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Vermicomposting of sewage sludge: Effect of bulking materials on the growth and reproduction of the earthworm *Eisenia andrei*

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Summary

The effect of different residual bulking agents (paper, cardboard, grass clippings, pine needles, sawdust and food wastes) in mixtures with sewage sludge (1:1 dry weight) on the growth and reproduction of *Eisenia andrei*, Bouché 1972 was studied in small-scale laboratory experiments with batches of sixty earthworms. The maximum weight achieved and the highest growth rate were attained in the mixture with food waste (755±18 mg and 18.6±0.6 mg day^{-1} respectively) whereas the smallest size and the lowest growth rate was achieved in the mixture of sewage sludge with sawdust (572±18 mg and 11±0.7 mg day^{-1} respectively). The earthworms showed much higher reproductive rates in the paper and cardboard mixtures (2.82±0.39 and 3.19±0.30 cocoons earthworm^{-1} week^{-1} respectively) compared to the control with sewage sludge alone (0.05±0.01 cocoons earthworm^{-1} week^{-1}).

Key words: *Eisenia andrei*, earthworms, sewage sludge, bulking agents, growth rate, reproduction

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Introduction

Research into the potential use of earthworms to break down and manage sewage sludge began in the late 1970s (Hartenstein 1978) and the use of earthworms in sludge management has been termed vermicomposting or vermistabilization (Neuhauser et al. 1988; Loehr et al. 1984). It was demonstrated quite early, at a laboratory scale, that aerobic sewage sludge can be ingested by the earthworm *E. fetida* and egested as casts, and that in the process the sludge is decomposed and stabilized (i. e. rendered innocuous) about three times as fast as non-ingested sludge, apparently because of the increases in rates of microbial decomposition in the casts. During this process objectionable odors disappear quickly, relative to non-earthworm ingested sludge, and there is a marked reduction in populations of the pathogenic microorganisms *Salmonella enteriditis, Escherichia coli,* and other Enterobacteriaceae. Although most of the sludge produced in sewage plants is anaerobic, and when fresh can be toxic to *Eisenia fetida* and *Eisenia andrei,* after becoming aerobic it becomes readily acceptable to these species. Because all these processes have not been well studied, there are still many fundamental aspects that need to be researched, evaluated, and resolved to assure the success of such a process.

Mixing sewage sludge with other materials, e. g. garden wastes, food wastes, paper pulp sludge or other carbon-rich wastes, and composting the mixture using earthworms, can accelerate decomposition, due to maceration and mixing of such materials during passage through the earthworm gut and passage into earthworm casts. These bulking materials can also improve the C:N ratio by supplying C, and at the same time prevent N losses by ammonia volatilization (Domínguez et al. 1997).

It is well known that the food source influences not only the size of earthworm populations but also their growth and reproduction rates. There is abundant literature on the response of earthworms to different types of vegetable substrates in the field (Guild 1955; Barley 1959; King & Heath 1967; Swain 1979; Shipitalo et al. 1988), but there is a lack of information about the effect of diet on earthworms during the vermicomposting process.

In this paper, we investigate the effect that some organic bulking agents (paper, cardboard, grass clippings, pine needles, sawdust and food wastes) mixed with sewage sludge (1:1 dry weight) have on the growth and reproduction of *Eisenia andrei*.

Materials and Methods

Young specimens of *Eisenia andrei*, weighing 200–250 mg live weight, were obtained from a stock culture maintained in the laboratory at a temperature of 25°C in cow manure. Sewage sludge biosolids were obtained from a wastewater treatment plant in Fresno, CA. Sixty earthworms were placed in 2 litre plastic dishes and supplied 100 g of a mixture of sewage sludge with paper, cardboard, grass clippings, pine needles, sawdust and food wastes (1:1 dry weight) with the bulking materials chopped and sieved (< 2 mm). Aged sewage sludge (two weeks old) was used as control. The worms were supplied with additional 100 g of the different mixtures every week for the duration of the experiment which ended when weight losses of the earthworms were detected (6 weeks). Three replicates for each treatment were established. The weight of the worms (with full alimentary tracts), clitellum development and cocoon production (determined by hand-sorting) were measured weekly. To compare the growth rates of *E. andrei* in the different mixtures, it was necessary to find a common denominator for comparison. To achieve this, the growth rate (mg weight gained day^{-1} $worm^{-1}$) was calculated and the maximum weight achieved was divided by the number of days required to reach the maximum weight.

Chemical analyses of the mixtures

A subsample (approximately 10 g) of each mixture was dried at 105°C and ashed at 550°C to determine moisture and organic matter content, respectively. The pH and conductivity were determined using water diluted samples (1:10) and NH_4^+-N by using an automatic steam distilator (Buchi 650, Zürich).

Statistical analyses

One-way analysis of variance (ANOVA) and separation of means based on the least significant difference (LSD, $p \leq 0.05$) allowed determination of significant differences between growth and reproduction rates in the different mixtures. In order to analyse the underlying variation in the growth and reproduction of *E. andrei* in the different diet mixtures, the data were subjected to principal component analysis (PCA). The PCA was carried out using the correlation matrix of the biological parameters (maximum weight, growth rate, maturation time and cocoon production).

Results

The sewage sludge utilized in these experiments showed relatively low ammonia content (219 µg g^{-1}) and conductivity values (0.8 mS cm^{-1}) which are adequate for rearing *E. andrei* (Edwards 1988) and consequently earthworm survival was very high (98 %) in the control. Earthworm mortality was very low (< 2%) in all the mixtures except in the one with grass clippings where more than 40 % of the individuals died during the first week.

The growth curves of *E. andrei* in sewage sludge and in the mixtures with the different bulking materials are given in Figure 1. The earthworms reached their maximum weights in the mixture with food waste (755 ± 18 mg) at a growth rate of 18.6 ± 0.6 mg day^{-1} and the smallest size was attained in the mixture with sawdust (572 ± 18 mg) with a growth rate of 10.96 ± 0.74 mg day^{-1} (Table 1). After the initial biomass increment a stabilization and, later, weight loss were observed in all mixtures tested. Food limitation does not explain the weight loss observed because additional food was added every week.

Table 2 shows parameters related to the sexual development and reproduction of *E. andrei* in the different mixtures. The mean maturation size was significantly higher ($p < 0.05$) in the mixtures of sewage sludge with cardboard, grass clippings, pine needles and food than in the other three mixtures and in the control, although the time to reach it was the same in all mixtures (2 weeks). Figure 2 shows the total cocoon production in the sewage sludge and in the mixtures with the different bulking agents. Cocoon production was significantly higher in the mixtures of sewage sludge with paper and cardboard (2.82 ± 0.39 and 3.19 ± 0.30 cocoons $earthworm^{-1}$ $week^{-1}$ respectively) than in the other mixtures. The lowest cocoon productions were achieved in the control (sewage sludge) and in the mixtures with food waste and grass clippings (0.05 ± 0.01, 0.08 ± 0.02 and 0.23 ± 0.04 cocoons $earthworm^{-1}$ $week^{-1}$ respectively).

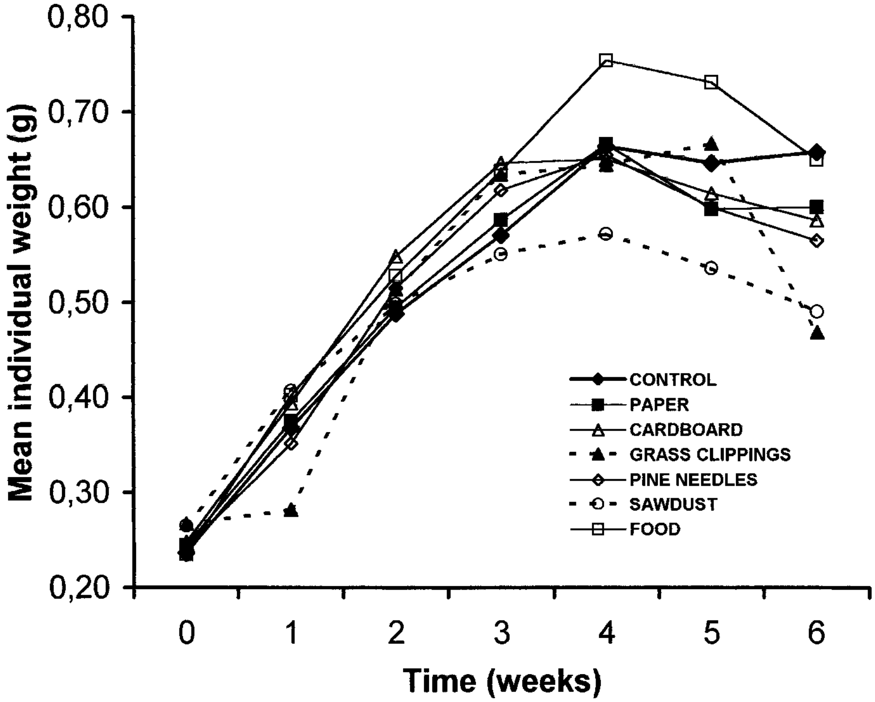

Fig. 1. Growth of *E. andrei* fed on sewage sludge (control) and mixtures (1.1 d.w.) with paper, cardboard, grass clippings, pine needles, sawdust and food waste (n = 3)

Table 1. Growth of *E. andrei* in the different diet mixtures

DIET MIXTURES[1]	Maximum weight achieved (mg) ± S.E.[2]	Growth rate (mg day^{-1})
Sewage sludge	674 ± 11[a 3]	15.63 ± 0.42[a]
SS + paper	667 ± 03[a]	15.09 ± 0.56[a]
SS + cardboard	656 ± 07[a]	16.14 ± 1.32[ad]
SS + grass clippings	672 ± 52[a]	14.46 ± 1.38[ac]
SS + pine needles	655 ± 22[a]	14.75 ± 0.41[a]
SS + sawdust	572 ± 18[b]	10.96 ± 0.74[b]
SS + food	755 ± 18[c]	18.58 ± 0.59[d]

[1] SS: Sewage sludge
[2] Standard error
[3] Treatments followed by the same letters are not significantly different ($p \leq 0.05$)

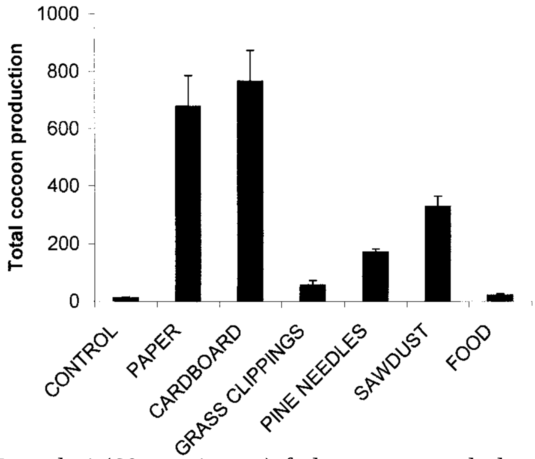

Fig. 2. Total cocoon production of *E. andrei* (60 specimens) fed on sewage sludge and in the different diet mixtures (n=3)

Table 2. Sexual development of *E. andrei* in the different diet mixtures

DIET MIXTURES[1]	Mean maturation size (g) ± SD[2]	Time (days)	Cocoons earthworm^{-1} week^{-1}
Sewage sludge	488 ± 07[a 3]	15	0.05 ± 0.01[a 3]
SS + paper	494 ± 18[a]	15	2.82 ± 0.39[b]
SS + cardboard	548 ± 15[b]	15	3.19 ± 0.30[b]
SS + grass clippings	593 ± 60[b]	15	0.23 ± 0.04[c]
SS + pine needles	515 ± 16[b]	15	0.70 ± 0.05[d]
SS + sawdust	488 ± 14[a]	15	1.37 ± 0.10[e]
SS + food	534 ± 08[b]	15	0.08 ± 0.02[a]

[1] SS: Sewage sludge
[2] Standard error
[3] Same letters correspond to not significant differences ($p < 0.05$)

Principal component analysis (PCA, Fig. 3) revealed the presence of two main factors accounting for 83,36 % of the total variance observed. The first factor (PC1) of the PCA explains 46.49 % of the environmental variance, which represents the growth of *E. andrei* in the different mixtures and is highly correlated with the growth rate (0.96, Table 3). The PC1 separates carbon rich mixtures (paper, cardboard, pine needles and food) from the control and the mixtures with sawdust and grass clippings, reflecting therefore, the food quality (Fig. 3). The second factor (PC2) of the PCA accounts for 36,87 % of the environmental variance and it was defined as the effect of reproduction, explained by cocoon production that is high and negatively correlated with PC2 (-0.84, Table 3). It separates the control and the mixtures of sewage sludge with grass clippings and food waste from the mixtures with sawdust, pine needles, paper and cardboard.

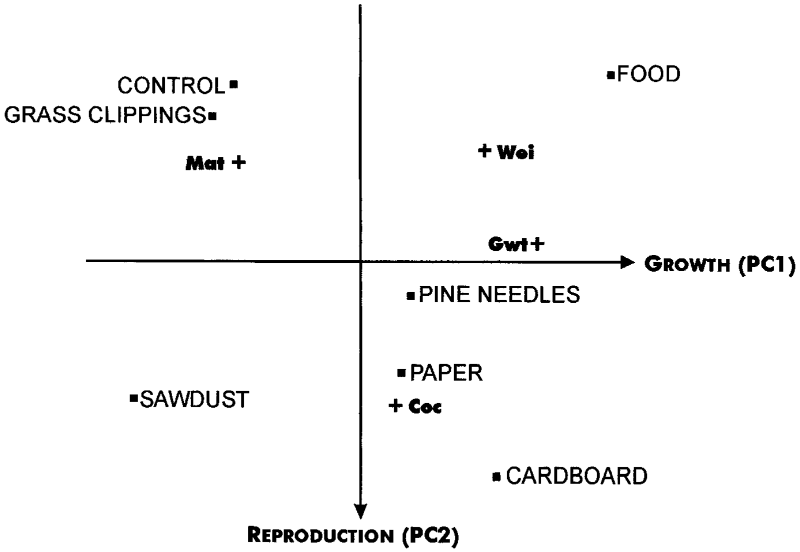

Fig. 3. Position of the the different treatments in the plan defined by factorial axes. PC1 represents 46.5 % of total inertia and PC2 36.87 %

Table 3. Factor loadings of the Principal Component Analysis of the five biological traits used to evaluate the "vigor" of *Eisenia andrei* fed on sewage sludge

	Growth (PC1)	Reproduction (PC2)
Maturation time (Mat)	-0.666	0.575
Maximum weight (Wei)	0.682	0.652
Growth rate (Gwt)	0.956	0.107
Cocoon production (Coc)	0.194	-0.842
Eigenvalue	1.859	1.475
Variance	46.49	36.87

Discussion

The bulking agent had a much important effect on the reproduction than on the growth of *E. andrei* and the maximum growth and reproduction rates were not obtained in the same mixtures (Fig. 3). Only the mixture of sewage sludge with food waste showed a significantly higher growth rate than the control (sewage sludge alone) due probably to an increase in the amount of microorganisms that provide a suitable nutrient source.

Comparing our results with others in literature, it can be easily seen that our higher growth rates (between 15 and 18.6 mg earthworm^{-1} day^{-1}) in the earthworms fed on the mixtures of sewage with paper and food wastes are similar to those previously reported for sewage and slurry mixtures, pointing out the high potential of sewage sludge for vermicomposting (Edwards 1988). Neuhauser et al. (1980) found that *E.*

fetida gained weight at rates which were dependent on population density and food type. When fed 250 g of horse manure at densities between 3 and 16 per dish, rates of growth varied between 19 and 7 mg earthworm^{-1} day^{-1}. Graff (1974) and Watanabe and Tsukamoto (1976) obtained growth rates of 60-80 mg per week for *E. fetida*. Reinecke et al. (1992) reported growth rates of 7 mg earthworm^{-1} day^{-1} for *E. fetida* fed on cattle manure for 150 days. Hartenstein and Hartenstein (1981) achieved rates of 14 mg earthworm^{-1} day^{-1} for *E. fetida* hatchlings fed on wet activated sludge. Cluzeau et al. (1992) obtained growth rates of only 4.5 mg earthworm^{-1} day^{-1} in batch culture for immature *E. andrei* fed on horse manure and peat. Elvira et al. (1996), growing 8 individuals of *E. andrei* on a mixture of fresh cow manure and straw, found greater growth rates (16.75 mg day^{-1}) and maximum weights (0.59 g) than in cow manure (12.25 mg day^{-1} and 0.43 g respectively).

Reinecke and Viljoen (1990) in studies with *E. fetida* reared in cow manure and Domínguez and Edwards (1997) studying the growth and reproduction of *E. andrei* in pig manure found that, when grown at different population densities, the earthworms in the crowded dishes grew more slowly and with a lower final bodyweight, although the total weight of earthworm biomass produced per unit of waste was greater. It is pertinent to note this because of the comparatively high population density in our batches.

In relation to sexual development, Neuhauser et al. (1980) suggested that food availability and population density determined the time to reach sexual maturity for earthworms. The time needed for clitellum development varies in direct relationship with nutrient abundance. This makes it difficult to compare our results with those of previous works using different organic substrates and taking longer periods of time to reach sexual maturity (e.g. Venter & Reinecke 1988; Edwards 1988; Haimi 1990; Elvira et al. 1996).

Our results confirm the general rule, also reported in the literature, establishing a direct relationship between the weight of *E. andrei* and the clitellum development so that the minimum weight for maturation is approximately 0.4 g (Mitchell 1983; Reinecke & Venter 1985; Venter & Reinecke 1988).

The marked differences between rates of cocoon production in the different mixtures must be related to the quality of the bulking agent. The maximum rates of cocoon production were obtained in the mixtures with cardboard and paper (3.19±0.3 and 2.82±0.4 cocoons earthworm^{-1} week^{-1}) respectively, values which are similar to those recorded for *E. fetida* (3.8 cocoons earthworm^{-1} week^{-1}) (Edwards 1988) and for *E. andrei* (3.08 cocoons earthworm^{-1} week^{-1}) (Haimi 1990).

The fact that the control showed a very low reproductive rate indicates that this sewage sludge is a much better substrate for vermicomposting when mixed with other materials; the use as bulking agents of grass clippings and food waste does not adequately improve cocoon production.

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