Hindawi Publishing Corporation
Applied and Environmental Soil Science
Volume 2011, Article ID 519485, 10 pages
doi:10.1155/2011/519485

Research Article

Effects of Biosolids at Varying Rates on Earthworms (*Eisenia fetida*) and Springtails (*Folsomia candida*)

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Received 17 September 2010; Accepted 29 October 2010

Academic Editor: Robert Edwin White

Land spreading is a major option internationally for the disposal/use of treated sewage sludge (biosolids), but effects of this practice on soil organisms are largely unknown. This study investigated the effects of biosolids on two soil invertebrate species, earthworms (*Eisenia fetida*) and Collembola (*Folsomia candida*), in laboratory tests. Five biosolids from different sewage works were assessed at rates equivalent to 0, 2, 5, 10, and 20 t ha^{-1}. Biosolids applied at 2 and 5 t ha^{-1} did not cause mortality of adult earthworms but did at 10 and 20 t ha^{-1}. At 5, 10 and 20 t ha^{-1}, all biosolids had significantly fewer juvenile worms relative to controls. Increasing the rates from 2 to 10 t ha^{-1} did not impact on the number of adult Collembola, but at 20 t ha^{-1} there were significantly fewer adults. There were significantly fewer juvenile Collembola recorded for biosolids applied at the 2 t ha^{-1} when compared with controls, and also when biosolids were applied at 5, 10, and 20 t ha^{-1} relative to 2 t ha^{-1}. Some significant difference between biosolids were observed, but generally, negative effects were not related to heavy metal concentrations in biosolids. It is recommended that possible detrimental mechanisms (e.g., ammonia production, lack of oxygen) be investigated in future work. It is concluded that biosolids, applied at legal, low rates (about 2 t ha^{-1}) are unlikely to be detrimental to earthworms or adult Collembola but can be detrimental to Collembola reproduction.

1. Introduction

The application of exogenous organic matter to agricultural land is considered to be one of the most serious anthropogenic pressures on soils in the European Union [1]. European law and Irish law [2–4] aim to promote the recycling of municipal sludge in agriculture and to set standards to protect the environment and food safety.

Treated sludge of an appropriate standard is termed "biosolids". By 2015, towns of more than 2000 people in Ireland will be obliged to have sludge treatment plants complying with adopted standards. Over the next decade it is estimated that 150.000 tonnes of dry solids will be produced annually [5] which is more than a doubling of current production, mirroring international trends. The only means currently of disposing of this material is to apply it to agricultural land. In Ireland, land spreading of exogenous organic matter is increasing rapidly because alternative disposal options have recently been eliminated (sea dumping) or are soon to be precluded (landfill); a third option, incineration, is not available, at least in the medium term. Because this material is a relatively new form of organic material, considered more innocuous than raw sewage sludge produced prior to the introduction of regulatory standards, its effects on soil biota are unknown.

European legislation that regulates sewage sludge amendments in agriculture land [2] or influences indirectly their use [6] consider just a chemical approach, imposing heavy metal limit values and or apply nitrate/phosphorus regulations. Despite the existence of standardized protocols (*Eisenia fetida, Folsomia candida,* and Enchytraeids), biological assays are not mentioned even in the third draft of the working document on sludge [7].

The earthworm *Eisenia fetida* and the Collembola *Folsomia candida* are considered excellent test organisms for studying the effects of organic amendment in the soil ecosystem because of their direct exposure and their sensibility to pollutants [8–11]. The stimulation of soil biota revealed in some field studies using agronomic dosage of biosolids [12] is probably linked to the enhancement of soil fertility, especially due to the contribution of the organic matter. However, in some laboratory investigations the application of such wastes has caused inhibitory effects on soil invertebrates [13].

The objective of this study was to investigate the effects of biosolids from various sources in Ireland applied at different rates on two sensitive indicator invertebrate soil species, *Eisenia fetida* and *Folsomia candida,* under laboratory conditions. Since available test protocols are not specifically designed for organic waste materials, the study also included methodological developments, for example, is it necessary to provide an organic food source in the earthworm test?

2. Materials and Methods

2.1. Test Substrate. Biosolids from five treatment plants (sources) throughout Ireland were investigated for their effects on the earthworm *Eisenia fetida* (Savigny, 1826) and the Collembolan *Folsomia candida* (Willem, 1902). The sources of biosolids were Biosolid 1 from Ringsend, Dublin, Biosolid 2 Dungarvan, Waterford, Biosolid 3 Little Island, Cork, Biosolid 4 Dunlickey, Limerick, and Biosolid 5 Osberstown, Kildare. All biosolids were collected during July 2007 and stored in sealed 160 litre plastic drums. A sixth biosolid high in Se obtained from Pueblo, Colorado, USA, was also investigated for comparative purposes for its effects on *F. candida.* Drying temperatures and dryer type used in the production of each biosolid are given in Table 1. Heavy metal analysis of each biosolid was obtained by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2.2. Test Organisms. Adult *Eisenia fetida* worms were obtained from a vermicompost unit managed at University College Dublin (Ireland). Animals were reared in a controlled environment cabinet (CEC) at 20 ± 2°C and 12 : 12 photoperiod, in a breeding substrate of 50% organic cattle dung (defaunated by drying) and 50% *Sphagnum* peat. The medium of pH 6 to 7 was maintained at approximately 60% water holding capacity (WHC). Worms were maintained in this breeding substrate for a period of 12 weeks and were the source of cocoons which provided worm cohorts of the same age and development stage for tests. Test worms had well-developed clitellae and were 40 days old. Ten worms, selected at random from a population of more than 2000, were weighed and used for each individual replicate of each biosolid.

A culture of *F. candida* was obtained from M. T. Fountain, University of Reading, UK. Laboratory reproduction and rearing of *F. candida* was in accordance with The International Standards Organization (ISO) protocol (ISO. 1999 *11267*, [14]) and those of Fountain and Hopkin [15]. Collembola were cultured in glass Petri-dishes 8.5 cm diameter and 1 cm deep on a moist substrate (approximately 0.4 cm deep) of 20 : 1 plaster-of-paris : graphite (calcium sulphate dehydrate : charcoal) in the laboratory at room temperature. Collembola were fed weekly with dry yeast (Type II Sigma-Aldrich-YSC2) and the substrate was kept moist by spraying with distilled water at intervals of 48 h.

2.3. Mortality and Reproduction. The artificial substrate used in tests comprised *Sphagnum* peat 10% (sieved through 5 mm mesh), 20% kaolin, and 70% quartz sand (80% particle size 0.2 to 2 mm) (ISO *1998* 11268-2 [16], ISO *1999* 11267 [14]). For worms, plastic containers (10 × 10 × 10 cm) with perforated snap-on lids were filled with substrate to a depth of 70 mm for each test replicate (640 mL added). The WHC of the substrate was adjusted to 68% using distilled water and the pH to 6.0, using calcium carbonate. This WHC was maintained throughout the period of the test. Containers with substrate were left without lids for 48 h prior to the addition of 10 worms per container. Worms were fed weekly with ground, surface-applied cow dung (5 g dry weight). The treatments investigated included biosolids mixed with substrate at rates equivalent to 2, 5, 10, and 20 t ha−1 dry matter. In the case of biosolids 4 and 5, the 2 t ha−1 rate was omitted. Additionally, Biosolid 1 was also investigated at all rates with and without cow dung as food. Control treatments comprised substrate without biosolid. Replication was 6-fold. The test parameters measured were (i) pre- and posttrial weights of each group of 10 worms per replicate, (ii) worm mortality posttrial, (iii) reproduction: following the removal of worms, containers with substrate were placed in the CEC for a further 28 days after which the number of juvenile worms in each container was recorded following collection by wet sieving of substrate.

For Collembola, screw top plastic containers (6 cm high and 3.7 cm diameter) were filled with substrate to a depth of approximately 3 cm for each test replicate (50 mL added). The soil substrate pH was adjusted to pH 6.0 using calcium carbonate. Five rates of biosolids equivalent to 0, 2, 5, 10 and 20 t ha−1 were mixed with the soil substrate and the pH readjusted to 6 where necessary. Additionally, every biosolid was also investigated at 2 t ha−1 rate but placed on the substrate surface. The substrate biosolid was moistened, using distilled water, so that no free water was visible when the soil was compressed [17]. Ten, 10 to 12 day old *F. candida* were placed in each container and provided with 3 mg of dry yeast (Type II Sigma-Aldrich-YSC2) as food. Replication was 6-fold. Containers were placed in a controlled environment cabined (CEC) for 28 days at 20°C in a 16 : 8 h light : dark regime. Relative humidity was maintained at approximately 100% by spraying the inside of container lids with distilled water at 48 h intervals. The food supply was replenished on day 14 by adding a further 3 mg of dry yeast. After 28 days the number of adult and juvenile *F. candida* in each container was recorded following their recovery by water floatation in plastic containers (26 × 15 × 10 cm).

2.4. Data Analysis. The data were analysed using general linear model procedures [18]. The data comparing Biosolid 1 at various rates and with or without cow dung as feed had a two by five factorial design and was analysed using ANOVA.

Table 1: Biosolid production temperatures (°C), dry matter content, and dryer type.

	Biosolid 1	Biosolid 2	Biosolid 3	Biosolid 4	Biosolid 5	Biosolid 6
Dryer temp.	350–450	350–450	118–175	275–325	120–130	Air temp
Targeted dry matter	>94	>94	>94	>94	>94	—
Measured dry matter	95.4	97.5	89.6	94	95.1	82.1
Dryer type	Rotating drum	Rotating drum	Thin-film evaporator	Rotating drum	3-stage Belt	Filter-press

Data on adult worms and Collembola were analysed using Friedman analysis of variance by rank with sources of biosolids treated as blocking factors. Worm weight data was log transformed and worm pretrial weights were included in analysis (ANOVA) as covariates. Analysis of data on juvenile worms and Collembola entailed Box-Cox transformations. A group variable was used to combine the single control with the blocked data in the case of juvenile worms, while for Collembola each biosolid had a control. Possible effects of chemical elements on juvenile worms and Collembola were investigated using chemical measurements as covariates in place of the block factor. Linear and quadratic effects and their interaction with the treatment factor were examined.

3. Results

3.1. Effect of Presence and Absence of Cowdung on Earthworms. The effect of presence and absence of cowdung, as a food, with various rates of Biosolid 1 on *E. fetida* are given in Table 2. There was a significant interaction between cowdung and rate of biosolid on adult worm numbers ($P < .0001$), weight ($P < .0001$), and number of juvenile worms ($P < .0001$) produced. The mean number of worms recovered where cowdung accompanied the various rates of biosolid was 9.9, while that without cowdung was 8.3 worms. The latter values differed significantly. There was no difference between treatments in worm weight pre-trial (Table 2), but data analysis of worm weight posttrial included individual pre-trial weights (as a covariate) since a relationship was found between these values. The absence of dung significantly ($P < .0001$) reduced worm weights when compared with that for worms receiving dung. Similarly, there was a significant effect of rate of biosolid on worm weight. Comparing posttrial worm weights for dung and no-dung at the various rates showed significant differences between controls ($P < .0001$), 2 t ha−1 rate ($P = .001$) and 20 t ha−1 rate ($P = .0003$). The mean number of juvenile worms produced where cowdung accompanied the various rates of biosolid was 55.3, while that without cowdung was significantly fewer with only 7.8 juveniles recorded.

3.2. Earthworms. Increasing the rate of application of the five biosolids resulted in a significant ($P = .0009$) reduction in earthworm numbers (Table 3). Comparing worm numbers recovered from the 2 t ha−1 and 5 t ha−1 rates, for the 5 biosolids, showed no significant effect on worm mortality. However, for comparisons between the 5 t ha−1 and 10 t ha−1 rates a significant ($P = .016$) reduction in numbers for the higher rate was recorded. The 2 t ha−1 rate had significantly more worms than the 10 t ha−1 rate. Similarly, the 20 t ha−1 rate had significantly ($P = .03$) fewer worms relative to that for the 10 t ha−1 rate. An examination of worm numbers for Biosolids 1 and 5 showed no significant impact due to increasing rate of application. In the case of Biosolids 2, 3, and 4, there was a significant ($P \leq .0001$) negative effect due to increasing rate.

The pre-trial weights of worms, which were randomly selected, did not differ significantly between rates or biosolids (Bonferroni grouping by means). However, the final weights of worms for the various biosolids treatments were significantly ($P = .03$) lower than worm weights from untreated controls. The increased application rate of biosolids as well as source of biosolid had significant effects ($P < .0001$ and .005) on posttrial worm weights.

In the combined analysis, biosolids from the five sources had significantly ($P < .0001$) fewer juveniles than controls (Table 3). Juvenile numbers were significantly ($P < .0001$) reduced with increasing rates of biosolids, and there was also a significant ($P < .0001$) effect for the various biosolids (i.e., a source effect). The effect of application rate on juvenile worms was determined by combining each rate for the five biosolids. This showed no difference in juvenile numbers between untreated and 2 t ha−1 rate, while the 5 t ha−1, 10 t ha−1, and 20 t ha−1 rates had significantly fewer juveniles ($P < .0001$ to .007). Only three of the five biosolids were investigated at the 2 t ha−1 rate, nevertheless, the results show that the 5, 10, and 20 t ha−1 rates had significantly ($P < .0001$) fewer juveniles than that for 2 t ha−1. The 5 t ha−1 rate has significantly ($P < .0001$) more juveniles than either 10 t ha−1 or 20 t ha−1 rates and the 10 t ha−1 rate had significantly ($P = .0002$) more juveniles than the 20 t ha−1 rate. In general, comparing the number of juvenile worms recovered from biosolids for each of the five sources with untreated controls showed that Biosolid 1 did not differ from that for the control while biosolids 2, 3, 4, and 5 all had significantly ($P < .0017$) fewer juvenile worms. Using P values to explore the block effect showed that Biosolids 2 and 3 had significantly fewer juveniles than Biosolid 1 and Biosolid 3 which had significantly fewer juveniles than Biosolids 4 and 5.

3.3. Collembola. There was no significant difference in the number of adult Collembola recovered between surface and soil mixing for the six biosolids at the 2 t ha−1 rate. Increasing the rate of biosolid application from the six sources significantly ($P < .0001$) reduced the number

Table 2: The effect of rate of Biosolid 1on *Eisenia fetida* adult mortality, body weight, and number of progeny when provided with cowdung as food or in the absence of dung (mean of 6 replicates, 10 worms per replicate).

Biosolid rate t ha^{-1}	Worm No. pre-trial	Worm No. posttrial	Worm wt. g pre-trial	Worm wt. g posttrial	No. juvenile worms
Cowdung provided					
0	10	10.0	0.3106	0.3311	68.2
2	10	10.0	0.2942	0.3202	95.2
5	10	9.8	0.2920	0.3404	81.0
10	10	10.0	0.3024	0.3773	32.3
20	10	9.8	0.3092	0.4735	0.0
sed		0.129	0.0115	0.0188	12.88
No dung					
0	10	10.0	0.3102	0.2043	1.2
2	10	10.0	0.3121	0.2665	15.5
5	10	10.0	0.3162	0.3408	21.7
10	10	10.0	0.3060	0.4413	0.7
20	10	1.7	0.3025	0.3652	0.0
sed		0.3887	0.0173	0.0189	2.911
Dung effect (*P*-value)		—	.254	<.0001	—
Biosolid rate effect (*P*-value)		—	.963	<.0001	—
Mean value + cowdung		9.93^{a}	0.3017^{a}	—	55.33^{a}
Mean value − cowdung		8.33^{b}	0.3094^{a}	—	7.8^{b}

^{a,b}Bonferroni grouping; values with different superscripts differ significantly.
sed: Standard Error of the Difference between Means.

Table 3: The effect of rate of application, t ha^{-1}, compared for each of five Biosolids on *Eisenia fetida* adult mortality, weight, and progeny (Mean of 6 replicates, 10 worms per replicate).

Biosolid	Rate t ha^{-1}	Worm No. posttrial	Worm wt g pre-trial	Worm wt g posttrial	No. juvenile worms
Untreated control	0	10.0	0.3106	0.3311	68.2
1	2	10.0	0.2942	0.3202	95.2
	5	9.8	0.292	0.3405	81.0
	10	10.0	0.3025	0.3773	32.3
	20	9.8	0.3092	0.4735	0.0
sed		0.136	0.0123	0.0199	13.50
2	2	10.0	0.2838	0.3231	107.0
	5	10.0	0.3100	0.3629	10.5
	10	8.5	0.2954	0.3849	0.0
	20	0.0	0.2917	0.0	0.0
sed		0.438	0.0105	0.0160	9.342
3	2	10.0	0.3122	0.3260	44.0
	5	10.0	0.3005	0.3125	6.3
	10	6.2	0.3000	0.4323	0.0
	20	0.0	0.3118	0.0	0.0
sed		0.902	0.00869	0.0621	4.554
4	5	9.7	0.3020	0.4444	46.8
	10	9.8	0.3008	0.4042	7.0
	20	3.8	0.3210	0.4557	0.0
sed		1.20	0.0119	0.0509	6.562
5	5	10.0	0.3046	0.3418	49.8
	10	10.0	0.2837	0.3513	1.2
	20	9.5	0.3152	0.3710	0.7
sed		0.279	0.0207	0.0253	7.864

sed: Standard Error of the Difference between Means.

Table 4: The effect of rate of application, t ha^{-1}, compared for each of six biosolids on the number of adult *Folsomia candida* and on the number of juveniles (mean of 6 replicates, 10 Collembola per replicate).

Biosolid	Rate, t ha^{-1}	Adult No. posttrial	No. juvenile Collembola
1	0	10.0	348.5
	2-surface	9.83	200.83
	2	9.5	142.33
	5	9.67	55.83
	10	7.83	40.17
	20	0.0	0.0
sed		0.3717	19.857
2	0	10.0	252.0
	2-surface	9.83	54.5
	2	8.5	20.67
	5	6.17	6.17
	10	0.0	0.0
	20	0.0	0.0
sed		0.5607	38.371
3	0	10.0	432.17
	2-surface	9.67	84.33
	2	9.0	41.17
	5	5.67	0.0
	10	0.0	0.0
	20	0.0	0.0
sed		1.0205	35.471
4	0	10.0	387.67
	2-surface	9.67	137.67
	2	8.33	125.83
	5	9.17	3.83
	10	4.5	0.0
	20	0.0	0.0
sed		0.7812	38.906
5	0	10.0	301.17
	2-surface	9.67	139.83
	2	9.5	103.83
	5	7.83	21.83
	10	7.5	0.0
	20	5.0	0.0
sed		0.8608	26.954
6	0	10.0	359.5
	2-surface	9.5	298.17
	2	9.83	354.17
	5	7.83	22.83
	10	8.67	12.33
	20	9.67	9.17
sed		1.0214	40.965

sed: Standard Error of the Difference between Means.

of Collembola (Table 4). A similar significant effect was obtained when the 2 t ha−1 surface application rate was omitted. Comparing the number of Collembola obtained from untreated controls with 2 t ha−1 rate mixed with soil showed the latter significantly ($P < .0001$) reduced Collembolan numbers. There was no significant difference in numbers between 2, 5, and 10 t ha−1 rates. However, the 20 t ha−1 rate had significantly ($P = .0002$) fewer Collembola when compared with the 10 t ha−1 rate.

Production of juvenile Collembola was a more sensitive parameter than adult mortality in determining effects of biosolids. Data analysis for combined biosolids at each rate showed there was a significant ($P < .0001$) reduction in juvenile Collembola in response to increase rate of biosolids application. There was also a similar significant difference between juvenile numbers for various biosolids. Each of the four rates of biosolids (combined sources) had significant ($P < .0001$) fewer juvenile Collembola when compared with the untreated controls. Relative to the 2 t ha−1 rate, the remaining rates of 5, 10, and 20 t ha−1 had significant reduced numbers of juveniles. There was no significant difference in juvenile numbers between the 5 t ha−1 and 10 t ha−1 rates. However, there were significantly ($P = .001$) fewer juveniles obtained at 20 t ha−1 when compared with 5 t ha−1 rate. The difference in juvenile numbers between the 10 t ha−1 and 20 t ha−1 rates was not significant. Comparing the effects of each of the six biosolids on the number of juvenile Collembola obtained showed that Biosolid 1 and 6 did not differ significantly but each had significantly ($P <$ .0001 to .008) more juveniles than Biosolids 2, 3, 4, and 5. Comparing the 2 t ha−1 rate for the six biosolids when surface applied and mixed with soil showed there were significantly ($P = .037$) fewer juvenile Collembola where the biosolid was mixed with the soil. Overall, most adult and juvenile worms and Collembola were associated with Biosolid 5 followed by biosolids 1, 4, 2, and 3, respectively.

3.4. Chemical Analysis. The chemical analysis of biosolids is given in Table 5. Comparing the highest and lowest concentrations of elements for each of the five Irish produced biosolids showed greatest differences, 30 to 34-fold, for aluminium, iron, and tin followed by nickel and lead having 8-fold and 5-fold differences, respectively, while remaining elements differed between 1.5− and 4.5−fold. Biosolid 5 had highest concentrations of 12 of the 23 elements measured, Biosolids 2 and 3 had highest concentrations of 7 and 5 elements, respectively, while Biosolids 1 and 4 had highest concentrations of 3 and 2 elements, respectively. Relative to Irish biosolids, biosolid 6 (Colorado, USA) had higher levels of selenium, cadmium, arsenic, barium, copper, and magnesium. Of these, selenium was 77-fold greater, cadmium 16-fold, and the remaining elements between 2- and 5-fold greater than Irish biosolid while beryllium was particularly low in Biosolid 6.

Arsenic and barium were found to have a significant negative relationship ($P = .007$ and $P = .004$) with juvenile worm numbers. Organic matter content of biosolids was found to have a significant positive relationship with juvenile worm numbers and the effect was linear with rate. In the case of juvenile Collembola, cadmium and sulphur had a

Table 5: Chemical analysis of Biosolids.

Chemical element	Biosolid 1	Biosolid 2	Biosolid 3	Biosolid 4	Biosolid 5	Biosolid 6
mg kg^{-1}						
Aluminium	9963	164038	25963	5514	31202	—
Antimony	3	2	3	3	4	—
Arsenic	2	3	3	3	4	10.5
Barium	114	99	228	222	234	332
Beryllium	<0.5	0.2	<0.5	<0.5	<0.5	<0.001
Cadmium	<0.5	0.8	1	0.6	0.8	16.3
Chromium	11.1	17	18	37	25	8.7
Cobalt	2	3	4	3	3	—
Copper	221	308	236	260	202	530.7
Iron*	2.12	7	7	5	72	—
Lead	38	157	79	57	31	54.3
Manganese	139	116	194	188	224	250
Nickel	9	15	20	23	71	31.1
Selenium	2	0.96	2	1	2	154.7
Silver	3	2	0.94	2	0.8	—
Tin	10	9	12	8	238	—
Zinc	301	547	681	355	403	83.4
Sulphur	5800	9900	7900	6300	10300	—
Nitrogen	36600	45400	39100	38400	30766	—
Phosphorous	11500	12400	15600	12800	28300	—
Potassium	1430	6430	4900	3060	2430	—
Magnesium	2530	5930	6500	3700	4060	—
Sodium	2360	5330	3410	1660	1500	—
Organic matter %	86.3	69.3	70.6	78.1	55.4	—

*Values for Iron are mg g^{-1}.

significant negative relationship, while silver was found to have a positive relationship. Data analysis for combined biosolids showed that selenium did not impact on juvenile Collembola despite the high concentration of this element in Biosolid 6. As with juvenile worms, organic matter content had a significant positive relationship with juvenile Collembolan numbers.

The phosphorous concentration of the five Irish biosolids determined the maximum amount at which biosolids could be applied to agricultural land [4]. In accordance with this legislation the respective maximum rates at which biosolids 1, 2, 3, 4, and 5 could be applied to an Index 3 soil was in the range 0.9 to 2.2 t ha−1 for cereals and 0.7 to 1.7 t ha−1 for grassland (Table 6). The phosphorous threshold was more critical than the heavy metal threshold [3] for which zinc was the limiting factor. The (theoretical) permitted rates for biosolids 1 to 5, respectively, using zinc as the determining parameter would be 25, 14, 11, 21, and 19 t ha−1.

4. Discussion

4.1. Method Development. Ecotoxicological methods using soil animals were originally developed for pesticide testing [19]. Recently, these tests have been applied to the evaluation of certain solid waste materials, in particular organic materials [9, 10, 20, 21]. The study reported here suggests that standardized tests are useful for testing biosolids at low application rates, but there are potential pitfalls at high rates. The high mortality of animals observed at high biosolids rates needs to be interpreted carefully since it may be related, at least in part, to the artificial conditions of the experiment and not toxicity *per se* of materials tested. These particular conditions include release of ammonia and decrease in pH [22], lack of oxygen [23], and proliferation of mould (authors' unpublished observations). Previous studies confirmed the high toxicity for earthworms at high levels of organic fertilisation (slurry and thermally dried sewage sludges), in laboratory trials [24–26] and in field work [27, 28]. Future work on the development of these methods for testing organic waste materials should monitor these conditions to establish if they can have deleterious effects on the test animals. This is also important since application rates and location in the soil can be comparable to field conditions, that is, detrimental mechanisms discovered under laboratory conditions may also exist in field soils after biosolids applications.

The use of suitable food and the correct position in the soil profile minimises the mortality of animals in laboratory cultures [29]. In our earthworm experiment, access to cow dung improved adult survival and reproduction, confirming its suitability for ecotoxicological studies [30].

Table 6: The maximum rates, t ha^{-1}, at which Biosolids could be applied to grassland and cereal crops grown in soils of varying soil indices.

	Biosolid 1	Biosolid 2	Biosolid 3	Biosolid 4	Biosolid 5
Nitrogen Winter wheat					
Index 1 (190 kg ha^{-1})	5.2	4.2	4.9	4.9	6.2
Index 2 (140 kg ha^{-1})	3.8	3.1	3.6	3.6	4.6
Index 3 (100 kg ha^{-1})	2.7	2.2	2.6	2.6	3.3
Index 4 (60 kg ha^{-1})	1.6	1.3	1.5	1.6	2.0
Nitrogen Spring barley					
Index 1 (135 kg ha^{-1})	3.7	3.0	3.5	3.5	4.4
Index 2 (100 kg ha^{-1})	2.7	2.2	2.6	2.6	3.3
Index 3 (75 kg ha^{-1})	2.0	1.7	1.9	2.0	2.4
Index 4 (40 kg ha^{-1})	1.1	0.9	1.0	1.0	1.3
Nitrogen Grassland normal and high stocking rates					
Normal (170 kg ha^{-1})	4.6	3.7	4.3	4.4	5.5
Phosphorous Winter wheat and Spring barley					
Index 1 (45 kg ha^{-1})	3.9	3.6	2.9	3.5	1.6
Index 2 (35 kg ha^{-1})	3.0	2.8	2.2	2.7	1.2
Index 3 (25 kg ha^{-1})	2.2	2.0	1.6	2.0	0.9
Index 4 (0 kg ha^{-1})	0.0	0.0	0.0	0.0	0.0
Phosphorous Grassland normal stocking rate					
Index 1 (39 kg ha^{-1})	3.4	3.1	2.5	3.0	1.4
Index 2 (29 kg ha^{-1})	2.5	2.3	1.9	2.3	1.0
Index 3 (19 kg ha^{-1})	1.7	1.5	1.2	1.5	0.7
Index 4 (0 kg ha^{-1})	0.0	0.0	0.0	0.0	0.0

It is recommended to maintain the provision of this food source because otherwise the tests would induce starvation. In the case of Collembola, adult *F. candida* produced more offspring when biosolids were applied at the soil surface compared with treatments where it was mixed into the soil. This finding suggests that surface-applied biosolids may represent a more easily located and concentrated source of food for Collembola. This concurs with the beneficial effect of organic wastes on *F. candida* as observed by [31].

It is generally recognised that reproduction is more sensitive to toxicants than adult mortality [31, 32]. This study confirmed that the most useful measure is the offspring number for both earthworms and springtails. However, adult mortality is also a useful measure of the sensitivity of springtails to biosolid applications, reflecting the higher sensitivity of springtails in comparison to earthworms. This has already been reported for these species by Natal-da-luz et al. [9].

4.2. Toxicity of Biosolids. Pollutant content and physicochemical characteristics of the biosolids-artificial soil mixture influence the results of toxicity tests of a complex material such as biosolids [33]. In these materials, inorganic elements such as Zn, Cr, Cd, and Pb are considered pollutants because at certain rates they can affect the mortality, growth, or reproduction of the animals tested. The pH, moisture and organic matter content are important physicochemical characteristics of this mixture because they can modify pollutant effects and animal behaviours [22]. Results of the worm reproduction test revealed that Biosolid 2, 3, and 4 had negative effects at increasing rate of application on mortality (equivalent to 10 and 20 t ha−1 rates) as well as reproduction (from rate 2 or 5 t ha−1), whereas Biosolid 1 and 5 produced a negative response only on reproduction, Biosolid 1 from 10 t ha−1 and Biosolid 5 from 5 t ha−1. The three more toxic biosolids did have greater concentrations of Pb than Biosolid 1 and 5, however, the Pb concentration was probably not high enough to induce toxic effects on mortality and cocoon production. This interpretation is supported by Garg et al. [34] who determined the effects of Pb on the survival of *E. fetida* in a standard artificial soil. After 45 days a mortality of 57–68% was observed at 500–2500 mg Pb kg^{-1} dry weight (DW). Spurgeon et al. [35] found a reduction in cocoon production at concentrations of 2000 mg Pb kg^{-1}. These values are much higher than the values measured for Biosolids 2, 3, and 4 (max 157 mg Pb kg^{-1} biosolid 2). The Zn contents of Biosolids 2 and 3 were substantially greater compared to those of the other biosolids. However, Zn could not be the reason for the greater toxicities of Biosolids 2 and 3 since the concentration of this element in Biosolid 5 was greater than that for Biosolid 4, with the latter recorded as having a greater negative impact on juveniles worms.

The application of biosolids at 2 t ha−1 did not show any negative effects on adult worm mortality and reproduction. However, comparing Biosolid 1, 2, and 3 on the basis of juvenile worms showed Biosolid 3 to be significantly more detrimental than Biosolids 1 or 2. Moreira et al. [8] and Natal-da-luz et al. [9] found no effects on either mortality or growth of *Eisenia* spp. at 6 t ha−1 of sewage sludge, suggesting the absence of toxicity a lower rates of these materials.

The increased deleterious effects with increasing rates of all Irish biosolids observed for Collembola confirm the trends recorded for earthworms. However, Collembola seem to be more sensitive than worms because they were negatively affected by all Irish sources with increasing rates for both mortality and reproduction. Also in the present tests, Biosolids 2 and 3, 4 were more deleterious than 1 and 5 since they were toxic at lower rates of applications. In the reproduction study, Biosolids 2 and 3 were more toxic that remaining Biosolids. As outlined earlier, Pb did have greater concentrations in Biosolids 2 and 3. According to the literature, the observed toxicity cannot be attributed to the level of Pb. In a study of Greenslade and Vaughan [36] using *F. candida*, the EC_{50} for the effects of Pb on reproduction in OECD artificial soil was about 20 times higher (2560 mg lead kg^{-1} DW) than the Pb content of the most concentrated soil-biosolid 2 mixture (157 mg lead kg^{-1} biosolid). Fountain and Hopkin [15] calculated an EC_{50} of 580–3160 mg Pb kg^{-1} DW for the effect of Pb on reproduction.

The fact that survival and reproduction decreased with increasing biosolid concentration is supported by Domene et al. [37] who evaluated the effects of four different kinds of wastes (dewatered, composted or thermally dried sludges and dried pig slurry) on survival and reproduction of *F. candida*. In the latter study, reproduction was not inhibited by aerobic thermally dried sludges at EC_{50} 5.3 mg kg^{-1} DW, nor was there inhibition for anaerobically digested sludges at Pb concentrations of 10.4 and 19 mg kg^{-1} DW. Crouau et al. [22] observed a significant reduction in juvenile numbers only at high sewage sludge concentrations (50% of sludge in test soil), however no effect was detected on mortality. Results for Biosolid 6 showed a broadly similar trend as that for Irish biosolids for *F. candida* reproduction but no mortality was observed at any rate of application. These results were somewhat surprising as it was anticipated that the high concentration in Se (154.7 mg kg^{-1} biosolid) might have impacted on Collembolan mortality. However, the statistical analysis of data on juvenile Collembolan mortality and selenium content of biosolids did not show any relationship.

Spurgeon et al. [35] and Spurgeon and Hopkin [38] found respective Zn EC_{50} of 276 and 462 mg kg^{-1} DW for cocoon production by *E. fetida*. However, no negative effects on growth rate were recorded at 400 mg Zn kg^{-1} DW by Spurgeon et al. [35]. Van Gestel et al. [39], studying *E. andrei* in artificial soil with a pH of 6.2, reported an EC_{50} for effects on cocoon production of 512 mg Zn kg^{-1} DW. In the case of springtails, Smit and Van Gestel [40] found an EC_{50} for the effect on *F. candida* reproduction of 261 mg Zn kg^{-1} DW. Based on the latter investigations, a deleterious effect would have been expected in the present study at the highest concentrations of the soil mixtures, where the concentration of Zn was of the same order of magnitude as the reported EC_{50} value. Again, in the present study no statistical relationship was established between Collembolan mortality and Zn content.

Metals in our study occurred as compounds formed in organic materials, whereas many studies added them in salt form which is much more soluble. For instance, Fischer and Molnár [41], from a study with *E. fetida* in peat and horse manure, reported significant effects of aluminium chloride on cocoon production at a concentration of 4850 mg Al kg^{-1} DW. Van Gestel and Hoogerwerf [42] determined the effects of three different Al salts on *E. andrei* survival in artificial soil at different pH levels. In artificial soils with pH of between 6.7 and 7.2, aluminium chloride appeared to be most toxic with LC_{50} of 1000 mg Al kg^{-1} DW, whereas aluminium oxide did not affect earthworm survival at concentrations of 5000 mg Al kg^{-1} DW. The same authors exposed earthworms to soils treated with aluminium sulphate for 6 weeks and found that at pH 7.3, aluminium affected cocoon production at 320 and 1000 mg Al kg^{-1} DW. These values are much lower than the values of aluminium of the five biosolids (between 5514 and 164038 mg Al kg^{-1} biosolids), however, the current study does not establish that Al was one of the factors that caused high toxicity at high rates of biosolid application.

Interpretation of the effects of As in this study presented similar problems. Fisher and Koszorus [43] tested the effect of 68 mg kg^{-1} DW of As (as potassium arsenate) on growth and reproduction of *E. fetida*. The number of cocoons produced per worm showed the highest sensitivity to As with a 56% reduction at the test concentration. This value is much higher than the values of As in the 5 biosolids tested in our study, nevertheless, the As content of biosolids was found to have a significant negative relationship with juvenile worm numbers but not with numbers of juvenile Collembola. Greenslade and Vaughan [36], using *F. candida* to evaluate the toxicity of As(III) and As(V) in artificial soil found EC_{50} values of 3 mg As(III) kg^{-1} DW for reproduction and EC_{50} of 119 mg As (V) kg^{-1} DW for survival. Crouau and Moïa [44] found a significant effect of As (sodium arsenate) on *F. candida* reproduction even at the lowest concentration tested, 2.2 mg As kg^{-1} DW.

4.3. Implications for Land Spreading. In compliance with Irish and European legislation [3, 4] the P content of biosolids was found to be the critical component in determining the maximum amount at which biosolids can be applied to land. In the assumed case of an Index 3 soil, the maximum allowable rate was 2.2 t ha−1 for cereals and 1.7 t ha−1 for grassland (Table 6). Applying each of the five biosolids at 2 and 5 t ha−1 did not negatively impact on numbers of adult worms in laboratory tests. However, of the three biosolids investigated at the 2 t ha−1 rate, biosolid 3 did have a negative effect on the numbers of juvenile worms while biosolid 1 and 2 had no effect. In the case of *F. candida* the combined data for the five biosolids at 2 t ha−1 showed a significant negative effect on adults, but data analysis for individual biosolids showed that at the 2 t ha−1 rate there was no significant reduction in adult numbers when compared with untreated controls. However, the 2 t ha−1 rate had significantly fewer juvenile Collembola than controls. It is concluded from these laboratory-based assays that biosolids, applied at realistically low rates (about 2 t ha−1) are unlikely to be detrimental in the short term to earthworms or adult Collembola, but they may be detrimental to juvenile Collembola. Further, these results cannot be used to predict the possible long-term effects of continuous land spreading of biosolids.

Funding

Funded by a TEAGASC Walsh Fellowship.

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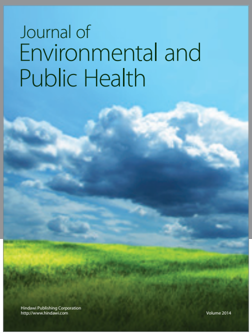
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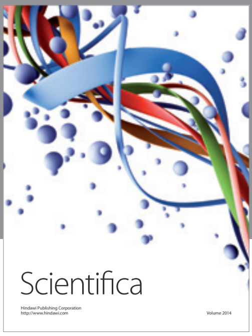
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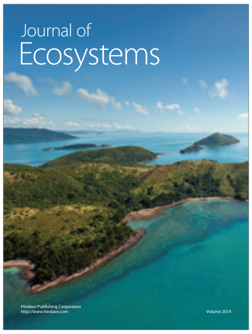
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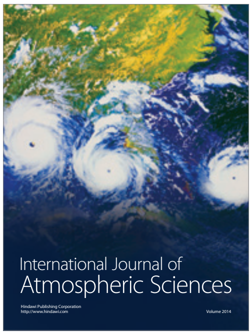
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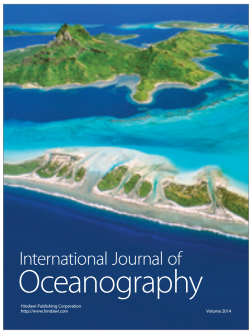
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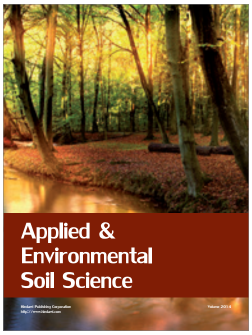
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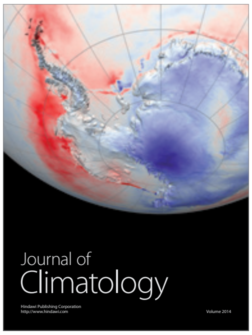
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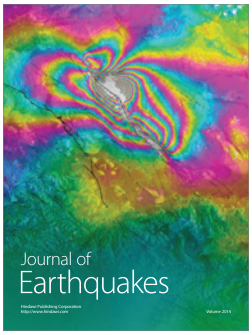
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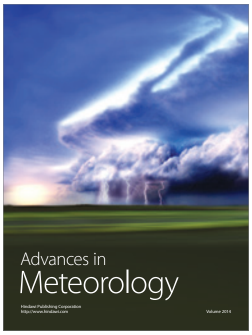
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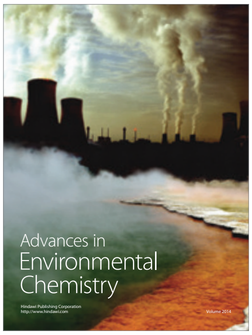
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