Ecological Risk Assessment of Organic Waste Amendments Using the Species Sensitivity Distribution from a Soil Organisms Test Battery

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ABSTRACT

Safe amendment rates (the predicted no-effect concentration or PNEC) of seven organic wastes were estimated from the species sensitivity distribution of a battery of soil biota tests and compared with different realistic amendment scenarios (different predicted environmental concentrations or PEC). None of the wastes was expected to exert noxious effects on soil biota if applied according either to the usual maximum amendment rates in Europe or phosphorus demands of crops (below 2 t DM ha^{-1}). However, some of the wastes might be problematic if applied according to nitrogen demands of crops (above 2 t DM ha^{-1}). Ammonium content and organic matter stability of the studied wastes are the most influential determinants of the maximum amendment rates derived in this study, but not pollutant burden. This finding indicates the need to stabilize wastes prior to their reuse in soils in order to avoid short-term impacts on soil communities.

Capsule: Ecological Risk Assessment of Organic Waste Amendments

1. INTRODUCTION

The use of organic wastes in soils is an increasing management option in the European Union. For sewage, such increase is mainly due to the rising amount of sludge produced, the increasingly stringent controls on landfilling, the public opposition to incineration, and the ban on disposal at sea (Schowanek et al. 2004, Thornton et al. 2001). Preference for the reuse of organic wastes in agricultural land with respect to other management options is also due to the benefits of this practice. Organic amendments enhance soil fertility by adding nutrients, it is a cheaper option that allows reduction in the use of fertilisers, improves the soil structure, the water retention and the resilience to erosion, and it is an inexpensive solution of management of organic wastes (Schowaneck et al. 2004). However, the side effects on the soil-dwelling organisms exposed to wastes' pollutant burden are often neglected (Bünemann et al. 2006), despite their central role in soil agroecosystems functioning (Giller et al. 1997, Neher 1999).

The amount of organic wastes applied to an agricultural land is generally dictated by their nutrient content (nitrogen and phosphorus) and by the crop demands. Furthermore, several criteria for environmental protection exist in Europe to ensure a minimum waste quality before waste application, in order to ensure its long-term sustainable use on land. However, quality relies exclusively on the waste total pollutant content. Sewage sludge amendments are regulated by the European Directive 86/278/EEC (European Council Directive 1986), which compels the raw sludge stabilization and sets heavy metals limit values

for sludge reuse on soil. There is no specific legislation for the reuse on agricultural land for non-sewage organic wastes, although it might be limited by their pathogen and heavy metal content when placed on the market as fertilisers (according to each member state transpositions of the EC Regulation No 2003/2003 (European Commission 2003b) and the EC Regulation No 1774/2002 (European Commission 2002)). The European Directive 91/676/EEC (European Council Directive 1991), which concerns the protection of waters against pollution caused by nitrates from agricultural sources, can also limit the use of wastes in soil. Hence, only chemical assays are considered to limit the use of organic wastes in soil, despite their disadvantages compared to biological assays (Crouau et al. 2002).

A great variety of wastes are currently produced in the European Union as a result of the spread of wastes treatment technologies which minimize their volume, and increase their hygienization and ease of handling. Composting of raw wastes or aerobic/anaerobic digestion of raw sludges, followed by composting or thermal drying of the resultant products, are the most common treatments to achieve such goals. It has been shown that such treatments have consequences on the wastes' physical, chemical, and biological parameters (Schowanek et al. 2004), but not much is known about the impact on soil organisms of these different products. The published studies on the effects of organic wastes on soil biota range from laboratory studies to field effects. No harmful effects on the croplands soil fauna have been reported when wastes are applied at agronomic rates, but ecological risk can not be excluded for all wastes or for higher application rates.

Ecological risk assessment has been defined as the process of estimating the likelihood that a particular event will occur under a given set of circumstances (Maltby 2006), which can be used both as a tool for taking decisions in current situations and for predicting future risks. In any risk assessment, the first step is to derive, the “predicted no-effect concentration” (PNEC). This is the concentration at which no harmful effects on the environment are expected. PNEC values are then compared with the “predicted environmental concentrations” (PEC) in the studied soil in order to calculate the “risk quotient” (RQ= PEC/PNEC), which is used to determine the ecological risk (when RQ>1).

In this study the safe amendment rates of different wastes estimated from a set of laboratory toxicity data obtained from a soil bioassay battery, are compared with several realistic amendment scenarios in order to determine the ecological risk amendments with such wastes.

The aims of the present study were (a) to assess the suitability of the species sensitivity distribution method for estimating organic wastes safe amendment rates, (b) to compare the estimated rates with plausible amendment rates in agricultural soils according to different scenarios based on crop demands and usual amendment rates in the European Union, and (c) to determine the relationship between waste composition and ecotoxicity, and also the influence of waste treatments and post-treatments on their ecotoxicity.

2. METHODS

2.1. Organic wastes

Seven materials were selected in order to represent the variety of organic wastes currently generated in Europe and used as amendment (Table 1): two dewatered sewage sludges (AED and AND, obtained from an aerobic and anaerobic digestion of raw sludge respectively), two composted sewage sludges (AEC and ANC, from composting of the aerobic and anaerobic sludge respectively), two thermally-dried sewage sludges (AET and ANT, obtained from the drying at temperatures around 140°C of the aerobic and anaerobic sludge respectively), and a thermally-dried pig slurry (SLT). Wastes treatments and post-treatments are summarized in Table 1.

Dewatered sludge is the final product of wastewater treatment, by aerobic or anaerobic digestion, followed by dewatering. This process reduces the sludge volume and pathogen content, and increases its stability. Some treatment plants carry out additional sludge post-treatments in order to enhance hygienization and to further reduce water content, composting and thermal drying being the most common technologies. In the dewatered sludges used in this study (AED, AND), dewatering was carried out by centrifugation.

Both sludge composts (AEC, ANC) were produced with a sludge-pine wood chips mixture (1:4.5 v/v). AEC was composted in a heap for 50 days, with continuous tumbling of the heap during the first month, followed by weekly turning. ANC was composted for 15 days in a rotatory tunnel with air injection. At the end of the composting period, composts were sieved to 1 cm.

Thermally-dried sludges (AET, ANT) were prepared by placing dewatered sludge for 45 minutes in a heated rotatory cylinder and by injecting hot air, which provided a temperature between 130 to 150ºC. Thermally dried pig slurry

(SLT) was obtained by anaerobic digestion of raw slurry followed by thermal drying in a rotatory tunnel at 130ºC.

In the laboratory, each waste was dried at 60ºC for 48-72 hours, depending on its initial water content, and then ground and sieved (<2mm). These steps were unavoidable in order to ensure the homogeneity and accuracy of the lower test concentrations. These materials were used for characterization and for the preparation of the soil-waste mixtures used in the bioassays.

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. (2007).

2.2. Effects on soil microorganisms

The substrate-induced respiration (SIR) was used as endpoint, measured according to the OECD Guideline 217 (Organisation for Economic Co-operation and Development 2000) and using glucose as substrate. The soil used in this bioassay was the top-layer (20 cm) of a freshly collected sandy soil from Serra de Prades (Tarragona, Spain). The soil had a water pH of 6.3 (1:2.5 v/v), an organic matter content of 0.5%, and a sandy texture (69% sand, 22% silt and 10% clay). The soil was sieved to 2 mm and, taking into account its original moisture content, was used for soil-waste mixtures preparation. Eight waste concentrations were tested (0, 10, 21.2, 44.8, 94.9, 201, 425, and 900 g kg^{-1}). Each replicate consisted in a 1.2 liter-capacity plastic container covered with a lid to avoid desiccation and filled with 500 g of wet soil-waste mixture. Moisture content of mixtures was adjusted to 50% of their maximum water holding capacity. Three replicates were prepared for each concentration. Containers

159 stored in the dark at 22ºC for 28 days and aerated three times per week to
160 avoid anoxic conditions. As indicated in the OECD Guideline 217, a 40-g
161 subsample (wet weight) of each replicate was mixed with 0.2 g of glucose, and
162 glucose-induced respiration rates were measured during 12 consecutive hours
163 according to Anderson (1982). The glucose-induced respiration rates were
164 expressed as carbon dioxide released (mg CO_2/kg dry soil/h). The mean
165 respiration rates were measured at day 1 (acute toxicity) and day 28 (chronic
166 toxicity) in the treated soil samples and a percentage of respiration was
167 calculated in comparison to that in controls.
168
169 **2.3. Effects on plants**
170 Effects on seedling emergence and growth of three plant species (*Brassica*
171 *rapa*, *Lolium perenne*, and *Trifolium pratense*) were assessed according to the
172 OECD Guideline 208 (Organisation for Economic Co-operation and
173 Development 2003). Experiments were performed in artificial soil, prepared as
174 indicated in the OECD Guideline 207 (Organisation for Economic Co-operation
175 and Development 1984). A preliminary assay using germination as endpoint (0,
176 1, 10, 100, 1 000 g kg^{-1}) was used to determine a range of concentrations for
177 the definitive assay, consisting of six concentrations in a geometric progression.
178 Soil-waste mixtures were adjusted and maintained at 60% of their water-holding
179 capacity during the assay. Each replicate consisted of a 250 ml plastic cup filled
180 with 100 g of soil-waste mixture (dry weight), and five replicates were prepared
181 for each concentration. Then, ten seeds were introduced in each replicate,
182 which were maintained in a 16:8 h light/dark and 15/21ºC period at 70% relative
183 humidity. Seedling emergence percentage was determined when 50% of the

184 seeds in controls germinated. At this point, 5 seedlings per replicate were
185 retained, and the remaining plants were removed. After 28 days, seedling
186 growth was measured as shoot length.
187
188 **2.4. Effects on collembolans**
189 Waste toxicity values were calculated from the raw data of Domene et al.
190 (2007). Effects on the reproduction of the soil collembolan *Folsomia candida*
191 were determined based on International Organization for Standardization (ISO)
192 Guideline 11267 (1999). The assay was performed in artificial soil-waste
193 mixtures. Twelve concentrations were tested (0, 1, 2, 3.9, 7.9, 15.8, 31.6, 63.1,
194 126, 251, 501, and 1 000 g kg^{-1}). Soil-waste mixtures were adjusted at around
195 60% of their water-holding capacity. Five replicates per concentration were
196 prepared in sealed 100 ml-flasks. Ten individuals 10 to 12 days-aged were
197 introduced in each flask. Replicates were aerated twice a week and maintained
198 in the dark at 21ºC. The animals were fed with 3 mg of yeast at the start of the
199 assay and after 14 days. The number of surviving adults and juveniles was
200 determined at day 28. Each replicate was flooded with water to float the adults
201 and juveniles. A dark dye was added to facilitate counting and a photograph
202 was taken. Adults and juveniles were counted using the image treatment
203 software ImageTool 3.0, distinguishable by their clearly different sizes.
204
205 **2.5. Effects on enchytraeids**
206 Effects on the reproduction of the soil enchytraeid *Enchytraeus crypticus*
207 were determined according to ISO Guideline 16387 (International Organization
208 for Standardization 2004). The assay was also performed in artificial soil-waste

mixtures. Ten concentrations were evaluated (0, 2.5, 5, 10, 25.1, 63.1, 158, 398, 700, and 1 000 g kg^{-1}). Water content of soil-waste mixtures was adjusted to 60% of their water-holding capacity. Four replicates per concentration were prepared, each in 150 ml-flasks filled with 30 g of the test substrate. Ten adults (clearly identified by the clitella) were introduced in each flask. The animals were fed with 25 mg of ground oat at the start of the assay, and weekly thereafter. Replicates were aerated twice a week and maintained in the dark at 21ºC. The number of surviving adults and juveniles was determined after 28 days.

2.6. Effects on earthworms

The earthworm *Eisenia andrei* was used, as indicated in ISO Guideline 11268-2 (International Organization for Standardization 1996), to determine effects reproduction and fresh weight of the individuals. The assay was carried out in artificial soil, using eight test concentrations (0, 10, 25.1, 63.1, 158, 398, 700, and 1 000 g kg^{-1}). The soil-waste mixtures water content was adjusted to 60% of their water holding capacity. Each replicate consisted of a 1000 ml-container covered with a perforated lid that allowed aeration filled with 500 g (dry weight) of moist soil-waste mixture. Ten clitellated individuals of synchronized age (4 weeks difference at most), and 267 to 598 mg weight, were placed in each container. Animals were fed with 5 g cooked oat flakes at the start and weekly thereafter. Replicates were maintained in a 16:8 light:dark photoperiod, 70% of relative humidity and a constant temperature of 21ºC for 28 days. Fresh weight of adults was measured at after 14 and 28 days of exposure. At day 28, adults were counted and removed from the test substrate,

and the replicates were incubated 28 more days in order to allow juveniles to emerge and grow. After this period, each replicate was placed in a water bath at a temperature of 60ºC. After 20 minutes, juveniles appeared at the substrate surface and were collected and counted.

2.7. Data treatment

For all bioassays and sublethal endpoints, the “effective concentration” for 20% inhibition (EC20) was calculated using Statistica 6.0, together with its 95% confidence intervals. Values were calculated from suitable regression models (exponential, Gompertz, hormesis, linear or logistic), chosen on the basis of the best fit to the data, and according to the criteria indicated in Stephenson et al. (2000). For *E. andrei*, the “no observed effect concentration” (NOEC) for fresh weight after 14 days of exposure was calculated instead of EC20 by means of the Bonferroni test, since for most wastes, inhibition with respect to the controls always was lower than 20%.

2.8. Wastes ecological risk assessment

2.8.1. PNEC estimation

PNEC is the concentration below which an ecosystem is not expected to suffer an unacceptable damage, according to a predefined acceptable effect level (LCx, NOEC, ECx) on different organisms. In the present study, selection of species, endpoint, and acceptable effect level for the risk assessment of organic wastes were based on the recommendations of European Commission (2003a) and Traas (2001). Chronic toxicity data for each waste were obtained

from different taxonomic groups: primary producers (three plant species), consumers (collembolans, enchytraeids, and earthworms), and decomposers (microorganisms). The acceptable effect level in this study was defined as the EC20 rather than NOEC for each endpoint for two main reasons. First, ECx is more reliable than NOEC, given the higher statistical robustness of the first approach (Moore and Caux 1997, Jager et al. 2006). Second, a 20% reduction of an endpoint may be considered a realistic value of maximum tolerable inhibition, given that several authors have reported that NOEC values are equivalent to a response inhibition of 5 to 30% with respect to the control (Hoekstra and van Ewijk 1993, Pack 1993, Moore and Caux 1997).

Among the available approaches to the PNEC estimation, we selected the species sensitivity distribution method (SSD) according to Aldenberg and Jaworska (2000). This method assumes that the acceptable effect level (sensitivity) of the different species in an ecosystem follows a probability function called “species sensitivity distribution”. Then, from a limited number of species, and assuming that they are a random sample of the whole ecosystem, an acceptable effect level for all the ecosystem’s species can be estimated (Van der Hoeven 2004). PNEC values for each waste were estimated, from the set of chronic toxicity values from different bioassays presented in Table 2, by means of the software ETX 2.0 (Van Vlaardingen et al. 2004). After checking the data normality, the program calculates a normal distribution of the entered toxicity data and provides the SSD. From this distribution, the program estimates the hazardous concentration (HC5) and its two-sided 90% confidence interval, which is selected in this study to represent the PNEC. The HC5 is the estimated 5th percentile of the distribution, which represents the concentration expected to

284 be protective of the 95% of the species of an ecosystem. PNEC values obtained
285 in the laboratory (g kg^{-1}) were converted to amendment rates in agricultural soils
286 (t DM ha^{-1}) assuming an ideal agricultural soil with a 20 cm plough layer, and a
287 density of 1.25 g cm^{-3}.
288
289 *2.8.2. PEC estimation and risk characterization*
290 The predicted environmental concentrations (PEC) were also estimated
291 assuming an ideal soil with a 20 cm plough layer, and a density of 1.25 g cm^{-3}.
292 Different PEC values were determined according to several scenarios. A first
293 group of scenarios was based on different agronomical demands of N and P for
294 different crops obtained from Johansson et al. (1999). More precisely, we
295 estimated for each waste the amendment rates that supply 100 kg N ha^{-1} (oat
296 and spring barley), 150 kg N ha^{-1} (wheat), 10 kg P ha^{-1} (oats, spring wheat,
297 spring barley, and winter wheat), and 20 kg P ha^{-1} (peas, and sugar beet).
298 Amendment rates according to these N and P demands were calculated from
299 the hydrolysable-N and total P values of the original wastes (see Domene et al.
300 2007). Finally, the last scenario was based on the median maximum
301 amendment rate in the European Union (2 t DM ha^{-1}) according to European
302 Commission (2001). Then, for each waste and scenario, the ecological risk
303 quotient was calculated (RQ=PEC/PNEC). Risk was considered as acceptable
304 if RQ was below 1.
305
306 **2.9. Relationship between waste parameters and PNEC values**
307 Physico-chemical properties of the wastes in this study (dry matter, water
308 holding capacity, pH, electrical conductivity, organic matter, stable organic

309 matter, total N, non-hydrolyzable N, hydrolysable N, NH_4-N, P and K) were
310 measured together with the heavy metal (Cd, Cr, Cu, Hg, Ni, Pb, Zn) and the
311 organic pollutant contents (polychlorinated dibenzodioxins and dibenzofuranes
312 [PCDD/F], polychlorinated biphenyls [PCB], di(2-ethylhexyl)phthalate [DEHP],
313 nonylphenols [NPE], polycyclic aromatic hydrocarbons [PAH], and linear
314 alkylbenzene sulphonates [LAS]). The values of each parameter in each waste
315 together with the analysis methods used have been already published in
316 Domene et al. (2007).

317 The contribution of the waste composition to the estimated PNEC in the
318 different wastes was assessed by means of Pearson correlation of the PNEC
319 values with the concentration of each individual pollutant, the sum of heavy
320 metal concentrations, the sum of organic pollutant concentrations, the sum of
321 persistent organics (PAH, PCB, and PCDD/F), the sum of non-persistent
322 organics (DEHP, LAS, and NPE), the sum of all pollutant concentrations, and
323 each physico-chemical parameter. All the correlations were calculated with the
324 log-transformed values using SPSS 13.0.

325

326 **3. RESULTS**

327

328 **3.1. Wastes toxicity**

329 As shown in Table 2, the soil substrate-induced respiration (SIR) test was
330 not sensitive to wastes, since no inhibition was observed with the exception of
331 pig slurry (SLT) at day 1. In addition, we failed in obtaining valid outcomes for
332 reproduction of the earthworm *E. andrei*, since the number of juveniles in
333 controls was below 30 in most wastes, which is not acceptable according to the

334 ISO Guideline 11268-2 (International Organization for Standardization 1996).
335 We also failed in finding an effect on fresh weight after 28 days since no
336 significant inhibition was found, probably due to the high variability between
337 replicates. On the contrary, inhibitory effects on fresh weight after 14 days of
338 exposure were observed in most of the wastes. Concerning plant, enchytraeid,
339 and collembola tests, all the assessed sublethal endpoints were sensitive to
340 wastes (Table 2), as they were inhibited with increasing waste concentration.
341 Fauna reproduction was generally more sensitive to wastes compared to plant
342 endpoints. However, the fauna sensitivity differed depending on the waste.
343 Collembola reproduction was the most sensitive endpoint to composted and
344 thermally dried sludges, while enchytraeid reproduction presented a high
345 sensitivity to dewatered sludges.

346 Most of the bioassays and sublethal endpoints were significantly correlated
347 (Pearson $p<0.05$). The exceptions were EC20 for reproduction in *F. candida*,
348 uncorrelated with the results in the remainder bioassays and EC20 for
349 reproduction in *E. crypticus*, not correlated with EC20 for germination in *B. rapa*
350 and *L. perenne*.

351 Concerning the data of Table 2 that were finally used for the PNEC
352 derivation, soil microbial respiration was only used for this purpose in the case
353 of pig slurry. In addition, given our concerns about earthworm test results, and
354 despite of the fact that inhibitory effects on fresh weight after 14 days of
355 exposure were observed in most of the wastes, we did not use this endpoint for
356 PNEC derivation. The remainder data presented in Table 2 were used for
357 PNEC calculation. In the specific case of plants, and in order not to include
358 more than one endpoint for the same plant species (emergence and growth),

359 we used only the most sensitive endpoint for each species for the PNEC
360 calculation (according to Janssen et al. 2004).
361
362 **3.2. Risk characterization**
363 Derived PNEC (HC5) for each waste and PEC values in the different
364 scenarios are presented in Table 3. Datasets obtained from the different
365 bioassays followed a normal distribution in all the wastes (Anderson-Darling
366 test). Highest PNEC values were found for composted sludges. Aerobic
367 dewatered sludge and aerobic thermally dried sludge were the most toxic as
368 may be concluded from the lower PNEC values.
369 The data for the different fertilization scenarios, showed that if the
370 amendment rates of the studied wastes were based on N or P crop demands,
371 application rates of all wastes would usually be below 7 t DM ha^{-1} (Table 3), and
372 in the range of the maximum application rates in different European countries
373 (0.5-10 t DM ha^{-1}) (European Commission 2001).
374 Risk quotients for the different scenarios indicated that no harmful effects on
375 soil ecosystems were likely to occur if wastes were applied according to crop
376 demands of P (Table 4). Furthermore, using the median maximum amendment
377 rate in Europe (2 t DM ha^{-1}), no risk for soil ecosystems should be expected for
378 the studied wastes. On the contrary, risk should be expected for some wastes
379 (AET and AND) when applied to crops with low N demands, while even higher
380 risk should be foreseen for some wastes (AET, AND, SLT) in crops with high N
381 demands (Table 4).
382
383

3.3. Relationship between waste parameters and PNEC

No significant relationships were found between PNEC values and concentrations of single pollutant or pollutant group in wastes. Similarly, no correlations were found for physicochemical properties of wastes, to the exception of the significant negative correlation between PNEC and ammonium content ($r = -0.766$, $p = 0.045$), and the marginal positive correlation between PNEC and stability of wastes ($r = 0.753$, $p = 0.051$) (Figure 1).

The lack of correlation with pollutant burden and the general correlation of toxicity with parameters related to waste's stability (ammonium content and hydrolysable nitrogen) has also been found for most of the bioassays and endpoints ($p<0.05$). The only exception was the reproduction inhibition in *F. candida*.

4. DISCUSSION

4.1. Quality assessment of organic amendments in Europe

Several external inputs (mineral fertilisers, organic amendments, microbial inoculants, and pesticides) are applied to agricultural soils to maximise productivity and economic returns. However, side effects of such amendments on soil organisms are not usually taken into account (Bünemann et al. 2006).

The current transposition to some member states of the European Union legislation concerning sewage sludge and organic wastes which are considered as fertilizers, allows and encourages the reuse in agricultural land of organic wastes with low pollutant content. More precisely, the heavy metals content is taken into account both for sewage sludges (European Council Directive 1986),

409 and organic wastes considered as commercial fertilizers (depending on the
410 exact transpositions to the member states of the Regulation (EC) No 2003/2003
411 (European Commission 2003b). The nitrogen content of wastes may also limit
412 these amendments in vulnerable zones (European Council Directive 1991).
413 However, it is widely accepted that chemical methods have important limitations
414 to predict waste effects on soil organisms (Crouau et al. 2002). They do not
415 account for all current potential pollutants in wastes, and give no indications
416 about bioavailability, interactions between pollutants, secondary products or
417 final effects on the soil dwelling organisms and soil ecosystem.

418 Hence, the main problem is not the use of wastes in soil per se, but the lack
419 of ecologically relevant methodologies to monitor the quality and environmental
420 safety of wastes when used as amendments, given the soil limited resistance to
421 pollution (Kördel and Römbke 2001). For this reason, ecotoxicological criteria
422 should be included, in addition to chemical methods, for monitoring organic
423 waste quality.

424

425 **4.2. Use of test batteries in risk assessment**

426 Contaminants, or mixtures of contaminants, have markedly differential
427 effects on the populations of different soil-dwelling species. This highlights the
428 importance of including different species in a battery of bioassays for any
429 ecological risk assessment (Van Gestel et al. 2001).

430 Test batteries have been widely used in aquatic ecotoxicology (Davoren et
431 al. 2005, Mariani et al. 2006), but are still scarce in soil ecotoxicity evaluation.
432 This approach has been used to evaluate polluted sites (Achazi 2002), to
433 assess the effectiveness of remediation treatments (Mendonça and Picado

2002, Molina-Barahona et al. 2005), to provide data for derivation of non harmful chemical concentrations in soil (Lock and Janssen 2003, Kuperman et al. 2006, Römbke et al. 2006) and to assess sewage sludge quality (Renoux et al. 2001, Robidoux et al. 2001).

There are no universal rules for suitable species selection, and furthermore, there is no agreement about the most suitable endpoints to predict effects on ecosystems. For some ecologists, maintenance of the ecosystem structure is the main aim, and any loss in population size, species diversity or genetic diversity is detrimental. For others, changes in the ecosystem structure are not critical if functions are preserved. Both approaches have been experimentally evaluated, and also the linkage between them (Wentsel et al. 2003). Hence, the use of a set of endpoints reflecting both structural and functional effects of pollutants on ecosystems would be the best choice. McMillen et al. (2003) recommend a minimum test battery including tests with plants, soft-bodied invertebrates and soil arthropods together with tests designed to assess ecosystem functions such as decomposition or nitrification. Based on this set of species and endpoints, a coarse vision of potential effects on the ecosystem structure and function is possible. Achazi (2002), concluded that a proper assessment of soil pollution was achieved using a test battery using both aquatic and soil tests (microbial activity and ammonium oxidation, earthworm and collembolan reproduction, and plant germination and growth). In the present study, different bioassays were selected in order to take into account both functional (microbial respiration) and structural endpoints (the performance of several soil organism). Plant emergence and growth, collembolan and enchytraeid reproduction, and earthworm fresh weight showed to be sensitive to

459 different organic wastes. Microbial respiration was not inhibited in most of the
460 wastes, so was not always useful as endpoint (Table 2). This unexpected result
461 could be attributed to opportunistic taxa that replaced the extinct taxa in a
462 higher extent with increasing concentrations or to the contribution of waste
463 microorganisms.
464
465 **4.3. Species sensitivity distribution in risk assessment of organic wastes**
466 The SSD is increasingly used to complement or replace arbitrary
467 assessment factors in chemicals risk assessment (Grist et al. 2002). Its
468 application is recommended by public organisations in Denmark, The
469 Netherlands, and Canada (Jensen et al. 2001) and also, recently, by the
470 European Union (European Commission 2003a). This methodology was initially
471 developed by Kooijman (1987), and has been modified and improved by several
472 authors (Posthuma et al. 2002). The method uses acceptable pollutant effect
473 levels (LCx, NOEC, ECx) for a chemical for a limited number of species to
474 determine an exposure level or concentration below which the ecosystem
475 species will not suffer unacceptable damages. The method assumes that the
476 available set of toxicity data for different species is randomly drawn from all
477 species potentially present in the ecosystem (Van der Hoeven 2004).
478 There are several limitations of the SSD methods for deriving soil quality
479 criteria or assessing ecological risk, and this is why these methods have been
480 criticized for their low ecological relevance in terms of the species and
481 endpoints selected (Forbes and Calow 2002, Duboudin et al. 2004, Van der
482 Hoeven 2004), exposure time (Jager et al. 2006), and methodology and sample
483 size (Van Straalen 2002, Duboudin et al. 2004). In addition, it has been

484 indicated that SSD methods do not take into account the modifying influence of
485 biotic and abiotic interactions acting in real ecosystems (Van Straalen and
486 Bergema 1995). This lack of ecological relevance has been supported by
487 Roessink et al. (2006). However, most of the published studies have shown that
488 the hazardous concentration values calculated using SSD for single-species in
489 the laboratory lead to harmful concentrations similar to those observed in field
490 studies at the community and ecosystem levels (Sloof et al. 1986, Versteeg et
491 al. 1999, Smit et al. 2002, Hose and van den Brink 2004, Schroer et al. 2004).
492 In the present study, the safe amendment rates derived from SSD according
493 to the methodology of Aldenberg and Jaworska (2000), showed clearly different
494 values for the different studied wastes, indicating its suitability for comparative
495 purposes (Table 3). However, its use for the prediction of harmful effects in real
496 situations can not be confirmed as we lack a field validation of the predictions of
497 this study.
498
499 **4.4. Relevancy of the estimated safe amendment rates**
500 All the studied organic wastes could be applied to soils according to pollutant
501 limit values of the Directive on Sludge (European Council Directive 1986)
502 except for AET and AED (given their high Pb concentrations).
503 According to the risk quotient determined in our study for different scenarios
504 (Table 4), none of the wastes is expected to exert noxious effects on the soil
505 biota if applied according to the median maximum amendment rate for
506 agriculture in the European Union (2 t ha^{-}) or according to P low and high crop
507 demands (always below 1 t DM ha^{-1}). On the contrary, if amendment is based
508 on low or high N crop demands, risk should be expected for the most toxic

509 wastes (AET, AND, and SLT). In addition, and despite the lack of risk of AED
510 and ANT, their safe amendment rates (PNEC) are within or around the range of
511 the maximum application rates allowed in different European countries (0.5-10 t
512 DM ha^{-1}) (European Commission 2001), something that indicates a likely
513 potential risk of these wastes. This is especially significant in the case of AND
514 and SLT, which could be used on soils according to the Directive on Sludge
515 (European Council Directive 1986) despite their predicted toxicity. The high risk
516 of pig slurry amendments with respect to other wastes agrees with the results of
517 Diez et al. (2001), who indicate negative effects of pig slurry amendment at 3.6 t
518 DM ha^{-1} (dry weight) on *F. candida* reproduction in laboratory tests. On the
519 contrary, they did not find noxious effects on plants and enchytraeids.

520 A non exhaustive selection of studies on the ecotoxicological effects of
521 organic waste amendments on soil biota is presented in Table 5. As a general
522 pattern, no harmful effects on crops and soil biota have been reported in field
523 studies when wastes are applied below the maximum amendment rates allowed
524 in the European Union (0.5-10 t DM ha^{-1}). No effects have been found below 20
525 t DM ha^{-1} (Krogh et al. 1997), but on the contrary, noxious effects of sewage
526 sludge to soil biota in the field have been reported above 187.5 DM t ha^{-1}
527 (Andrés 1999, Barrera et al. 2001). Other studies have reported effects at lower
528 amendment rates in the laboratory (8.6 t DM ha^{-1} in Krogh et al. 1997, and 9 t
529 DM ha^{-1} in Andrés and Domene 2005), and also bioaccumulation in earthworms
530 at even lower concentrations in the field (Matscheko et al. 2002). The
531 magnitudes of amendments causing harm to soil biota (Table 5) are in
532 accordance with results from the present study. On the other hand, it is
533 noticeable that safe amendment rates of the composted sludges in this study

534 (with predicted safe amendment rates of 21 and 55 t DM ha^{-1}) are much greater
535 than the amendment rates based on the crop demands.
536 Despite this, these conclusions are only valid for the short-term, as they are
537 based on one-month studies at most with a limited number of species.
538 Furthermore, the toxicity data used for deriving the safe amendment rates were
539 obtained in the laboratory using OECD artificial soil and require a field
540 validation.
541
542 **4.5. Relationship between waste parameters and safe amendment rates**
543 In our study, no significant correlations were found between pollutant
544 concentration and safe amendment rates. This finding indicates the failure of
545 chemical methods in predicting effects on organisms and the need for including
546 ecotoxicological criteria in legislation. It is worthy of notice that ammonium
547 content and waste stability are the most influential determinants of the
548 maximum amendment rates derived in this study (Figure 1). The more stabilized
549 a waste, the lower is its ammonium content, the lower is its toxicity and the
550 higher the safe amendment rate. The coupled behaviour of both parameters
551 (stability and ammonium content) is not casual, since waste stabilization implies
552 a higher recalcitrant organic matter content and a lower amount of hydrolyzable
553 nitrogen and of ammonium release (Witter and Lopez-Real 1988, Martins and
554 Dewes 1992). During decomposition of wastes in soil, nitrogen losses are
555 initially mainly as ammonium and ammonia. This also explains the marginal
556 correlation between hydrolysable nitrogen and safe amendment rates. These
557 results agree with published reports on phytoxicity of amendments with non
558 stabilized organic wastes (Zucconi et al. 1981, Pascual et al. 1997, Atiyeh et al.

559 2000, Huang et al. 2004, Zmora-Nahum et al. 2005) and specifically attributed
560 to ammonium (Katayama et al. 1985). This pattern has also been indicated for
561 soil fauna after organic amendments (Neher 1999) or application of nitrogen
562 fertilizers (Seniczak et al. 1994).

563 Results from this study point out the importance of stabilization treatments
564 like composting prior to the use of organic wastes in soils, as decomposition of
565 low stabilized wastes generate noxious substances like ammonia, phenols, and
566 organic acids (Déportes et al. 1995). The relationship between these
567 parameters and the level of toxicity may be strong enough in the short-term to
568 exceed and mask the differences in pollutant burden existing between of
569 different wastes.

570

571 **CONCLUSIONS**

572

573 The SSD method, as used in the present work, is suitable for comparative
574 purposes of the risk assessment of organic wastes, as demonstrated by clearly
575 differentiated results for different wastes. Predictions for real field situations
576 should to be validated by empirical validation. If the predicted safe amendment
577 rates of the studied wastes are realistic, the median maximum amendment rate
578 in Europe (2 t DM ha^{-1}) and amendments based on P crop demands are safe.
579 On the contrary, some wastes of this study may produce harmful effects if
580 applied according to N crop demands (only slightly above 2 t DM ha^{-1} in some
581 wastes).

582 The toxicity of waste, and therefore the safe amendment rate, is not related
583 mainly to its pollutant burden, at least in the short-term, but primarily to its lack

584 of stability and to noxious compounds such as ammonium, which is released
585 during decomposition of waste in soil. Waste stabilization appears in this study
586 as a suitable treatment to decrease the short-term impact of organic waste on
587 soil biota, therefore to allow its safe reuse application to soil.
588
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592
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Figure 1. Correlation of log-transformed values of stability (%) and ammonium content (%) in wastes with PNEC (tonnes DM ha^{-1}). PNEC is based on the results (EC20 values) of bioassays with a battery of soil organisms applied to seven different organic wastes.

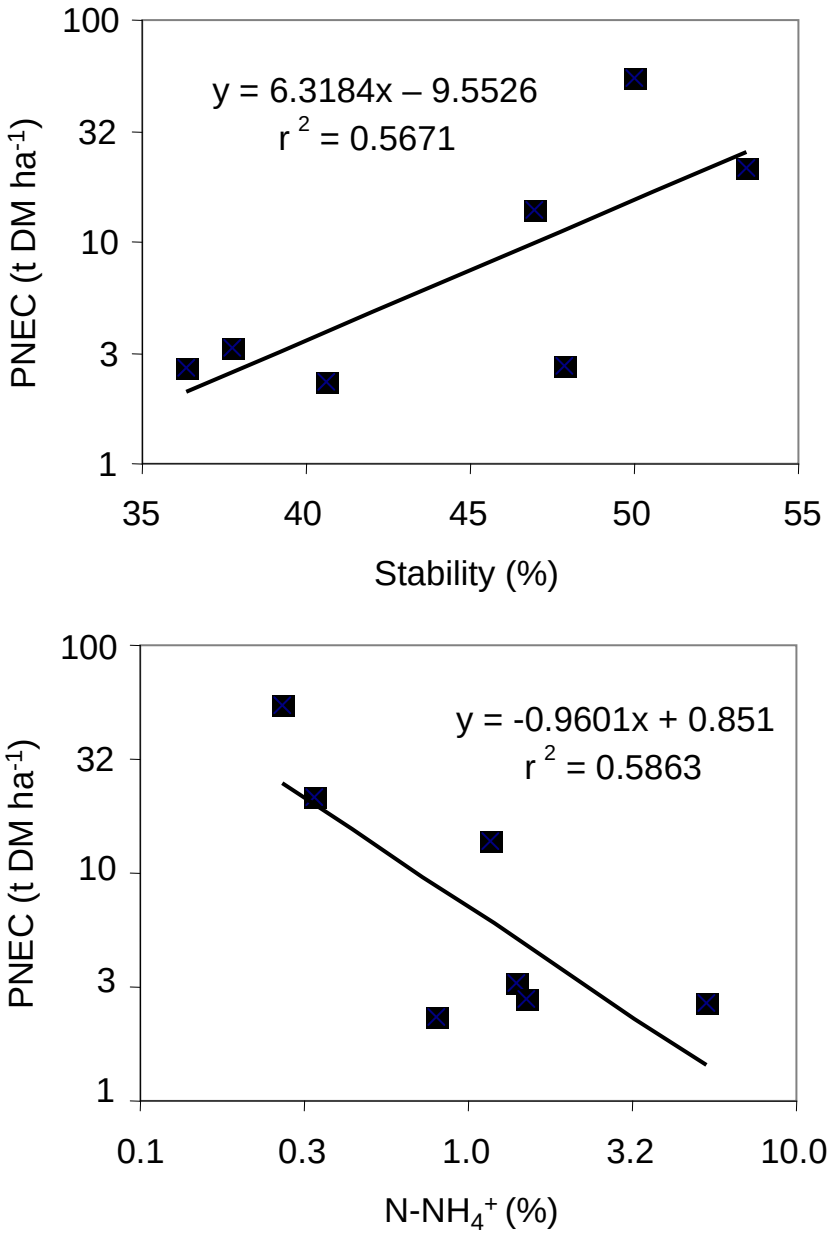

Table 1. Origin, treatments and post-treatments of the organic wastes used for ecotoxicity testing using a battery of soil organisms; WWTP = wastewater treatment plant; WTP = waste treatment plant.

Waste	Origin	Treatment	Post-treatment
AED	Banyoles WWTP	Aerobic digestion, dewatering	None
AEC	Banyoles WWTP	Aerobic digestion, dewatering	Composting in tunnel
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. Effects on sublethal endpoints of different organic wastes, measured as EC20 in OECD artificial soil and a natural soil in the case of soil microorganisms respiration. EC20 values are expressed as g kg^{-1} and presented with their 95% confidence intervals. Blank cells indicate a lack of inhibition. Earthworm's fresh weight was measured after 14 days of exposure and expressed as NOEC; reproduction of collembolans and enchytraeids and seedling growth was measured after 28 days; emergence was measured around 7 days (when 50% of the seeds in controls had emerged). For waste abbreviations see Table 1.

Species	Endpoint	AEC	AED	AET	ANC	AND	ANT	SLT
Microorganisms	Respiration	-	-	-	-	-	-	1.6 (1.5, 1.7)
Eisenia andrei	Fresh weight	158	-	-	158	63.1	25.1	10
Enchytraeus crypticus	Reproduction	551 (248, 1221)	1.4 (0.9, 2.0)	5.8 (0.8, 25.2)	98.2 (44.5, 215)	1.6 (1.2, 2.2)	17.5 (12, 25.2)	2.3 (1.5, 3.5)
Folsomia candida	Reproduction	26.3 (4.5, 134)	7.9 (5.8, 10.8)	1.1 (0.7, 1.5)	12.1 (5.6, 25.2)	14 (10.8, 18.0)	6.7 (4.5, 9.9)	18.1 (6.4, 48.4)
Brassica rapa	Emergence	193 (161, 230)	11.6 (5.8, 19.5)	16.4 (13.0, 20.1)	585 (535, 639)	58.9 (27.4, 117)	13.4 (7.6, 21.2)	7.7 (6.1, 9.5)
Brassica rapa	Growth	206 (91.1, 453)	8.1 (6.5, 9.8)	18.5 (6.4, 39.3)	586 (535, 643)	74.5 (55.5, 98.8)	39.5 (25.9, 58.3)	18.6 (14.8, 22.8)
Lolium perenne	Emergence	203 (197, 209)	26.8 (21.4, 33.1)	28.4 (21.4, 33.1)	612 (541, 693)	75.8 (35.5, 129.6)	35.1 (29.4, 62.3)	8.7 (6.5, 11.2)
Lolium perenne	Growth	223 (217, 228)	13.6 (10.9, 16.6)	19.0 (15.9, 22.4)	594 (445, 791)	72.4 (54.8, 108)	33.3 (28.6, 38.6)	17.3 (14.4, 20.6)
Trifolium pratense	Emergence	161 (128, 203)	16.7 (14.1, 19.6)	15.5 (11.3, 20.5)	367 (313, 431)	16.3 (10.3, 24.0)	17.7 (14.5, 21.4)	4.8 (1.69, 4.14)
Trifolium pratense	Growth	183 (173, 192)	13.1 (9.8, 17.0)	15.6 (13.1, 204)	177 (154, 204)	14.1 (10.2, 18.7)	17.1 (13.3, 21.5)	4.5 (0.9, 11.2)

Table 3. Estimated PNEC (HC5, the hazardous concentration protecting 95% of the species), derived from the EC20 values of a battery of soil organisms, and PEC values for each waste, expressed as tonnes of waste (dry weight) per hectare of soil. Low and high crop demands of N and P from Johansson et al. (1999). Median maximum amendment rates from European Commission (2001). For waste abbreviations see Table 1.

Waste	PNEC	PEC (tones DM ha^{-1})				
	(tones DM ha^{-1})	Low N demand (100 kg N ha^{-1})	High N demand (150 kg N ha^{-1})	Low P demand (10 kg P ha^{-1})	High P demand (20 kg P ha^{-1})	EU median maximum amendment rate
AEC	54.6	4.44	6.67	0.45	0.91	2
AED	3.3	2.17	3.26	0.49	0.98	2
AET	2.3	2.41	3.61	0.49	0.98	2
ANC	21.4	13.2	19.74	0.35	0.70	2
AND	2.8	3.79	5.68	0.30	0.60	2
ANT	13.9	2.87	4.30	0.34	0.68	2
SLT	2.7	1.94	2.91	0.49	0.98	2

Table 4. Risk quotient (RQ=PEC/PNEC) for each waste and scenario, expressed as tonnes of waste per hectare of soil (dry weight). Risk is acceptable when RQ is below 1. For waste abbreviations see Table 1.

Waste	Low N demand (100 kg N ha^{-1})	High N demand (150 kg N ha^{-1})	Low P demand (10 kg P ha^{-1})	High P demand (20 kg P ha^{-1})	EU median maximum amendment rate
AEC	0.08	0.12	0.01	0.02	0.04
AED	0.66	0.98	0.15	0.30	0.60
AET	1.03	1.54	0.21	0.42	0.85
ANC	0.61	0.92	0.02	0.03	0.09
AND	1.36	2.04	0.11	0.21	0.72
ANT	0.21	0.31	0.02	0.05	0.14
SLT	0.72	1.08	0.18	0.36	0.74

Table 5. Reported effects of organic wastes on soil biota obtained from laboratory and field studies. When explicit information was not available in the reference, waste concentrations were converted to an equivalent field amendment rate assuming an ideal soil with a 20 cm plough layer, and a density of 1.25 g cm^{-3}.

Reference	Site	Waste	tonnes DM ha^{-1}	Effect on soil biota
Andrés & Domene 2005	Laboratory	Sewage sludge	9-23	Decrease in faunal density and disturbance of trophic structure.
Andrés (1999)	Restored land	Sewage sludge	375	Impoverishment of the community structure and decrease in the soil oribatid diversity.
Barrera et al. (2001)	Restored land	Sewage sludge	187.5-375	Increase in the adult and juvenile density of two earthworm species (*Allobophora chlorotica*, *Nicodrilus caliginosus*).
Diez et al. (2001)	Laboratory	Pig slurry	>3.6	Significant decrease in the reproduction of the collembolan *Folsomia candida*.
Krogh et al. (1997)	Agricultural land	Cattle manure, sewage sludge	3.5-21	No harm to crops, microarthropods, or earthworms.
Krogh et al. (1997)	Laboratory	Cattle manure, sewage sludges	8.6-25.2	Effects on reproduction of *Folsomia fimetaria*.
Matscheko et al. (2002)	Agricultural land	Sewage sludge	≥1	PBDE and PCB bioaccumulation in earthworms.
Pernin et al (2006)	Laboratory	Sewage sludge spiked with copper	150	Noxious effects on the mesofauna community structure.
Petersen et al. (2003)	Agricultural land	Sewage sludge, household compost	3.6-14.9	No harmful effects on crops.
Renoux et al. (2001)	Laboratory	Sewage sludge	≥20	Noxious effects on plants (*Hordeum vulgare, Lactuca sativa*) and earthworms (*Eisenia andrei*).