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Tropical Endogeic Earthworm Population in a Pollution Gradient with Weathered Crude Oil

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ABSTRACT

For decades, toxicity studies on earthworms have focused only on the influence of heavy metal while there is little data on the biomarker´s response to organic contaminants. Most of the soil toxicity tests have been carried out using epigeic earthworm; however, exposure and effect of these species to soil contamination is sometimes questioned. Thus, current knowledge on the sensitivity and ability of tropical endogeic earthworms to survive in hydrocarbon-polluted soils is limited. The present study aimed to determine the earthworm populations present in soil polluted with weathered crude oil. Earthworm diversity, abundance and biomass, as well as the physical and chemical properties of soil and total oil hydrocarbon content, were determined along a pollution gradient. The findings reveal that the chemical quality of soil decreases significantly with soil depth, and that oil-hydrocarbons are dispersed across the pollution gradient. Earthworm abundance and biomass largely depend on the pollution gradient and are related to soil texture and nutrient content. Three earthworm endogeic species were recorded in this study, with the exotic *Pontoscolex corethrurus* as the dominant species. We conclude that tropical endogeic earthworms may be potentially useful in ecotoxicological and biostimulation studies, and may have more ecological relevance than using epigeic earthworms. Further studies are needed to elucidate the hypotheses.

Key words: Biomarker, ecotoxicology, ecosystem engineers, soil quality, environmental services

INTRODUCTION

Oil industry has changed the landscape worldwide, reducing the environmental services provided by biodiversity, soil and water (Chaineau *et al*., 2003; Culbertson *et al*., 2008; Wang *et al*., 2010). Southeastern Mexico, the Cinco Presidentes Oil Field has recorded the highest number of oil spills, causing soil pollution and reducing the area and productivity of both natural ecosystems and agroecosystems (Zavala-Cruz *et al*., 2005; Adams *et al*., 2008).

Weathered crude oil is defined as oil that has been exposed to environmental conditions in and on the ground. The residence time of hydrocarbons in sediments may range from 3 to 40 years but the degradation rate in the tropics can be increased by the presence of nutrients, light, high temperatures and oxygen, all of which allow high rates of bacterial breakdown and photochemical oxidation (Bojes and Pope 2007; Culbertson *et al.*, 2008). Some properties of soil, such as clay content and type, organic matter content and hydrogen ion concentration (pH) favor the fixation of polluting chemicals, thereby affecting the persistence of toxic chemicals in the soil (Chaineau *et al.*, 2003; Zavala-Cruz *et al.*, 2005; Bojes and Pope, 2007; Adams *et al.*, 2008). For example, oil spills in clayey marshlands take many years to recover, whereas coarse soils recover promptly. This suggests that hydrocarbon polluted soils undergo physical, chemical and biological degradation processes that translate into a reduction in the current and potential capacity of soil to produce goods and services (Sanchez-Hernandez, 2006).

Among the ecosystem engineers, earthworms are a key functional group of the edaphic system, mainly for their beneficial effect on soil structure and function. The biological activity of these organisms (burrowing and feeding) exerts a positive influence on soil structure, water infiltration, organic matter dynamics, distribution and abundance of microorganisms, nutrient release, plant growth and degrade and accumulate heavy metals and hydrocarbons (Lee, 1985; Brown *et al.*, 1999; Lavelle and Spain, 2001). Earthworms occur in multiple soil types, are sensitive to physicochemical parameters, are the food source for a large variety of animals and have a short life-cycle and size that makes them suitable for field and laboratory handling (Paoletti, 1999; Rombke *et al.*, 2005).

The tropical epigeic species *Eisenia fetida* and the endogeic *Aporrectodea caliginosa* have been used in most ecotoxicological studies (Dorn and Salanitro, 2000; Schaefer *et al.*, 2005; Sanchez-Hernandez, 2006) for assessing the effect of certain heavy metals (e.g., Cu, Cd, Zn and Pb); however, studies on the response of earthworms to organic pollutants such as hydrocarbons are scarce (Ma *et al.*, 1995; Schaefer *et al.*, 2005; Geissen *et al.*, 2008). In addition, earthworms should be evaluated in the field to validate them as early-warning indicators of negative ecological consequences (Sanchez-Hernandez, 2006; Suthar *et al.*, 2008). In this regard, there is limited knowledge about the ability of endogeic tropical earthworms species (which live in and consume soil) to survive in hydrocarbon-polluted soils (Geissen *et al.*, 2008). Therefore, investigating the potential use of tropical endogeic earthworms as bioremediators of polluted soil and assessors of ecological risk is key. The purpose of the present study was to investigate the presence of earthworms in soil contaminated with weathered crude oil.

MATERIALS AND METHODS

Study area: The study was conducted in the area of influence of the Cinco Presidentes Oilfield (18°124' 50" N and 94°00' 45" W), located in the municipality of Huimanguillo, Tabasco, Mexico. The relief consists of fluvial plains and marshes with clayey and silty sediments. The climate is warm and humid with mean annual temperature and precipitation of 26.0°C and 1800 mm, respectively. Coconut and pastures are cultivated in this area for copra and livestock, respectively. It is also common to find patches of lowland evergreen forest, palm trees, mangroves and hydrophilous vegetation (Zavala-Cruz *et al.*, 2005).

In this oil field a sampling site was selected with a crude oil spill from the Battery No. 5 separation pit (water and gas removed from crude oil), which has weathered for 20 years (Zavala-Cruz *et al.*, 2005).

Earthworm sampling: The sampling site has a dystri-hotric anthrosol soil (Zavala-Cruz *et al.*, 2005). Three 50 m transects with a spacing of 5 m were marked from the source of the crude oil spill (Battery 5 pit) in order to establish a pollution gradient including five levels: A, B, C, D and E (10, 20, 30, 40 and 50 m, respectively). Five monoliths (25×25×40 cm in depth) were built in each transects at 10 m intervals, resulting in a total of 15 monoliths; that is, three replicates (monoliths) per pollution level. Each monolith was analyzed according to the TSBF method of macrofauna separation (Anderson and Ingram, 1993) and stratified into four layers, each 10 cm deep. Earthworms from each of the four monolith layers were collected manually and fixed in 4% formaldehyde which was subsequently replaced by 70% alcohol in the laboratory (Anderson and Ingram 1993). Subsequently, earthworms were sorted according to species and age-class, weighed and counted. The following age-classes were recognized: clitellate (adults with clitellum), juveniles (without sexual characteristics) and cocoons.

Soil chemical characteristics: Soil sample of 1 kg was collected from the four depths of each monolith in order to determine the physical (clay, silt and sand, field capacity) and chemical (pH, electrical conductivity, organic matter, total nitrogen, phosphorus, potassium and cation exchange capacity) characteristics of soil based on the TSBF method (Anderson and Ingram, 1993). In addition, another 250 g sample of soil was collected to quantify the content of Total Petroleum Hydrocarbons (TPH) as per the Mexican Official Standard NOM-138-SEMARNAT/SS-2003 (SEMARNAT, 2005).

Statistical analysis: A database was constructed with the information recorded. The following statistical analyses were performed using the software Statistica (StatSoft, 1999): (a) Two away ANOVA, to test differences in earthworm abundance and biomass and age-class among the pollution gradient (five levels) and vertical distribution (four layers), (b) Tukey's multiple comparison test (HSD, $p<0.05$) of all the variables mentioned above and (c) Pearson's correlation, to determine the relationship between earthworm demography and soil physical and chemical characteristics and earthworm demography and total hydrocarbon content. Original data was transformed (square root) to achieve a better fit to the normal distribution required for the parametric analyses performed.

RESULTS

This section presents in a population gradient the physical-chemical characteristics of the soil, the content of total petroleum hydrocarbon, the abundance and biomass of earthworms and the relationship between these variables.

Soil physical characteristics: In the analyses of the variation of texture with soil depth, the silt and sand varied significantly (Table 1). The highest percentage of silt was found in the first and uppermost layer of soil, followed by the second, with no difference between the third and fourth layers (Table 2). In contrast, the lowest proportion of sand was found in the first soil layer, with intermediate values in the second and third layers and peaking in the fourth layer. The amount of clay was similar throughout the fourth layers.

Nutrient content in soil: The p analysis revealed that Organic Matter (OM), total N, P, K and CEC varied significantly with soil depth (Table 1). Organic matter decreased significantly with soil depth in each of the four soil layers (Table 2). Total N content varied significantly from intermediate levels between the uppermost two and the deepest two layers, respectively. P content was low across the soil profile, being significantly higher in the uppermost layer relative to the other three. K was significantly higher in the first layer than in all other levels. Finally, CEC significantly decreased with soil depth.

Total Petroleum Hydrocarbons (TPH): Total Petroleum Hydrocarbons (TPH) varied significantly with the pollution gradient (Table 1). The results presented in the Fig. 1 show that the highest and lowest hydrocarbon content in soil were found in levels B and E, 20 and 50 m away from the spill, respectively; TPH displayed intermediate concentrations in levels A, C and D (10, 20 and 40 m). In 11 of 15 monoliths (73.3%) were found an average concentration

Table 1: Significance level of the ANOVA of soil characteristics and earthworm parameters across the gradient, profile and their interactions of anthrosol soil polluted with weathered oil

	Gradient pollution		Soil depth		Gradient × soil depth	
	F	P	F	P	F	P
Soil characteristics						
Clay	1.330	0.276	0.958	0.422	1.138	0.358
Silt	2.175	0.089	6.053	0.001	1.824	0.077
Sand	2.400	0.068	3.600	0.022	0.800	0.603
pH	2.341	0.071	0.271	0.846	0.385	0.961
Organic matter	2.108	0.976	13.695	0.001	0.392	0.958
Total N	1.491	0.223	24.428	0.001	0.574	0.849
P	2.153	0.092	11.277	0.001	0.299	0.985
Total K	0.287	0.884	15.577	0.001	0.321	0.981
CIC	1.123	0.359	10.061	0.001	0.402	0.955
TPH	2.879	0.039	0.857	0.473	1.492	0.182
Earthworm parameters						
Abundance	1.403	0.245	4.460	0.008	0.648	0.788
Biomass	1.262	0.295	8.704	0.001	0.722	0.721

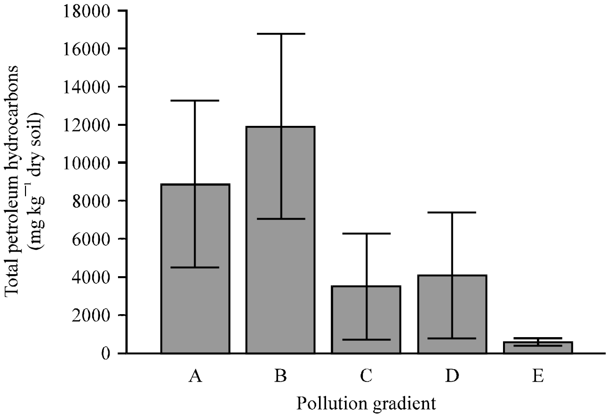

Fig. 1: Dispersion of total petroleum hydrocarbons in the pollution gradient of an anthrosol soil from Battery 5 of the Cinco Presidentes Oilfield. A: 10, B: 20, C: 30, D: 40 and E: 50 m. Vertical lines indicate standard error

Table 2: Physical and chemical properties of soil across the profile of anthrosol soil polluted with weathered oil

	Depth (cm)			
Parameter	0-10	10-20	20-30	30-40
Silt (%)	5.17 ± 1.78^b	4.24 ± 2.29^{ab}	3.44 ± 1.37^a	2.84 ± 1.56^a
Sand (%)	86.79 ± 1.85^a	87.85 ± 2.09^{ab}	88.25 ± 1.54^{ab}	88.72 ± 1.39^b
Clay (%)	8.04 ± 0.77^a	7.91 ± 0.69^a	8.31 ± 1.14^a	8.44 ± 1.23^a
pH (H_2O)	5.74 ± 0.34^a	5.89 ± 0.42^a	5.76 ± 0.80^a	5.89 ± 0.73^a
OM (%)	3.74 ± 1.17^c	2.50 ± 1.65^{bc}	1.56 ± 1.48^{ab}	0.86 ± 0.38^a
Nt (%)	0.13 ± 0.02^b	0.12 ± 0.04^b	0.07 ± 0.03^a	0.05 ± 0.02^a
P (cmol k^{-1})	3.87 ± 0.95^b	2.99 ± 1.08^a	2.48 ± 0.49^a	2.28 ± 0.38^a
K (cmol k^{-1})	0.12 ± 0.04^b	0.07 ± 0.02^a	0.06 ± 0.01^a	0.06 ± 0.01^a
CEC (cmol k^{-1})	4.45 ± 1.59^c	3.32 ± 1.54^{bc}	2.59 ± 1.05^{ab}	1.86 ± 0.63^a

In each rows, different letters indicate differences significant at Tukey test ($p<0.05$)

of 1013.01 mg kg^{-1} dry soil (from 99.7 to 8569.9) and in the rest (at levels A, B, C and D of the transect two) there was 35946.74 mg kg^{-1} dry soil (range from 31321.8 to 40506.6).

Earthworm populations: Three earthworm species were observed, belonging to the families Pontoscolecidae, Glossoscolecidae and Acanthodrilidae. The dominant species was the exotic earthworm *Pontoscolex corethrurus*, followed by *Glossodrilus* sp. and *Dichogaster saliens*. The exotic earthworm *P. corethrurus* was present in all levels of the pollution gradient, whereas the *Dichogaster saliens* was present only in levels B and D. The abundance and biomass of earthworms was similar along the hydrocarbon pollution gradient (Table 1). Earthworm abundance and biomass was higher in levels B and C, intermediate in levels D and E and low in level A (Fig. 2).

Vertical distribution: Earthworm abundance and biomass decreased significantly with soil depth (Table 1). At the pollution gradient levels A, D and E, all earthworms were found in the topsoil (Table 3). In contrast, at levels B and C the relative abundance of earthworms were in the first, second and third soil layers. The population of *P. corethrurus* was distributed into these three soil layers. Populations of the *Glossodrilus* sp. and *Dichogaster saliens* were found only in the soil surface.

Age classes: Juveniles were the most abundant age class across the pollution gradient (Table 4). Juvenile abundance and biomass varied significantly between levels A, B, C, D and E. In contrast, the abundance and biomass of adults were similar across pollution levels and these were absent in level A. The presence of cocoons in levels B, C and E were negligible and these were absent from levels A and D.

Relationship with soil physical and chemical properties of: The physical and chemical properties of the soil showed no correlation with the pollution gradient (Table 5). In contrast, soil depth was significantly and positively correlated with sand and negatively correlated with

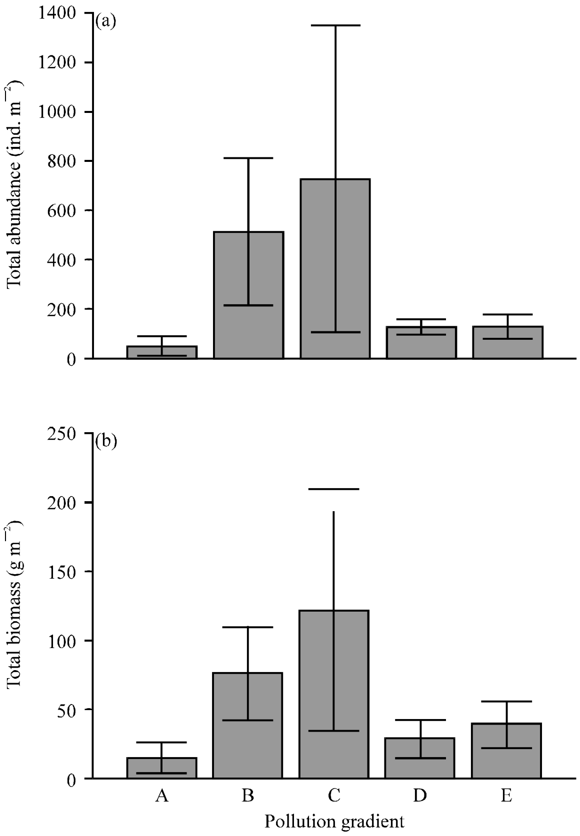

Fig. 2(a-b): (a) Total earthworm abundance (b) biomass , in the pollution gradient of an anthrosol soil from Battery 5 of the Cinco Presidentes Oilfield. A: 10, B: 20, C: 30, D: 40 and E: 50 m. Vertical lines indicate standard error

Table 3: Mean abundance and biomass of earthworm populations found in the pollution gradient of the anthrosol soil

		Pollution gradient				
Parameters	Depth (cm)	A	B	C	D	E
Abundance	0-10	48.0±69.7	416.0±433.2	480.0± 681.3	128.0±57.7	128.0±106.9
	10-20	0.0	74.7±72.2	240.0± 401.9	0.0	0.0
	20-30	0.0	21.3±18.5	5.3 ±9.2	0.0	0.0
	30-40	0.0	0.0	0.0	0.0	0.0
Biomass	0-10	14.2±19.1	63.9 ±46.8	85.9± 95.7	28.7±24.1	39.2±32.7
	10-20	0.0	10.9±10.4	35.9± 55.6	0.0	0.0
	20-30	0.0	1.2±1.1	0.3± 0.3	0.0	0.0
	30-40	0.0	0.0	0.0	0.0	0.0

OM, total N, P, CEC, K, silt and EC (in decreasing order). Regarding the abundance and biomass of earthworms, these parameters displayed a significant negative correlation with sand and depth (Table 6) and a positive correlation with total N, K, MO, silt and P (in decreasing order).

Table 4: Mean abundance and biomass by age class of the earthworm population in the pollution gradient of anthrrosol soil

		Pollution gradient				
Parameter	Age class	A	B	C	D	E
Abundance	Adult	0.0	53.3±48.9	48.0±69.7	16.0±27.7	26.7±24.4
	Juveniles	48.0±69.7	416.0±499.1	672.0±999.7	112.0±69.7	90.7± 64.7
	Cocoons	0.0	42.7±48.9	5.3±9.2	0.0	10.6±9.2
Biomass	Adult	0.0	23.3±21.4	21.2±29.7	7.6±13.2	12.2±11.9
	Juveniles	14.2±19.1	52.2±49.7	100.8±121.0	21.1±24.9	26.7±24.1
	Cocoons	0.0	0.5±0.5	0.1±0.1	0.0	0.2±0.1

Table 5: Pearson's correlation coefficient between the pollution gradient and the physical and chemical properties of anthrosol soil

		Gradient		Depth	
Properties	Parameter	R	P	R	P
Physical	Clay	-0.02	0.87^{NS}	0.22	0.10^{NS}
	Silt	0.08	0.54^{NS}	-0.53	0.000**
	Sand	-0.07	0.59^{NS}	0.43	0.001**
Chemical	pH	0.26	0.06^{NS}	0.02	0.87^{NS}
	EC	0.00	0.99^{NS}	-0.31	0.02*
	MO	0.08	0.55^{NS}	-0.88	0.000**
	Nt	0.13	0.34^{NS}	-0.82	0.000**
	P	0.10	0.48^{NS}	-0.74	0.000**
	K	0.14	0.30^{NS}	-0.62	0.000**
	CEC	0.11	0.43^{NS}	-0.73	0.000**
	TPH	-0.23	0.09^{NS}	-0.4	0.76^{NS}

*,**Significance level: $p<0.01$, 0.001, respectively, NS: Not significant

Table 6: Pearson's correlation coefficient between earthworm abundance and biomass and the physical and chemical properties of the anthrosol soil

		Abundance		Biomass	
Properties	Parameter	R	P	r	P
Distribution	Gradient	-0.04	0.78^{NS}	0.03	0.98^{NS}
	Depth	-0.39	0.002**	-0.49	0.000**
Physical	Clay	0.03	0.84^{NS}	-0.05	0.69^{NS}
	Silt	0.28	0.03*	0.33	0.01*
	Sand	-0.31	0.01*	-0.32	0.01*
Chemical	pH	-0.06	0.63^{NS}	-0.04	0.74^{NS}
	EC	-0.02	0.86^{NS}	-0.02	0.87^{NS}
	MO	0.27	0.03*	0.34	0.007**
	Nt	0.38	0.003**	0.44	0.000**
	P	0.22	0.09^{NS}	0.27	0.03*
	K	0.31	0.01*	0.37	0.003**
	CEC	0.19	0.14^{NS}	0.25	0.051^{NS}
	TPH	-0.03	0.79^{NS}	-0.01	0.94^{NS}

*,**Significance level: $p<0.01$, 0.001, respectively, NS: Not significant

DISCUSSION

In tropical as opposed to temperate environments, hydrocarbons are degraded at a faster rate due to better edaphoclimatic conditions (Sanchez-Hernandez, 2006). However, the weathered hydrocarbons derived from oil activities become more recalcitrant through time (Bojes and Pope, 2007). This study indicates that oil content varied markedly across the pollution gradient. Sites with low and high TPH concentrations (604.57 and 11885.11 mg kg^{-1}, respectively) were found 50 and 20 m away from the battery, respectively, i.e., hydrocarbons have dispersed both away from the spill (horizontally) and down into the soil column (vertically). In this regard, TPH levels ranging between 550 and 35108 mg kg^{-1} have been recorded in sites adjacent to Battery five and other batteries at the *Cinco Presidentes* Oilfield, which display pollution liabilities of more than 20 years (Zavala-Cruz *et al*., 2005; Adams *et al*., 2008). TPH levels at the La Venta Oilfield range from 115000 to 323000 mg kg^{-1} (Rivera-Cruz *et al*., 2002); levels ranging from 860 to 214515 mg kg^{-1} have been observed in other tropical regions of southern Mexico (Adams *et al*., 2008; Perez-Armendariz *et al*., 2010; Uribe-Hernandez *et al*., 2010).

Arenosols soil are relatively young, characterized by little or no profile development, homogeneous sands and coarse texture, all of which contribute to explain the high permeability and low water and nutrient-storage capacity (Zavala-Cruz *et al*., 2005; IUSS Working Group/WRB, 2006). The results of this study reveal that fertility varied significantly within soil; that is, the surface layer displayed the highest silt and nutrient content (OM and total N), while the other three vertical layers showed the highest proportion of sand. The pH was significantly negatively associated with the presence of hydrocarbons, while electrical conductivity had a significant and positive association.

Among disturbed environments (agroecosystems), grasslands favor the growth and development of earthworm populations (Lee, 1985; Fragoso *et al*., 1999; Marichal *et al*., 2010). In Mexico, 72% of the 120 earthworm species described are endogeic (Fragoso *et al*., 1999). In the present study, hydrocarbon polluted soil where grass and coconut palm tree are grown supports an earthworm community consisting of three endogeic species; similar results have been reported for other southeastern Mexico's grasslands (Ortiz-Ceballos and Fragoso, 2004; Huerta *et al*., 2005). Of the three species recorded in this study, *P. corethrurus* was especially abundant (85%). Juvenile earthworms comprised the best represented age class. Abundance and biomass figures recorded here for this species in the present investigation were similar to those reported for other grasslands of southeastern Mexico (Fragoso *et al*., 1999; Ortiz-Ceballos and Fragoso, 2004; Huerta *et al*., 2005), as well as for other tropical environments in the world (Lee, 1985; Marichal *et al*., 2010). However, earthworm abundance and biomass varied broadly (48.0-725.3 ind m^{-2} and 14.2-100.8 g m^{-2}, respectively), likely as a result of the spatial fertility or from the dispersion of hydrocarbons. That is, the abundance of earthworms was significantly associated with the physical (positive with silt and negative with sand) and chemical (positive with nutrients) soil characteristics.

Both earthworm abundance and hydrocarbon content varied significantly across the pollution gradient. These two variables were positively associated; the highest earthworm abundance and TPH occurred at 20 and 30 m of the spill, with 512.0 and 725.2 ind m^{-2} and 11885.2 and 3530.1 mg^{-1} kg TPH, respectively. This suggests that the ability of earthworms (mainly *P. corethrurus*) to colonize soils contaminated with hydrocarbons may be mediated by the following mechanisms: (a) Avoidance of contaminated microsites (De Silva and van Gestel, 2009; Natal-da-Luz *et al*., 2009) (b) Genetic plasticity to tolerate pollutants (Pena-Castro *et al*., 2006) and (c) Genetic resistance induced by mutagenic hydrocarbons (Wilding *et al*., 2006; Hirano and Tamae, 2010). Nevertheless, the type and amount of hydrocarbons observed may not be toxic (Uribe-Hernandez *et al*., 2010). Alternatively, soil fertility is likely to mitigate any toxic effects, as happens when organic amendments are applied (Ma *et al.*, 1995; Shakir and Weaver, 2002; Schaefer *et al.*, 2005; Wang *et al.*, 2010). This is plausible, given that earthworms were abundant (77.5%) in the topsoil and virtually absent (22.5%) in the three deeper layers. The above can be further investigated through laboratory tests to assess the effect of different types of polycyclic aromatic hydrocarbons (Ma *et al.*, 1995; Rombke *et al.*, 2005).

Earthworms are regarded as excellent bioindicators of soil quality (Paoletti, 1999; Sanchez-Hernandez, 2006). Their limited mobility facilitates proper monitoring of the impact of pollutants, changes in land use and agricultural practices (Paoletti, 1999). Endogeic species may possess a greater ability to tolerate pesticides, herbicides and heavy metals than epigeic ones (Paoletti, 1999). It is also known that epigeic earthworms may degrade or accumulate heavy metals (Ceccanti *et al.*, 2006; Elaigwu *et al.*, 2007; Maleri *et al.*, 2008) and hydrocarbons (Ma *et al.*, 1995; Shakir and Weaver, 2002; Contreras-Ramos *et al.*, 2009). Therefore, the results suggest that the earthworm *P. corethrurus* may be potentially useful in the same or to a larger extent than the species traditionally used in ecotoxicological and bioturbation studies (OECD, 2010; Buch *et al.*, 2011). This is because its biological activity (casting, burrowing and feeding) may contribute either directly or indirectly (through interactions with bacteria, roots and nutrients) to degrade hydrocarbons. Furthermore, its biological activity is easy to observe, no special expertise is required to identify this species (calciferous glands and large white or pink cocoons) and cultivating it is easy both in the field and the laboratory (Brown *et al.*, 1999; Ortiz-Ceballos *et al.*, 2009; Buch *et al.*, 2011). Further studies are needed to elucidate the hypotheses.

CONCLUSION

Based on the above, it is concluded that the endogeic earthworms (*P. corethrurus*, *Dichogaster saliens* and *Glossodrilus* sp.) tolerate the presence of weathered crude oil, and its presence is significantly associated with soil fertility. These species may be suitable bioindicators when contaminants occur at soil depths where epigeic earthworms are rarely found. Tropical earthworms may have an important potential role as endogeic bioindicators of ecological risk assessment (exposure and effects) of contaminated soils by the oil industry and other human activities that affect the environmental services of soil.

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