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Earthworm Populations in an Aged Hydrocarbon Contaminated Soil

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ABSTRACT

Hydrocarbon contamination is known to reduce the soil microfauna, but little is known about the sensibility of endogeic earthworms towards it. The aim of this study was to investigate how the earthworm biomass and abundance was affected by a petroleum contamination. Endogeic earthworms were studied in an anthrosol soil contaminated with hydrocarbons after an oil spill in ‘Cinco Presidentes’ (Tabasco, Mexico) 20 years ago. Monoliths of 0.5×0.5×0.4 m were taken along a transect at 10, 20, 40 and 80 m from the origin of the oil spill. Each monolith was divided in four layers (0-0.1, 0.1-0.2, 0.2-0.3 and 0.3-0.4 m) and the number of earthworms, soil characteristics and the concentration of polycyclic aromatic hydrocarbons (phenanthrene, anthracene and benzo(a)pyrene (BaP) were determined. BaP was detected, while anthracene and phenanthrene were not found. On average, the total abundance of the earthworms was 319 individuals m^{-2} with an average biomass of 43 g m^{-2}, but their abundance was not correlated with the presence of BaP. Three different species of earthworms were detected with *Pontoscolex corethrurus*, an endogeic earthworm, the most abundant (75%). Based on the results obtained, the presence of *P. corethrurus* in the contaminated site indicated its tolerance towards BaP and its possible use in the remediation of hydrocarbon contaminated soils.

Key words: Benzo(a)pyrene, bioremediation, ecotoxicology, endogeic earthworms, *Pontoscolex corethrurus*

INTRODUCTION

In the southeast of Mexico, the petrochemical industry activities have contaminated the environment, principally in the state of Tabasco, where oil spills has been reported frequently, with damage to fauna and flora (Perez-Armendariz *et al*., 2010). Oil spills caused by ruptures of oil ducts have polluted soils, reducing soil quality. Polycyclic aromatic hydrocarbons (PAHs) found in crude oil are composed by two or more aromatic fused-rings (Haritash and Kaushik, 2009) they are toxic and persistent in the environment and some can remain in the soil for a long time (Bojes and Pope, 2007). Among the 16 PAHs considered as priority pollutants for their toxicity to the environment and humans, benzo(a)pyrene (BaP) is potentially carcinogenic and mutagenic (USEPA, 1987). Although phenanthrene and anthracene have been reported to have no genotoxic and carcinogenic effect (Moody *et al.*, 2001), they have been found to be toxic to fish and algae (Sutherland *et al.*, 1992).

Earthworms have different important functions in the soil. They improve soil structure and contribute to organic matter decomposition and nutrient cycling. Earthworms burrow through the soil, excrete mucus, while their castings concentrate nutrients and soil microorganisms. Consequently, they improve the physical and chemical characteristics of a soil (Richardson *et al.*, 2009). Numerous studies have been done to investigate the effect of earthworms on soil fertility, but their sensibility to hydrocarbon contamination and their ability to survive in these polluted environments is limited (Geissen *et al.*, 2008).

Earthworms have been studied in different ecosystems of the southeast of Mexico (Ortiz-Ceballos and Fragoso, 2004; Huerta and Van der Wal, 2012), but few studies included hydrocarbon-contaminated sites (Trujillo, 2010). Earthworms can tolerate PAHs contamination, but their survival is affected when contamination reaches critical concentrations (Contreras-Ramos *et al.*, 2006; Tejada and Masciandaro, 2011). As such, their abundance in a contaminated soil might be used as an indicator of the level of pollution (De Silva *et al.*, 2009). Additionally, earthworms that survive in contaminated areas might be used to remediate contaminated sites. The study reported here investigated the earthworm population in an anthrosol soil from Campo Petrolero Cinco Presidentes (Tabasco, Mexico) contaminated 20 years ago. The objectives of this study were to i) to characterize the hydrocarbon contaminated soil, ii) to identify and count the earthworms at 10, 20, 40 and 80 m from the source of the contamination in the 0-0.1, 0.1-0.2, 0.2-0.3 and 0.3-0.4 m soil layers and iii) determine if the contamination has an effect on the earthworm abundance and biomass.

MATERIALS AND METHODS

Sampling site: The sampling site was located in Cinco Presidentes in the state of Tabasco, Mexico (17°45' and 18°12' NL and 93°45' and 94°08' WL) at 10 m.a.s.l. The sampling site was near an artificial lake and the sampled soil was contaminated due to an oil spill 20 year ago. The weather is wet with a mean annual temperature of approximately 26°C. The natural vegetation is composed of palm trees, bushes, lowland floodplains with mangroves and hydrophytes, grassland and multiple or perennial farming. The annual average precipitation is 1800 mm. The main activities in this area are oil extraction, agriculture and livestock farming.

Earthworm sampling: On the border of the artificial lake, three transects were made away from the contamination source. In each transect, a monolith (0.5×0.5×0.4 m) was taken at 10, 20, 30, 40 and 80 m from the contamination source. Each monolith was divided in four 0.1 m layers, resulting in a total of 60 soil samples (three transects, five sampling points and four layers) (Anderson and Ingram, 1993). Earthworms were extracted manually from each soil sample. The earthworms were collected and kept in glass beakers containing 4% formaldehyde.

In the laboratory, the formaldehyde was substituted by alcohol (75%), morfo species were separated, grouped by age as adults (clitellum present) or juveniles (without clitellum), counted and weighed. Number of species, abundance (individuals m^{-2}) and biomass were determined.

From each soil sample (n = 60), one kg of sample was taken to determine soil characteristics. The particle size distribution, pH, organic material, total nitrogen (N), extractable phosphorus (P) and total potassium (K) content were determined. All the techniques for the physical and chemical parameters mentioned above are included in the Official Mexican Norm (Norma Official Mexicana NOM-021-SEMARNAT, 2000) and they are based on the Soil Science Society of America methods of soil analysis (SSSA, 1996). Additionally, a 200 g sub-sample was taken to analyse the content of anthracene, phenanthrene and BaP. The PAHs were extracted from soil (Song *et al.*, 1995) and determined by an Agilent 4890D gas chromatograph (GC). Details of the technique used to extract the PAHs can be found in Contreras-Ramos *et al.* (2008). Briefly, 1.5 g of soil was weighted and mixed with 3 g anhydrous sodium sulphate and 10 mL acetone was added shaken on a vortex and sonicated for 20 min. The PAHs extracted with acetone were separated from the soil by centrifugation at 13 700×g for 15 min. The same procedure was repeated three times and the filtered extracts were concentrated to 2 mL and then analyzed for phenanthrene, anthracene and BaP by GC.

Statistical analysis: Significant difference between soil characteristics, earthworm populations and concentrations of anthracene, phenanthrene and BaP as a result of distance from the contamination source, soil depth and their interaction were determined by analysis of variance (ANOVA) and based on the least significant difference using the general linear model procedure ($p<0.05$) (SAS, 1989). Soil and earthworm characteristics and concentrations of BaP were separately explored with a principal component analysis (PCA) using PROC FACTOR (SAS, 1989). Phenantrene and anthracene were not found in the studied zone. The matrix of 11 columns (abundance and biomass of earthworms, particle size distribution (sand, loam and clay), pH, organic material, total N and K, extractable P and concentration of BaP and 60 lines (rows) was used for principal component analysis.

RESULTS

Physical and chemical parameters of anthrosol soil: The organic material, total N, extractable P, showed significant differences within the soil profile between 0-0.1 and 0.3-0.4 m and mostly at 30, 40 and 80 m from the contamination source (Table 1). In the profile and at that distance, the organic material decreased significantly from an average of 20 to 5 g kg^{-1}, while total N decreased from 1.4 to 0.5 g kg^{-1} and extractable P from 1.9 to 0.7 mg kg^{-1}.

The pH and extractable p were significantly affected by the distance from the source of contamination (gradient) ($p<0.05$ and $p<0.0001$), total N, extractable P and total K by depth ($p = 0.0079$, $p = 0.003$ and $p = 0.0009$), but none of the soil characteristics was affected by the interaction between depth and gradient (Table 2). Only clay, loam and sand content were significantly affected by gradient, depth and their interactions (gradient×depth) ($p>0.0001$).

Concentrations of polycyclic aromatic hydrocarbons in soil: Only BaP was detected in the contaminated soil. Its concentration was significantly larger at 10 m (37 mg kg^{-1}) of the source of contamination than at other distances and significantly higher in the 0-0.1 m layer (39.3 mg kg^{-1}) than in the other layers (Fig. 1a, b). The BaP concentration in the soil decreased significantly with depth and gradient. Distance from the contamination source, depth and their interactions had a significant effect on the concentration of BaP ($p<0.0001$) (Table 2).

Table 1: Characteristics of an anthrosol soil along a transect, i.e. distance from the contamination source and in different soil layers, at Cinco Presidentes, Tabasco Mexico

		Distance from the contamination source (m)					
Soil characteristics	Soil layer (m)	10	20	30	40	80	MSD[a]
pH	0-0.1	5.5 A[b] a[c]	6.1 Aa	6.0 Aa	5.8 Aa	5.9 Aa	0.9
	0.1-0.2	5.3 Aa	5.6 Aa	5.9 Aa	5.8 Aa	5.9 Aa	0.8
	0.2-0.3	5.3 Aa	5.7 Aa	6.2 Aa	6.0 Aa	6.1 Aa	1.1
	0.3-0.4	5.3 Aa	5.7 Aa	6.1 Aa	6.0 Aa	6.1 Aa	1.3
	MSD	1.3	1.5	0.6	0.6	0.6	
Organic material (g kg^{-1})	0-0.1	32 Aa	42 ABa	20 Aa	24 Aa	18 Aa	73
	0.1-0.2	30 Aa	36 Ba	11 Ba	15 ABa	11 ABa	71
	0.2-0.3	25 Aa	53 ABa	5 BCa	9 Ba	7 Ba	105
	0.3-0.4	29 Aa	57 Aa	4 Ca	8 Ba	5 Ba	122
	MSD	73	19	6	13	8	
Total nitrogen (g kg^{-1})	0-0.1	0.8 Aa	0.9 Aa	1.3 Aa	1.5 Aa	1.4 Aa	1.0
	0.1-0.2	0.9 Aa	0.5 Aa	0.9 ABa	1.3 Aa	0.9 ABa	1.1
	0.2-0.3	0.8 Aa	0.9 Aa	0.6 Ba	0.8 Aa	0.5 Ba	1.0
	0.3-0.4	1.2 Aa	0.4 Aa	0.4 Ba	0.8 Aa	0.4 Ba	1.4
	MSD	1.6	1.4	0.6	0.8	0.7	
Extractable phosphorus (mg kg^{-1})	0-0.1	4.6 Aa	2.2 Aa	1.8 Aa	2.1 Aa	2.1 Aa	3.6
	0.1-0.2	3.6 Aa	1.5 Aa	1.2 Ba	1.3 Aa	1.4 ABa	2.6
	0.2-0.3	2.8 Aa	1.2 Aa	0.8 Ba	0.9 Aa	0.6 Ba	2.4
	0.3-0.4	2.3 Aa	1.2 Aa	0.8 Ba	0.9 Aa	0.6 Ba	2.3
	MSD	5.4	1.8	0.5	1.4	1.3	
Total potassium (cmol kg^{-1})	0-0.1	0.23 Aa	0.21 Aa	0.20 Aa	0.16 Aa	0.13 Aa	0.24
	0.1-0.2	0.08 Aa	0.10 Aa	0.09 ABa	0.11 Aa	0.11 Aa	0.12
	0.2-0.3	0.10 Aa	0.13 Aa	0.06 Ba	0.10 Aa	0.05 Aa	0.17
	0.3-0.4	0.06 Aa	0.16 Aa	0.08 ABa	0.09 Aa	0.06 Aa	0.20
	MSD	0.21	0.26	0.12	0.14	0.13	

[a]MSD: Minimum significance difference, [b]Values with the same capital letter are not significantly different between the soil layers (between the rows) ($p<0.05$), [c]Values with the same letter are not significantly different for the distance from the contamination source (between the columns) ($p<0.05$)

Table 2: Effect of distance from the contamination source (gradient), soil layer (depth) and their interactions on soil and earthworm characteristics

		p-value	
Soil characteristics	Gradient	Depth	Gradient×depth
Clay	<0.0001	<0.0001	<0.0001
Loam	<0.0001	<0.0001	<0.0001
Sand	<0.0001	<0.0001	<0.0001
pH	0.0003	0.82030	0.9442
Organic material	0.0594	0.93840	0.9999
Total nitrogen	0.1804	0.00790	0.2549
Extractable phosphorus	<0.0001	0.00300	0.9989
Total potassium	0.4185	0.00090	0.8408
Benzo(a)pyrene	<0.0001	<0.0001	<0.0001
Earthworm parameters			
Abundance	0.2748	<0.0001	0.7485
Biomass	0.1284	<0.0001	0.3188

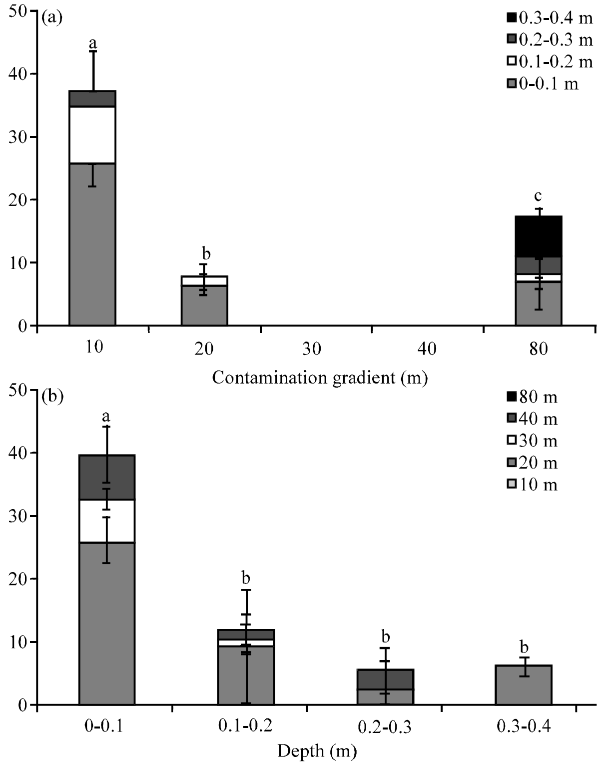

Fig. 1(a-b): Benzo(a)pyrene concentration in (a) Contamination gradient and (b) Different depths of the anthrosol soil. The different letters mean significant differences ($p<0.05$) and the vertical lines indicate the standard deviation

Earthworm communities: Three endogenic earthworm species were found. *Pontoscolex corethrurus* (Pontoscolecidae family), Glossodrilus sp. (Glossoscolecidae family) and *Dichogaster saliens* (Acanthodrilidae family). The abundance of *Pontoscolex corethrurus* was the highest (75%), with 88% of the total earthworm biomass. *Glossodrilus* sp. contributed 21% to the total number of earthworms and 11% to the total biomass, while the *Dichogaster saliens* contributed only 4% of the total number and 1% of the total biomass. The number of *P. corethrurus* (431 individuals) juveniles was higher than the number of adult forms (289 individuals). *Glossodrilus* sp. also had more juvenile (153) than adult forms (50), whereas the specie *Dichogaster saliens* had a lower number of juvenile (14 individuals) than adult forms (22 individuals).

The abundance (density) and biomass of earthworms was significantly larger in the 0-0.1 m layer than in the other layers, except 80 m from the origin of the contamination were it was similar in the 0-0.1 and 0.1-0.2 m layer ($p<0.05$) (Table 3). Depth had a significant effect on abundance and biomass of earthworms ($p<0.0001$), but not the distance from the contamination or the interaction between depth and gradient (Table 2).

Correlation and principal component analysis: The abundance and biomass of the earthworms was positively and significantly correlated to the TN and TK and silt content in soil ($p<0.05$) (Table 4). Also, the concentration of BaP was significantly correlated with the distance and soil depth ($p<0.05$) (Table 2). The abundance and biomass of the earthworms was negatively and significantly correlated to the sand content in the soil ($p<0.05$).

Table 3: Characteristics of the earthworms sampled along a transect or distance from the contamination source and in different soil layers

Earthworm characteristics	Soil layer (m)	Distance from the contamination source (m) 10	20	30	40	80	MSD[a]
Abundance	0-0.1	175 A[b] b[c]	112 Ab	312 Aa	364 Aa	145 Ab	114
	0.1-0.2	17 Bb	15 Bb	39 Bb	108 Bab	119 Aa	70
	0.2-0.3	4 Bb	1 Bb	4 Bb	39 BCa	20 Bab	19
	0.3-0.4	0 Bb	0 Bb	1 Bb	23 Ca	0 Bb	10
	MSD	50	26	74	83	78	
Biomass	0-0.1	16.2 Ac	23.8 Abc	36.6 Ab	66.8 Aa	14.0 ABc	20.0
	0.1-0.2	2.2 Bc	3.3 Bbc	4.9 Bbc	13.8 Bab	19.5 Aa	11.0
	0.2-0.3	0.4 Bb	0.1 Bb	0.7 Bb	4.6 Ba	4.6 Ba	3.0
	0.3-0.4	0.0 Bb	0.0 Bb	0.3 Bb	4.1 Ba	0.0 Bb	2.0
	MSD	5.6	4.5	8.9	18.5	12.3	

[a]MSD: Minimum significance difference, [b]Values with the same capital letter are not significantly different between the soil layers (between the rows) ($p<0.05$), [c]Values with the same letter are not significantly different for the distance from the contamination source (between the columns) ($p<0.05$)

Table 4: Pearson correlation coefficients between soil characteristics, concentration of benzo(a)pyrene (BaP) and earthworm abundance and biomass

Characteristic	pH	Organic material	Total N	Extractable P	Total K	BaP	Earthworm Abundance	Biomass	Clay	Silt
Organic material	<0.0001 [a] (-) [b]									
Total N	0.0005 (-)	0.0003 (+) [c]								
Extractable P	<0.0001 (-)	0.0014 (+)	0.0002 (+)							
Total K	0.3334 (+)	0.6790 (+)	0.4671 (+)	0.4806 (+)						
BaP	0.3195 (-)	0.7600 (+)	0.5190 (-)	0.0003 (+)	0.0124 (+)					
Abundance	0.5355 (+)	0.7637 (+)	0.0020 (+)	0.0828 (+)	0.0001 (+)	0.2756 (+)				
Biomass	0.7786 (+)	0.7336 (+)	0.0013 (+)	0.1488 (+)	0.0078 (+)	0.9232 (+)	<0.0001 (+)			
Clay	0.0210 (+)	0.3220 (-)	0.8911 (+)	0.2653 (-)	0.4115 (+)	0.8785 (-)	0.0590 (+)	0.0747 (+)		
Silt	0.0038 (-)	0.0131 (+)	0.0380 (+)	<0.0001 (+)	0.0018 (+)	0.0085 (+)	0.0084 (+)	0.0398 (+)	<0.0001 (-)	
Sand	0.0378 (+)	0.0182 (-)	0.0105 (-)	<0.0001 (-)	<0.0001 (-)	0.0022 (-)	<0.0001 (-)	0.0004 (-)	0.5647 (+)	<0.0001

[a]Prob<\|r\| under Ho: Rho, 0 (SAS., 1989), [b](-) is a negative correlation, [c](+) is a positive correlation, N: Nitrogen, P: Phosphorus, K: Potassium, BaP: Benzo(a)pyrene

The PCA revealed that most soil samples were grouped in the upper right quadrant (positive or small negative PC1 and a positive PC2) or in the lower left quadrant (negative PC1 and a negative PC2) (Fig. 2). Soils in the upper right quadrant are generally from the top layer and generally found near the origin of contamination, while those in the lower left quadrant are generally from the deeper soil layers and farther away from the origin of the contamination. Soils in the upper right quadrant have a positive PC1, i.e. higher in silt content, soil organic material content and total N and a positive PC2, i.e. high in BaP and total K. Soils in the lower left quadrant have opposite characteristics, i.e. higher in sand and clay content and high in pH.

DISCUSSION

The concentration of phenanthrene in crude oil is on average 146 mg kg^{-1}, anthracene 4.3 mg kg^{-1} and BaP 1.5 mg kg^{-1} (Kerr and Melton, 1999). Although, the BaP concentration in crude oil is the lowest, it was that only component that was found in the soil 20 years after contamination. BaP is more resistant to microbial degradation than both phenanthrene and

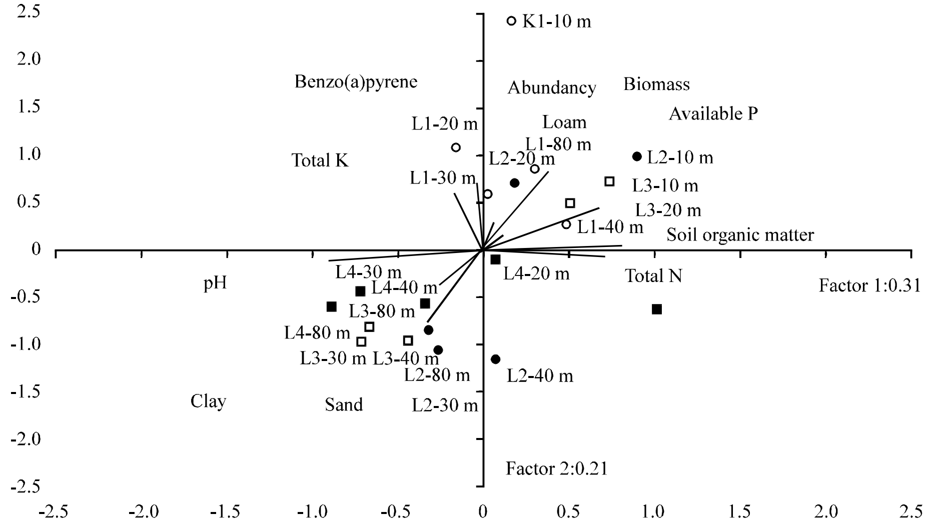

Fig. 2: Loadings and scores of principal component analysis (PCA) from the component 1 and 3 of contamination gradient at different depths defined as L1 (0-0.1 m); L2 (0.1-0.2 m); L3 (0.2-0.3 m) and L4 (0.3-0.4 m) and the parameters physicals, chemicals and earthworms populations evaluated

anthracene (Lily *et al.*, 2009). BaP might also be less availability for microorganisms in soil limiting its removal (Opuene *et al.*, 2007). The average concentration found at the sampling site was 3.1 mg BaP kg^{-1}, with values ranging from 0 to 29 mg kg^{-1}. Some values exceed the maximum amount permissible for soils with farming activity (2 mg kg^{-1}) (USEPA, 1993; CCME, 1999). Similar values (8.3-33.6 mg kg^{-1}) have been reported earlier for BaP concentrations in soil at Cinco Presidentes (Cram *et al.*, 2004). In other industrial coastal areas of Mexican, the BaP concentration in soil was 1.65 mg kg^{-1} (Iturbe *et al.*, 2004). Similar concentrations (1.3-3.0 mg kg^{-1}) have been reported at contaminated sites in New England (Bradley *et al.*, 1994) and Nigeria (0.4-1.13 mg kg^{-1}) (Duke and Albert, 2007). In this study, maximum BaP concentrations were found in the top soil layer. Hydrocarbons are absorbed mainly on the organic material, which has a polarity more similar to that of hydrocarbons. The organic material content was higher in the top soil layer so the amount of BaP leached out to the lower soil layers was small (Cram *et al.*, 2004; Adams *et al.*, 2009).

The abundance and biomass of the earthworms found in this study was generally higher than reported for other pastures in the southeast of Mexico (Ortiz-Ceballos and Fragoso, 2004; Huerta and Van der Wal, 2012) and for other tropical regions of the world (Nunes *et al.*, 2006; Marichal *et al.*, 2010). A previous study in the same area found 210 earthworm m^{-2} and 38.1 g biomass m^{-2} (Trujillo, 2010). More earthworms (319 individuals m^{-2}) with a larger total biomass (43.1 g m^{-2}) were found in this study. As such, no negative effect on the earthworm population was found of the oil spill that occurred 20 years ago.

Pontoscolex corethrurus is often found in pasture soils of sub-tropic and tropic regions (Lavelle and Gilot, 1994), e.g. central and southeastern Tabasco (Mexico), in the south of Brazil and south west of Colombia (Huerta *et al.*, 2006; Nunes *et al.*, 2006; Marichal *et al.*, 2010). The high abundance of *P. corethrurus* in this study suggested better adaptative partenogenetic strategies, faster growing and a higher tolerance to a wide range of different soil conditions compared to the other two morfo-species (Lavelle *et al.*, 1987). This might allow them to tolerate hydrocarbons better than other earthworms. Considering their particular characteristics and the presence and high abundance of this species in many human alternated ecosystems, such as cropping systems, pastures and urban areas (Brown *et al.*, 2006), may indicate their relative tolerance to various contaminants in the soil (Buch *et al.*, 2011). In ecotoxicology study, the absence of earthworms has been indicative of contamination, as these organisms are highly sensible to toxic compounds (De Silva *et al.*, 2009). In this study, however, a high abundance of *P. corethrurus* in the contaminated soil indicated a certain tolerance towards hydrocarbons and the potential this species might have in remediation of hydrocarbon-contaminated soil.

Several studies have been reported on the importance of earthworms in the removal of hydrocarbons (Geissen *et al.*, 2008; Sinha *et al.*, 2008; Contreras-Ramos *et al.*, 2009; Tejada and Masciandaro, 2011). The majority of these studies were done in regions with a temperate climate and few investigations have been done in tropical regions. Additionally, most studies with earthworms in PAHs contaminated soils have been done with epigeic species, such as *E. fetida* and *Eisenia andrei*, while endogeic or anecic earthworms might be more important due to their ecological characteristics. Epigeic earthworms are species that live above the mineral soil surface, typically in the litter layers of forest soils, anecic earthworms live in permanent vertical burrows in mineral soil layers, while endogeic earthworms make horizontal non-permanent burrows, mainly in the uppermost 0.1-0.15 m of soil (Bouche, 1977). In tropical environments, endogeic earthworm species are dominant (Nunes *et al.*, 2006). At the contaminated site, the endogeic earthworm *P. corethrurus* was the most abundant (75%) contributing 88% of the biomass. The presence of the endogeic earthworm *P. corethrurus* indicated that it was able to live in contaminated environments, in these sense further studies are necessary to evaluate its tolerance and potential to remove PAHs from soil.

The PCA analysis showed that abundance and biomass of the earthworms was directly related to the soil nutrient content (i.e., total N, soil organic material, extractable P) and that they preferred higher amounts of loam in the soil. This might indicate that the earthworms preferred a lighter soil, but not a sandy or clayey, rich in organic material with high amounts of nutrients. These characteristics were found generally in the top soil layers so more earthworms were found there. The abundance of earthworms was generally higher near the source of contamination so that a possible effect of hydrocarbons on the earthworms was minimal after 20 years.

CONCLUSION

The endogeic earthworm *P. corethrurus* was the dominant earthworm species in the studied tropical soil contaminated by an oil spill 20 years ago. The larger abundance and biomass of *P. corethrurus* in the top 0.1 m layer was due to it being richer in organic material than the lower soil layers and having a lighter soil texture. The abundant presence of *P. corethrurus* in the contaminated soil with BaP suggested a tolerance to PAHs and its potential in soil remediation.

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