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Feeding Inhibition in the Soil Collembolan *Folsomia candida* as an Endpoint for the Estimation of Organic Waste Ecotoxicity

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ABSTRACT

Despite the increasing quantities of organic wastes that are being reused in soils, there are few studies that focus on the selection of bioassays for the ecotoxicological risk assessment of organic wastes to soils. In the present study, differences in feeding inhibition in the soil collembolan *Folsomia candida* were evaluated as an ecotoxicological endpoint for the assessment of risk to soils amended with polluted organic wastes. 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. A clear dose response was observed for this parameter, with an increase in percentage of individual feeding inhibition with increased doses of organic wastes. More significantly, feeding inhibition correlated highly with mortality and reproduction inhibition in the different wastes. Composted sludges displayed the lowest toxicity, followed by thermally-dried sludge and dewatered sludge. Thermally-dried pig slurry showed the highest toxicity for feeding, with lower EC50 values than the lowest dose tested. Among waste physicochemical parameters and pollutants, low organic matter stability appeared to be the main predictor of potential adverse effects on soil fauna, as it correlated significantly with feeding inhibition and mortality. Furthermore, feeding inhibition tests were run over a short exposure time (less than 7 d), which, together with the results obtained, makes this bioassay a good screening tool for organic waste toxicity.

KEYWORDS: *Folsomia candida*, Feeding inhibition, Reproduction, Survival, Ecotoxicity

INTRODUCTION

In recent decades, increase in the production of sewage sludge in the European Union, as a result of the implementation of Directive 91/271/EEC, has mainly been recycled to land, over other options such as landfill or incineration ([1]; http://ec.europa.eu/environment/waste/sludge/problems.htm). Despite the undeniable benefits of organic waste amendments in terms of soil fertility, concerns may arise regarding waste with a higher pollutant content, which may have deleterious effects on soil ecosystems and hence, counteract their contribution to soil fertility. Despite the importance of soil fauna for soil ecosystems [2], only a few studies have focused on the harmful effects of sludge amendments to soil invertebrates in the field. None of them have reported noxious effects of sludge at agronomical dosages ([3]; http://www2.mst.dk/Udgiv/publications/1997/87-7810-865-9/pdf/87-7810-865-9.pdf, [4,5]). On the other hand, several laboratory studies have shown significant effects on soil fauna [3,6]. Despite the extent of soil reuse and the existence of limit values for some pollutants in sludge for this practice, there is still a paucity of studies which focus on the selection of laboratory assays to determine the ecotoxicological risk of wastes for soil inhabiting organisms. Among the few attempts that have been conducted are those involving plants and earthworms [7-9], and collembolans [3,10-12].

Reproduction and growth are the most common sublethal endpoints in soil ecotoxicity testing due to their ecological relevancy. However, they require a great effort in terms of time and handling, which in recent decades has encouraged research aimed at finding alternative endpoints which provide similar information but require less experimental effort [13-15]. Reproduction and growth are the ultimate objectives in a long line of processes that start with biochemical and physiological events [16], and endpoints at the biochemical, physiological or behavioral level might then be used as a complement to these traditional endpoints as they are early-warning indicators which generally provide results faster. This is the main reason for the attempts to relate reproduction or growth with biomarkers [17,18] or avoidance responses [19]. However, it has been indicated that behavioral responses, together with growth and reproduction, only show a weak correlation with effects at higher biological levels. Furthermore, it has also been indicated that biomarkers rarely provide useful predictions of effects at higher biological levels [20,21]. This is why endpoints at the individual level such as reproduction, growth or behavioral responses are preferred to those at lower biological levels and considered as more ecologically relevant. An alternative to conventional individual endpoints might be feeding inhibition, a parameter that has been recognized as a general stress response to toxicants and demonstrated on a variety of aquatic species [15,22,23], although not much is known about its importance in soil organisms. Reduced feeding from artificially-polluted food has been reported in *F. candida* by several authors [24-26], although this pattern has never been tested for polluted organic wastes, despite the evidence in this species of active feeding on these materials [10,27].

Feeding inhibition, either during or after exposure to contaminants, has been used as an ecotoxicological endpoint in a variety of organisms, mainly aquatic but also in terrestrial species. Feeding inhibition during exposure has been especially demonstrated in the freshwater cladoceran, *Daphnia magna*, for a variety of pollutants [14,28-31], and other aquatic invertebrates [13,32-35]. Other authors have detected feeding inhibition behavior in several crustaceans [15,23,36], and fish [37] when relocated to uncontaminated environments, after previous exposure to contaminants (so-called post-feeding inhibition).

On the other hand, only a few studies have addressed feeding inhibition in terrestrial organisms, and with contradictory results. Several authors have shown feeding inhibition with exposure to pollutants in nematodes [38,39] and collembolans [24-26]. However, no feeding inhibition was observed in isopods when leaves contaminated with the herbicide trifluralin were offered as food [40].

Despite the relative abundance of literature using this endpoint, the exact mechanisms involved in feeding inhibition as a response to pollution are still unknown. More precisely, it is not clear whether it is due to direct avoidance of pollutants in food or an indirect physiological or biochemical effect of toxicants that finally impact on feeding activities, or both.

It has been suggested that direct avoidance of contaminated food occurs in collembolans [24-26], soil nematodes [38], and *D. magna* [41]. The importance of preingestive inhibition may be high in collembolans as it has been demonstrated that their food preferences are linked to odor [42]. Furthermore, regurgitation of polluted food in *D. magna* has also been reported [30]. Feeding behavior may also be indirectly affected through physiological and biochemical effects. The persistence of feeding inhibition after exposure to contaminants is the main evidence of this mechanism. The explanation is that pollutants are able to affect the organism’s biochemistry and physiology, disturbing its sensorial reception, enzymatic activities, and metabolism, with unavoidable influences on feeding behavior and other parameters. Several studies have demonstrated this mechanism in daphnids [15,28] and polychaetes [43]. Other studies have even specifically linked feeding inhibition with a decrease in acetyl cholinesterase (AChE) levels due to pollutant levels, as demonstrated in crustaceans [32], fishes [44], and birds [45].

In summary, feeding inhibition seems to be a complex response involving components which are not easy to separate. On the one hand, pollutants may directly reduce consumption through active avoidance of polluted food, but conversely, they can disrupt feeding mechanisms through effects on biochemical and physiological processes.

The aim of this work was to evaluate the suitability of feeding inhibition in *F. candida* as an ecotoxicological endpoint to assess potential risks of soil amendments with polluted organic wastes. To achieve this goal, different wastes and exposure times were tested, and feeding inhibition values were compared with effects of survival and reproduction inhibition in the same species in terms of sensitivity, correlation of responses and workload.

METHODS

Test organisms

The *F. candida* culture used in the tests was raised in our laboratory, and was initiated four years ago from cultures of the Institute of Ecological Science of the Vrije Universiteit (Amsterdam, The Netherlands). Breeding of individuals was performed in polyethylene containers of 17.5 x 12.5 x 7.5 cm, with a 1 cm layer of wet substrate made of a mixture of plaster of Paris and charcoal (9:1

v/v). Cultures were kept in darkness in a climatic chamber at a constant temperature of 21±1ºC. Every two months the substrate was renewed and the density of individuals was reduced to avoid overcrowding. Synchronized cultures were used in the tests, prepared as described in the International Organization for Standardization (ISO) Guideline 11267 [46].

Test substrate

Artificial soil prepared as documented by the ISO Guideline 11267 [46] but removing peat from the mixture. By doing this it was ensured that reported consumptions in artificial soil-waste mixtures corresponded only to waste ingestion, as in a preliminary test with conventional artificial soil it was shown that individuals consumed peat from the substrate, a fact that would disturb the assessment of feeding behavior. The test substrate was composed of 78% quartz sand and 22% kaolin. A suitable amount of calcium carbonate was added to provide a pH of approximately 6±0.5. In all tested doses of the organic wastes, the water content of the mixture was adjusted to 50% of the water-holding capacity of the artificial soil.

Organic wastes

One dried pig slurry and six sewage sludges, with different origins, and subjected to different treatments and post-treatments, were selected in order to provide a wide range of waste properties to evaluate the sensitivity of the bioassay described in this work (Table 1). All wastes were dried at 60ºC for 48 to 72 h, depending on the initial humidity, and then ground and sieved (<2mm). This step was unavoidable in order to ensure the accuracy of the low doses tested in most of the wastes. The resulting samples were used both for the preparation of soil-waste mixtures and for waste characterization. Details of physicochemical properties and concentrations of metals and organic pollutants in the wastes together with methods for their characterization are described in Domene et al. [12].

Sludge composts used in the present study were produced from a 1:4.5 (v/v) mixture of dewatered sludge and pinewood chips. For the anaerobic sludge, composting was carried out with pine splinters in a tunnel with air injection for 15 d, while aerobic sludge was composted in a heap for 50 d using chips from recycled wood and furniture as a bulking agent. At the end of the process, both composts were sieved to 1 cm to remove approximately 90% of the chips. This post-treatment decreased the total, hydrolysable and ammonium nitrogen content of the initial sludge, while increasing organic matter stability.

Thermal drying was carried out by placing dewatered sludge in a heated rotary cylinder and injecting hot air, which subjected the sludge to a temperature of approximately 130 to 150ºC for 45 min. With this post-treatment, pollutant levels did not change significantly, but $N\text{-}NH_4$ levels decreased and electrical conductivity increased.

Pig slurry was obtained from a treatment plant where the raw slurry is subjected to anaerobic digestion followed by thermal drying at 130ºC, producing a final product with a dusty appearance. The final product showed high electrical conductivity, high levels of hydrolysable nitrogen and ammonium, and low organic matter stability.

Experimental setup

A 4-d preliminary assay was performed in order to define the range of doses for each organic waste to be tested in the definitive assay. The doses used in this preliminary assay were 1, 10, 50, 100, 300, 500, 700, 1000 g Kg^{-1}. Based on these results, a definitive concentration range was defined for each waste, taking as definitive the range of concentrations with 20% to 80% of individuals showing feeding inhibition. In the definitive test, five doses in an arithmetic progression were defined for each waste, but always within the 1 to 500 g Kg^{-1} range as maximum. Each dose tested was composed of 5 replicates and an additional replicate for the determination of water content, pH and electric conductivity. The test was carried out using 50 ml polyethylene containers filled with only 5 g of wet substrate mixture in order to maximize retrieving of the individuals and their observation. 15 individuals (10 – 12 d old) were placed in each test container. Three exposure times (2, 4, and 7 d) were defined for each waste and concentration. The number of individuals was determined in a preliminary experiment that showed that feeding patterns were similar regardless of the number of individuals tested (10, 15, and 20). The number of individuals selected (15) is considered high enough to minimize the impact of possible individual mortality or inability to refloat some of the animals, but low enough to allow direct and simultaneous counting of all the individuals in the test container.

Observation of feeding behavior

It has been suggested that *F. candida* ingests sludge from test substrate when this is available [10,27]. Since gut content in *F. candida* is easily observed in vivo, given the lack of pigment in its cuticle [26], individuals feeding on the organic wastes could be easily assessed at the end of the test by the presence of dark content in the gut. Direct observation of the individuals was possible after flooding the test container, allowing the animals to float on the surface of the water (Fig. 1).

Data treatment

The 50% effective concentration (EC50) values and their associated 95% confidence intervals were estimated by probit analysis using Minitab 13.2 software (Minitab, State College, PA, USA). A logistic distribution was assumed to carry out probit analysis as it displayed the best adjustment to the data.

After calculations, EC50 values for feeding in each waste after 2, 4, and 7 d of exposure were compared by Pearson correlation with the 50% lethal concentration values (LC50) and EC50 reproduction values for *F. candida*, obtained from the same wastes in a previous study [12]. These values were obtained in accordance with ISO Guideline 11267 [46] with some modifications to adapt it to waste testing, and were derived using linear and non-linear regression models according to Stephenson et al. [47]. EC50 feeding values were also compared by Pearson correlation with all the physicochemical parameters and pollutants assessed for each waste in order to detect any significant relationship. All correlations in the present study were carried out with log-transformed values.

RESULTS

Feeding behavior

Retrieving of individuals with the substrate used averaged 75%. Losses should be attributed mainly to an inability to flotate all the individuals in this type of substrate rather than to mortality, since losses of individuals in replicates were similar with increasing waste doses and also because these were not expected to affect survival according to the LC50 values found by Domene et al. [12] (Table 2). The main reason may be a lack of peat in the substrate, which gave it a less crumbly structure. This made flotation of individuals more difficult in comparison with conventional artificial soil. For the reasons already indicated, we assumed that this phenomenon did not affect the test results.

Feeding inhibition increased with increasing doses of the different wastes, presenting a linear or linear-like form depending on waste and exposure time tested (data not shown, see an example in Fig. 2). Mean feeding inhibition rates in the lower test doses varied between 12 and 34% depending on the waste, while at the highest tested doses mean rates were between 68 and 95%. The exception was pig slurry (SLT), which showed an inhibition of approximately 72% at the lowest tested dose (1 g Kg^{-1}). This inhibition continued to increase towards the highest dose (data not shown). An additional experiment (results not presented) showed that the feeding inhibition of this waste remained very high even at concentrations as low as 0.1 g Kg^{-1}. As no feeding EC50 could be calculated for this waste, we excluded this waste for comparisons with reproduction and survival inhibition values.

The EC50 feeding values of the different wastes generally did not overlap in their 95% confidence intervals, suggesting a satisfactory precision of the method (Table 2), and were below 5 g Kg^{-1} in the aerobic dewatered (AED) and thermally-dried (AET) sludges, while the anaerobic dewatered (AND) and thermally-dried (ANT) sludges showed values below 30 g Kg^{-1}. On the other hand, composted sludges showed the highest EC50 values, approximately 165 to 300 g Kg^{-1} for ANC, and 423 to 456 g Kg^{-1} for AEC.

Feeding rates for each waste and concentration showed no significant differences between times tested (analysis of variance [ANOVA], $p > 0.05$), indicating that the response of this endpoint remains nearly constant within one week of exposure.

Comparison of endpoints

Feeding inhibition in *F. candida* correlated highly with effects on survival and reproduction (Table 3). This correlation was maintained throughout the different exposure times, both with survival and reproduction. Maximum correlation was found with feeding inhibition after 7 d of exposure and reproduction. This relationship is interesting given the lack of correlation between survival and reproduction in the present study ($r = 0.636$, $p = 0.174$). Furthermore, it is also worth noting that feeding behaviour was usually inhibited at lower waste concentrations than those affecting mortality, with the exception of AEC. Conversely, feeding was usually inhibited at concentrations above those affecting reproduction (except AED and AET). It is also noteworthy that, despite being less sensitive than reproduction, feeding inhibition was more reliable, as it displayed narrower confidence intervals.

Influence of waste properties on feeding behavior

The EC50 feeding values correlated positively and significantly with organic matter stability of wastes throughout the experimental period. Furthermore, significant negative correlation was found with ammonium at 7 d, and marginally significant at 4 d. Finally, a significant negative relationship was found with total nitrogen after 4 d of exposure, although the relationship also appeared marginally significant at 2 d (Table 3). These correlations agree with a previous study, where LC50 values in *F. candida* were correlated with the same properties [12].

DISCUSSION

Relevance and sensitivity of feeding inhibition

Feeding inhibition is a suitable endpoint for ecotoxicity testing because it is a general stress response to exposure to toxicants in a variety of species [15,22,23]. Whether feeding inhibition is an avoidance response to polluted food or a result of physiological or biochemical processes finally impacting on feeding behavior, the reduction of food intake has an impact at least at the individual level. Acquisition and allocation of energy determines developmental rate, growth rate, fecundity, and survival. Hence, any disturbance in the energy allocation to any of these processes, is expected to be also translated into effects at the population level [14,15,34,48,49]. For example, Maltby [50] showed that feeding inhibition in the amphipod, *Gammarus pulex*, correlated both with lethality and reproduction. This pattern has been confirmed in the freshwater rotifer, *Brachionus calcyflorus* [13]**.** Barata and Baird [14] also concluded for *Daphnia magna* that chemicals affecting endpoints like feeding rate or viability of eggs were predictive of effects in traits like reproduction and survival, given the influence of the former endpoints on the latter. However, Lopes et al. [51] found no correlation between feeding inhibition and survival in *Daphnia longispina* when exposed to copper. Results from our study suggest a link between feeding inhibition in the first week of exposure and reproduction and survival after a month.

Furthermore, some studies have suggested that feeding inhibition might be an ecologically relevant parameter, since impacts of pollutants on this endpoint have correlated with changes at the community and ecosystem levels. Maltby et al. [33] found that in situ feeding rates of the aquatic crustacean, *Gammarus pulex*, correlated significantly with stream macroinvertebrate diversity and detritus processing. On the other hand, McWilliam and Baird [15] found no correlation between postexposure feeding depression of *Daphnia magna* and macroinvertebrate community structure. No studies are available for terrestrial ecosystems, but given the key role of soil fauna in facilitating microbial decomposition and nutrient turnover [52], effects at the individual level may provoke effects at higher biological levels [34].

In the present study, feeding inhibition of *F. candida* in the set of wastes studied correlated highly with mortality and reproduction inhibition, indicating the relevance of this endpoint for estimating effects on other more commonly used endpoints which require a longer experimental period. Furthermore, EC50 values for feeding also remained significantly constant during the first week of exposure. This may permit a reduction of the exposure time to only two days while still providing relevant information on waste toxicity. In addition, feeding inhibition values displayed narrower confidence intervals than survival and reproduction inhibition values, showing the high reliability of this endpoint. An explanation might be the lower chance of mortality (due to the feeding test running for a shorter period), and/or the best adjustment of the model used for feeding data with respect to those used for survival and reproduction.

When inhibition values were compared, it was apparent that feeding behavior was generally inhibited at lower waste concentrations than mortality, while feeding was usually inhibited at concentrations above those affecting reproduction. This trend is usual for lethality in studies with aquatic organisms exposed to pollutants [13,14,23,29]. However, there is no agreement on what is typical with respect to reproduction. Some published studies have shown that reproduction is generally affected at lower concentrations than feeding [13,5], while others have reported inhibition at similar concentrations [14,39] or the opposite relationship [54].

Suitability of **F. candida** *for feeding inhibition bioassays*

Among the most commonly used soil test species, *F. candida* is especially recommended for the assessment of feeding inhibition responses, as it is possible to observe the consumption of some food sources, in this case organic wastes, as a dark gut content due to the lack of pigment in its cuticle [26]. Like other collembolans, *F. candida* molts throughout its entire life cycle. During molting the whole cuticle and gut epithelium is completely regenerated, and at this stage individuals stop feeding. This may interfere with the observation of feeding activities [55]. Despite this fact, we assumed that this phenomenon is negligible for the purposes of this study as a synchronized culture was used for the experiments, which should reduce any variability in results caused by these molting events. In addition, the rate of non-feeding individuals in the lower waste concentrations is similar, independent of the waste type and exposure time (approximately 25%, data not reported), suggesting a low bias on the results of moulting stages. These rates are in accordance with the 20% reported by Thimm et al. [55] in mixed-age cultures, which were assigned by the authors to moulting individuals. Moreover, the feeding rates observed offer an accurate assessment of the instantaneous situation of individuals, as the period between ingestion and excretion of food boluses in this species lasts only 35 minutes in similar experimental conditions to those used in our study [55].

The consumption of sewage sludge has already been suggested for *F. candida* [12] and *F. fimetaria* [27]**.** However, this consumption seems to be important only when an alternative clean food source is not available. In a previous unpublished work we observed that individuals of *F. candida* showed lower consumption rates of sewage sludge when yeast was available, in accordance with its near relative *F. fimetaria* [3]. This behavior agrees with findings from other authors who reported avoidance of contaminated food in this species [24-26]. Filser and Hölscher [24] also showed that the appeal of potato bait decreased with copper concentration. Pedersen et al. [25] found that both *F. candida* and *F. fimetaria* consumed less contaminated yeast with increasing concentrations of copper. Fountain and Hopkin [26] reported a lower feeding rate for *F. candida* with increasing concentrations of heavy metals in yeast offered as food. These authors also demonstrated an active avoidance of polluted food, as a higher percentage of individuals fed on culture substrate with increasing concentrations of metals in yeast. The authors attributed this behavior to an attempt to use the substrate as an alternative food source, demonstrating the existence of mechanisms of contaminant recognition in this species.

The use of feeding inhibition as an endpoint has the main advantage of providing results more quickly than other endpoints and generally requires simpler experimental setups [13-15]. However, no endpoint could be generally considered to be better than others, as each chemical has its main mode of action and each species may present a different sensitivity to a given parameter. Despite this, feeding inhibition seems to be a general response to a variety of pollutants with different modes of action and toxicities, making this parameter a sensitive and robust response for use as an ecotoxicological endpoint [15].

Parameters involved in feeding inhibition behavior

Results from this study indicate the suitability of feeding inhibition in *F. candida* as a sensitive endpoint for organic waste ecotoxicity testing, as it was clearly affected at different doses depending on the waste. We have no evidence about the main mechanism involved in the feeding inhibition observed in the present study. However, by not adding an alternative food source to test containers we forced individuals to interact with the waste offered. By doing this we obtained an integrative response, regardless of whether direct avoidance of polluted food and/or biochemical and physiological disruption of feeding behavior were the main cause.

Composted sewage sludges inhibited feeding at much higher doses when compared to the effects obtained with dewatered and thermally-dried sludges.

On the other hand, it was not possible to assess the EC50 feeding values of the thermally-dried pig slurry as they were below 1 g Kg^{-1}, given the higher toxicity of this sludge with respect to the other wastes [12].

The main factor influencing feeding patterns in this species was the organic matter stability of wastes, a parameter which reflects ease of decomposition. This relationship has already been pointed out in a previous study [12], where a strong correlation was found between this waste parameter and mortality in this species, although no significant relationship was found with reproduction. During decomposition, the breakdown of the more labile fraction of organic matter decreases the relative quantities of total nitrogen, mainly through ammonia losses [56]. Soil amendments with organic wastes with a low degree of stability may cause problems for soil biota, as during their decomposition there is a release of secondary metabolites such as ammonium, phenols, and organic acids, among other adverse effects [57]. Correlation between degree of waste stability and toxicity has been widely reported for plants [58], and also for soil fauna [2]. Furthermore, a correlation with the degree of stability may also reflect the already mentioned degradation of the less persistent organic pollutants in wastes through composting [59,60]. Whatever the reason, according to results from this study, organic matter stability appears as the best predictor for anticipating potential adverse effects for soil fauna derived from soil amendments, at least in the short term.

CONCLUSION

Feeding inhibition of *F. candida* is a suitable endpoint to assess the ecotoxicological risk of organic waste amendments in soils. It showed different responses to different wastes, and presented a clear dose-response relationship with increasing waste doses. More significantly, feeding inhibition was correlated highly with mortality and reproduction inhibition in the different wastes. Besides providing equivalent information, results involving feeding inhibition could be obtained over a shorter exposure time (less than 7 d) in comparison with other more conventional endpoints like reproduction. Results from this study indicate the value of this bioassay as a screening tool for organic waste toxicity.

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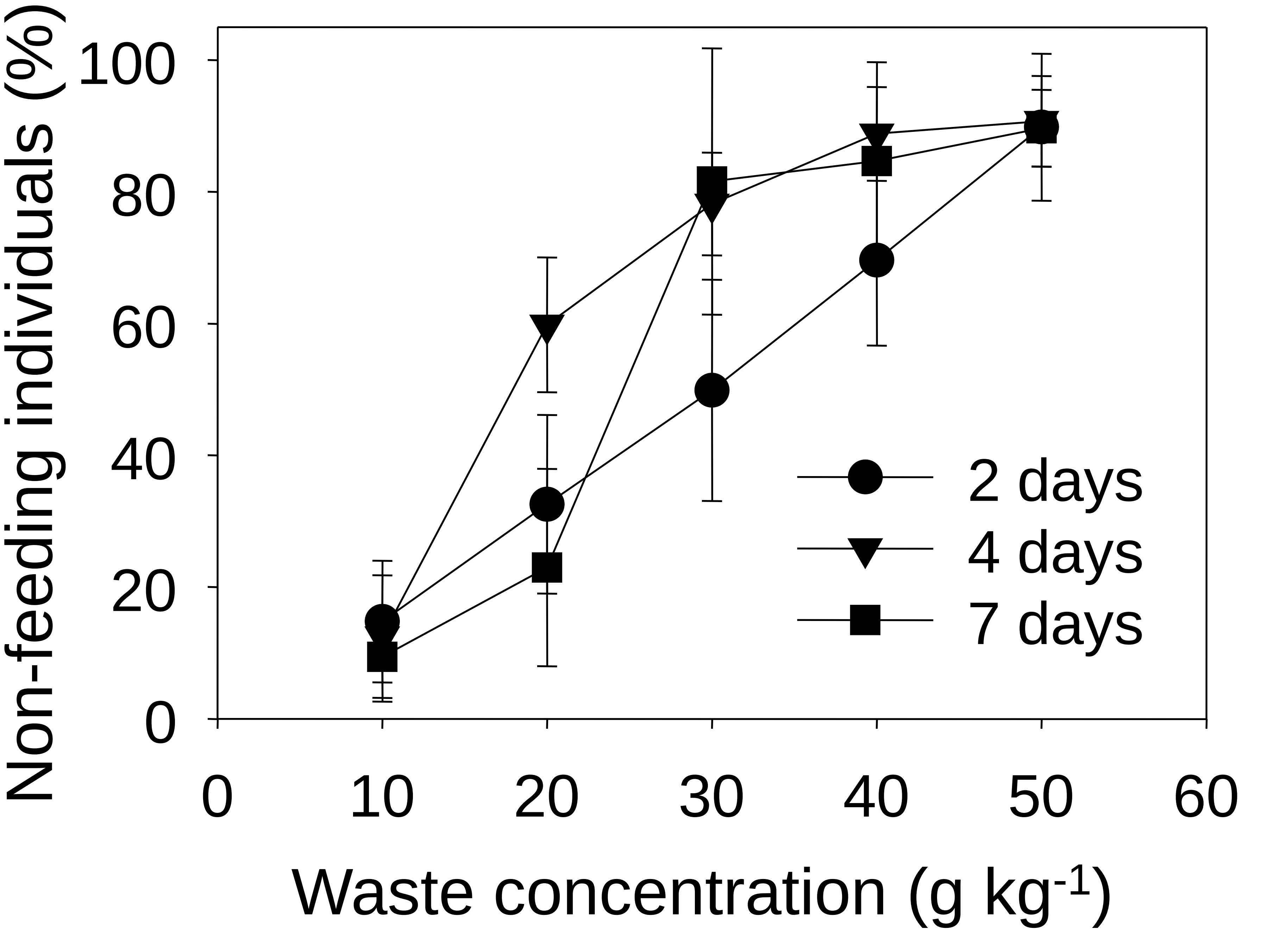
FIGURE LEGENDS

Figure 1. Group of *Folsomia candida* removed from a test chamber by flotation. Four individuals have the ingested waste clearly visible in their gut.

Figure 2. Feeding inhibition values in *Folsomia candida* exposed to increasing concentrations of the anaerobic thermally-dried sewage sludge (ANT) after 2, 4, and 7 d of exposure. Bars indicate standard deviation. n = 5.



Non-feeding individuals (%)
0
20
40
60
80
100
0
10
20
30
40
50
60
Waste concentration (g kg-1)
2 days
4 days
7 days

Table 1. Origin, 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. Feeding inhibition values (EC50, 2, 4, and 7 d), plus survival (LC50) and reproduction (EC50) (28 d) inhibition, after exposure to contaminated waste. Values expressed as g Kg^{-1} (dry wt). * = from Domene et al. [15]. See Table 1 for waste abbreviations; EC50 = concentration of waste estimated to reduce the outcome in a sublethal endpoint rate by 50% compared to the control; LC50 = concentration of waste estimated to prove lethal at the end of the test by 50% compared to the control.

Waste	Feeding EC50, 2 d	Feeding EC50, 4 d	Feeding EC50, 7 d	LC50, 28 d*	Reproduction EC50, 28 d*
AEC	445.7 (435.3, 456.2)	455.9 (445.7, 466.0)	423.6 (411.1, 436.0)	252.3 (221.7, 287.2)	206.9 (36.8, 1141.8)
AED	3.9 (3.7, 4.2)	3.6 (3.4, 3.9)	4.4 (4.2, 4.6)	43.9 (34.1, 56.6)	10.0 (3.8, 23.8)
AET	1.7 (1.1, 2.2)	2.9 (2.7, 3.2)	3.9 (3.7, 4.1)	44.0 (37.4, 51.7)	5.3 (2.8, 9.4)
ANC	299.1 (290.3, 308.2)	281.5 (264.2, 304.0)	164.6 (155.2, 173.6)	833.8 (626.4, 1109.7)	28.7 (17.7, 46.0)
AND	18.1 (16.9, 19.3)	26.1 (25.2, 27.1)	20.5 (18.8, 22.0)	154.4 (133.7, 178.3)	16.4 (14.7, 18.2)
ANT	27.3 (26.2, 28.5)	20.0 (19.2, 20.8)	24.7 (24.0, 25.5)	85.6 (72.3, 101.3)	10.4 (7.5, 14.2)
SLT	-	-	-	23.7 (20.2, 27.8)	19.4 (3.8, 86.4)

Table 3. Pearson's correlations of values of feeding inhibition (EC50) *Folsomia candida* after 2, 4, and 7 d of exposure with survival (LC50), reproduction EC50, and some physicochemical parameters. Correlation analyses were carried out with log-transformed values. EC50 = concentration of waste estimated to reduce the outcome in a sublethal endpoint rate by 50% compared to the control; LC50 = concentration of waste estimated to prove lethal at the end of the test by 50% compared to the control. *n* = 6.

	Feeding EC50, 2 d	Feeding EC50, 4 d	Feeding EC50, 7 d
LC50, 28 d	$r = 0.890$, $p = 0.017$	$r = 0.908$, $p = 0.012$	$r = 0.858$, $p = 0.029$
Reproduction EC50, 28 d	$r = 0.865$, $p = 0.026$	$r = 0.880$, $p = 0.021$	$r = 0.905$, $p = 0.013$
Organic matter stability	$r = 0.897$, $p = 0.015$	$r = 0.913$, $p = 0.011$	$r = 0.884$, $p = 0.019$
NH_4-N	$r = -0.773$, $p = 0.071$	$r = -0.801$, $p = 0.056$	$r = -0.817$, $p = 0.047$
Total nitrogen	$r = -0.802$, $p = 0.055$	$r = -0.829$, $p = 0.041$	$r = -0.764$, $p = 0.077$