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Avoidance behaviour of two eco-physiologically different earthworms (*Eisenia fetida* and *Aporrectodea caliginosa*) in natural and artificial saline soils

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ABSTRACT

We studied the avoidance behaviour of *Eisenia fetida* and *Aporrectodea caliginosa* in OECD artificial soil spiked with NaCl and in natural saline soil (of varying ionic constitutions) collected from Robertson Experimental Farm (ROBS) in Western Cape, South Africa. For each organism, the ecotoxicological test was performed using a two-chamber test over a period of 48 h. The results showed that in the OECD soil, the avoidance EC50 (the concentration/electrical conductivity at which there is effect on 50% of the organisms) for *A. caliginosa* of 667 mg kg^{-1} NaCl was lower than 1164 mg kg^{-1} for *E. fetida*. Similarly in ROBS soil, the avoidance EC50 for *A. caliginosa* of 0.26 dS m^{-1} was lower than 0.56 dS m^{-1} in *E. fetida*. These results indicated that *A. caliginosa* showed better avoidance to salinity than *E. fetida* irrespective of soil types or ionic constitution. When compared with literature data, EC50 values in avoidance tests were either lower or comparable to those of reproduction, which was the most sensitive life-cycle parameter. The only exception was the EC50 value for avoidance of *E. fetida* in natural soil which was higher than for reproduction suggesting that the predictive value of the avoidance test for this species might be lower in natural soils. The variation in sensitivities of these earthworms could be as a result of differences in their eco-physiology. These findings suggest the relevance of the avoidance test as a suitable screening method showing first tendencies of saline stress on the habitat function of soils.

1. Introduction

Salinisation of soil is a rising problem in most arid and semi arid areas (Sumner, 1995). Poor irrigation and drainage management are normally the main causes of salinisation and as the water table rises, salts dissolved in the groundwater, move to the soil surface and accumulate through capillary action (Rietz and Haynes, 2003) Much information is available on the effects of salinity on physico-chemical properties of soil (Sumner, 1995), and on plants (Ramoliya et al., 2004; Kadukova and Kalogerakis, 2007), and knowledge of its effects on beneficial soil organisms is building up gradually.

Salinity affects the growth and survival of microorganisms (Lippi et al., 2000; Rietz and Haynes, 2003; Yuan et al., 2007), and soil enchytraeids (Hobel et al., 1992). It has been reported that NaCl in excess of 0.5% wet weight may be harmful to earthworms in activated sludge (Hartenstein et al., 1981). Khalaf El-Duweini and Ghabbour (1965) reported that high salinity resulting from excessive irrigation can limit earthworm populations in some situations. Fischer and Molnar (1997) found that mortality was significant at 100 mM NaCl in organic manure and peat substrates, and cocoon production ceased totally while growth was negatively affected at concentrations below or in excess of 60 mM NaCl. Recently, Owojori et al. (2008) found a 28-d LC50 of 5436 mg kg^{-1} NaCl and 28-d EC50 for growth and cocoon production of 4985 and 2020 mg kg^{-1} NaCl, respectively, for the earthworm *Eisenia fetida* in an Organisation for Economic Co-operation and Development (OECD) substrate. These authors concluded that earthworms would be severely affected at salt concentrations considered to be safe for many plants.

Since most agricultural lands are either under the threat of salinisation or likely to be so in the future (Williams, 2001), there is a need for rapid soil screening methods for salinity in affected areas. Avoidance tests are increasingly regarded as a quick method for determining the potential harmfulness of contaminated soil (Lukkari et al., 2005). It has been suggested that avoidance behaviour could be used as a meaningful indicator since the endpoints measured can sometimes be related to life-cycle effects (Amorim et al., 2008a). In the case of earthworms, avoidance of contaminated soils would have serious ecological impact since they are a major component in many soils and help to enhance nutrient turnover and soil aeration (Edwards and Bohlen, 1996).

Earthworms have chemical receptors in their prostomium, and possess high locomotory capacities; they can therefore sense pollutants and avoid polluted soils (Stephenson et al., 1998). Avoidance behaviour of earthworms to various chemicals has been reported by many authors (e.g. Lukkari et al., 2005; Garcia et al.,

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2008) and appears very promising as a quick assessment method of risk posed by a contaminated soil. When avoidance behaviour is shown by earthworms, it is often at concentrations lower than those affecting life-cycle parameters, indicating its relevance as predictive marker of impending effect at individual and population levels (Garcia, 2004; Lukkari et al., 2005). As of now, the test has been shown to be suitable for many pollutants and mixtures of pollutants (ISO, 2006). In a few cases, non avoidance of earthworms to certain chemicals (organophosphate pesticides and lead nitrate) has also been reported (Hodge et al., 2000; Reinecke et al., 2002). For salinity, only tangential evidence exist that they show avoidance in sandy soil soaked with several dilutions of sea water (Piearce and Piearce, 1979).These authors had performed their test before the first draft of the OECD guideline (1984) was proposed. Since they did not use a standard OECD soil or natural soil, direct comparison of data collected in the avoidance test with those collected in acute and chronic tests (for which substantial information is already available) is not possible.

Although there is paucity of information on the avoidance response of earthworm species to salinity, available information showed variation in response of worms belonging to different ecological groups (Piearce and Piearce, 1979). This study therefore aimed to compare the avoidance behaviour of two eco-physiologically different earthworms, *E. fetida*, a suitable laboratory species also known for its composting abilities, and *Aporrectodea caliginosa*, a species found in most forest, agricultural and garden soil. The specific aims were to compare the avoidance behaviour of these two earthworms in substrates of different soil properties and ionic constitutions as well as to ascertain if the avoidance response is as sensitive as the most sensitive life-cycle parameter identified in parallel studies (Owojori et al., 2008, in press).

2. Materials and methods

2.1. Test organisms

E. fetida is an epigeic (litter-dwelling) earthworm species. It inhabits only organic matter – rich locations, such as animal manure or compost heaps. Since it is mainly litter-dwelling, it lives on the soil surface or in the upper reaches of the mineral soil (Edwards and Bohlen, 1996). *A. caliginosa* is an endogeic species, and one of the most common earthworms in fertile forest, agricultural and garden soils. It burrows its way through the soil, taking its nutrition mainly from organic matter. Through its burrowing and feeding activity, it mixes surface and deeper soil layers, having a central role in the formation of fertile mull soil structure (Edwards and Bohlen, 1996).

E. fetida specimens used for this study were taken from a culture kept in the laboratory of the Ecotoxicology Group, University of Stellenbosch, South Africa since 1992. Adult worms of between 300 and 600 mg were used in the experiments. *A. caliginosa* specimens were collected at grassland close to Eerste River in Stellenbosch, Western Cape, South Africa by digging and hand-sorting. The species occurs abundantly in this area which had no known history of pesticide use. These soils are not known to be saline. Only adult worms of between 400 and 800 mg with fully developed clitella were selected for use in the experiment. They were not required to be from a synchronized culture (ISO, 2006).

2.2. Test soil

Two test soils were used in this experiment: OECD soil (OECD) and Robertson soil (ROBS).

The OECD soil was prepared as described by OECD Guideline (2004). It consisted of 70% sand, 20% kaolin clay and 10% sphagnum peat by dry weight. The pH was adjusted to 6.0 ± 0.5 by $CaCO_3$. The maximum water holding capacity (WHC) was 65%. ROBS soil was collected from a farmland at Robertson Experimental Farm, Robertson, Western Cape, South Africa (33°50′.028″S, 19°53′.492″) E (33°50′S, 19°50′E). The collection, preparation and treatment of this soil have previously been described by Owojori et al. (in press). Two bulk soil samples, initially identified based on differences in electrical conductivity (EC) were collected from the top 10 cm layer. These were brought to the laboratory, air dried, sieved (2 mm) and thoroughly mixed individually, after carefully removing the surface organic materials and fine roots. The EC of these soil samples were determined as described in Soil Survey Staff (1996). A soil-water extract (1:5; w/v) was made and measurements were taken with a conductivity meter (SM 802 pH/EC/TDS Meter, Spraytech). Soils with intermediate EC were prepared by serial dilution. This soil had a pH ranging from 9.4 in the undiluted soil to 9.2 in the most diluted soil. Organic matter (OM) content was <1%, clay 6%, and maximum WHC was 36%. A full description of the physico-chemical properties of these soil treatments could be found in Owojori et al., in press.

2.3. Test procedures

The two earthworm species were each tested separately in both OECD and ROBS soils using similar salt concentrations. The worms were acclimatized for 24 h in the respective soils using either unspiked soil (OECD) or lowest salinity soil (ROBS). For the OECD soil, samples were spiked with technical grade NaCl (artificial sea salt) purchased from Royal Salt Company Ltd., Parow East, South Africa in the following concentrations: 0, 500, 1000, 2000, 4000 mg kg^{-1} corresponding to 0.12, 0.26, 0.43, 0.77, 1.31 dS m^{-1} respectively. The concentrations were chosen to include those used by Owojori et al. (in press). The total amount of salt required for each concentration was added at once into deionised water and mixed with the total volume of soil for that concentration to achieve 55% of the WHC for each soil. For ROBS soil, that is a natural saline soil, the EC of the soil was adjusted to 0.08, 0.30, 0.52, 1.03, 1.33 dS m^{-1} to include EC values used by Owojori et al. (2008). The soil was moistened with deionised water to achieve 55% of the maximum WHC of the soil. The inclusion in this study of these salt concentrations (for OECD soil) and these EC values (for ROBS soil) was done to allow comparisons with the results of previously mentioned authors. After the soils were prepared, they were allowed to equilibrate for 2 d before being used in the experiments.

The avoidance test was performed as described by ISO (2006). Treated soils were introduced into the corresponding plastic containers. The cylindrical plastic containers (115 cm area, 10 cm height) were divided into two sections by drawing a line on the outside and labelling it with the name of the corresponding treated soil. Using a piece of plastic fitted transversally as a divider in the vessel, one half of the vessel was filled with saline soil, the other filled with control soil. For control purposes, control soils (undiluted soil with lowest EC value) were used on both sides of the divided containers. The volumes of soil used were 300 g and 250 g (dry wt) for the ROBS and OECD soils, respectively, on each half of the container. Five replicates were used for each treatment. After the soils were introduced, the plastic divides were removed and 10 adult earthworms were placed on surface at the dividing line between the two halves of each test container. In total, 500 worms were used for both tests. The vessels were then closed with transparent, perforated lids. The tests were carried out in a climate chamber at 20 ± 1 °C with a light/dark cycle of 16/8 h for all treatments. The animals were not fed during the test. At the end of the test period of 48 h, the control and the contaminated soil sections were carefully separated by inserting the plastic divide and the number of earthworms was counted in each section of the vessels.