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Characteristics of earthworms (*Eisenia fetida*) in PAHs contaminated soil amended with sewage sludge or vermicompost

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ABSTRACT

Earthworms can be used to remove polycyclic aromatic hydrocarbons (PAHs) from soil, but this might affect their survival and they might accumulate the contaminants. Sterilized and unsterilized soil was contaminated with phenanthrene (Phen), anthracene (Anth) and benzo(a)pyrene (BaP), added with or without *Eisenia fetida*, sewage sludge or vermicompost. Survival, growth, cocoon formation and concentrations of PAHs in the earthworms were monitored for 70 days. Addition of sewage sludge to sterilized or unsterilized soil maintained the number of earthworms and their survival was 94%. The addition of sludge significantly increased the weight of earthworms 1.3 times compared to those kept in the unamended soil or in soil amended with vermicompost. The weight of earthworms was significantly lower in sterilized than in unsterilized soil. Cocoons were only detected when sewage sludge was added to unsterilized soil. A maximum concentration of 62.3 μg Phen kg^{-1} was found in the earthworms kept in sterilized soil amended with vermicompost after 7 days and 22.3 μg Phen kg^{-1} when kept in the unamended unsterilized soil after 14 days. Concentrations of Phen in the earthworms decreased thereafter and $\leq$2 μg kg^{-1} after 28 days. A maximum Anth concentration of 82.5 μg kg^{-1} was found in the earthworms kept in sterilized soil amended with vermicompost and 45.8 μg Anth kg^{-1} when kept in the unamended unsterilized soil after 14 days. A maximum concentration of 316 μg BaP kg^{-1} was found in the earthworms kept in sterilized soil amended with vermicompost after 56 days and 311 μg BaP kg^{-1} when kept in the unsterilized soil amended with vermicompost after 28 days. The amount of BaP in the earthworm was generally largest after 28 days, but after 70 days still 60 μg kg^{-1} was found in *E. fetida* when kept in the sterilized soil amended with sewage sludge. It was found that *E. fetida* survived in PAHs contaminated soil and accumulated only small amounts of the contaminants, but sewage sludge was required as food for its survival and cocoon production.

1. Introduction

Recently, removal of organic contaminants from soil has been investigated using organic materials, such as glucose, sawdust, manure, sewage sludge, compost and vermicompost, as biostimulants (Namkoong et al., 2002; Hafidi et al., 2008). Biostimulation of soil with organic or inorganic fertilizers introduces additional nutrients into a contaminated ecosys-

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tem, increases the population of the indigenous microorganisms and as a consequence the removal of the contaminants (Pankrantz, 2001). Sewage sludge when added to a contaminated soil might accelerate removal of hydrocarbons (Namkoong et al., 2002), but the organic material that it contains can also be used as a source of nutrients for earthworms (Contreras-Ramos et al., 2005). Earthworms are known to accelerate removal of hydrocarbons from soil (Contreras-Ramos et al., 2006).

Earthworms are found in a wide range of soils and may represent 60–80% of the total soil biomass (Bouché, 1992). Earthworms accumulate many lipophilic organic pollutants from the surrounding soil environment, passive absorption through the body wall and also by intestinal uptake during the passage of soil through the gut. This accumulation increases as the concentration of the pollutant in the soil environmental increases (Belfroid et al., 1995). Autochthonous microorganisms degrade hydrocarbons (Johnsen et al., 2005), but if earthworms are added to soil, they will improve aeration, and stimulate microbial activity, thus increasing biodegradation. Additionally, earthworms excrete mucoproteinaceous slime and products of nitrogen metabolism which are easily metabolized by microorganisms, thereby stimulating their activity and growth (Scheu, 1991).

Few studies have focused on the effect that contamination of a soil with polycyclic aromatic hydrocarbons (PAHs) might have on the weight, cocoon production and survival of earthworms and on the amount of PAHs accumulated by them. In this work, earthworms were added to a sterilized or unsterilized soil contaminated with phenantrene (Phen), anthracene (Anth) and benzo(a)pyrene (BaP) and amended with or without sewage sludge or vermicompost and kept under aerobic conditions at 22 ± 2 °C for 70 days. Additional earthworms were kept in sludges or vermicompost for 70 days. The objectives of this study were (i) to monitor the survival, cocoon production and weight of *Eisenia fetida* when added to sterilized or unsterilized soil contaminated with Phen, Anth and BaP amended with sewage or vermicompost and (ii) determine the amount of PAHs in the earthworms.

2. Materials and methods

2.1. Materials used

Hydrocarbons were obtained from Sigma (USA) with purity >96% for Phen, >99% for Anth and >97% for BaP. Acetone was obtained from J.T. Baker (USA) with purity 99.7%.

2.2. Vermicompost and earthworms used

Reciclagua (Sistema Ecológico de Regeneración de Aguas Residuales Ind., S.A. de C.V.) in Lerma, State of Mexico (Mexico) treats wastewater from different sources. Ninety percent of the sewage biosolids were from different industrial origin mainly from textile industries and the rest from households. The waste from each company must comply with the following guidelines: biological oxygen demand (BOD) less than 1000 mg dm^{-3}, lipids content less than 150 mg dm^{-3}, phenol content less than 1 mg dm^{-3} and not contain organic contaminants. The wastewater is aerobically digested in a reactor and the biosolids obtained after the addition of a flocculant is passed through a belt filter. Ten kilograms of aerobically digested industrial biosolids were sampled three times aseptically in plastic bags after passing through the belt filter.

E. fetida was cultivated in the sewage sludge and the obtained vermicompost while survival, weight and cocoon production were monitored for 70 days. After 7, 14, 28, 56 and 70 days, the earthworms were removed from the soil and counted. The soil was 3-mm sieved and the cocoons counted on the sieve.

Adult earthworms were used in the experiment, with an average weight of 0.26 g $earthworm^{-1}$ and with a developed clitellum. Details of the sewage sludge and vermicompost can be found in Contreras-Ramos et al. (2005). Briefly, the obtained vermicompost was mature and stable, low in heavy metal concentrations and pathogen-free.

2.3. Soil characteristics

Details of the sampling site, located near the ex-convent of Acolman in the State of Mexico (Mexico) can be found in Betancur-Galvis et al. (2006). The sandy loam soil (Gee and Bauder, 1986, USDA modified soil texture triangle) with water holding capacity (WHC) 68%, pH in water 6.6 (Thomas, 1996), organic C content 18 g kg^{-1} soil (Amato, 1983), inorganic C 0.7 g kg^{-1} soil (Nelson and Sommers, 1996), and total N 0.84 g kg^{-1} soil (Bremner, 1996) contained 220 g clay kg^{-1}, 140 g silt kg^{-1} and 640 g sand kg^{-1}. Three plots of ca. 400 m^2 were sampled at random from an agricultural field of 1 ha. Thirty samples were collected by augering the top 0–15 cm layer of each plot. The soil from each plot was pooled, taken to the laboratory and separately 5-mm sieved. As such, three soil samples were obtained of more than 10 kg soil.

2.4. Sterilized soil with earthworms

Fifty grams sub-samples of soil of each plot were added to 162 120-ml glass flasks. One-third of the soil samples (54) were amended with 5% sewage sludge on a dry weight base, one-third with 5% vermicompost on a dry weight base and the rest were left unamended. The soil was mixed and adjusted to 70% WHC by adding distilled water, found to be the optimum water content when earthworms are added (Contreras-Ramos et al., 2005). The flasks were sterilized three times for 30 min with an interval of a day with pressurized steam at 121 °C supplied by an autoclave (Wolf and Skipper, 1996). Phen, Anth and BaP were dissolved in acetone and added to soil at a concentration of 100 mg kg^{-1} Phen, 500 mg kg^{-1} Anth and 50 mg kg^{-1} BaP under sterile conditions.

Five adult earthworms were weighed and added on top of each soil sample. As a whole treatments were (1) sterilized soil contaminated with PAHs and added with earthworms, (2) sterilized soil contaminated with PAHs and added with earthworms and sterilized sewage sludge, and (3) sterilized soil contaminated with PAHs, and added with earthworms and sterilized vermicompost.

Three samples from each treatment were taken at random, extracted for PAHs with acetone and analyzed on