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# VERMICOMPOSTING OF SLUDGES FROM PAPER MILL AND DAIRY INDUSTRIES WITH *EISENIA ANDREI*: A PILOT-SCALE STUDY

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**Abstract**
*We studied vermicomposting with* Eisenia andrei *of sludges from a paper mill mixed with cattle manure in a six-month pilot-scale experiment. Initially, a small-scale laboratory experiment was carried out to determine the growth and reproduction rates of earthworms in the different substrates tested. In the pilot-scale experiment, the number of earthworms increased between 22- and 36-fold and total biomass increased between 2·2- and 3·9-fold. The vermicomposts were rich in nitrogen and phosphorus and had good structure, low levels of heavy metals, low conductivity, high humic acid contents and good stability and maturity. These sludges could be potentially useful raw substrates in larger commercial vermicomposting systems, and would reduce the costs related with the exclusive use of different types of farm wastes as feed for earthworms.*


*Key words:* Cattle manure, chemical composition, dairy sludges, *Eisenia andrei*, organic matter, paper mill sludges, physical properties, pilot-scale, vermicomposting.

## INTRODUCTION

The paper-pulp, paper-mill and dairy industries are an important part of the economy in many European Union countries. These industries are among the greatest consumers of water and also the greatest loading generators of industrial solid wastes, such as fly ash, bark, silt, general mill sweepings, inorganic precipitates and solid sludges from complex wastewater treatment facilities. The sludges are frequently incinerated or disposed of in landfill sites. Both methods lead to the loss of a profitable resource and have obvious environmental and economic disadvantages.

The Department of Agroecology at the Zaidin Experimental Station, CSIC (Granada, Spain), in collaboration with the Department of Environment and Natural Resources of the University of Vigo, Spain, undertook a two-year project to study earthworm biotechnology on laboratory- and pilot-scales. The final goals were to obtain stabilized paper-mill and dairy sludges, and end products (vermicomposts) appropriate for agricultural purposes. Previous studies had shown that these sludges could not be used alone as profitable feeding media for epigeic earthworm growth (Butt, 1993; Elvira *et al.*, 1995; Gratelly *et al.*, 1996). Therefore, sludges from paper-mill industries need to be mixed with other nitrogen-rich organic wastes in order to provide nutrients and an inoculum of microorganisms (Hartenstein, 1978; Butt, 1993; Elvira *et al.*, 1996a, b; Kavian and Ghatnekar, 1991). Similarly, sludges from dairy industries also need to be mixed with other organic residues to improve their structure and balance the nutrient contents in the mixture (Gratelly *et al.*, 1996). Earlier studies dealt with small-scale laboratory experiments and until now no information about the utilization of these sludges in larger experiments has been available. Such information is required if these sludges are to be used as raw materials for commercial vermicomposting systems.

We present the results of a six-month experiment, on a pilot scale, of vermicomposting with *Eisenia andrei* (Bouché, 1972) of sludges from the paper-mill and dairy-processing industries, mixed with cattle manure. The technical viability of this system was studied by determining the growth and reproduction of earthworms in each mixture, as well as the physical and chemical changes in the different substrates. Initially, a small-scale laboratory experiment was carried out to determine the viability of this species in the different substrates assayed.

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## METHODS

### Earthworms, substrates and treatments

Clitellated and non-clitellated specimens of *E. andrei* were obtained from the San Agustin Vermiculture Company, a commercial earthworm grower near Granada, Spain. Paper-mill sludge (PS) (pH 8·2; total organic carbon (TOC) 119 g kg$^{-1}$; total kjeldahl nitrogen (TKN) 2·7 g kg$^{-1}$; C/N 45) was obtained from a wastewater treatment plant of a paper-mill company (Torras-Papel, Motril, Granada). Dairy sludge (DS) (pH 7·1; TOC 234 g kg$^{-1}$; TKN 43 g kg$^{-1}$; C/N 5·4) was collected from a wastewater treatment plant in the dairy processing industry (Puleva, Granada). Six treatments containing these sludges and/or cattle manure were investigated. The composition of each treatment is shown in Table 1.

### Laboratory-scale experiment

150 g dry weight (dw) of each substrate detailed in Table 1 was placed in each of five 600 cm$^3$ cylindrical plastic containers. Five non-clitellated earthworms weighing between 0·05 and 0·15 g each were added per container. The moisture content of the substrate in each container was kept at 80–85% throughout the experimental period and the pots were kept in darkness at 25°C. Earthworm mortality, weight gain, clitellum development and cocoon production were measured weekly for 70 days.

### Pilot-scale experiment

For the pilot-scale experiment, four 2 m$^2$ vermicomposting beds composed of a wooden frame set on a 5% slope were lined with a net (2 mm mesh) and a layer of plastic with an outlet for leachate drainage underneath. The four beds were constructed in a 50 m$^2$ non-controlled greenhouse. Each bed was filled with a layer (10 cm high) containing 90 kg fresh weight (125 l) of one of the substrates detailed in Table 1, except PS and DS. Each bed was inoculated with 1500 clitellated earthworms with a body weight of 0·7–0·8 g each. During the vermicomposting period (six months, from December to June), 90 kg of the corresponding substrate was added after two and four months. The moisture content of the substrate in each bed was kept at 80–85% by microsprinkler irrigation throughout the vermicomposting period.

After three months and at the end of the vermicomposting period three random cores of 30 × 30 cm were sampled from each bed. In each core the number of clitellated and non-clitellated earthworms, their weights and the number of cocoons was recorded. In addition, after three months 50 cocoons in each bed were collected and each was placed in a petri dish between two sheets of moist filter paper. These cocoons were incubated in darkness at 25°C and the number of hatchlings per cocoon was counted. These hatchlings were subsequently inoculated into the beds. At the end of the experimental period, after the earthworms were removed the contents of each core were thorougly mixed and air-dried.

**Table 1. Compositions of the different treatments investigated**

| Treatments | Substrates |
|---|---|
| M (Control) | Cattle manure |
| PS | Paper-mill sludge |
| DS | Dairy sludge |
| PS:M | 1 part paper-mill sludge : 4 parts cattle manure (vol : vol) |
| DS:M | 1 part dairy sludge : 4 parts cattle manure (vol : vol) |
| PS:DS:M | 1 part paper-mill sludge : 1 part dairy sludge : 3 parts cattle manure (vol : vol : vol) |

### Analytical methods

In the initial and final substrates of the pilot-scale experiment the following parameters were analyzed: particle size distribution, bulk and particle density ($D_b$, $D_p$), pH, electrical conductivity (EC), TOC, TKN, total phosphorus (TP) and total potassium (TK) (MAPA, 1986). Total heavy metals (Fe, Mn, Cu, Zn, Cd, Ni and Pb) were determined using an IL Model 357 atomic absorption spectrophotometer, after digestion of the samples with $HNO_3$:$HClO_4$ (2:1). Organic matter fractions were determined according to Sequi *et al.* (1986). After extracting organic carbon with 0·1 M $Na_4P_2O_7$–0·1 M NaOH solution (total extractable carbon, TEC), humified and non-humified fractions were separated by precipitating humic acid (HA) at pH <2 and loading the soluble fraction onto columns packed with insoluble polyvinylpyrrolidone (Aldrich, chromatography-grade cross-linked PVP). The non-humified fraction (NH) was not retained and the fulvic acid fraction (FA) was retained. Total organic carbon was determined in TEC, NH, HA and FA. Humification parameters were calculated as follows: HA/FA ratio, humification index (HI = NH/HA+FA (Sequi *et al.*, 1986)) and degree of humification (DH% = [(HA+FA)/TEC] × 100 (Ciavatta *et al.*, 1988). Finally, water-soluble carbon (WSC) was extracted from the samples with distilled water at a ratio of 1:10.

## RESULTS AND DISCUSSION

### Growth and reproduction of *E. andrei* in the laboratory-scale experiment

Figure 1 and Fig. 2 show, respectively, the growth and cumulative cocoon production of *E. andrei* in the six different feeding treatments. No mortality of earthworms was observed in any treatment. In the control (M), biomass increased (8·40 ± 0·59 mg

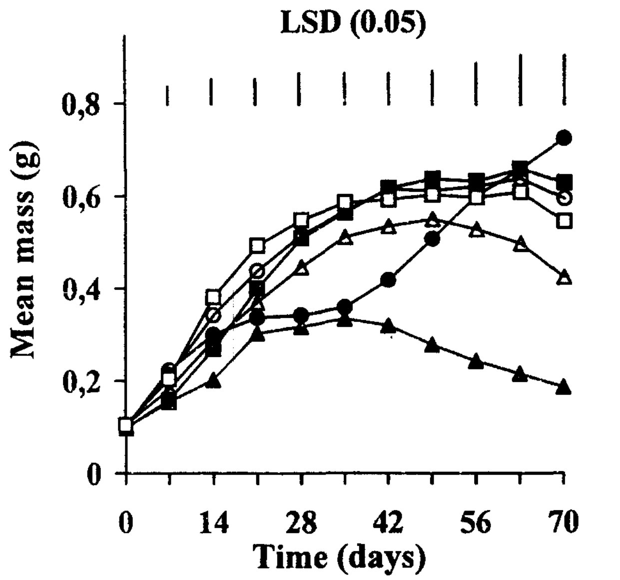


**Fig. 1.** Growth of *E. andrei* during the laboratory-scale experiment in the different treatments (see Table 1) (■, M; ●, PS; ▲ DS; ○, PS:M; △, DS:M; □, PS:DS:M).

earthworm$^{-1}$ day$^{-1}$) (mean ± standard deviation) to a maximum mean weight of 0·66±0·04 g earthworm$^{-1}$ at day 63. All individuals developed a clitellum before day 42, but subsequently the reproductive condition was lost by 36% of the worms. At the end of the experimental period, 20 cocoons per container were counted (0·69 cocoons clitellated per week).

The mean weight of *E. andrei* increased slowly in the PS at a median rate of 8·94±0·35 mg worm$^{-1}$ day$^{-1}$ during the experimental period, reaching a maximum mean weight of 0·73±0·03 g earthworm$^{-1}$ after 70 days. However, the percentage of clitellated

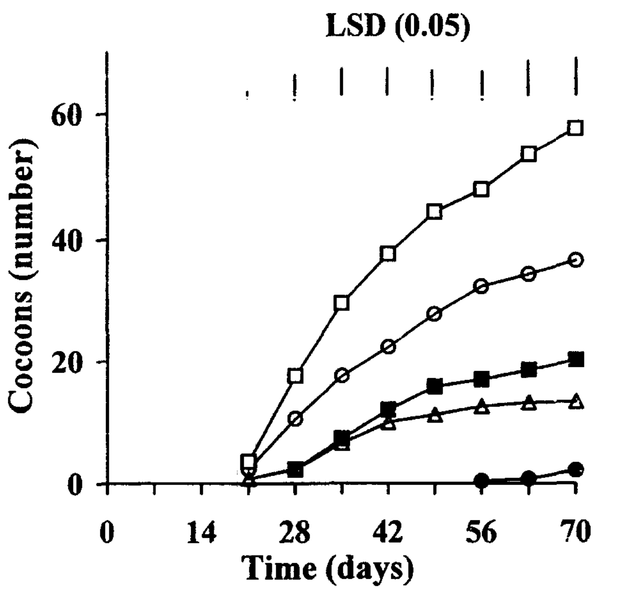


**Fig. 2.** Cumulative cocoon production of *E. andrei* during the laboratory-scale experiment in the different treatments tested (■, M; ●, PS; ▲, DS; ○, PS:M; △, DS:M; □, PS:DS:M).

earthworms was markedly lower (72% at the end of the experimental period) and only 3·6 cocoons were produced per container (0·33±0·16 cocoons clitellated per week). DS supported the slowest growth rates. After 70 days a mean weight of 0·19±0·01 g earthworm$^{-1}$ was reached (maximum 0·34±0·01 g earthworm$^{-1}$ at day 35), with no development of the clitellum by the end of the experimental period. The low rates of reproduction in the PS and DS treatments suggest that these sludges cannot be used alone as substrates for *E. andrei* but must be supplemented with other organic materials.

In treatments consisting of a mixture of paper sludge or/and dairy sludge with cattle manure, the growth dynamics of earthworms were similar to that observed in the control (M), although after 70 days the mean weights of *E. andrei* in these mixtures were lower than in M. This lower growth of *E. andrei* was significantly ($P<0·05$) more pronounced when they were fed with the mixture of dairy sludge and cattle manure. The maximum mean weight was reached around day 56 (0·55±0·05 g earthworm$^{-1}$ in DS:M; 0·64±0·01 g earthworm$^{-1}$ in PS:M) and the growth rates ranged from 8·04±0·12 mg worm$^{-1}$ day$^{-1}$ in PS:M to 9·62±0·27 mg worm$^{-1}$ dcay$^{-1}$ in PS:DS:M. In contrast to the control (M), all individuals grown in treatments containing paper-mill sludge (PS:M and PS:DS:M) developed their clitellum before day 35 and maintained their reproductive condition throughout the experimental period. As a consequence, the number of cocoons per container was significantly higher after 70 days in these treatments (37 in PS:M and 58 in PS:DS:M; $P<0·05$), with mean reproductive rates of 1·02±0·19 (PS:M) and 1·64±0·32 (PS:DS:M) cocoons clitellated per week. In contrast only 92% of earthworms in treatment DS:M developed a clitellum before day 35 and most of them (80%) had lost their reproductive condition by the end of the experimental period: only 13 cocoons per container were counted (0·46±0·12 cocoons clitellated per week).

### Growth and reproduction of *E. andrei* in the pilot-scale experiment

After three months, the number of earthworms had declined slowly in all treatments (Table 2). This decrease was most pronounced in treatment PS:M. Most of the earthworms in all treatments retained their clitellum and had a mean weight between 1·1 and 1·2 g higher than at the beginning of the experiment (0·7–0·8 g). Maximal cocoon production was significantly higher in treatments M and PS:M (*c.* 6200; $P<0·05$) than in the other treatments. In general, cocoon production rate was lower in the pilot-scale experiment than in the laboratory-scale experiment. Finally, no significant differences were observed in the numbers of hatchlings per cocoon from the different substrates, which ranged from 3·9 hatchlings per cocoon in M to 4·7 hatchlings per

**Table 2. Numbers of earthworms, percentage of clitellated earthworms, total worm biomass, number of cocoons and hatchlings per cocoon during the vermicomposting period in the pilot-scale study**

| | After three months | | | | After six months | | | |
|---|---|---|---|---|---|---|---|---|
| | M | PS:M | DS:M | PS:DS:M | M | PS:M | DS:M | PS:DS:M |
| Worms (number per bed) | 1259a | 1133a | 1178a | 1178a | 53640a | 3620c | 33460c | 45350b |
| Clitellated worms (%) | 97·6a | 64·4d | 74·8c | 89·3b | 1·42c | 1·43c | 3·61a | 2·74b |
| Total biomass (g per bed) | 1346a | 889d | 1141c | 1278b | 3227b | 2470c | 2517c | 4284a |
| Cocoons (number per bed) | 6288a | 6192a | 4481b | 2800c | 667a | 252c | 496b | 304c |
| Clitellated weight (g) | 1·1a | 1·1a | 1·1a | 1·2a | 0·43a | 0·36a | 0·34a | 0·42a |
| Hatchlings/cocoon | 3·9a | 4·3a | 4·3a | 4·7a | nd | nd | nd | nd |

Means in the same row at three and at six months followed by the same letter are not significantly different ($P<0{\cdot}05$).
nd = not determined.

cocoon in PS:DS:M. These figures were slightly higher than those found in other studies (Sheppard, 1988; Reinecke and Viljoen, 1991).

At the end of the vermiocomposting period the number of earthworms had increased between 22- and 36-fold and the total biomass had increased between 2·2- and 3·9-fold (Table 2). In general, the greatest increase was recorded in the control bed (M) and the lowest in the PS:M and DS:M treatments. In all substrates, from 96 to 99% of total earthworms were non-clitellated earthworms that had hatched between three and six months previously.

At pilot scale, growth rate and cocoon production by *E. andrei* were generally lower than at laboratory scale. This may be due to differences in the conditions between the two sets of experiments, i.e. age of earthworms (non-clitellated in the case of small-scale, clitellated in pilot-scale cultures), culture system, population density (rather higher in small-scale pots) and, especially, temperature. In the laboratory-scale experiment the temperature was kept at 25°C, whereas in pilot-scale beds the temperature changed markedly (Fig. 3). According to some authors (Tsukomoto and Watanabe, 1977; Hartenstein and Bisesi, 1989), the best growth and

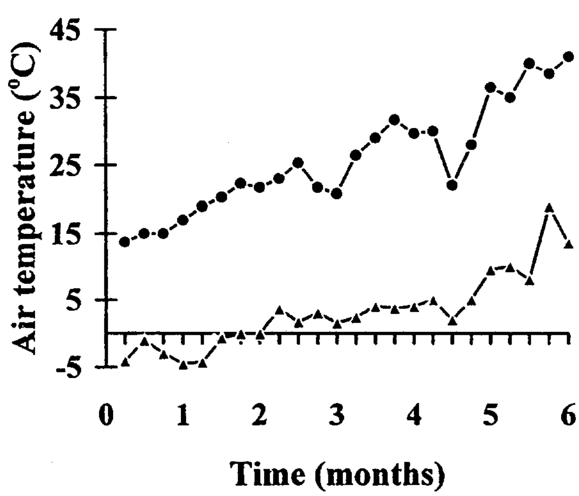


**Fig. 3.** Maximum (●) and minimum (▲) air temperatures during the vermicomposting period in the pilot-scale study.

reproduction rates of *E. andrei* and other *Eisenia* species occur in a temperature range of 20–25°C, whereas temperatures lower than 5°C or higher than 30°C often lead to a high degree of mortality. Minimum temperatures below 5°C were reached in this pilot-scale study during the first three months (Fig. 3), which could explain the earthworm mortality (between 16 and 24%) and the smaller cocoon production. Clitellated earthworm mortality may also reflect the adaptation of these oligochaeta to the different substrates assayed (Haimi and Huhta, 1986). The negative influence of temperature on earthworm population during the experimental period was not very pronounced, as demonstrated by the number of cocoons laid and their high fecundity, and by the spectacular increase in the number of earthworms in the beds.

**Changes in physical and physicochemical properties of the substrates**

The vermicomposting process appreciably modified the physical and physicochemical properties of the different substrates tested (Table 3). Organic matter in the different substrates was fragmented, significantly increasing the percentage of particles <5 mm in size (73 to 90% of the total) and the bulk density, as a consequence of substrate transit through the earthworm gut (Fayolle, 1985). This mechanical action increased the surface:volume ratio, thus augmenting the microbial activity in the substrates (Hartenstein and Hartenstein, 1981). The lower pH recorded in the final products might have been due to the production of $CO_2$ and organic acids by microbial activity during the process of bioconversion of the different substrates in the beds (Haimi and Huhta, 1986). A decrease in pH may be an important factor in nitrogen retention, as this element is lost as volatile ammonia at high pH values (Hartenstein and Hartenstein, 1981). These changes, together with the significantly lower ($P<0{\cdot}001$) conductivity of the end products, suggest that the vermicomposts obtained in this study are potentially suitable as potting media, although the pH would need to be corrected for use with ornamental cultures (Verdonck *et al.*, 1987).

**Table 3. Physical and physicochemical properties of the substrates at the beginning (B) and end (E) of the vermicomposting period in the pilot-scale study**

| | M | | PS:M | | DS:M | | PS:DS:M | |
|---|---|---|---|---|---|---|---|---|
| | B | E | B | E | B | E | B | E |
| Particle size (%) | | | | | | | | |
| >5 mm | 65a | 18b | 83a | 17b | 63a | 27b | 75a | 10b |
| 5–2 mm | 22b | 36a | 12b | 38a | 25b | 38a | 17b | 43a |
| <2 mm | 13b | 45a | 5b | 45a | 12b | 35a | 8b | 47a |
| $D_p$ (g cm$^{-3}$) | 1·7a | 1·8a | 1·8a | 2·0a | 1·5a | 1·9b | 1·8a | 1·9a |
| $D_b$ (g cm$^{-3}$) | 0·28b | 0·55a | 0·39b | 0·56a | 0·33b | 0·54a | 0·43b | 0·71a |
| pH | 8·8a | 7·9a | 8·6a | 8·1b | 8·3a | 7·8a | 8·3a | 7·9b |
| EC (dS m$^{-1}$) | 3a | 0·46b | 2·1a | 0·41b | 3a | 0·45b | 2a | 0·42b |

In each treatment, means at the beginning and the end of the experimental period followed by the same letter are not significantly different ($P<0·05$).
$D_p$ = particle density; $D_b$ = bulk density; EC = electrical conductivity.

**Changes in organic matter fractions of the substrates**

Because of the combined action of microorganisms and the earthworms, a large fraction of the organic matter in the initial substrates was lost as $CO_2$ (between 20 and 43% as TOC) by the end of the vermicomposting period, whereas the percentage of total extractable carbon increased significantly (Table 4). Decreases in TOC and increases in TEC were less evident in treatment PS:DS:M. In the final products, the carbon content of humic acids increased significantly ($P<0·05$), whereas carbon content in fulvic acids remained constant and non-humified-carbon content and water-soluble carbon decreased significantly. As a consequence of these modifications, all final products had low levels of water-soluble carbon (<1·5% from TOC), low C/N ratios (<17), high HA/FA (>5), low humification indexes (<0·3) and a high degree of humification (>75%) (Table 4). These values imply that the entire mass of organic matter contained in the initial substrates was rapidly stabilized and was well humified. The final products had high humic acid contents and their humification parameters (C/N, HA/FA, HI, DH%) were similar to those found for organic matter extracted in other well-matured composts and vermicomposts (Ciavatta *et al.*, 1993; Elvira *et al.*, 1996a).

**Changes in elemental composition of the substrates**

TKN and TP had increased significantly ($P<0·05$) by the end of vermicomposting period, probably because of mineralization of the organic matter (Table 5). In comparison with the control (M), the use of dairy sludge (DS:M) tended to increase the inorganic fertilizer value of the end products. Likewise, total metal concentrations were higher in the vermicomposts than in the initial substrates, although the figures recorded were lower than the maximum levels allowed in sludges for agricultural soils (Council of the European Commission, 1986). These increases were more appreciable for Fe, Zn and Ni in the control treatment (M). On the other hand, TK concentration in the initial substrates had

**Table 4. Organic matter fractions and humification parameters in the substrates at the beginning (B) and end (E) of the vermicomposting period in the pilot-scale study**

| | M | | PS:M | | DS:M | | PS:DS:M | |
|---|---|---|---|---|---|---|---|---|
| | B | E | B | E | B | E | B | E |
| TOC (g kg$^{-1}$) | 325a | 225b | 266a | 151b | 303a | 225b | 229a | 185b |
| HA (g kg$^{-1}$) | 10b | 37a | 10b | 34a | 13b | 38a | 6b | 21a |
| FA (g kg$^{-1}$) | 7a | 5a | 6a | 4a | 7a | 5a | 6a | 4a |
| NH (g kg$^{-1}$) | 13a | 8b | 13a | 6b | 16a | 8b | 14a | 7b |
| WSC (mg kg$^{-1}$) | 9·6a | 2·4b | 7·7a | 2·1b | 8·8a | 2·3b | 5·5a | 1·9b |
| C:N | 27a | 15b | 23a | 16b | 27a | 13b | 41a | 13b |
| TEC/TOC (%) | 10b | 22b | 13a | 24b | 14a | 24b | 12a | 17b |
| HA:FA | 1·4b | 7·4a | 1·7b | 8·5a | 1·8b | 7·6a | 1b | 5·2a |
| HI | 0·74a | 0·20b | 0·83b | 0·18a | 0·8b | 0·18a | 0·85b | 0·28a |
| DH% | 52a | 84b | 46a | 81b | 46a | 81b | 42a | 78b |

All data are expressed on dry matter basis.
In each treatment, means at the beginning and the end of the experimental period followed by the same letter are not significantly different ($P<0·05$).
TOC = total organic carbon; TEC = total extractable carbon; NH = non-humified fraction; HA = humic acids; FA = fulvic acids; TSC = total soluble carbon; HI = humification index; DH = degree of humification.

**Table 5. Elemental composition of the substrates at the beginning (B) and end (E) of the vermicomposting period in the pilot-scale study**

| | M | | PS:M | | DS:M | | PS:DS:M | |
|---|---|---|---|---|---|---|---|---|
| | B | E | B | E | B | E | B | E |
| TKN (g $kg^{-1}$) | 12b | 15a | 11a | 12a | 11b | 17a | 7b | 14a |
| TP (g $kg^{-1}$) | 4·2b | 5·7a | 3·9b | 5·9a | 7·3a | 7·7a | 4·7b | 6·1a |
| TK (g $kg^{-1}$) | 31a | 8·3b | 23a | 7·6b | 25a | 7·6b | 18a | 5·3b |
| Fe (g $kg^{-1}$) | 6·1b | 9·6a | 6·9b | 7·5a | 7·4b | 9·3a | 5·4b | 6·4a |
| Mn (mg $kg^{-1}$) | 198b | 240a | 180a | 190a | 298a | 218b | 123b | 148a |
| Cu (mg $kg^{-1}$) | 34a | 36a | 31b | 34a | 39b | 43a | 32b | 35a |
| Zn (mg $kg^{-1}$) | 108b | 155a | 110a | 108a | 198a | 198a | 123b | 165a |
| Pb (mg $kg^{-1}$) | 14a | 15a | 15a | 13a | 13b | 18a | 10b | 19a |
| Ni (mg $kg^{-1}$) | 23b | 30a | 25b | 29a | 25b | 37a | 25b | 30a |

All data are expressed on dry matter basis.
In each treatment, means at the beginning and the end of the experimental period followed by the same letter are not significantly different ($P<0{\cdot}05$).
Cadmium concentrations in the different organic samples were below the reliable detection limits of the analytical procedure used.
TKN = total kjeldahl nitrogen; TP = total phosphorus; TK = total potassium.

decreased significantly ($P<0{\cdot}05$) by the end of the vermicomposting period. This probably reflects leaching of this soluble element by the excess water that drained through the mass. This effect was noted by Benitez *et al.* (1996), who observed that the leachates collected during the vermicomposting processes had high potassium concentrations and could be considered potential potassium fertilizers for agricultural purposes.

## CONCLUSIONS

The information presented here should provide a sound basis for the management of sludges from paper mill and dairy industries, mixed with other organic wastes, in large commercial vermicomposting systems. Although the best results in the pilot-scale study were obtained when earthworms were fed exclusively with cattle manure, known to be one of the best natural feeds for earthworms, *E. andrei* also grew and reproduced favorably in mixtures of manure with paper-mill sludge and/or dairy sludge. Moreover, the vermicomposts obtained in this study were rich in total nitrogen and phosphorus and had good physical properties, low levels of heavy metals, low conductivity, high humic acid contents and optimum stability and maturity. These characteristics make these vermicomposts useful as soil conditioners, healthy organic fertilizers and, if pH is corrected, as peat substitutes in potting media. Finally, the use of paper-mill sludges and dairy sludges as raw materials in the vermicomposting systems can potentially help to convert these wastes into value-added materials, avoid their widespread disposal and corresponding environmental and economic drawbacks, and reduce the costs related to the exclusive use of different types of farm wastes as feeds for earthworms.

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