TITLE: Ecotoxicological assessment of organic wastes using the soil collembolan *Folsomia candida*

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ABSTRACT

The reproduction test with the collembolan *Folsomia candida* is used as a tool to evaluate the ecotoxicological potential of organic wastes currently applied to soil. Seven organic wastes (dewatered sewage sludges, thermally-dried sewage sludges, composted sewage sludges, and a thermally-dried pig slurry) were tested. These wastes had different origins, treatments, and pollutant burdens, and were selected as a representative sample of the wide variety of wastes currently generated. *F. candida* showed varied sensitivity depending on the waste, but also depending on the endpoint assessed. Reproduction was more sensitive than survival, although no correlations between reproduction and physico-chemical parameters and pollutant burden could be found. On the other hand, mortality was directly related to the lack of stability of wastes, probably reflecting the toxicity of the decomposition of secondary end-products such as ammonium. Body length was not shown to be a sensitive endpoint for waste testing, as it was neither affected nor even stimulated by waste concentrations.

Organic matter, pH, and electrical conductivity varied with waste concentration in soil-waste mixtures, although their effect on collembolan performance was expected to be low and part of the complex effect exerted by wastes when applied to real soils. Selection of the water content is the most problematic aspect in waste testing, as it may affect the performance of test organisms. In this study a qualitative approach for water content selection in waste testing was considered to be the most suitable.

Treatment of wastes affected composition and toxicity. Composting of sewage sludge increased its stability, compared to the initial sludge, but decreased its non-persistent organic pollutant burden and toxicity. On the other hand, thermally-dried

wastes from sludge and pig slurry displayed high toxicity, mainly attributable to their low stability. The results from the study indicate the inability of chemical methods to predict the effects of complex mixtures on living organisms with respect to ecotoxicity bioassays, but also the need for stabilization treatments of organic wastes prior to their reuse in soils.

KEYWORDS: *Folsomia candida*, survival, reproduction, body length, organic wastes, stability

1. INTRODUCTION

The amount of sewage sludge produced in the European Union has increased dramatically in recent years due to the implementation of Directive 91/271/EC. This increase will mainly be managed through its reuse in agricultural soil, despite our poor understanding of the impact of this management option. There is a large amount of experimental evidence which suggests that this practice may enhance soil fertility, but there are also well-known associated environmental risks, including pathogens, nitrate pollution of ground waters, and inputs of heavy metals and organic pollutants (Düring and Gath, 2002).

To date, experimental results of sludge application to agricultural soils indicate a low level of risk for crops, but little is known about its effects on soil biota, a critical element in soil functioning (Giller et al., 1997). Harmful effects on soil invertebrates have been found in laboratory experiments (Krogh et al., 1997; Andrés and Domene, 2005), but some field experiments have shown that soil biota are stimulated when sludge is added to soil at agronomic rates (Krogh and Pedersen, 1997; Petersen et al., 2003).

Measuring pollutant concentration by chemical methods is the most common way to estimate the toxicity of pollutants and wastes, despite the development of biological methods in recent decades and their advantages over chemical methods. For example, the European Union regulation restricts the reuse of sewage sludge in soil taking into account limit values for six heavy metals (Directive 86/278/EC), but no biological tests are mentioned, even in the third draft of the Working Document on Sludge (European Communities, 2000). Furthermore, methods to assess the direct toxicity of solid wastes are not available despite the existence of standardized protocols for single chemicals using terrestrial organisms.

Crouau et al. (2002) concluded that the standardized Collembola reproduction test ISO 11267 (1999) was suitable for this purpose. They also pointed out that reproduction in this species may be affected not only by pollutant content but also by physico-chemical characteristics of waste such as pH, moisture and organic matter content. As a result, bioassays applied to organic wastes were not easy to interpret as two contradictory effects occurred at the same time. On the one hand, the organic matter in residues may have a stimulatory effect on soil organisms, while on the other hand the pollutant burden may exert inhibitory effects (Krogh et al., 1997; Andrés and Domene, 2005). Furthermore, parameters such as water availability or pH may also contribute to the biological effects observed.

The main aim of this study was to assess the suitability of the *F. candida* reproduction test as a tool for the ecotoxicological assessment of organic wastes which are to be applied to soils. Special attention was devoted to the special characteristics of waste testing, which involves variation in the physico-chemical properties of the soil-waste mixtures as the waste concentration increases. In addition, the influence of the origin, treatment, and composition of organic wastes on the ecotoxicological response of *F. candida* were be studied.

2. METHODS

2.1. Test species

The strain of *F. candida* used in our experiments was provided by the Institute of Ecological Science of the Free University of Amsterdam. Cultures were raised in polyethylene containers 17.5 x 12.5 x 7.5 cm. The substrate consisted of a 1 cm layer of a wet mixture of plaster of Paris and charcoal (9:1 v/v). Cultures were raised in darkness in a climatic chamber at a constant temperature of 21±1ºC. The substrate was renewed and the density of individuals was reduced every two months to avoid overcrowding.

2.2. Organic wastes

In order to represent a variety of organic wastes currently applied to agricultural soils, we selected seven types of waste: two dewatered sewage sludges, two composted sewage sludges, two thermally-dried sewage sludges, and a thermally-dried pig slurry. Treatments and post-treatments of the wastes differed as summarized in Table 1.

Physico-chemical properties, heavy metal and organic pollutant contents of the wastes are recorded in Table 2. Dry matter, water holding capacity, water pH, electrical conductivity, total nitrogen, and organic matter were measured according to EN 12880 (2000), ISO 11267 (1999), EN 13037 (1999), EN 13038 (1999), EN 13342 (2000) and EN 12879 (2000), respectively.

Non-hydrolyzable (stable) organic matter and non-hydrolyzable nitrogen were measured as a percentage of organic matter and nitrogen remaining in the sample residue after acid hydrolysis, as described in Rovira and Vallejo (2002). This method removes the more labile fraction of an organic substrate, mainly consisting of polysaccharides and proteins. Hydrolyzable nitrogen was calculated by subtracting the content of non-hydrolyzable nitrogen from total nitrogen content. N-NH_4 was measured on the distillates obtained from fresh samples.

Elemental analysis of P, K, Cd, Cr, Cu, Hg, Ni, Pb and Zn was carried out by ICP-MS according to ISO 11885 (1996). Polychlorinated dibenzodioxins and dibenzofuranes (PCDD/F) were measured with HRGC-HRMS, polychlorinated biphenyls (PCB) by HRGC-ECD, di (2-ethylhexyl) phthalate (DEHP) and nonylphenols (NPE) by HRGC-MS. Polycyclic aromatic hydrocarbons (PAH) and linear alkylbenzene sulphonates (LAS) were determined by HPLC with fluorescence and UV detectors, respectively. Values for each pollutant group were expressed as indicated in the third draft of the Working Document on Sludge (European Communities, 2000). Hence, DEHP, LAS, PCDD/F values represent total values. NPE include nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups. PAH are the sum of acenapthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, and indeno(1, 2, 3-c, d)pyrene. PCB is the sum of the polychlorinated biphenyl congeners number 28, 52, 101, 118, 138, 153 and 180.

It should be noted that each type of waste came from a different batch, and hence, besides differences resulting from contrasting treatments and post-treatments, values for individual pollutants may also be different in wastes from the same plant, given temporal changes in wastewater composition. Despite that, some of the physico-chemical characteristics of wastes changed too dramatically with post-treatments for this to be attributed exclusively to batch differences.

The current final product of wastewater treatment is dewatered sludge, obtained from aerobic or anaerobic digestion followed by centrifugation. Sludge stabilization and dewatering is compulsory prior to its application to the soil, as this process reduces pathogen content and volume. Some wastewater plants perform additional sludge post-treatments, the most common of which are composting and thermal drying. Sludge composts of this work were produced by mixing dewatered sludge with pine wood chips (1:4.5, v/v). For the original anaerobic sludge, composting was carried out in a tunnel with air injection for fifteen days at the wastewater plant itself. For the aerobic sludge, composting was performed in a heap. Components of the heap were well mixed every two days by tumbling the first four weeks, and then every week until the end of the composting period (50 days). At the end of this period, both composts were sieved to 1 cm. Composting decreased total, hydrolyzable and ammonium nitrogen content, and increased organic matter stability compared to dewatered sludge. Composting also resulted in a reduced concentration of non-persistent organic pollutants (DEHP, NPE and LAS)(Table 2).

Thermal drying was carried out by placing dewatered sludge in a heated rotary cylinder and injecting hot air, which provided a temperature of around 130-150ºC for 45 minutes. This treatment reduced the $N\text{-}NH_4$ content of dewatered sludge, but increased its electrical conductivity, and did not decrease pollutant levels with respect to dewatered sludge, with the exception of DEHP.

Pig slurry was obtained from an anaerobic digestion of raw slurry followed by thermal drying at 130ºC, a treatment that provided a waste characterized by high electrical conductivity, high hydrolyzable nitrogen and $N\text{-}NH_4$ content, and low organic matter stability (easily mineralizable).

For the analysis of the wastes, and for the preparation of soil-waste mixtures for the bioassays, each waste was dried at 60ºC for 48-72 hours depending on its initial water content, and then ground. These steps were unavoidable in order to ensure the homogeneity and accuracy of the lower test concentrations.

2.3. Test preparation

The experiment was performed as indicated in the standard test ISO 11267 (1999), although several modifications were performed to the protocol in order to adapt it to the experimental aims and waste properties. These changes were based on an unpublished preliminary work that showed that effects on different individual parameters (survival, reproduction, and body length) may occur at quite different concentrations. First, the range-finding assay was not performed and testing was reduced to a single assay. This allowed simultaneous observations of the endpoints studied in each waste. Twelve test concentrations were used: 0, 1, 2, 4, 7.9, 15.8, 31.6, 63.1, 125.9, 251.2, 501.2 and 1000 g kg^{-1} (w/w) of waste in a mixture with OECD artificial soil. Second, given that water holding capacity (WHC) of wastes was higher than the artificial soil, water content should be increased with increasing concentrations in soil-waste mixtures in order not to affect the performance of test organisms. A possible approach to this problem might be to provide the same % of the WHC in all the test concentrations, but that might require, before any waste bioassay, a prior assessment of WHC for every test concentration. In this study we took an alternative approach in order to provide a more workable method, which makes any previous work unnecessary. Hence, the suitable moisture for each concentration was determined by the addition of small amounts of water in order to provide the optimum moisture for soil-waste mixtures. As indicated in ISO 17512 (2005), such optimum water content is defined as that when no standing water or free water appears when the soil is compressed. In controls, that point was achieved with humidity around 50-60% (w/dw), and around 55-75% in the highest waste concentration. Using these criteria, all test concentrations had a similarly wet appearance and a crumbly structure.

Each replicate consisted of a 125 ml polyethylene container filled with 30 g of wet test substrate, with a lid that allowed sealing. For each concentration 5 replicates were prepared, as well as an additional replicate to determine changes in pH, electrical conductivity, water content, and water availability (measured as soil water potential) at the end of the test period. The pH and electrical conductivity were measured in a 1:5 soil-water extract obtained according to ISO 10390 (2005) using a Crison MicropH 2000 pHmeter, and a Crison Conductimeter 522 (Crison, Barcelona, Spain), respectively. The soil water potential was measured with a WP4 dew point potentiometer (Decagon Devices, Pullman, USA).

The physico-chemical characteristics of the soil-waste mixtures used in the bioassays are summarized in Fig. 1. As waste concentration increased, the pH values decreased by 1.5 units at most from controls to the highest concentration, while the electrical conductivity markedly increased from intermediate concentrations. The moisture content was similar at most of the concentrations, but was slightly higher at the higher waste concentrations, as more water was added in order to provide the optimum moisture content. Water potential values also remained nearly constant at lower waste concentrations, but decreased markedly at intermediate concentrations. This was mainly explained by the high solute concentration provided by wastes, as a significant correlation between log transformed values of electrical conductivity and water potential was detected (Pearson, $r = 0.814$, $P<0.001$).

2.4. Test performance

Ten 10-12 day-old individuals of *F. candida* were placed in each container together with 3 mg of granulated dry yeast. Yeast was added again on the 14th day. Containers were kept in the dark at 21±1ºC for 28 days, and were opened twice a week. During this period, individuals reached sexual maturity and produced offspring.

At the end of this period, the containers were flooded with water to float the adults and juveniles. A dark dye was added to facilitate counting and a photograph was taken. Adults and juveniles were counted using the image treatment software ImageTool 3.0, and they were distinguished by their clearly different sizes. The mean body length of adults per replicate was measured from the anterior end of the head, between the antennae, to the posterior end of the abdomen, as described by Folker-Hansen et al. (1996).

Relative survival (100*number of adults in replicates/ mean number of adults in controls), relative reproduction (100*number of juveniles in replicates/ mean number of juveniles in controls), and relative body length (100*mean body length of adults in replicates/ adults mean body length in controls) were calculated.

2.5. Data treatment

LC50, LC20, EC50, and EC20 were calculated for each type of waste using Statistica 6.0. These values and their 95% confidence intervals were calculated from suitable regression models (exponential, Gompertz, hormesis, linear or logistic). The choice of the model was based on the best fit to the data, based on Stephenson et al. (2000).

In order to find out which individual pollutant, pollutant group, or physico-chemical parameter might be responsible for the observed effects in the whole set of wastes, we calculated Pearson correlation coefficients for the toxicity values (LCx, ECx) with respect to the individual pollutant concentrations in wastes, the sum of heavy metal concentrations, the sum of organic pollutant concentrations, the sum of persistent organics (PAH, PCB, and PCDD/F), the sum of non-persistent organics

(DEHP, LAS, and NPE), the sum of all pollutant concentrations, and individual values for physico-chemical parameters. Pearson correlation coefficients were calculated using SPSS 11.0.

Additionally, we estimated the individual pollutant concentrations at the LC50 and EC50 and we compared them with LC/EC50 values collected from the literature, in order to check if any pollutant was on a range above that expected to exert harmful effects on collembolans.

3. RESULTS

3.1. Toxicity test results

Test results complied with the validity requirements of ISO 11267 (1999), as in controls the number of surviving individuals was over 8 (8.6±0.2), and more than 100 juveniles (348±197) were present. Mean body length of individuals in controls at the end of the assays was 1.59±0.04 mm.

The results of the toxicity tests are shown in Fig. 2. Survival and reproduction decreased with increasing waste concentrations, survival being the least sensitive. It is also noteworthy that reproduction usually showed a higher variability between replicates than survival. On the other hand, body growth was either not affected by waste concentration or was even slightly stimulated. For most of the studied wastes, body growth increased in parallel with the decrease in reproduction.

LCx and ECx values are shown in Table 3. No values were calculated for body length, since it was not affected. Survival was strongly inhibited by pig slurry (LC50 = 24 g kg^{-1}), but to a lesser extent in both composted sludges (LC50 = 252 and 834 g kg^{-1}) indicating their lower toxicity. Reproduction was hardly inhibited by aerobic thermally-dried sludge (EC50 = 5.3 g kg^{-1}), followed by aerobic and anaerobic dewatered sludge (EC50 = 10.0 and 10.4 g kg^{-1}). The lowest inhibition in reproduction was shown by anaerobic composted sludge (207 g kg^{-1}).

3.2. Waste composition and toxic effects

The comparison of LC50 and EC50 for individual pollutants obtained from the literature with their concentrations in test containers at LC50 and EC50 showed that none of those pollutants were present in concentrations which might be expected to affect survival (Table 4). However, some pollutants might affect reproduction (Table 5). More precisely, nonylphenol ethoxylates (including 4-nonylphenol) in some wastes (AEC, AND, ANT and AND) showed values above 2.9 mg kg^{-1}, which was reported to be EC50 for 4-nonylphenol on *F. candida* (Greenslade and Vaughan, 2003). Also Zn was present in a range that could affect reproduction in AEC according to Fountain and Hopkin (2005). Finally, LAS concentrations were close to those expected to affect reproduction in AEC, ANT, and AND, according to Jensen et al. (2001a).

Pearson coefficients of toxicity values (LCx, ECx) with concentration of individual pollutants and the sum of concentrations of pollutant groups showed a general lack of association. On the other hand, toxicity values showed significant correlation with physico-chemical properties, such as the positive correlation between survival and stable organic matter (LC50 r = 0.953, LC20 r = 0.947), and negative correlations were found for survival with total nitrogen (LC50 r = -0.971, LC20 r = -0.968), hydrolyzable nitrogen (LC50 r = -0.966, LC20 r = -0.963), and N-NH_4 content (LC50 r = -0.794, LC20 r = -0.801). In contrast, no significant correlations were found between reproduction values and waste physico-chemical properties.

4. DISCUSSION

4.1. Effects of treatment on waste properties

The changes in waste properties resulting from composting observed in this study coincide with similar published papers. Composted sludge shows a high degree of organic matter stability, since during this post-treatment there is a loss of the more labile fractions through microbial degradation (Grube et al., 2006). In this aerobic process, part of the less persistent organic pollutants (DEHP, LAS, and NPE) are also degraded. This fact has already been reported for DEHP (Bagó et al. 2005), LAS (Sanz et al., in press), and NPE (Déportes et al., 1995). Despite this, NPE levels in the dewatered sludges studied were so high that composting was not able to reduce their concentration in composted sludges below the limit value of 50 mg kg-1 laid down in the draft of the Working Document on Sludge (European Communities, 2000). This agrees with the opinion that NPE is the most harmful group for the environment of all the non-persistent organic pollutants. On the other hand, heavy metals and more persistent organic pollutants maintained or increased their concentrations with composting, as has already been pointed out by Déportes et al. (1995).

As far as we know, no comparative studies on the effect of thermal drying on the pollutant burden of organic wastes have been published. We did not observe great differences in pollutant contents with respect to dewatered sludge, even for PCDD/F, which has been observed to increase during the thermal dewatering process (Blazer and Pluschke, 1994). Furthermore, it is worth pointing out the lower DEHP level in thermally-dried sludge, already mentioned by Bagó et al. (2005) and attributed to steam distillation during the drying process.

Treatments applied to pig slurry produce a waste product with high values of hydrolyzable nitrogen, ammonia, K and Zn, an extremely high electrical conductivity, and a low proportion of stable organic matter.

4.2. Influence of physico-chemical variation in soil-waste mixtures

Crouau et al. (2002) have already pointed that the actual toxicity of wastes is not easy to assess since pH, organic matter and water content may also affect the test organisms as well as the pollutant burden. The effects of pH and organic matter are waste-characteristic but, on the other hand, the water content provided to soil-waste mixtures may exert some influence on the observed results by its direct effects on organisms and indirect effects on pollutant bioavailability.

4.2.1. Organic matter, pH, and electrical conductivity.

Increased waste concentrations exerted contradictory effects on *F. candida*. On the one hand, waste inhibited survival and reproduction at higher concentrations, but stimulated reproduction at lower concentrations. Such behavior shows the contradictory effects of polluted organic wastes, the organic matrix of which acts simultaneously as a nutritional resource and as a source of toxicity (Krogh et al., 1997; Andrés and Domene, 2005). The presumed nourishing effect of organic matter from wastes has been confirmed in an unpublished study that showed that *F. candida* ingested sludge from the test substrate, as has already been shown by Krogh and Pedersen (1997) and by Scott-Fordsmand and Krogh (2004) for *F. fimetaria*. Nevertheless, sludge consumption rates were lower when yeast was available as an additional food source. Since yeast was quickly consumed after its addition to the test replicates, it is likely that individuals use organic wastes as an alternative food resource. This observation indicates that for this species, when organic wastes are tested, exposure could be mediated both by cuticle contact and consumption, as suggested by Krogh and Pedersen (1997).

The observed decrease in pH with waste concentration was unlikely to influence the results, as has already been pointed out by Crouau et al. (2002). The variation in pH between controls and concentrations with effects on survival or reproduction was always less than 1 pH unit (Fig. 1), too low a variation to affect survival, and reproduction according to Crommentuijn et al. (1997), and Crouau et al. (1999). However, pH variation may influence pollutant bioavailability, although its variation in this study was within a range not expected to affect its uptake, according to Sandifer and Hopkin (1996) and Crommentuijn et al. (1997), but also given the nature of most of the pollutants contained in wastes, sorbed to waste particles.

Electrical conductivity also showed very large increases as waste concentration increased, although this in itself did not explain the observed effects on collembolans, as there was a lack of association between conductivity and toxicity.

In summary, organic matter content, electrical conductivity, and to a lesser extent pH, may in themselves affect survival and reproduction of *F. candida* as will slight differences in pollutant bioavailability. Nevertheless, these influences should be considered as part of the complex effects of wastes on soil biota when applied to real soils rather than as a disturbing factor for the interpretation of ecotoxicological results.

4.2.2. Water content and water availability

Water content is the most problematic parameter in waste testing given that the water holding properties of wastes are usually higher than that of soil. This makes it difficult to select a suitable water content without a previous case-by-case knowledge of the maximum water retention properties of soil-waste mixture concentrations, which would make any waste bioassay largely unworkable for current use. In this study, an alternative approach was used, as water content was qualitatively provided to soil-waste mixtures in order to provide a similarly wet and crumbly structure to all test mixtures. This approach is similar to that suggested to provide an optimum humidity of test substrate in a recent standardized protocol for earthworms (ISO 17512-1, 2005). To verify the suitability of such an approach we measured the soil water potential of test mixtures, which is the most realistic and most relevant measure of water availability for collembolans (Holmstrup et al., 2001). According to several authors (Holmstrup, 1997) *F. candida*'s survival is not significantly affected at relative air humidities over 98.5%, which is equivalent to a soil water potential of -2.04 MPa, below the permanent wilting point for plants (-1.5 MPa). In our test concentrations, such values were only attained for most wastes at concentrations over 750 g Kg^{-1}, much higher than the concentrations affecting survival, and especially reproduction (Table 3), which in turn is mainly due to the high solute content provided by the wastes rather than water deficiency. Toxic effects generally appeared at a range of concentrations with water potentials below -1 MPa, very close to those of the controls, and not expected in themselves to affect the performance of individuals. These findings support the idea that the soil environment is highly buffered with respect to desiccation, since air in soil pores is always near to saturation whenever soil has a moist appearance (Hillel, 1971). On the other hand, water availability differences may influence pollutant bioavailability. We lack a direct measure of pollutant bioavailability and hence any remarks about this would be premature. Nevertheless, we considered this possibility to be very limited given the little variation in water availability in the range of concentrations with effects and the already detected low influence of water content variation in pollutant toxicity in this species (Van Gestel and Van Diepen, 1997).

4.3. Sensitivity of *F. candida* endpoints to wastes

F. candida is a suitable species for waste testing due to its easy culture and manipulability, and sensitivity to pollutants (Greenslade and Vaughan, 2003), but also because it is a representative species of soil collembolans, a group which performs key functions in soil ecosystems (Fountain and Hopkin, 2005).

All *F. candida* biological endpoints reacted to organic waste, although with different patterns. Survival showed a continuous decrease with waste concentration increase, usually at much higher concentrations than those affecting reproduction. Reproduction increased over the controls at the lowest concentrations, indicating hormetic and/or trophic effects of wastes, followed by an inhibition at higher doses of waste. Furthermore, reproduction showed higher variability between replicates than survival. This has already been noticed for this species (Crouau and Cazes, 2003). On the other hand, body length was not sensitive to waste concentration, as it was unaffected, or only slightly affected, at the highest concentrations with survivors. This lack of sensitivity to pollution agrees with the work of Folker-Hansen et al. (1996) for two collembolan species, although other studies support the sensitivity of this endpoint for collembolans (Scott-Fordsmand and Krogh, 2004). In the present study, stimulation of body length was coupled with inhibition in reproduction. This may be explained by the previously demonstrated negative trade-off between reproduction and growth in other insects (Ernsting et al., 1993).

4.4. Waste composition and toxic effects

Despite the fact that wastes showed concentrations for one or more pollutants above the limit values of the Working Document on Sludge (European Communities, 2000), NPE was the only pollutant group with overall high concentrations in all tested wastes, with levels over the 50 mg kg^{-1} mentioned in the draft (European Communities, 2000) (Table 2). Surfactants can affect soil microorganisms and invertebrates by dissolving biomembranes (Jensen, 1999), but NPE are also thought to have estrogenic effects and hence to affect the reproduction of invertebrates (Oehlmann and Schulte-Oehlmann, 2003). Toxic effects of NP on reproduction of *F. candida* (EC50 = 2.9 mg kg^{-1}) (Greenslade and Vaughan, 2003), and also on the reproduction and survival of *F. fimetaria* around 40 and 99-140 mg kg^{-1}, respectively (Scott-Fordsmand and Krogh, 2004) have been reported. According to the estimated NPE concentrations shown in Table 5, only some of the wastes showed concentrations above 2.9 mg kg^{-1}. Furthermore, no correlation was found between survival or reproduction and NPE levels. Likewise, none of the remaining pollutants or pollutant groups could be directly related to the observed effects, and hence none of them in themselves were able to account for toxic response. This agrees with the extended consideration of chemical methods, compared with bioassays, as unsuitable for the prediction of ecological risk to soil organisms of the complex pollutant burden of wastes, as already pointed out by Crouau et al. (2002).

On the other hand, some physico-chemical properties of the wastes showed a correlation with the observed effects. More precisely, LC50 and LC20 values were positively correlated with stable organic matter (ease of decomposition) and negatively correlated with total nitrogen, hydrolyzable (labile) nitrogen, and ammonium, although no correlation appeared with EC50 reproduction values. The more stabilized a waste is, the higher the proportion of recalcitrant organic matter, and the lower the amount of total, hydrolyzable nitrogen and ammonium released (Martins and Dewes, 1992). This is the reason why survival is correlated with all these parameters, as all of them reflect the stability of wastes.

The negative correlation between the stability of wastes and their toxicity has been widely reported for plants (Pascual et al., 1997). It has been suggested that phytotoxicity was mediated by the release of ammonium, phenols, and organic acids during waste degradation, but also by competition between plants and microorganisms for available nitrogen, and by the decrease in soil oxygen levels (Déportes et al., 1995). Ammonia was directly related in this work to the observed negative effects on the survival of collembolans, as has already been shown for plants in soils amended with non-stabilized composts (Katayama et al., 1985), but also for short-term reductions of soil fauna density after the application of nitrogenated fertilizers (Seniczak et al., 1994) or organic wastes (Neher, 1999). On the other hand, reproduction inhibition was not significantly associated with waste stability, despite the fact that the more stabilized wastes, composted sludges, had a lower impact on reproduction. This pattern suggests that this endpoint, besides being more sensitive than survival, is affected in a different way by the waste that was tested. Hence, reproduction probably reflects the combined effect of waste physico-chemical properties and pollutant burden of wastes, providing more integrative information.

5. CONCLUSIONS

F. candida shows differential sensitivity depending on the type of waste, but also depending on the endpoint assessed. Reproduction is far more sensitive than survival, as it is affected at lower waste concentrations, while body length is a non-sensitive endpoint for waste testing. Pollutant burden alone is not able to predict the ecological risk of organic wastes to soil organisms, since neither the concentrations of single pollutants nor the sum of concentrations of pollutant groups can be related with the observed toxic effects. On the other hand, collembolan mortality is clearly explained by the stability of wastes, which is probably related to releases of secondary metabolites with decomposition, mainly ammonium. In contrast to survival, none of the physico-chemical parameters explains the effects on reproduction, as this endpoint is likely to reflect the combined effects of the physico-chemical parameters and pollutant burden of wastes.

Soil-waste mixtures vary in their organic matter, pH, and electrical conductivity with increasing concentrations, but it would be better to consider them as contributors to the observed effects rather than disturbing factors, as these factors also act in real situations. On the other hand, selection of water content is a problematic step in waste testing, as it needs to be adjusted in order to ensure that water content does not affect test organism performance. In this study, a qualitative approach for the choice of optimum water content is validated as suitable for water content selection.

Treatment of sewage sludge changes its composition and toxicity, especially with composting, which increases its stability, decreases the non-persistent organic pollutant burden, and decreases toxicity. Thermal drying increases toxicity, which is attributable to a decrease in waste stability promoted by high temperatures. It is also worth pointing out the high toxicity of thermally-dried pig slurry, which is mainly due to its low stability.

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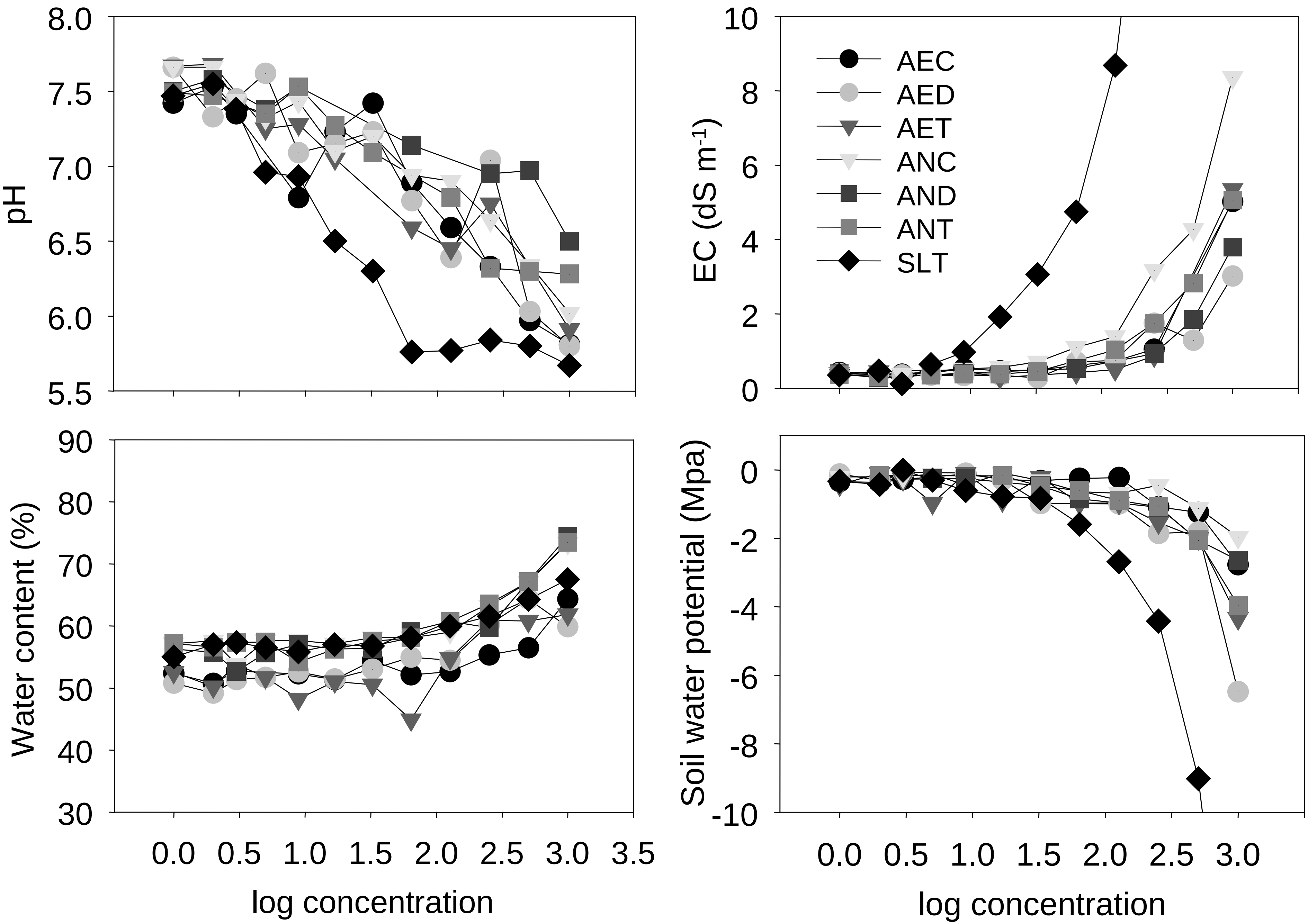
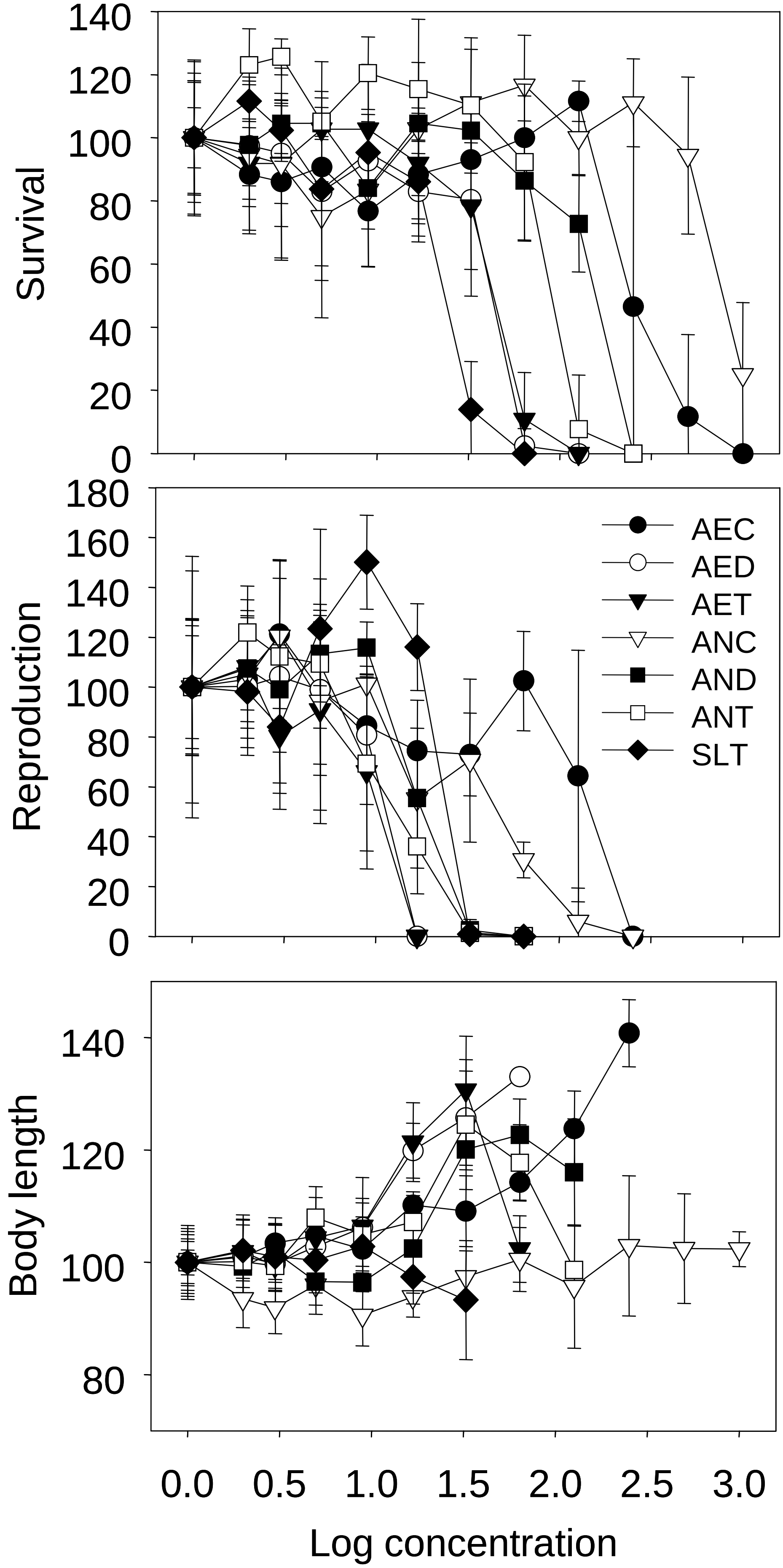
FIGURE CAPTIONS

Figure 1. Changes in physicochemical parameters in soil-waste mixtures with increasing waste concentration. Concentrations are expressed as log [1+concentration], in g Kg-1. See Table 1 for waste abbreviations.

Figure 2. Mean values for survival, reproduction, and body length of *Folsomia candida* with increasing concentrations of wastes in soil-waste mixtures. Effects on endpoints are expressed as a percentage with respect to controls. Concentrations are expressed as log [1+concentration], in g Kg-1. Bars indicate standard deviation. See Table 1 for waste abbreviations.



pH
EC (dS m-1)
AEC
AED
AET
ANC
AND
ANT
SLT
Water content (%)
Soil water potential (Mpa)
log concentration
log concentration



Survival
Reproduction
Body length
Log concentration
AEC
AED
AET
ANC
AND
ANT
SLT
0
20
40
60
80
100
120
140
160
180
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Table 1. Treatments and post-treatments of the organic wastes.

Waste	Origin	Treatment	Post-treatment
AED	Banyoles WWTP	Aerobic digestion, dewatering	None
AEC	Banyoles WWTP	Aerobic digestion, dewatering	Composting in vessel
AET	Banyoles WWTP	Aerobic digestion, dewatering	Thermal drying
AND	Blanes WWTP	Anaerobic digestion, dewatering	None
ANC	Blanes WWTP	Anaerobic digestion, dewatering	Composting in heap
ANT	Blanes WWTP	Anaerobic digestion, dewatering	Thermal drying
SLT	Juneda WTP	Anaerobic digestion, dewatering	Thermal drying

Table 2. Physicochemical properties, heavy metal and organic pollutant contents of the organic wastes (* = over the limit value in the 3rd draft of Working Document on sludge (European Communities 2000), ** = over the limit value of the current Directive on sludge (86/278/EEC); *w.w.* = wet weight; *d.w.* = dry weight). See Table 1 for waste abbreviations.

Parameter	**Units**	**AEC**	**AED**	**AET**	**ANC**	**AND**	**ANT**	**SLT**
Dry matter	g kg^{-1} (*w.w.*)	449	150	945	470	199	844	865
WHC	% (*w.w.*)	74.4	63.9	74.7	64.9	64.8	67.9	55.9
pH	water, 1:5 (v/v)	7.8	8.1	6.9	7.2	8.4	7.2	6.4
Electrical conductivity	dS/m, 25ºC	1.2	1.5	3.57	4.2	2.25	6.22	64.65
Organic matter	g kg^{-1} (*d.w.*)	622	684	687	551	566	668	612
Stable organic matter	%	50.1	37.8	40.4	54.2	47.7	46.7	36.6
N	g kg^{-1} (*d.w.*)	39.5	62.4	60.6	23.7	38.8	53.3	62.5
Non-hydrolyzable N	g kg^{-1} (*d.w.*)	17.0	16.4	19.1	16.1	12.4	18.4	10.9
Hydrolyzable N	g kg^{-1} (*d.w.*)	22.5	46.0	41.5	7.6	26.4	34.9	51.6
NH_4-N	g kg^{-1} (*w.w.*)	2.7	14.0	8.0	3.4	15.1	11.6	52.9
P	g kg^{-1} (*d.w.*)	22.0	20.4	20.5	28.6	33.6	29.2	20.4
K	g kg^{-1} (*d.w.*)	3.6	1.9	2.2	4.4	2.3	2.5	55
Cd	mg kg^{-1} (*d.w.*)	1.0	1.3	1.3	3.5	3.2	3.1	<0.7
Cr	mg kg^{-1} (*d.w.*)	345	55	30	53	54	127	15
Cu	mg kg^{-1} (*d.w.*)	294	624	645	798	933	833	780
Hg	mg kg^{-1} (*d.w.*)	0.67	1.33	0.95	2.13	2.51	2.25	0.12
Ni	mg kg^{-1} (*d.w.*)	59	80	53	76	64	45	29
Pb	mg kg^{-1} (*d.w.*)	1196**	3940**	3747**	92	78	85	<20

Zn	mg kg^{-1} (*d.w.*)	843	956	952	1028	988	890	2060
DEHP	mg kg^{-1} (*d.w.*)	10	61	27	22	143	71	1
LAS	mg kg^{-1} (*d.w.*)	298	816	331	214	3240*	5572*	60
NPE	mg kg^{-1} (*d.w.*)	86*	153*	76*	158*	513*	573*	54*
PAH	mg kg^{-1} (*d.w.*)	0.1	0.4	0.3	1.6	1.1	1.4	0.05
PCB	mg kg^{-1} (*d.w.*)	0.015	0.034	0.029	0.041	0.023	0.029	<0.007
PCDD/F	ngTEQ kg^{-1} (*d.w.*)	16	15.6	13.7	12.4	7.7	13.2	0.3

Table 3. Toxicity values for survival (LCx) and reproduction (ECx) of *F. candida* expressed in g kg^{-1}, with 95% confidence intervals enclosed in parentheses. See Table 1 for waste abbreviations.

Waste	**LC50**	**LC20**	**EC50**	**EC20**
AEC	252 (222, 287)	210 (81, 546)	207 (37, 1142)	26 (4.51, 134)
AED	44 (34, 57)	35 (26, 46)	10 (3.8, 24)	7.9 (5.8, 11)
AET	44 (37, 52)	32 (25, 40)	5.3 (2.8, 9.4)	1.1 (0.7, 1.5)
ANC	834 (626, 1110)	665 (384, 1152)	29 (18, 46)	12 (5.6, 25)
AND	154 (134, 178)	114 (97, 133)	16 (15, 18)	14 (11, 18)
ANT	86 (72, 101)	63 (50, 79)	10.4 (7.5, 14)	6.7 (4.5, 9.9)
SLT	24 (20, 28)	18 (14, 23)	19 (3.8, 86)	18 (6.4, 48)

Table 4. Published LC50 values for the effect of single pollutants on *F. candida*, and equivalent concentrations of these products in studied wastes at the LC50 level. All values reported were from *F. candida* with the exception of LC50 for PAH, DEHP, NPE, and LAS, obtained from *Folsomia fimetaria*, and LC50 for PCDD/F, obtained from Collembola as a group. PCDD/F are expressed as ng TEQ kg^{-1}. See Table 1 for waste abbreviations.

Pollutant	LC50 (mg kg^{-1})	Reference	Pollutant equivalent concentration (mg kg^{-1})						
			AEC	AED	AET	ANC	AND	ANT	SLT
Cd	850	Crommentuijn et al. 1993	0.2	0.1	0.1	2.9	0.5	0.3	0.02
Cr	-	-	87.0	2.4	1.3	44.2	8.3	11.2	0.4
Cu	1810	Greenslade and Vaughan 2003	74.2	27.4	28.4	665.4	143.7	73.8	18.5
Hg	-	-	0.17	0.06	0.04	1.78	0.39	0.20	0.00
Ni	-	-	14.9	3.5	2.3	63.4	9.9	4.0	0.7
Pb	980-2900	Bongers et al. 2004	301.7	173.0	164.8	76.7	12.0	7.5	0.5
Zn	5150	Lock and Janssen 2001b	212.7	42.0	41.9	857.1	152.1	78.8	48.8
PCB	>204 (PCB153)	Aldrich and Daniel 2003	0.004	0.001	0.001	0.034	0.003	0.003	<0.001
PAH	67-1025	Sverdrup et al. 2002	0.02	0.02	0.01	1.33	0.17	0.12	0.001
DEHP	>5000	Jensen et al. 2001b	2.5	2.7	1.2	18.3	22.0	6.3	0.02
NPE	99-140 (NP)	Scott-Fordsmand and Krogh 2004	21.7	6.7	3.3	131.7	79.0	50.7	1.23
LAS	>793	Holmstrup and Krogh 2001	75.2	35.8	14.6	178.4	499.0	493.4	1.4
PCDD/F	>10 (OCDD)	van Straalen et al. 1995	4.04	0.68	0.60	10.34	1.19	1.17	0.01

Table 5. Published EC50 values for the effect of single pollutants on *F. candida*, and equivalent concentrations of these products in studied wastes at the EC50 level. Values for NP from *Folsomia fimetaria*. PCDD/F are expressed as ng TEQ kg^{-1}. See Table 1 for waste abbreviations.

Pollutant	EC50 (mg kg^{-1})	Reference	Pollutant equivalent concentration (mg kg^{-1})						
			AEC	AED	AET	ANC	AND	ANT	SLT
Cd	51-780	Fountain and Hopkin 2005	0.21	0.01	0.01	0.10	0.05	0.03	0.01
Cr	604	Lock and Janssen 2002a	71.4	0.5	0.2	1.5	0.9	1.3	0.3
Cu	250-1480	Fountain and Hopkin 2005	60.83	6.2	3.4	22.8	15.23	8.7	15.1
Hg	3.26	Lock and Janssen 2001a	0.14	0.01	0.01	0.06	0.04	0.02	0.00
Ni	476	Lock and Janssen 2002b	12.2	0.8	0.3	2.2	1.0	0.5	0.6
Pb	580-3160	Fountain and Hopkin 2005	247.4	39.3	19.9	2.6	1.3	0.9	0.4
Zn	50-2088	Fountain and Hopkin 2005	174.4	9.5	5.0	29.4	16.2	9.3	40.0
PCB	-	-	0.003	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
PAH	-	-	0.021	0.004	0.002	0.046	0.018	0.015	0.001
DEHP	>5000	Jensen et al. 2001b	2.1	0.6	0.1	0.6	2.3	0.7	0.02
NPE	2.9 (NP)	Greenslade and Vaughan 2003	17.8	1.5	0.4	4.5	8.4	6.0	1.0
LAS	91	Jensen et al. 2001a	61.7	8.1	1.7	6.1	53.0	58.00	1.2
PCDD/F	-	-	3.3	0.2	0.1	0.3	0.1	0.1	0.01