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Development of phytotoxicity indexes and their correlation with ecotoxicological, stability and physicochemical parameters during passive composting of poultry manure

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Abstract

Both raw and composted poultry manure is applied as soil amendment. The aims of this study were: 1) to develop phytotoxicity indexes for organic wastes and composts, and 2) to assess the correlation among phytotoxicity indexes, ecotoxicological endpoints and stability and physicochemical parameters during passive composting of poultry manure. Six 2-m^3 composting piles were constructed and four parameter groups (physicochemical and microbiological parameters, ecotoxicological endpoints, and biological activity) were determined at four sampling times during 92 days. Extracts were used to carry out acute toxicity tests on *Daphnia magna*, *Lactuca sativa* and *Raphanus sativus*. Composting decreased average toxicity 22.8% for the 3 species and *D. magna* was the most sensitive species. The static respiration index decreased from 1.12 to 0.46 mg O_2 g OM h^{-1} whilst organic matter reduced by 64.1% at the end of the process. *Escherichia coli* colonies remained higher than values recommended by international guidelines. The *D. magna* immobilization test allowed the assessment of possible leachate or run-off toxicity. The new phytotoxicity indexes ($RGIC_{0.8}$ and $GIC_{80\%}$), proposed in this study, as well as salinity, proved to be good maturity indicators. Hence, these phytotoxicity indexes could be implemented in monitoring strategies as useful ecotoxicological tools. Multivariate analyses demonstrated positive correlations between ecotoxicological endpoints (low toxicity) and biological activity (stability). These two parameter groups were associated at the final sampling time and showed negative correlations with several physicochemical parameters (organic and inorganic contents). The final poultry manure compost was rendered stable, but immature and, thus, unsuitable for soil amending.

***Keywords*:** *Daphnia magna*, *Lactuca sativa*, maturity, phytotoxicity, *Raphanus sativus*, stability.

1. Introduction

Poultry production is a quantitatively important agro industry worldwide. World production of eggs has increased 24.98% between 2001 and 2011, according to statistics from Food and Agriculture Organization of the United Nations. During the same period, egg production in Argentina soared 101.7% (FAOSTAT, 2013). Consequently, a large volume of waste (poultry manure) is an inevitable side effect of this increase.

Commonly, raw poultry manure is applied to farmland as organic amendment (Bolan et al., 2010). This waste contains nutrients (N, P, K), heavy metals (As, Pb, Zn, Ni, Cd, Cu, Mn), xanthophylls, antibiotics, antiprotozoals, antioxidants, mold inhibitors, probiotics, polychlorinated phenols, tetrachlorodibenzo-*p*-dioxin and hormones (Frank et al., 1988; Jackson et al., 2003) that may impact negatively the ecosystem through leaching and runoff. These adverse effects on ecosystem can be circumvented with low-cost composting. Composting minimizes the concentration of phytotoxic substances, controls the spread of pathogens, improves storage and handling of waste, and reduces unpleasant odors (Edwards and Daniel, 1992). The quality of the compost may have either a positive or negative impact on both soil fertility and plant health. Nutrients loss depends on several factors such as aeration, moisture content, temperature and carbon-to-nitrogen (C:N) ratio (Ogunwande et al., 2008). The initial C:N ratio is the most widely used parameter for deciding composting methodology. Poultry manure contains high nitrogen content. Therefore, the degradation process may be improved adding carbon-rich materials (Petric et al., 2009). High initial C:N ratio causes longer composting time (Tuomela et al., 2000), whereas low initial C:N ratio generates higher emission of volatile gases and leachates (Tiquia and Tam, 2000b). Co-composting of poultry manure with other agricultural wastes improves the physicochemical characteristics and reduces the phytotoxicity (Rizzo et al., 2013). Composting may be aerated by passive or active systems (Ogunwande, 2011). Passive aeration is an effective inexpensive treatment system for co-composting poultry manure, poultry litter and sawdust, according to Ogunwande and Osunade (2011), and is more cost-effective than active aeration systems in terms of initial capital investment, operation, maintenance, and operator training costs (Solano et al., 2001).

The use of toxicity tests on aquatic and terrestrial organisms allows the integral assessment of the waste before disposal. Several authors have reported acute toxicity on several organisms exposed to raw (Gupta and Kelly, 1992; Gupta et al., 1997) and composted poultry manure (Komilis and Tziouvaras, 2009). *D. magna* has demonstrated a good sensitivity to assess toxicity of landfill leachate (Matejczyk et al., 2011; Pablos et al. 2011), hazardous wastes (Pablos et al., 2009) and municipal solid waste leachate (Isidori et al., 2003; Bortolotto et al., 2009). Germination index (GI) is the phytotoxicity index used commonly to assess toxicity from complex solid samples, such as waste or compost. However, there is a lack of ecotoxicological tools in the literature when a material demonstrates high toxicity. In addition, integral monitoring strategies have not previously been used to study a passive aeration composting of poultry manure with low quantities of carbon-rich materials. The aims of this study were: 1) to develop phytotoxicity indexes for waste or compost samples, and 2) to assess the correlation between ecotoxicological endpoints with both stability and physicochemical parameters during passive aeration composting of poultry manure. A seed toxicity test was used to assess effects on a terrestrial plant since our objective was to develop the phytotoxicity indexes. On the other hand, *D. magna* test was selected to assess possible leachate or run-off toxicity, since it is a standard toxicity test widely used in monitoring programs of different kind of samples.

2. Materials and methods

2.1 *Composting*

2.1.1 *Experiment*

Poultry manure was collected in an automatized farm of the Zucami® type, located in Hurlingham, Argentina. Six 2-m^3 (1m x1m x1.2m) composting piles were built mixing poultry manure with dry grass

(7:3 v/v) in an experimental field of the National Institute of Agricultural Technology (INTA), Hurlingham, Argentina. Composting piles had an initial C:N ratio of 24.6 ± 3.6:1 and moisture content of 70.6 ± 3.2% wb. In addition, wood chips were added as bulking agent. A static pile with a passive aeration system and V-shaped pipe configuration was used as composting method, according to Ogunwande (2011). A pipe with 35-mm diameter perforations was used, as recommended by Ogunwande and Osunade (2011).

Experimental design consisted of a completely randomized statistical design with 6 composting piles (n=6) and repeated measurements. Each composting pile was the experimental unit (*n* = 6 piles). Sampling was done by quartering from each pile at days 0, 14, 56 and 92 (*n* = 24), according to standardized specifications (USDA and USCC, 2001).Three sub-samples were taken from each composting pile and at each sampling time and were kept at 4 ºC until analysis.

2.1.2 *Physicochemical and microbiological characterization*

The following physicochemical parameters were evaluated: ambient and pile temperature, moisture content (MC), organic matter (OM), total organic carbon (TOC), total Kjeldhal nitrogen (TN), C:N ratio, soluble organic carbon (SC), total phosphorous (TP), soluble phosphorous (SP), major cations, metals, pH, and electrical conductivity (EC), according to standard methods (USDA and USCC, 2001). The major cations (Ca, Mg, K, Na) and metals (Zn, Mn, Cu) were quantified using an atomic absorption spectrophotometer (Varian model 220 A). The percentages of OM and TN losses were calculated using Eq. 1 and 2, according to Paredes et al. (2000).

$$\mathrm{OM\,loss}(\%) = 100 - 100 \times \left[\frac{X_1(100 - X_2)}{X_2(100 - X_1)} \right] \qquad \text{Equation 1}$$

$$\mathrm{TN\,loss}(\%) = 100 - 100 \times \left[\frac{X_1 N_2}{X_2 N_1} \right] \qquad \text{Equation 2}$$

where N_1 and N_2 are the initial and final TN percentages, and X_1 and X_2 are the initial and final ash percentages, respectively.

Commercial kits (Rida Count®) were used for microbiological characterization to determine total coliforms, *E. coli* and *Salmonella* spp. (CFU g^{-1}) provided by R-Biopharm AG.

Biological activity was measured using the static respiration index (SRI) (Iannoti et al., 1993; USDA and USCC, 2001). This technique is a static respiration stability assessment method which is performed in mesophilic temperatures (37 °C) with sealed 500 mL flasks. An electrochemical dissolved O_2 electrode is placed in the headspace of the flask and records the O_2 air concentration drops within the flask. Oxygen uptake rate (OUR) is finally expressed in mg O_2 g^{-1} OM h^{-1} and is calculated via the slope of the O_2 concentration drop. The SRI is the maximum averaged OUR calculated during a 24 hour period (after the initial lag time).

2.2 *Toxicity tests*

In order to simulate the mixture of water-extractable substances present in leachate or runoff, aqueous extracts were prepared mixing a dry sample with deionized water (1:10 w/v). These extracts were stirred at room temperature (23±2 ºC), according to a procedure described by Tiquia et al. (1996).

2.2.1 *Organisms*

Two species of plants and an aquatic crustacean were used as test organisms. A non-chemically treated seed lot of lettuce (*L. sativa* variety “Carilauquen INTA”) and radish (*R. sativus* variety “Puntas blancas”) were obtained from the experimental stations of the INTA, located in La Consulta and San Juan cities, Argentina, respectively. Seeds were kept in a dry environment at 4 ºC.

The aquatic crustacean *D. magna* was reared in a laboratory of ecotoxicology (IMYZA, INTA). The population of daphnid was fed 3-4 times per week with a mixture of several species of algae, under controlled conditions (23 ± 2 ºC and 16L:8D). Dechlorinated and aerated tap water (pH = 8.1 ± 0.3; EC = 642 ± 24 μS cm^{-1}) was used as culture medium.

2.2.2 *Seed germination and root elongation test*

Seed germination and root elongation tests were carried out at 22 ± 2 ºC in darkness for 120-h, according to standardized protocols (Sobrero and Ronco, 2004). Experimental design consisted of 10 treatments (i.e. 9 different concentrations of the extracts and a control group) per composting pile and per sampling time (*n* = 240) using triplicates. The extract concentrations used in the tests ranged from 0.5% to 100% v/v (0.5, 1; 5, 10, 20, 40, 60, 80, and 100%); deionized water was used as a negative control and zinc chloride solutions as positive control. A total of 15 tests were conducted using lettuce and 18 tests using radish. Fifteen seeds of each the species (radish and lettuce) were exposed to 4-mL of each of the nine extract concentrations and control water in 90-mm diameter Petri dishes lined with filter paper (Munktell AB Box 300, SE-790 20 GRYCKSBO, Sweden). A total of 10800 seeds of each species were used in these experiments. The quality controls used were percentage of germination over 90%, coefficient of variation for root elongation below 30%, in negative controls, whilst Zn (zinc chloride) was used as a reference toxic in positive controls. The zinc chloride concentrations at each positive control were: 18.75, 37.5, 75, 150, 300 mg Zn L^{-1}.

Toxicity endpoints assessed were seed germination and root elongation (Inhibition concentration 50 [IC_{50}, no-observed-effect concentration [NOEC], lowest-observed-effect concentration [LOEC], relative growth index [RGI], and germination index [GI]). Alterations in germination and normal development of seedlings were recorded. The root elongation length was used to calculate RGI (Eq. 3) (Alvarenga et al., 2007). RGI values between 0 and 0.8 are categorized as inhibition of root elongation (I), values greater than 0.8 and less than 1.2 as no-significant-effect (NSE), and values greater than 1.2 as stimulation of root elongation (S) (Young et al., 2012). The number of germinated seeds and root elongation length were used to calculate GI (Eq. 4) (Zucconi et al., 1981). GI values lower than 80% were considered to indicate inhibition (Tiquia et al., 1996).

$$\mathrm{RGI} = \frac{\mathrm{RLS}}{\mathrm{RLC}} \qquad \text{Equation 3}$$

$$\mathrm{GI}\ (\%) = \frac{\mathrm{RLS}}{\mathrm{RLC}} \times \frac{\mathrm{GSS}}{\mathrm{GSC}} \times 100 \qquad \text{Equation 4}$$

where RLS is the radicle length of the sample, RLC is the radicle length of the control, GSS is the number of germinated seeds in the sample and GSC is the number of germinated seeds in the control. Two phytotoxicity indexes ($\mathrm{RGIC}_{0.8}$ and $\mathrm{GIC}_{80\%}$) are proposed herein to assess the maturity of composted manure. $\mathrm{RGIC}_{0.8}$ estimates the lowest extract concentration to get an inhibition of root elongation (RGI = 0.8). $\mathrm{GIC}_{80\%}$ estimates the lowest extract concentration to get a response of 80% in GI. The validation process of these new phytotoxicity indexes was conducted using published and unpublished data of our group from several types of samples. Phytotoxicity indexes were applied to data of four samples of compost and two samples of effluents. The poultry manure derived compost (PMC) was produced after a period of 12 weeks, according to Rizzo et al. (2013). Poultry manure was mixed with corn bare cobs, sawdust and shavings. Composting piles were manually turned. The poultry litter and horse manure derived compost (PLHMC) was composted in an experimental field of the INTA after a period of 16 weeks, according to Riera et al. (2014). Poultry litter contained a mixture composed by poultry manure, feathers, spilled feed, and bedding material. Active aeration composting was obtained in manually turned bins. The municipal solid waste derived compost (MSW1) was obtained from a composting facility in Trenque Lauquen (Argentina). Organic fraction of MSW was separated at home and then composted in the plant for 16 weeks. Active aeration composting was conducted in manually and mechanically turned piles. Other municipal solid waste derived compost (MSW2) was obtained from a composting facility in Metropolitan Area of Buenos Aires (Argentina). Organic fraction of MSW was manually and mechanically separated within the plant. Active aeration composting was obtained in manually turned bins after a period of 11 weeks. The samples of the untreated and treated effluent were collected in the treatment system from an anaerobic bioreactor, according to Young et al. (2012). The anaerobic bioreactor was loaded daily with 35 kg of cereal residues and 125 L of treated effluents. Untreated and treated effluents were obtained from the inflow into the first treatment pond and the recirculated flow to the bioreactor respectively.

Values of the phytotoxicity indexes ($RGIC_{0.8}$ and $GIC_{80\%}$) were differentiated into two categories according to the toxicity effects observed:

- Inhibitory effects: ≤ 100%
- Non-inhibitory effects: > 100%

2.2.3 *D. magna immobilization test*

The *Daphnia* immobilization test was used to assess the acute toxicity from composting extracts (USEPA, 1996). Toxicity tests were carried out by triplicate. Experimental design consisted in 10 treatments for each composting pile and sampling time (*n* = 240). Extract concentrations used in the tests ranged from 0.1% to 80% v/v (0.1, 1, 4, 8, 15, 25, 40, 60, and 80%), and a negative control. Ten neonates less than 24-h of hatching were exposed during 48-h in a static system, containing 30-mL of each of nine the extract concentration or control water. A total of 7200 daphnids were used in these experiments. Toxicity endpoints assessed were effective concentration 50 (EC_{50}), NOEC, and LOEC. The quality controls used were immobilization under 10% in negative controls and Cr (potassium dichromate) as reference toxic in positive controls. The potassium dichromate concentrations at each positive control were: 0.1, 0.2, 0.3, 0.4 and 0.5 mg Cr L^{-1}.

2.3 *Statistical analysis*

The temporal variation of parameters was assessed by one-way ANOVA. When the *F* values of the ANOVA were significant ($p<0.05$), means were compared by the Tukey’s pair wise test. The influence of physicochemical parameters on the ecotoxicological endpoints and biological activity was also assessed by multivariate statistical procedures, such as principal component analysis (PCA) and correlation analysis (Pearson correlation coefficient).

3. Results and discussion

3.1 *Composting*

3.1.1 *Physicochemical characterization*

The variation of the ambient and pile temperature profiles showed a similar tendency from 40 days (Fig. 1). As was reported by other authors, two main phases can be seen in the temperature profile of composting piles. The average maximum temperature of the piles ranged between 40 and 46°C and lasted for five days, as shown in Fig. 1 (top). However, some piles reached a maximum of 60.5° C. The maturation phase started from day 40, when the temperature of the piles was similar to ambient temperature. Passive aeration systems reach lower temperatures than active aeration systems (Barrington et al., 2003). Silva et al. (2009) reported that co-composting of poultry manure with low quantities of carbon-rich materials (80:20 ratio) reached a maximum pile temperature lower than 40ºC. However, Ogunwande and Osunade (2011) compared three passive aeration composting of poultry manure that reached a thermophilic phase above 42 °C that lasted for approximately 20 days. However, this longer thermophilic phase could be due the initial composition of the composting piles. Ogunwande and Osunade (2011) evaluated composting with sawdust, poultry manure and litter. Poultry manure is characterized by a high relative density, whereas sawdust and poultry litter are materials with low density that could improve the total porosity of the mix. In this study, the initial composition had a high proportion of poultry manure (70%) which could have affected the porosity and thus oxygen diffusion. Both the organic and inorganic content decreased (OM = 34.8% and EC = 54.5%; $p<0.05$) during the biodegradation period. Other parameters showed a significant decrease as well, such as MC, TOC, SC, TN, Ca and Mg (Table 1). The MC was kept above 60% by manual irrigation. The pH was remained slightly alkaline from day 14, then increased and the final pH was less than 9. This increase could be attributable to proteolysis and high microbial activity during the first days of composting (Bustamante et al., 2008). Authors reported similar pH values using passive and active aeration systems (Ogunwande and Osunade, 2011; Rizzo et al., 2013). The high initial values of EC could be associated to the poultry diet (Bolan et al., 2010). Although EC decreased, the compost obtained had restrictions in use due to a high EC final value. Active aeration systems may reach a higher decrease of EC due to salt loss by higher leaching (Rizzo et al., 2013). Such high EC values in poultry manure compost were found by Komilis and Tziouvaras (2009).

The highest losses of OM and TN were registered during the first 14 days (Fig. 1-middle). The cumulative losses of OM and TN at day 92 were of 64.1 ± 2.1 % and 68.1 ± 10.4% respectively. Also, a positive correlation between OM and EC ($R^2 = 0.77$) was found. TN loss was associated with the gradual increase of pH during the first 14 days (52.1%), which could increase the volatilization of $N\text{-}NH_3^+$. Ogunwande (2011) compared three passive aeration systems and reported a TN loss of 38.1% until day 14, lower than those found in this study. Tiquia and Tam (2002) reported similar losses of TN (58%) using a forced-aeration system for composting of poultry litter. On the other hand, Parkinson et al. (2004) found a higher TN loss in active aeration system than in passive aeration system. Both moderate temperatures and presence of microbial groups that increase and / or maintain the pool of N, such as N-fixing and nitrifying bacteria (Paredes et al., 1996). It could have caused a decrease in TN loss during the mesophilic phase.

3.1.2 *Stability and microbiological contents*

The highest pile temperature and biological activity (SRI = 1.12 mg O_2 g OM h^{-1}) were recorded during the first 14 days (Fig. 1). The SRI showed a negative correlation with SC and Mn (R^2: 0.92 and 0.76 respectively; Table 3), whereas it showed a slight positive correlation with TP (R^2: 0.65; $p<0.05$). Low values of SRI at end of the process (day 92) indicated that biological stability (SRI ≤ 0.5 mg O_2 g^{-1} OM

h^{-1}) was reached. *Salmonella* spp. was not detected during composting. However, the high counts of total coliforms and *E. coli* observed in all piles and at all sampling times suggest that these pathogens survived the short thermophilic phase of the process, which indicates the low quality of the derived compost.

3.2 *Toxicity tests*

3.2.1 *Quality controls*

Results of the toxicity tests were acceptable according to the criteria established by the quality controls. In the seed tests, the coefficients of variation between the averages of root length in the negative controls were 19.6 and 23.3% for lettuce and radish, respectively, lower than that recommended in the test protocols. The IC_{50} average values of root elongation in the positive controls were 55.4 ± 16.9 (n = 15) and 82.6 ± 15.1 (n = 18) mg L^{-1} of Zn for lettuce and radish respectively. On the other hand, the average value of immobilized neonates of *D. magna* in the negative controls was 2.2%, lower than that recommended in the test protocols. The EC_{50} average value in the positive controls was 0.30 ± 0.07 (n = 21) mg L^{-1} of Cr.

3.2.2 *Exposure to extracts*

Toxicity tests carried out on terrestrial plant species (lettuce and radish) allowed determining the quality of the compost as a soil amendment, whereas on the aquatic organism (daphnid) allowed determining the potential toxicity of leachates or runoff. The three organisms exposed to aqueous extracts showed acute toxicity in all samples. Ecotoxicological endpoints of the test organisms at each sampling time can be found in Table 2. The average EC_{50} or IC_{50} of the 3 species was 8.29 ± 0.35% (n=18) in the initial sampling (day 0) and 31.12 ± 10.99% (n=18) in the final sampling (day 92). Composting reduced the average toxicity by 22.8% for the 3 species. The sensitivity of the organisms measured in terms of EC_{50} or IC_{50} was highest for daphnid, followed by lettuce and then radish. Rizzo et al. (2013) also found lettuce to be more sensitive to adverse effects than radish. Endpoints of immobilization (*D. magna*) and root elongation (*L. sativa* and *R. sativus*) exhibited toxic response in all samples. Delgado et al. (2013) reported high mortality on daphnid exposed to poultry manure leachates. Root elongation was an endpoint with most sensitivity than seed germination for the both plant species, as reported by Fuentes et al. (2004). Seed germination exhibited no toxic response in 17 and 33% of the samples for LOEC, NOEC and IC_{50} respectively at day14 and 56 for lettuce and at day 0 for radish. In addition, this endpoint exhibited no toxic response from day 14 for radish. Several authors have reported the genotoxicity of leachate landfill and compost extracts on terrestrial plants and bacteria (Cabrera et al., 1999; De Simone et al., 2005; Kwasniewska et al., 2012). Gupta and Kelly (1992) demonstrated that poultry litter leachate may induce mutagenicity using the Ames test. Further studies could focus on assessing the capability of composting to reduce the genotoxicity of poultry manure.

Insert Figure 1

3.2.3 *Phytotoxicity indexes*

RGI and GI have been used to assess the toxicity of composting samples (Tiquia and Tam, 2000a; Tiquia, 2010). The results obtained in the present study are similar to those reported by other authors. GI values in the 100% extract concentration were zero in 62.5% of the samples for lettuce (average GI value = 3.88%; *n*= 24) and in 16.6% of samples for radish (average GI value =21.07%; *n*= 24). Komilis and Tziouvaras (2009), for example, found GI values between 0 and 6% in extract concentration of 100% (raw extract) of poultry manure derived compost using radish, lettuce, pepper (*Capsicum* spp.), spinach (*Spinacia oleracea*), tomato (*Lycopersicon esculentum*), cress (*Lepidium sativum*) and cucumber (*Cucumis sativus*). If raw extract inhibits germination completely, RGI and GI lose their value as indexes. For this reason, Komilis and Tziouvaras (2009) had excluded GI data of poultry manure derived compost from correlation analysis. An alternative experimental strategy was proposed by Morel et al. (1985), who determined GI using three aqueous extract concentrations (10, 20 and 40% w/v) (Silva et al., 2009). However, this methodology cannot be used with any type of sample because the concentrations depend on the toxicity degree. Therefore, we propose to use $RGIC_{0.8}$ and $GIC_{80\%}$ as cut-off values to indicate the lowest concentration that induces inhibitory effects. Also, values lower than or equal to 100% indicate any toxicity degree from a sample or immaturity of the compost, whereas values greater than 100% indicate a non-toxicity degree from a sample or maturity of the compost. These new indexes allow the comparison between samples with different toxicity degrees, such as EC_{50}, IC_{50} or LC_{50}, which are commonly used in ecotoxicology. The use of several types of samples allowed to analyze the robustness of the indexes during the validation process (Table 3). Mature compost (MSW2) and treated effluent showed non-inhibitory effects, whereas immature compost (PMC, PLHMC and MSW1) and untreated effluent showed inhibitory effects.

The $RGIC_{0.8}$ values showed an increase between the initial and final sampling time for lettuce from 0.31 to 30.50%, and for radish from 0.06 to 52.74% (Fig. 1-bottom). The $GIC_{80\%}$ values showed an increase between the initial and final sampling time for lettuce from 0.42 to 54.34%, and for radish from 0.06 to 54.76% (Fig. 1-bottom). These values indicate that composting reduced toxicity. However, the maximum values of $RGIC_{0.8}$ and $GIC_{80\%}$ were lower than 100%. Therefore, the composting piles of this study did not reach full maturity. Further studies could incorporate these phytotoxicity indexes to assess several types of samples, such as effluents, surface water or solid waste extracts.

3.2.4 *Correlations*

Multivariate analysis can suggest a relationship between toxicity and the inorganic and organic content. An association between physicochemical parameters and ecotoxicological endpoints, including the initial and final sampling times (Fig. 2) was detected after applying Principal Components Analysis (PCA). The results of this analysis account for 67.6% of the variability of the data matrix. PCA showed two clear groups of parameters associated to each sampling time. High values of EC, carbon content (TOC and SC), TN, MC, OM and some cations (mainly Mn, Mg and Ca) were associated with the initial time, whereas high values of the ecotoxicological endpoints (low toxicity), pH and TP were associated with the final time.

Insert Figure 2

A correlation analysis was carried out between physicochemical and ecotoxicological parameters (Table 4). The EC_{50} for daphnid showed a negative correlation with SC, Mn and Ca (R^2: 0.73, 0.72, and 0.69 respectively). Also, correlations were observed between phytotoxicity endpoints of lettuce and physicochemical parameters. The highest R^2 values were obtained for IC_{50}, NOEC and LOEC of seed germination on lettuce. Pablos et al. (2011) suggested a relationship between electrical conductivity and increasing toxicity. Specifically, authors associated conductivity with the inhibition of root elongation on seeds of lettuce (Young et al., 2012). The IC_{50} showed a negative correlation with SC, Mn and TN (R^2: 0.86, 0.79, 0.71 respectively), whereas showed a positive correlation with both SRI and TP (R^2: 0.85 and 0.70 respectively). The lack of strong correlation between maturity and stability indexes was also observed in Oviedo et al. (2015) as well as in Komilis and Tziouvaras (2009). Komilis and Tziouvaras (2009) reported negative correlations between GI of cress and both TOC and TN. However, we found a negative correlation between $GIC_{80\%}$ of lettuce and Mn (R^2: 0.69). Both lettuce (R^2: 0.79) and radish (R^2: 0.98) was obtained a positive correlation between $RGIC_{0.8}$ and $GIC_{80\%}$. The lower correlation between these phytotoxicity indexes for lettuce could be attributable to a higher inhibition of seed germination.

5. Conclusions

1. The proposed monitoring strategy demonstrated the low effectiveness of passive aeration systems to treat poultry manure that is present in high percentages in composting piles (>70%).
2. Although the values of SRI, C:N ratio and OM loss indicated compost stability, *E. coli* colonies remained higher than the limits recommended by international guidelines.

3. The *D. magna* endpoints allowed the assessment of possible leachate or run-off toxicity, which showed positive correlations with phytotoxicity endpoints.
4. Multivariate analyses demonstrated positive correlations between ecotoxicological endpoints (low toxicity) and biological activity (stability). A PCA demonstrated that these two parameter groups were associated with final sampling time and showed negative correlations with several other physicochemical parameters (organic and inorganic contents). The latter were associated to initial sampling time.
5. The $RGIC_{0.8}$ and $GIC_{80\%}$ indexes and salinity indicated that the compost did not reach maturity. As a result, the final compost was not considered suitable for use as a soil amendment.
6. The newly proposed phytotoxicity indexes ($RGIC_{0.8}$ and $GIC_{80\%}$) could be used to assess toxicity from complex samples or could be implemented in monitoring strategies as useful ecotoxicological tools.

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Table 1. Mean (± SD) physicochemical and microbiological parameters of the six composting piles at each sampling time and limit values of final composts.

Parameter	0-d	14-d	56-d	92-d	Target value or range / upper limit	Reference
pH	6.8 ± 0.3 ^{a}	7.7 ± 0.3 ^{b}	7.6 ± 0.4 ^{b}	8.2 ± 0.4 ^{b}	6 - 8 / 9	WRAP (2011)
EC (mS cm^{-1})	17.6 ± 1.9 ^{a}	13.3 ± 5.5 ^{ab}	10.8 ± 2.9 ^{b}	8.0 ± 1.8 ^{b}	< 0.6 / 1.5	WRAP (2011)
C:N ratio	24.6 ± 3.6 ^{a}	31.6 ± 3.1 ^{a}	23.4 ± 3.6 ^{a}	24.8 ± 3.7 ^{a}	20:1	SENASA (2011)
OM (%)	70.1 ± 1.3 ^{a}	62.1 ± 3.0 ^{b}	51.1 ± 6.8 ^{c}	45.7 ± 2.7 ^{c}	≥ 15	SENASA (2011)
MC (%)	70.6 ± 3.2 ^{a}	67.8 ± 3.8 ^{a}	64.6 ± 4.9 ^{ab}	60.4 ± 4.2 ^{b}	35 - 40 / 50	WRAP (2011)
TOC (%)	35.0 ± 0.7 ^{a}	31.0 ± 1.5 ^{b}	25.5 ± 3.4 ^{c}	22.9 ± 1.3 ^{c}	-	-
TN (%)	1.4 ± 0.2 ^{a}	1.0 ± 0.1 ^{b}	1.1 ± 0.2 ^{b}	0.9 ± 0.1 ^{b}	NPK ≥ 6	SENASA (2011)
TP (mg g^{-1})	20.3 ± 3.1 ^{a}	n.d.	n.d.	24.8 ± 3.0 ^{a}	NPK ≥ 6%	SENASA (2011)
SC (%)	2.5 ± 0.3 ^{a}	n.d.	n.d.	1.2 ± 0.3 ^{b}	-	-
SP (mg g^{-1})	0.5 ± 0.1 ^{a}	n.d.	n.d.	0.5 ± 0.1 ^{a}	NPK ≥ 6%	SENASA (2011)
Ca (mg L^{-1})	79.8 ± 15.4 ^{a}	n.d.	n.d.	31.5 ± 40.8 ^{b}	> 1%	SENASA (2011)
Mg (mg L^{-1})	125.2 ± 30.3 ^{a}	n.d.	n.d.	70.9 ± 47.5 ^{b}	> 0.05 %	SENASA (2011)
K (mg L^{-1})	1636.6 ± 165.9 ^{a}	n.d.	n.d.	1695.3 ± 861.3 ^{a}	NPK ≥ 6%	SENASA (2011)
Na (mg L^{-1})	472.9 ± 39.9 ^{a}	n.d.	n.d.	468.9 ± 139.7 ^{a}	< 100 / 150	WRAP (2011)
Zn (mg L^{-1})	0.9 ± 0.5 ^{a}	n.d.	n.d.	0.6 ± 0.6 ^{a}	< 150 / 400	WRAP (2011)
Mn (mg L^{-1})	2.0 ± 0.5 ^{a}	n.d.	n.d.	1.1 ± 0.3 ^{b}	-	-
Cu (mg L^{-1})	1.5 ± 1.3 ^{a}	n.d.	n.d.	0.9 ± 0.7 ^{a}	< 50 / 100	WRAP (2011)
Total coliforms (CFU)	8.0x10^{6} ± 1.0x10^{7}	n.d.	n.d.	7.5x10^{6} ± 9.3x10^{6a}	-	-
E. coli (CFU)	1.9x10^{7} ± 1.1x10^{7}	n.d.	n.d.	7.5x10^{6} ± 9.1x10^{6a}	Absent / 1000	WRAP (2011)
Salmonella spp.	Absent	n.d.	n.d.	Absent	Absent / Zero	WRAP (2011)

Different letters indicate significant differences (p<0.05) among sampling times.

EC = Electrical conductivity; OM = Organic matter; MC = Moisture content; TOC = Total organic carbon; TN = Total Kjeldahl nitrogen; SC = Soluble carbon; TP = Total phosphorous; SP = Soluble phosphorous; n.d. = no data

Table 2. Mean (95% CI) ecotoxicological endpoints of the test organisms at each sampling time.

Endpoint	0-d	14-d	56-d	92-d
Lettuce				
Seed germination				
IC$_{50}$ (%)	23.5 [15.6-31.5] ^{a}	69.5 [61.5-77.4] **^{b}	60.0 [49.2-70.9] **^{bc}	46.8 [44.4-49.3] ^{c}
LOEC (%)	28.3 [17.7-39.0] ^{a}	70.0 [54.0-86.0] *^{b}	64.0 [50.6-77.4] *^{b}	50.0 [41.2-58.8] ^{ab}
NOEC (%)	14.2 [8.8-19.5] ^{a}	50.0 [34.0-66.0] *^{b}	44.0 [30.6-57.4] *^{b}	30.0 [21.2-38.8] ^{ab}
Root elongation				
IC$_{50}$ (%)	8.8 [3.6-14.0] ^{a}	45.4 [26.5-64.4] ^{b}	55.0 [39.8-70.2] ^{b}	32.9 [24.7-41.1] ^{ab}
LOEC (%)	7.8 [1.9-13.6] ^{a}	25.2 [10.7-41.3] ^{a}	38.3 [16.6-60.1] ^{a}	28.3 [17.7-39.0] ^{a}
NOEC (%)	3.6 [0.5-6.7] ^{a}	12.3 [3.9-20.7] ^{a}	24.2 [6.9-41.4] ^{a}	14.2 [8.8-19.5] ^{a}
Radish				
Seed germination				
IC$_{50}$ (%)	63.3 [47.7-78.8]**	n.t.	n.t.	n.t.
LOEC (%)	88.0 [73.7-100.0]*	n.t.	n.t.	n.t.
NOEC (%)	68.0 [53.7-82.3]*	n.t.	n.t.	n.t.
Root elongation				
IC$_{50}$ (%)	9.7 [4.4-15.0] ^{a}	51.8 [34.4-69.2] ^{b}	54.1 [39.5-68.7] ^{b}	41.6 [20.6-62.7] ^{ab}
LOEC (%)	12.0 [0.0-24.3] ^{a}	49.0 [21.2-76.8] ^{a}	54.2 [33.9-74.5] ^{a}	41.0 [11.6-70.4] ^{a}
NOEC (%)	5.5 [0.0-11.9] ^{a}	32.2 [8.1-56.3] ^{a}	36.8 [21.4-52.3] ^{a}	26.9 [3.0-50.8] ^{a}
Daphnid				
EC$_{50}$ (%)	6.7 [4.3-9.0] ^{a}	26.5 [16.6-36.4] ^{ab}	29.3 [20.1-38.6] ^{b}	28.5 [14.8-42.2] ^{b}
LOEC (%)	9.0 [5.0-13.0] ^{a}	27.6 [17.0-38.2] ^{a}	33.3 [16.4-50.2] ^{a}	28.2 [12.4-43.9] ^{a}
NOEC (%)	4.3 [1.8-6.8] ^{a}	16.8 [9.8-23.8] ^{a}	21.0 [9.0-33.0] ^{a}	17.3 [6.4-28.3] ^{a}

Different letters indicate significant differences ($p<0.05$) among sampling times.

* A total of 83% of the samples exhibiting a toxic response.

** A total of 67% of the samples exhibiting a toxic response.

n.t.: no toxicity response.

Table 3. Values of the new phytotoxicity indexes on several types of samples to validate the proposed methodology.

Type of sample	Waste origin	Treatment	Seed	RGIC$_{0.8}$	GIC$_{80\%}$	Reference
Immature compost (PMC)	Poultry manure	Active composting	Lettuce	19.67	16.11	Rizzo et al. (2013)
			Radish	34.40	21.77	
Immature compost (PLHMC)	Poultry manure	Active composting	Lettuce	35.99	30.88	Riera et al. (2014)
			Radish	24.63	20.32	
Immature compost (MSW1)	Organic fraction of MSW	Active composting	Lettuce	40.61	45.56	Unpublished data
			Radish	87.88	73.45	
Mature compost (MSW2)	Organic fraction of MSW	Active composting	Lettuce	108.76	104.19	Unpublished data
Untreated effluent	Cereal residues	Anaerobic biodigestion	Lettuce	18.02	24.77	Young et al. (2012)
Treated effluent				111.35	121.57	

PMC: poultry manure derived compost. PLHMC: poultry litter and horse manure derived compost. MSW1 and MSW2: municipal solid waste derived compost.

Table 4. Pearson correlation coefficients among various parameters measured at four sampling times at six composting piles (n = 24).

		Physico-chemical parameters								Stability	*D. magna*	*L. sativa (lettuce)*					*R. sativus (radish)*		
		Ash	OM or TOC	SC	Ca	Mg	Na	Mn	Cu	SRI	NOEC or LOEC	NOEC or LOEC r.e.	IC_{50} s.g.	NOEC or LOEC s.g.	$GRIC_{0.8}$	$GIC_{80\%}$	NOEC or LOEC r.e.	$GRIC_{0.8}$	$GIC_{80\%}$
Physico-chemical parameters	pH	n.s.	n.s.	-0.75*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	EC	-0.80**	0.77**	0.83**	n.s.	n.s.	n.s.	0.79*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ash		-0.78**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	OM or TOC			0.92**	n.s.	n.s.	n.s.	0.78*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	TN			0.87**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	SC				0.77*	n.s.	n.s.	0.78*	n.s.	-0.92**	n.s.	n.s.	-0.86**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ca					0.94**	n.s.	0.75*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	K						0.97**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Zn							n.s.	0.84**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Mn								n.s.	-0.76*	n.s.	n.s.	-0.79*	-0.91**	n.s.	n.s.	n.s.	n.s.	n.s.
Stability	SRI										n.s.	n.s.	0.85**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
D. magna	EC_{50}										0.90**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L. sativa (lettuce)	IC_{50} r.e.											0.85**	n.s.	0.78**	0.90**	0.82**	n.s.	n.s.	n.s.
	NOEC or LOEC r.e.												n.s.	n.s.	0.84**	0.91**	n.s.	n.s.	n.s.
	IC_{50} s.g.													0.83**	n.s.	n.s.	n.s.	n.s.	n.s.
	$GRIC_{0.8}$															0.79**	n.s.	n.s.	n.s.
R. sativus (radish)	IC_{50} r.e.																0.89**	0.81**	0.81**
	NOEC or LOEC r.e.																	0.87**	0.87**
	$GRIC_{0.8}$																		0.98**

Significant parameters are only shown (* $p<0.01$ and ** $p<0.001$).

n.s.: not significant; r.e.: root elongation; s.g.: seed germination; OM and TOC are shown together because they have the same correlation coefficient values; NOEC and LOEC are shown together because they have the same correlation coefficient value.

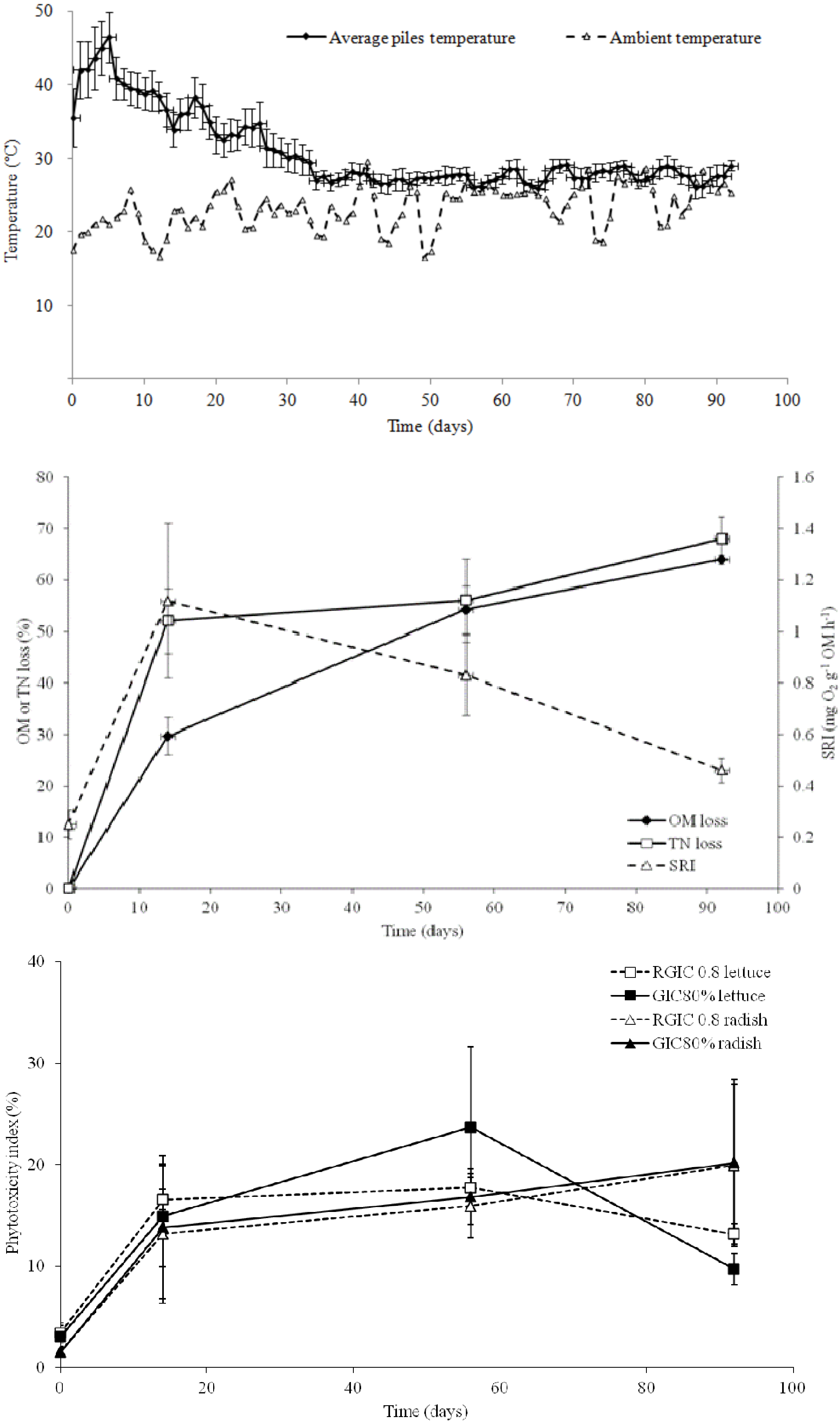
Temperature (°C)
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
Time (days)
Average piles temperature
Ambient temperature
OM or TN loss (%)
SRI (mg O$_2$ g^{-1} OM h^{-1})
80
70
60
50
40
30
20
10
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
Time (days)
OM loss
TN loss
SRI
Phytotoxicity index (%)
40
30
20
10
0
20
40
60
80
100
Time (days)
RGIC 0.8 lettuce
GIC80% lettuce
RGIC 0.8 radish
GIC80% radish

Figure 1.

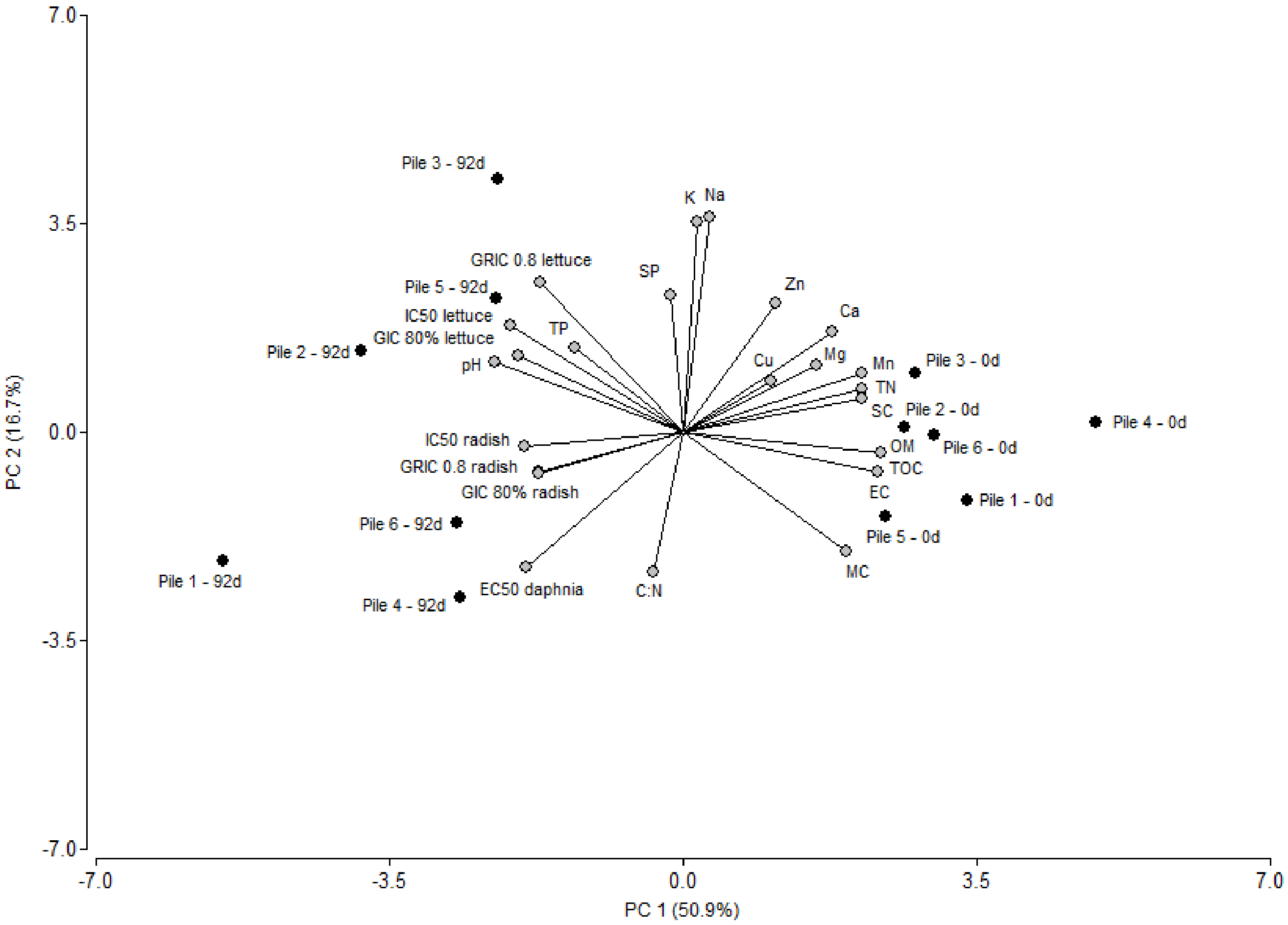
PC 2 (16.7%)
7.0
3.5
0.0
-3.5
-7.0
-7.0
-3.5
0.0
3.5
7.0
PC 1 (50.9%)
Pile 3 - 92d
K
Na
GRIC 0.8 lettuce
SP
Pile 5 - 92d
Zn
Ca
IC50 lettuce
TP
GIC 80% lettuce
Pile 2 - 92d
Cu
Mg
Mn
Pile 3 - 0d
pH
TN
SC
Pile 2 - 0d
Pile 4 - 0d
IC50 radish
OM
Pile 6 - 0d
GRIC 0.8 radish
TOC
GIC 80% radish
EC
Pile 1 - 0d
Pile 6 - 92d
Pile 5 - 0d
Pile 1 - 92d
MC
EC50 daphnia
C:N
Pile 4 - 92d

Figure 2.

Figure captions

Figure 1. (Top): Average temperature of ambient air and of the inside of the composting piles (average based on *n* = 6) error bars demonstrate standard deviations), (Middle): Average cumulative losses (±standard error) of the OM and TN (%) and average SRI (mg O_2 g^{-1} OM h^{-1}) during the composting period; (Bottom) Average values (±standard error) of the phytotoxicity indexes measured during the composting period.

Figure 2. Principal components analysis (PCA) shows the association between physicochemical parameters and ecotoxicological endpoints with respect to the first two components. Data of IC_{50} of lettuce and radish are only shown for root elongation. Black dots indicate each composting pile and sampling time.