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Using earthworms to test the efficiency of remediation of oil-polluted soil in tropical Mexico

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ABSTRACT

This study focuses on the medium-term effects of soil bioremediation on mortality and reproduction rates of *Eisenia fetida* (laboratory experiment) and of the tropical earthworm *Polypheretima elongata* (field experiment). We compared soils restored with the two bioremediation technologies landfarming (LF) and compost-bioremediation (BI) with control soils and with soils contaminated with 1% and 2% of petroleum. Control and restored soils both were fertile and showed low hydrocarbon contents. The mortality of *E. fetida* was not influenced by soil restoration and by contamination with 1% petroleum; it only increased in soils contaminated with 2% petroleum. However, the reproduction rate of *E. fetida* was significantly lower in the soils restored with LF and in those contaminated with 1% crude oil and significantly higher in the soils restored with BI. *P. elongata* showed the same reaction as *E. fetida*. We conclude that it is important to include reproduction or other sub-lethal tests for earthworms when estimating the efficiency of restoration techniques.

1. Introduction

In many countries where petroleum exploration is done, soils are contaminated with crude oil on a nearly monthly basis, due to the use of old exploration technologies. In SE Mexico, between 1993 and 1998, 686 oil spills occurred (CIMADES, 1999). A great number of restoration technologies exist to clean oil-contaminated soils. In the last decade, bioremediation technologies have been commonly used to restore oil-contaminated soils (Adriano et al., 1999). Bioremediation using different types of allochtonous or autochtonous organisms is an environmentally friendly and cost-effective approach to remove contaminants from the environment (Lynch and Moffat, 2005). Bioremediation accelerates the naturally occurring biodegradation of organic pollutants optimizing these processes via aeration and the addition of nutrients controlling pH, moisture content, and temperature (Atlas and Bartha, 1992).

Determining the efficiency of soil restoration has only looked at soil chemical parameters, i.e. hydrocarbon content after restoration (SEMARNAT, 2003). However, no information has been required concerning soil restoration as far as soil's function as a habitat for fauna and flora. Dorn et al. (1998) suggest the need to develop biological criteria for post-restoration soil quality as an alternative to arbitrary chemical criteria cleanup levels. This would show ecological risk for soils. Earthworms show a very high sensitivity to oil and heavy metal contamination (Dorn et al., 1998; Morgan and Morgan, 1992). Römbke et al. (2005) confirm the necessity to include bioindicators such as earthworms in studies about the soil quality. Individual and combined ecotoxicology tests have already been applied in studies assessing the efficiency of different types of soil bioremediation (Molina-Barahona et al., 2005; Plaza et al., 2005; Saterbak et al., 2000; van Gestel et al., 2001). The earthworm *Eisenia fetida* has been used as a test organism for different contaminants (OECD, 2004); however, tropical earthworm species have not yet been included in ecotoxicological research work. Therefore, it is important to expand on the use of endogeic tropical earthworm species, as they use soil resources differently from epigeics (as *E. fetida*); then it is relevant to use tropical earthworms in order to understand the impact of soils pollutants on tropical soils.

The aim of this study is to investigate the effect of two different bioremediation technologies (on site-compost-bioremediation (BI) and in situ landfarming (LF)) on the habitat function of the soil. Therefore, we tested the response of the compost worm *E. fetida* and the tropical, endogeic species *Polypheretima elongata* to soils restored with different technologies. Furthermore, we also

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tested the response of *E. fetida* to soil recently contaminated with 1% and 2% crude oil to determine the tolerance of this species to oil contamination. We assumed that earthworm mortality and reproduction rate may be sensible indicators for the efficiency of soil restoration.

2. Methods

2.1. Study area

The study was carried out in Tabasco State in tropical SE Mexico. The climate is generally warm and humid with an average annual precipitation of 3862 mm. Average annual temperature is 25.4 °C (INEGI, 2000). This zone has three seasons: a season with lower rainfall (April–May); a tropical rainy season (June–October); and a season with moderate rainfalls from November to March. Predominant soils in the area are Gleysols and Fluvisols, which have a clayey texture and are flooded by river inundations during the tropical rainy season. Parent material of all soils in the study region is homogeneous, characterized by loamy or clayey alluvial material (Palma-López and Cisneros, 2000). The experimental sites were located on Fluvisols.

2.2. Technologies of restoration, experimental design and soil sampling and analysis

In summer 2004, we selected experimental sites in two areas that had been contaminated with crude oil in 1999 and 2000 and that had been restored in 2002.

Two bioremediation technologies were applied. Restoring the contaminated soils with LF technology was processed in situ. The contaminated soil was homogenized and a desorbent was applied. Afterwards, an oxidant was applied, followed by inorganic NPK application to stimulate petroleum degradation by autochthonous microorganisms. The other technology, BI, was applied on site; i.e. the contaminated soil was removed to areas beside the field. The soil was homogenized and a commercial product of bacteria and compost was applied to stimulate oil degradation by allochthonous microorganisms (SEMARNAT, 2004).

We selected three replication plots for each restoration technology. Additionally, we selected three control plots which had never been contaminated or restored in the same areas, i.e. six control plots in total. We assumed that the control and restored soil before treatment showed the same characteristics due to the fact that the restored plots and their respective control plots were located on the same field sites. The plots of restoration were located in the area of the fields which was contaminated and afterwards restored; the control plots were located in the non-contaminated part of the same field sites.

Each experimental plot had a size of 100 m^2. For the characterization of soil properties, we took three soil samples of each plot at 0–30 cm depth, taking into consideration the treatment depth of 30 cm.

We analyzed the following soil physical–chemical properties using standard methods outlined in SEMARNAT (2002); pH(KCl), Corg. (Walkley and Black), Ntot (Kjeldahl), Ca-exchangeable, Pdisp. (Olsen), Cation exchange capacity (CEC) texture, DA. Furthermore, we analyzed the content of aliphatic (C10–C25) and aromatic hydrocarbons of the same soil samples following the methods described by US EPA (1996a, b). The hydrocarbons were extracted with soxhlet extraction during 8 h using dissolution of hexane and dichloromethane (1:1). Afterwards the samples were concentrated with rotary evaporation. We determined the aliphatic fraction using a FID gas chromatograph and the aromatic fraction using a mass spectrograph.

2.3. Earthworms experiment

The study was divided into two parts: a laboratory experiment and a field study. In summer 2004, we installed an ecotoxicology test in the laboratory to study the mortality and reproduction rates of *E. fetida* in the two restored soils (LF-R, BI-R). Results were compared with the two control soils (LF-C, BI-C) and with two variants contaminated with 1% (LF-Co-1, BI-Co-1) and 2% light crude oil (LF-Co-2, BI-Co-2).

We inserted the control and restored soils from the field plots, formally sieved to 2 mm, in glass containers. We mixed the soils of the three control plots and the three restored plots, respectively to get a total of 4 replication units per treatment. To apply the contaminated variants (LF-Co, BI-Co), we treated the two control soils (LF-C, BI-C) with 1% and 2% light crude oil which contained 60.6 mg kg^{-1} aromatic hydrocarbons and 3500 mg kg^{-1} aliphatic hydrocarbons (Table 1). We analyzed the content of aliphatic and aromatic hydrocarbons of the contaminated variant after 0, 1 and 15 days. We put 18 kg of each soil (control, restored, and contaminated soil) in four glass containers, for a total of 24 units.

The mortality test for *E. fetida* was carried out following the norm of ISO/DIS 15799 (ISO, 2001). We placed 30 adult *E. fetida* individuals into each container and put them in an open area covered by a roof to ensure that rain would not enter the containers. The number of surviving earthworms was determined after 14 days by hand-sorting; then a mortality rate was determined. Soil moisture was kept constant at field capacity. Air temperature varied between 22 and 28 °C, according to natural conditions.

Table 1
Experimental design of laboratory study ($n = 4$) (LF = landfarming, BI = compost-biorremediation)

Technology	Variant	Treatments
LF	C	Control soil of LF
LF	R	Soil restored with LF
LF	Co-1	Control soil of LF contaminated with 1% crude oil
LF	Co-2	Control soil of LF contaminated with 2% crude oil
BI	C	Control soil of BI
BI	R	Soil restored with BI
BI	Co-1	Control soil of BI contaminated with 1% crude oil
BI	Co-2	Control soil of BI contaminated with 2% crude oil

The reproduction test for *E. fetida* was carried out following the OECD Guideline 222 (OECD, 2004). However, we applied some modifications to the test, as we used natural soils (sieved to 2 mm) as substrate. We inserted 30 adult individuals in each glass container, and estimated the number of juvenile and adult individuals after 2 months to determine the rate of reproduction. The worms were fed daily with *Mucuna utilis* leaves, which we placed onto the soil surface.

To corroborate the results of the laboratory experiment, we conducted a field experiment to determine mortality and reproduction rate of the endogeic, most abundant earthworm species in the area, *P. elongata*. This species belongs to the family Megascolecidae. The field experiment was carried out from February to April 2006. In each field plot (six restored plots and six control plots in total), we installed 3 units of 0.75 m × 1.50 m. These units were delimited by metal barriers down to a depth of 50 cm.

In the beginning of the experiment we determined the abundance of the *P. elongata* population in the control and restored plots besides the units, hand-sorting four monoliths, each with size of 0.25 m^2 (Anderson and Ingram, 1993). In the control plots of BI, we determined a mean abundance of 19 adults and 37 juveniles per m^2. In the control plots of LF, we found a mean abundance of 23 adults and 43 juveniles per m^2. In the restored parcels, no earthworms were found. Based on the natural abundance determined in the corresponding control plots, we manually inserted the same abundance of *P. elonga* into the units of the restored plots. During the experiment, soil humidity was kept at about 30%. Sixty days later we extracted the earthworms by hand-sorting from the units of the restored control plots to determine the reproduction and mortality rate. The 2-month period of the experiment was determined due to the results of previous laboratory studies where we observed a reproduction period of 25–30 days.

2.4. Data analysis

We applied the Kolmogorov Smirnov test to test for normal distribution of the data. In case of normal distribution, we tested significant differences between the variants with the one-way ANOVA, followed by the Tukey test. In cases of non-normal distribution we applied the Kruskal–Wallis test followed by the Mann–Whitney *U*-test. We estimated correlations (Spearman) between physical–chemical soil properties and mortality and reproduction rates.

3. Results

3.1. Physical– chemical properties of the soils

The soils restored by LF showed significantly different soil physical–chemical properties compared to the control soils. The pH value, organic matter and Ca content were significantly higher, while the P content was significantly lower in the restored soils. The apparent density as well as the sand content was higher, and the clay content lower (Table 2a). The soils restored with BI also showed significantly different soil chemical properties compared to the control plots, such as significantly higher pH value and P content (Table 2a). However, soil physical properties did not show significant differences. The soils contaminated with