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Ecotoxicological evaluation of swine manure disposal on tropical soils in Brazil

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ABSTRACT

Swine production in Brazil results in a great volume of manure that normally is disposed of as agricultural fertilizer. However, this form of soil disposal, generally on small farms, causes the accumulation of large amounts of manure and this results in contaminated soil and water tables. To evaluate the effects of increasing concentrations of swine manure on earthworms, several ecotoxicological tests were performed using *Eisenia andrei* as test organism in different tropical soils, classified respectively as Ultisol, Oxisol, and Entisol, as well as Tropical Artificial Soil (TAS). The survival, reproduction and behavior of the earthworms were evaluated in experiments using a completely randomized design, with five replications. In the Ultisol, Oxisol and TAS the swine manure showed no lethality, but in the Entisol it caused earthworm mortality (LOEC=45 $m^3\ ha^{-1}$). In the Entisol, the waste reduced the reproductive rate and caused avoidance behavior in *E. andrei* (LOEC=30 $m^3\ ha^{-1}$) even in lower concentrations. The Entisol is extremely sandy, with low cation exchange capacity (CEC), and this may be the reason for the higher toxicity on soil fauna, with the soil not being able to hold large amounts of pollutants (e.g. toxic metals), but leaving them in bioavailable forms. These results should be a warning of the necessity to consider soil parameters (e.g. texture and CEC) when evaluating soil contamination by means of ecotoxicological assays, as there still are no standards for natural soils in tropical regions. *E. andrei* earthworms act as indicators for a soil to support disposal of swine manure without generating harm to agriculture and ecosystems.

1. Introduction

The huge increase in swine breeding and production in the Southern states of Brazil is the main reason for its 3rd place in the world for pork production (ABIPECS, 2012). The major problem with this activity is the generation of enormous amounts of manure that may become a dangerous pollutant of terrestrial and aquatic ecosystems instead of a beneficial fertilizer, in spite of its high content of plant nutrients. Today the most common alternative for the disposition of this waste is its application on agricultural soils as fertilizer, since it contributes to increase organic matter (OM) in soil and is active in the provision of plant nutrients. Therefore, when disposed of in an adequate manner, the manure does improve physical, chemical and biological soil characteristics (Scherer et al., 2007). However, its inadequate use in great concentrations generates environmental pollution and this is the major concern for pig farms due to the great geographical concentration and intensification of swine growing farms. In the soil, an intensive and prolonged disposition of swine manure causes the accumulation of elements as P, K, Cu and Zn, especially in the upper 0–5 cm layer (Scherer et al., 2010).

Pig feeds have high concentrations of Cu and Zn in order to minimize gastro-intestinal disorders (Corrêa et al., 2011) that produce great amounts of P excretion, which can reach 67% of the total supplied in the diet (Oliveira, 2006). The animals also excrete medications such as antibiotics administered as prophylaxis or disease treatment in urine and feces, or both, in an already partially metabolized form of the original compound (Regitano and Leal, 2010).

Although there is a growing concern about the environmental risks of this activity and a good number of studies and alerts on this subject in international literature (Scherer et al., 2010), the Brazilian effort on this subject is still very scarce and many aspects related to the soil as a recipient are still unknown.

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The evaluation of negative effects that livestock wastes (e.g. swine manure) may impose on the soil fauna, especially on earthworms, by means of standard soil ecotoxicological studies (ISO, 1993, 1998, 2008), has become widely accepted and obligatory in European countries (European Union Council Directive 91/689/EEC-European Community,1991). These methods complement the more traditional evaluations, such as chemical analyses. Ecotoxicology studies the effects of contaminants on standard soil faunal species (OECD, 1984; ISO, 1998) and the responses of these organisms to any contaminant, obtained in ecotoxicological assays, help to determine secure concentrations of contaminating substances in the environment.

Earthworms of the species *Eisenia andrei* are good indicators of soil quality (ISO, 1998). They are quite sensitive to the presence of certain polluting substances in the soil and are the most used standard organisms in ecotoxicological tests. Many studies using *E. andrei* for assessing the impact of pollutants have been published but to our knowledge none had the objective of testing the impact of swine manure on the soil community (Van Gestel and Hoogerwerf, 2001; César et al., 2008; Natal-da-Luz et al., 2011; Onuoha and Worgu, 2011; Dominguez-Crespo et al., 2012).

This study evaluated the effects of the addition of increasing concentrations of swine manure on four tropical soil types (Ultisol, Oxisol, Entisol and TAS) on the survival, reproduction, and avoidance behavior of *E. andrei,* with the objective of determining the highest secure deposition doses for swine manure that will not affect earthworms in soils.

2. Material and methods

2.1. Soil organisms to BE tested

Earthworms of the species *E. andrei* were grown in the laboratory in a mixture of dried and sieved horse manure, powdered coconut husk, and fine sand ($>50\%$ of grains measuring 0.05–0.2 mm), in the proportion 2:1:0.15 by dry weight (d.w.), respectively. All pre-existing animals in the mixture were killed by a defaunation process (Pesaro et al., 2003), consisting of three 48-h cycles of freezing and thawing. When necessary the pH of the mixture was corrected to values between 6 and 7, by addition of $CaCO_3$. Weekly, the earthworms were fed with a mixture of oat flakes and deionized water in the proportion of 2:1 (v/v) and only adult (clitellate) worms, with an individual body weight of 250–600 mg, were used in the assays. The temperature was 23 °C $\pm$ 2 with a photophase of 12 h, during the breeding of worms and acute or chronic toxicity tests.

2.2. Soils

Three natural soils were sampled at a depth of 0–0.20 m in forest areas without an agricultural history. These soils were classified as Ultisol, Oxisol and Entisol. Also, were used a standard artificial soil (TAS) (Table 1). The artificial soil used in our tests was an adaptation of the OECD standard artificial soil recommended by the guidelines of the Organization for Economic Co-operation and Development (OECD, 1984), called Tropical Artificial Soil (TAS). TAS is the substrate used for ecotoxicological evaluations in regions with tropical climate (Römbke et al., 2007; De Silva and Van Gestel, 2009; Alves et al., 2013), and consists of a mixture of fine sand ($>50\%$ of grains measuring 0.05–0.2 mm), kaolinitic clay (powdered kaolin), and powdered coconut husks, in a proportion of 70:20:10 d.w., respectively (Garcia, 2004). When necessary the pH of soils was corrected to 6.0 ± 0.5 with $CaCO_3$ and the moisture of natural soils and TAS was maintained at 60% of the water holding capacity (WHC), at the beginning of the experiment.

Table 1
Physical and chemical characterization of the four soils used in the tests – $n=3$ (means$\pm$standard deviation).

	Ultisol	Oxisol	Entisol	TAS
Clay (%)	49.7 ± 4.0	47.7 ± 3.2	7.3 ± 3.1	20 ± 0
CEC[a]	78 ± 13.2	129 ± 37.4	6.2 ± 5.5	77 ± 2.7
SB[b]	29.8 ± 5.3	25.9 ± 3.3	8.8 ± 2.3	65 ± 11.4
pH[c] (KCl)	5.6 ± 0	5.7 ± 0.1	5.8 ± 0.1	6 ± 0.1
OM (g dm^{-3})	36.3 ± 9.5	44.7 ± 9.7	1.5 ± 0.9	52 ± 2.7
P (mg kg^{-1})	16.3 ± 9.6	12.2 ± 6.7	$4.3\pm2.$	28 ± 9.1
K (mmolc dm^{-3})	6.8 ± 2.6	1.4 ± 0.6	2.2 ± 1.1	27 ± 1.7
Ca (mmolc dm^{-3})	17.3 ± 6.2	21 ± 5.2	3.1 ± 2.7	25 ± 10.2
Mg (mmolc dm^{-3})	5.7 ± 1.8	3.3 ± 1.3	6.2 ± 3.1	9 ± 0.4
H+Al (mmolc dm^{-3})	48.2 ± 8.5	97.2 ± 31.5	2 ± 0	12 ± 8.9
Cu (mg kg^{-1})	5.1 ± 3.9	9.4 ± 4.2	2.8 ± 2.7	0.2 ± 0
Zn (mg kg^{-1})	3.3 ± 0.2	1.4 ± 0.7	0.9 ± 0.3	6 ± 0.1
Fe (mg kg^{-1})	22 ± 7.8	64.6 ± 33.7	57.0 ± 14.5	1.9 ± 0.7

[a] CEC – cation exchange capacity.
[b] SB – Sum of Bases ($Ca^{2+}+Mg^{2+}+K^{+}$).
[c] Mean of pH values at the start of the tests.

For soil analysis, copper, iron and zinc were extracted from soils with a DTPA solution at pH 7.3 (Lindsay and Norvell, 1978) and determined by Atomic Absorption Spectroscopy (AAS). The available P, K, Mg and Ca were extracted by ion exchange resin and determined by a colorimetric method, Atomic Emission Spectroscopy (AES) and AAS, respectively (Van Raij et al., 2001). For organic matter (OM) a colorimetric method was used, following Van Raij et al. (2001).

2.3. Swine manure

The manure was sampled directly on the pig farm and was left to stabilize for a period of 120 days (CQFSRS/SC, 2004). The manure was originated from growing animals with a weight of 8–25 kg. During this growth period, the fodder contained high values of Zn and Cu (Gräber et al., 2005) because the animals presented gastric problems (Bertol and Brito, 1998), which gave some nutrient loss by means of excretion, therefore generating the worst-case scenario for the application of swine manure on soil. This is the most critical phase for the animal health nevertheless the animals used for sampling of manure received no medication. The analysis of chemical and physical attributes of swine manure showed 74.7% of moisture, 9.48% of total C, 7.95% of organic C, 0.73% of total N, 1.26% of total P, 0.42% of total K, pH (KCl) 7.3 and 164, 367, 4809 mg kg^{-1} of Cu, Zn and Fe, respectively.

Organic and total carbon were determined via dichromate digestion, total N by the Kjeldahl method, total P, K, Ca, Mg, Cu, Zn, Na and Fe according to USEPA no. 3051 (USEPA, 1998) with the flame photometer for K and Na, and the other elements by ICP-OES. The pH value was determined in 1 mol L^{-1} KCl (1:5 w/w).

2.4. Experimental procedures

The experiments were set up in a (4×5) completely randomized factorial design, with 4 soils and 5 swine manure concentrations, with five replicates for each treatment. For the acute toxicity test, in all the tested soils, the same five manure concentrations were used (0, 25, 50, 75 and 100 m^3 ha^{-1}) derived from the official recommendation of manure volumes to be used as soil fertilizer, presented in the Normative Instruction no. 11 (FATMA, 2009), that preconizes a maximum application of 50 m^3 of manure ha^{-1} year^{-1}. Based on the results of this first test, five sub-lethal concentrations were selected for each soil to be used to measure the reproductive (chronic test) and behavior (avoidance test) endpoints, according to the protocols ISO no. 11268-2 (ISO, 1998) and no. 17512-1 (ISO, 2008). In the Ultisol, Oxisol and TAS