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Macrofauna and mesofauna from soil contaminated by oil extraction

David García-Segura[a], Isis Maviel Castillo-Murrieta[a], Froylán Martínez-Rabelo[a], Antonio Gomez-Anaya[a], Jacobo Rodríguez-Campos[c], Benito Hernández-Castellanos[b], Silvia M. Contreras-Ramos[c], Isabelle Barois[a,*]

[a] Red de Ecología Funcional, Instituto de Ecología A.C. (INECOL), Carretera antigua a Coatepec 351, El Haya, Xalapa 91070, Veracruz, Mexico
[b] Facultad de biología, Universidad Veracruzana, Circuito Gonzalo Aguirre Beltrán s/n, Zona Universitaria, 91090 Xalapa, Veracruz, Mexico
[c] Unidad de Tecnología Ambiental, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. (CIATEJ), Av. Normalistas No. 800, Col. Colinas de la Normal. Guadalajara, A.P. 2-191, Guadalajara, 44270, Jalisco, Mexico

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ABSTRACT

Mexico is an oil producing country; the extraction of oil on land has left many sites with soil contaminated by oil spills. The aim of our study was to determine the impact of this contamination on the soil fauna; thus we compared the macro and mesofauna from a non contaminated soil to a moderately and highly polluted soil caused by oil extraction. Total petroleum hydrocarbons (TPH) and soil physicochemical characterization was determined. TPH results showed two areas: highly contaminated (8150 mg TPH/kg) and moderately contaminated (1800 mg TPH/kg). The macrofauna abundance was not significantly different between the sites. The Hymenoptera, Gastropoda, Isoptera and the earthworms were the most abundant groups. The Gastropoda population decreased with the increase of TPH concentration while other groups of macrofauna increased their density (ants, isopteran and earthworms). The mesofauna was significantly more abundant in the moderately contaminated area (50,500 Ind./m^2). The main groups present were the Acari, Collembola and ants. The Acari orders were present in similar proportions both in the control soil and in the medium and highly contaminated area, while the Collembola families varied in their proportion in the three areas. The diversity index showed that the moderately contaminated site was the more diverse both in macro and mesofauna. Many groups of fauna (earthworms, ants and Isoptera) were positively correlated to some petroleum hydrocarbons (PH), such as naphthalene. The Gastropoda and Acari were the groups that were most negatively correlated to the different hydrocarbons. The PCA differentiated significantly three groups, both in the case of macrofauna as well as for mesofauna. Earthworms were clearly associated with TPH specially the native species *Protozapotecia australis*. These results indicate that oil spills could be a source of food for soil organisms after oxygenation and weathering.

1. Introduction

The petrochemical industry is one of the most important activities in the world due to the great demand for hydrocarbons and their by-products for other industries. At the same time, oil extraction and associated activities have negative impacts on the environment, mainly in the form of spills or waste products, which affect the sea and soil, the flora and fauna. Mexico is the tenth largest producer of oil worldwide, with 2267 millions of barrels per day (PEMEX, 2016). All oil activities have as consequences the spreading of hydrocarbons to the soil. According to the last report, 159 spills in the soil were recorded as hydrocarbon pollution in Mexico, with a total of 1162 ha of contaminated soil in 2015 (PEMEX, 2016). The states with most spills recorded are Tabasco (40.2%), Veracruz (32.1%) and Chiapas (18.3%) (CNH, 2014). These spills have received little attention; they are rarely remediated and they can last for decades before any action is taken (PEMEX, 2015).

Oil hydrocarbons are toxic to organisms particularly to beneficial soil organisms, such as the meso- and macrofauna: acari, collembola, potworms, earthworms, etc. (Cébron et al., 2011; Reinecke et al., 2016; Sverdrup et al., 2001; Van Brummelen et al., 1996). These organisms have important functions in the maintenance of soil ecosystem services and they contribute as well in the form of physical engineering by creating channels and aerating the soil and biochemical engineering by promoting the decomposition of organic matter in the soil and causing interactions with fungi and bacteria (Lavelle et al., 2016). In this way, they are essential for maintaining the functionality of terrestrial

* Corresponding author.
E-mail address: isabelle.barois@inecol.mx (I. Barois).

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ecosystems. So, the abundance and diversity of soil organisms have been used as indicators of environmental impact and disturbance due to natural or anthropogenic activity, such as some microarthropods (Austruy et al., 2016) and especially collembolas (Ardestani and Van Gestel, 2014; Gimenes-Rieff et al., 2016). The macrofauna and some of their groups have also been used as bio-indicators to determine disturbance or contamination (Bedano et al., 2011; Paoletti, 1999; Rousseau et al., 2013; Ruiz et al., 2011; Velasquez et al., 2007; Wahl et al., 2012). Earthworms and collembola are sensitive to changes and have therefore been used for eco-toxicological tests (Römbke et al., 2006). However, there are few studies concerning meso- and macrofauna at contaminated sites or their tolerance to petroleum hydrocarbons both separately or together. The lower taxonomic resolution of groups can give a moderate quality of soil faunal function and diversity with reduced taxonomic effort and cost. Although a full identification of part of the community at high taxonomic resolution is required especially when you are looking for bio-indicator groups (Ekschmitt et al., 2003). Thus, the aim of this study was 1) to determine meso- and macrofauna groups at a contaminated site due to spills resulting from oil extraction and to observe their relation with total petroleum hydrocarbons (TPH) and specific hydrocarbons (PH) and 2) to observe the diversity of specific groups like earthworms, acari and collembola which are groups susceptible to be used as indicators of soil contamination by PH.

2. Materials and methods

2.1. Experimental site and soil sampling

2.1.1. Experimental site

The oil well 98 field Miguel Alemán, is located in the Totonaca region, near Papantla in the north of the state of Veracruz, Mexico whose coordinates are 20°27′22.6″ N and 97°20′8.5″ W. Its climate is warm with an average temperature of 24.4 °C, with abundant rain in summer, its annual average rainfall is 1010 mm and it is 50 m.a.s.l. Its original vegetation was tropical semi-deciduous forest. The surrounding vegetation now is secondary forest or grassland and citric plantations; the contaminated area is partly covered by grass. The main activities in the surrounding area are the oil industry, agriculture and livestock. In this region, the tar or “chapopote” as it was called by the pre-Colombian inhabitants was often found naturally on the soil surface, particularly during the last century but with intense oil extraction they were less obvious (Aguilera, 1980).

2.1.2. Soil and macrofauna sampling

In the area of oil well No. 98, where the oil extraction stopped 15 years ago, a contaminated site as well as a surrounding area free of contamination (which served as a control) was sampled. The contaminated site corresponded to the channel, which was used to lead the oil spill to a small dam that was made to retain it. The non-contaminated site (control) was set 100 m apart more or less parallel to the channel. At both sites a transect of 100 m was established and every ten meters a monolith (25 × 25 × 30 cm) was excavated and divided into three strata at a depth of 0–10, 10–20 and 20–30 cm following the method of the Tropical Soil Biology and Fertility programme (TSBF, Anderson and Ingram, 1993). A total of ten monoliths were sampled per site. Each monolith was reviewed manually at different depths and macrofauna were collected and placed in their respective plastic containers containing 70% alcohol; with the exception of earthworms which were kept in containers with formaldehyde at 4%. Afterwards, the taxonomic groups were identified, counted and weighed in the laboratory with the help of taxonomic keys (Dindal, 1990). In general, the organisms were identified up to the order level for insects and species level for earthworms (Oligochaeta).

2.1.3. Mesofauna determination

The mesofauna of the soil was sampled next to each of the monoliths from both transects with a cylinder measuring 5 cm in diameter and 10 cm tall (196.25 cm^3) at a depth of 0–10, 10–20 and 20–30 cm. The samples were transported in plastic bottles in order to protect the organisms until the mesofauna extraction with a homemade Berlese funnel (Karyanto et al., 2008). After the extraction, the mesofauna were counted and identified. Acari were identified to the level of order and collembola to the family level.

Deeper taxonomic identification in the majority of the groups was not done because keys, experience, time and money were not available; the only groups that had a higher taxonomic identification were those that could be susceptible to be used as indicators.

2.2. Soil characterization

One kilogram of soil was taken at each point from the transects where the monoliths were made to determine soil characteristics such as pH (H2O), electrical conductivity (EC), cation exchange capacity (CEC), available (P-Olsen) and total phosphorous (TP), organic (OC) and total carbon (TC), total nitrogen (TN), C/N ratio, moisture, field capacity, bulk density, texture, iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni), lead (Pb) and cadmium (Cd). All the techniques for the physical and chemical parameters were based on the Soil Science Society of America methods of soil analysis (Sparks et al., 1996) or according to the standardized Mexican protocols NOM-021-RECNAT-2000 (SEMARNAT, 2000). Available P was measured with Olsen method and total P with vanadate-molybdate method. Additionally, a sub-sample (200 g) from the soil obtained from the contaminated area in the ten points sampled was taken, stored at 4 °C until its analysis to measure the content of TPH and individual PH.

2.3. Total petroleum hydrocarbons (TPH) determination

The TPH were determined using gas chromatography with mass detector (GC-MS) according to the sonication extraction method 3550B of the Environmental Protect Agency (EPA, 2007) slightly modified. The extraction of TPH was made from 2 g of dry homogenized soil, mixed with 2 g of anhydrous $NaSO_4$ and placed into a 20-mL glass tube. 10 mL of dichloromethane (DCM) were added and mixed in a vortex and after that sonicated in an ultrasonic bath (Brason 5800/5510) at 40 °C for 30 min with vortex agitation every 5 min. After this, the solution was transferred into a 50-mL flask and 10 mL of DCM was added again to repeat the extraction process twice. The extracted solution was added to the same flask and left to evaporate up to 3 mL volume and then passed through a glass column containing 20 g of florisil® (Sigma) and Na_2SO_4 (1:1) (Sigma) to clean the extract. The column was conditioned with 25 mL of DCM and samples were eluted with 40 mL of DCM, filtered using a 45 μm nylon disk filter and concentrated to 1 mL.

The identification and quantification of individual alkanes and polycyclic aromatic hydrocarbons (PAHs) were measured by gas chromatography (GC–MS). The GC (Agilent Technologies model 6890) was equipped with a capillary column HP S1933 (30 m × 0.25 mm × 0.25 μm). The oven temperature was set at 50 °C for 1 min, increased until 120 °C at a rate of 25 °C/min after 10 °C/min until 160 °C, 6 °C/min until 240 °C and 2 °C/min until 315 °C and finally maintained at 315 °C for 10 min. The carrier gas was high purity helium at 1.2 mL/min. The injection volume was 1 μL in splitless mode at 300 °C. The selective mass detector was a quadrupole (Agilent Technologies Model 5975), with an electronic impact ionisation system at 70 eV and at 230 °C.

The identification of compounds was achieved with pure standards and the mass spectra with the NIST5.0/EPA/NIH (version 2.0 d) library of mass spectra. Standard curves for quantification were obtained by preparing a solution with a mixture of the different standard compounds of alkanes (Sigma, UST122) and PAH (Sigma, 69281) in