ions, which are weak bases causing ionic acidity (Garg *et al*. 2006). It is worth mentioning that pH has a decisive influence on the availability of nutrients to plants, and the efficiency with which plants can absorb nutrients (Adams 1995).

Organic matter content in sewage sludge was 47.31% and highest in cow dung (70.02%), followed by treatment A (60.70%). So it appears that cow dung additions controlled organic matter in the treatments (Table 2). It has been shown that a high percentage of organic matter favors growth of earthworms. When the earthworm population is high, the process of decomposition of organic matter is accelerated and the humification is carried out in a short time due to the action of microorganisms contained in the excreta of earthworms (Martínez, 1997). This utilization of organic matter may explain the lower organic matter content in vermicompost (treatment C) compared to sewage or cow dung (Table 2). Sewage sludge organic matter was lower than previously reported elsewhere

TABLE 1.
Treatments, survival and reproduction of *E. foetida* (n=3, average ± standard deviation)

Treatment	Sewage Sludge (%)	Cow Dung (%)	Adults	Juveniles (units)	Cocoons
A	70	30	55±21	19±5	136±50
B	80	20	50±34	18±7	151±17
C	90	10	40±33	4±1	55±19
D	95	5	0	0	0

Note: The initial population was 50 adult earthworms per treatment.

TABLE 2.
Physicochemical characteristics of the sewage sludge, cow dung, vermicompost and Mexican official guidelines

Parameter	Sewage Sludge	Cow Dung	Treatment A	Treatment B	Treatment C	Official Guidelines
Bulk density (g cm^{-3})	N.D.	N.D.	0.80±0.06a	0.65±0.06b	0.64±0.03b	0.40-0.90
Electrical conductivity (dS m^{-1})	2.64±0.03c	2.20± 0.00d	4.16±0.41a	3.28±0.18b	3.27±0.19b	≤ 4
pH	6.02±0.03a	7.38±0.15b	5.65±0.05d	5.80±0.04c	5.82±0.13c	5.50-8.50
Organic matter (%)	47.31±0.35e	70.02±0.39a	60.70±0.33b	56.83±0.80c	54.82±1.07d	20-50
Organic carbon (%)	27.44±0.20e	40.70±0.18a	35.21±0.19b	32.96±0.46c	31.80±0.62d	11-29
Total nitrogen (%)	3.19±0.03a	1.03±0.05e	1.86±0.21d	2.18±0.14c	2.55±0.06b	1-4
C:N	8.58±0.13d	39.51±2.25d	19.18±2.69a	15.17±1.17b	12.46±0.44c	≤ 20

(n=3, average ± standard deviation). Mexican official guidelines NMX-FF-109-SCFI-2007. N.D.: Not determined. N.A.: Not available. Different letters in each row indicate significant differences at $p < 0.05$.

(53.65%, Garrido *et al*. 2005b; 61.97% and 65-73%, Hogan *et al*. 2001, Carbonell *et al*. 2009). This lower initial organic matter in sewage and its biological utilization during vermicomposting probably explain why the vermicompost showed lower organic matter than the official guidelines (Table 2).

The sewage sludge contained the largest amount of total N (3.19%), followed by treatments C (2.55%), B, and A (Table 2). Differences between treatments were significant. Total N concentrations in these vermicomposts may be related to their slightly acid pH and ensuing N retention, otherwise lost as volatile ammonia at alkaline pH (Garg *et al*. 2006). Contrary to composting, vermicomposting avoids loss of nutrients such as NH_3 (Contreras-Ramos *et al*. 2005).

The available N, which is directly related to crop response (Miralles del Imperial *et al*. 2003), is shown in Figure 1. Vermicomposts A, B and C (689.66, 1081.69 and 2189.80 mg kg^{-1}, respectively) considerably improved over sewage sludge (162.72 mg kg^{-1}). This demonstrates the nutritional value of sludge in all chemical parameters (higher than cow dung except for organic matter and available potassium). But in addition, vermicompost seemed to induce a non-linear availability of N, as sludge content increased 5% between treatments while available N augmented 156.8% between A and B and 202.4% between B and C. All treatments were significantly different; available N augmented 17-fold from 0.5% of the sewage sludge to 8.57% in treatment C (treatments A and B augmented 5 and 10 times, respectively). This non-linear pattern is attributable to a synergism between earthworms and microorganisms whereby earthworms increase organic matter decomposition and accelerate the change of organic N to available N, thus improving the fertility of sewage sludge (Liu *et al*. 2005). Nitrogen mineralization by microflora is also quite intense in the gut of earthworms and continues for several hours in fresh casts (Mariani *et al.* 2007).

The average concentration of available P in sewage sludge was 1994.21 mg kg^{-1}, similar to vermicompost (Figure 1), and much higher than cow dung. Available P is reported elsewhere at 1239 mg kg^{-1} and 369-1511 mg kg^{-1} (Carbonell *et al*. 2009; Hogan *et al*. 2001). Several studies have found a higher content of available P in earthworm casts than in control samples without earthworms (Krishnamoorthy 1990; Guggenberger *et al*. 1996; Liu *et al*. 2005). Earthworms have been shown, after intestinal transit, to help desorb P from the solid phase of soil or waste; they also have a great impact on P mineralization as a result of faecal and bacterial phosphatase activity. Despite this acknowledged role in P mineralization, no increase in available P after vermicomposting was found, nor significant differences between treatments (Figure 1). A possible explanation is that in urban sewage sludge, P is expected as inorganic P from household detergents, which is not mineralized by microorganisms (Fassbender and Bornemisza 1994). To assess this assertion was beyond the scope of this study, but since 82% of all tropical soils in the Americas are phosphorus-deficient (Fassbender and Bornemisza 1994) fractionation was used to establish the chemical forms of P. Fractionation was carried out in sewage sludge and treatment C, the one with highest sewage sludge content (Figure 2). The highest share of P was found to be a slightly soluble fraction, which would indicate that P is immediately available to plants and/or easily lost through leaching or runoff (Penn and Sims 2002). However, as the pH of the vermicompost is acid, the second largest P fraction is bonded to Al in the insoluble fraction (Hedley *et al*. 1982); this diminishes the provision of P to plants under field conditions (Adams 1995).

Available K was highest in composted cow dung (4238 mg kg^{-1}); treatments A (highest cow dung content) and B were significantly different from C (Figure 3). There is evidence that earthworms help increase

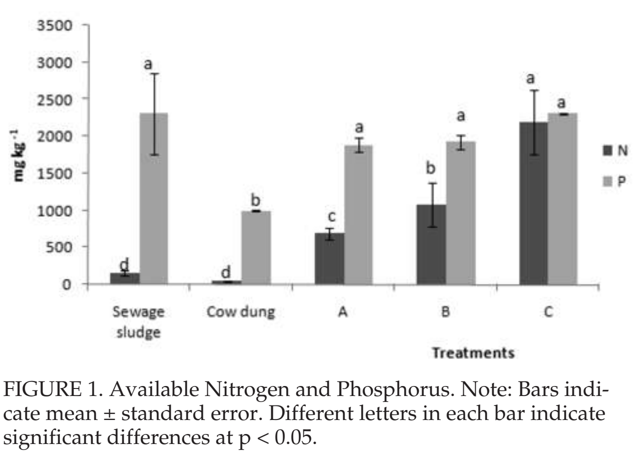

FIGURE 1. Available Nitrogen and Phosphorus. Note: Bars indicate mean ± standard error. Different letters in each bar indicate significant differences at $p < 0.05$.

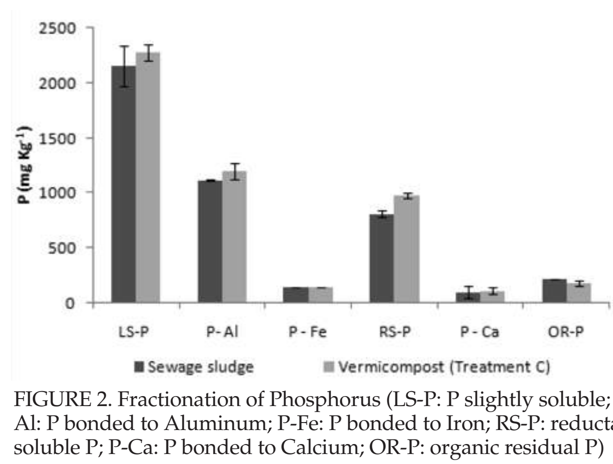

FIGURE 2. Fractionation of Phosphorus (LS-P: P slightly soluble; P-Al: P bonded to Aluminum; P-Fe: P bonded to Iron; RS-P: reductant soluble P; P-Ca: P bonded to Calcium; OR-P: organic residual P)

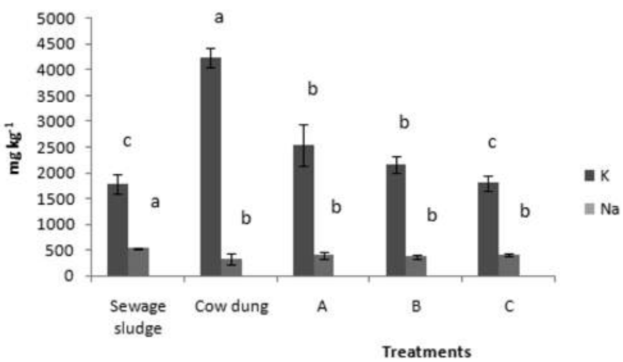

FIGURE 3. Available K and Na. Note: Bars indicate mean ± standard error. Different letters in each bar indicate significant differences at $p < 0.05$.

the concentration of K (Garg *et al*. 2006; Suthar and Singh 2008) and promote the formation of potassium-humic compounds, maintaining K available to plants (Rodríguez-Aragonés 1998).

As to available Na in the sewage sludge (528.76 mg kg^{-1}), it was not significantly different from treatment C (Figure 3), but differed from treatments A and B (398.39 and 377.61 mg kg^{-1}, respectively). Dayananda *et al*. (2008) suggest that sodium content can be a limiting factor in earthworm growth and reproduction, which could have been the case in this study. Also, high concentrations of this element inhibit plant growth and affect soil structure (Contreras-Ramos *et al*. 2005).

C:N ratios, one of the most widely used indexes of organic waste maturity, were between 12.4 and 19.1 (Table 2). Similar values are reported elsewhere (Bansal and Kapoor 2000; Atiyeh *et al*. 2000). Vermicomposting is associated to fast rates of decomposition and mineralization of the organic matter and accelerates the decrease of the C:N ratio (Kaushik and Garg 2003). A decline of C:N to less than 20, indicates an advanced degree of maturity in organic waste (Senesi 1989). Whereas some studies suggest that 70% of sewage sludge in the vermicompost feed presented the best stability and maturity (Contreras-Ramos *et al*. 2005), it was found here that up to 90% sewage sludge could be added, with good maturity.

All parameters in the treatments were in line with the vermicompost Mexican official guidelines (NMX-FF-109-SCFI-2007, shown in Table 2). It must be mentioned that these results apply to the use of domestic wastewater sewage sludge, but caution is warranted when using industrial sewage sludge due to the presence of toxic chemicals (heavy metals and organic chemicals such as pharmaceuticals, hormone disruptors, flame retardants or pesticides), involving a potential risk for soil organisms and plants (Carbonell *et al.* 2009).

In addition to reducing processing times, this study shows that *E. foetida* can accept fresh sludge as feed, if combined with at least 10% cow dung. This appears to be true even when the earthworms drastically change environments from horse dung to fresh sewage sludge, notwithstanding suggestions to adapt earthworms to experimental conditions (Fründ *et al*. 2010; Kaushik and Garg 2003). The results found here buttress the idea that earthworms play an important role in the availability and nutrient cycling in natural and agricultural ecosystems (Jiménez *et al*. 2003; Brossard *et al*. 1996; Decaëns *et al*. 1999; Garg *et al*. 2006).

Conclusions

Treatments of 70:30 and 80:20 ratios of sewage sludge to composted cow dung were the most suitable for the reproduction of *E. foetida*, and to some extent, for their survival. Furthermore, the earthworms tolerated a 90:10 ratio, but this affected survival and reproduction: adult survival decreased to 79%; juvenile and cocoon amounts were significantly lower suggesting substrate rejection by earthworms or toxicity around egg incubation time. This 90:10 treatment yielded the highest values of available N and P, and a slightly lower concentration of available K. Earthworm activity in mixtures of sewage sludge and cow dung could significantly improve available nutrients such as N and agronomic physicochemical characteristics. There were significant differences in the parameters due to different ratios of sewage sludge added to the mixture, except in P. The vermicomposts obtained were within the range recommended by the Mexican guidelines for a good quality fertilizer.

Our recommendation for sewage sludge treatment is to divide the vermicomposting into two lots: one devoted to earthworm production (with 20-30% cow dung content) and another one (with 10% cow dung) devoted to maximize the production of soil amender or fertilizer and to reuse higher amounts of sewage sludge.

Finally, this study suggests that sewage sludge and cow dung vermicomposted with *E. foetida* can improve fertility and nutrient availability, as well as earthworm population growth, compared with the sewage sludge alone.

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