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Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: Investigation of hydrocarbon degradation potential

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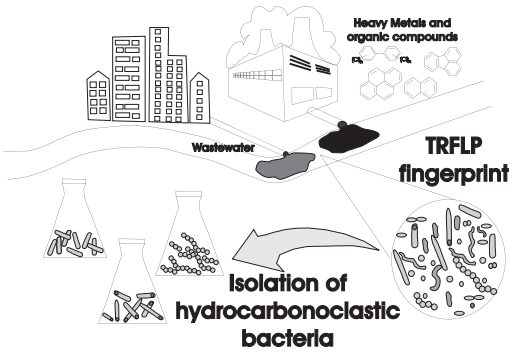
HIGHLIGHTS

- Elevated values of metals, PCBs and PAHs were found on Lerma River.
- TRFLP: high PAH and HM concentrations can affect the microbial diversity.
- 8 hydrocarbonoclastic strains were isolated and identified.
- 3 hydrocarbonoclastic consortia were found.
- The isolates and consortia present potential as bioremediation agents.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater contamination usually comes from runoff water or direct wastewater discharges to the environment. This paper presents a case study which reveals the impact of these types of contamination on the sediment bacterial population. A small stretch of Lerma River Basin, heavily impacted by industrial activities and urban wastewater release, was studied. Due to industrial inputs, the sediments are characterized by strong hydrocarbon concentrations, ranging from 2 935 to 28 430 $\mu g \cdot kg^{-1}$ of total polyaromatic hydrocarbons (PAHs). These sediments are also impacted by heavy metals (e.g., 9.6 $\mu g \cdot kg^{-1}$ of Cd and 246 $\mu g \cdot kg^{-1}$ of Cu, about 8 times the maximum recommended values for environmental samples) and polychlorinated biphenyls (ranging from 54 to 123 $\mu g \cdot kg^{-1}$ of total PCBs). The bacterial diversity on 6 sediment samples, taken from upstream to downstream of the main industrial and urban contamination sources, was assessed through TRFLP. Even though the high PAH concentrations are hazardous to aquatic life, they are not the only factor driving bacterial community composition in this ecosystem. Urban discharges, leading to hypoxia and low pH, also strongly influenced bacterial community structure. The bacterial bioprospection of these samples, using PAH as unique carbon source, yielded 8 hydrocarbonoclastic strains. By sequencing the 16S rDNA gene, these were identified as similar to *Mycobacterium*

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goodii, *Pseudomonas aeruginosa*, *Pseudomonas lundensis* or *Aeromonas veronii*. These strains showed high capacity to degrade naphthalene (between 92 and 100% at 200 mg·L^{-1}), pyrene (up to 72% at 100 mg·L^{-1}) and/or fluoranthene (52% at 50 mg·L^{-1}) as their only carbon source on in vitro experiments. These hydrocarbonoclastic bacteria were detected even in the samples upstream of the city of Salamanca, suggesting chronical contamination, already in place longer before. Such microorganisms are clearly potential candidates for hydrocarbon degradation in the treatment of oil discharges.

1. Introduction

The technological development of human society greatly increased the requirements for energy. Among the energy sources of major economical, political and social effects, the petroleum and other fossil fuels stand out. Besides being a limited and nonrenewable source, oil exploitation, transport, processing and use may have a high negative impact on the environment. As an example, the largest accidental oil spill in recent history, which occurred in the Gulf of Mexico on April–July 2010, resulted in the leakage of nearly 4.9 million barrels of light crude oil, contaminating extensive benthic coastal ecosystems (e.g., Kostka et al., 2011; Uhlmann, 2011). Moreover, untreated urban wastewaters or nutrient inputs may also impact ecosystems due to organic matter increase, which leads to eutrophication and anoxic conditions.

Petroleum is an extremely complex mixture of hydrocarbons, and this complexity directly affects its degradability, usually making it very persistent in the environment (Atlas, 1981). On the other hand, several studies have shown that there exist some hydrocarbon-degrading organisms, such as fungi and bacteria (Bartha and Atlas, 1977; Lu et al., 2011). Of special interest are the microorganisms with ability to use high molecular weight hydrocarbons as carbon sources, since these are usually resistant to biodegradation (Juhasz and Naidu, 2000; Lu et al., 2011; Baboshin and Golovleva, 2012). In addition, oil contamination can be abiotically eliminated from the environment depending on hydrological (stream) and physico-chemical local characteristics (temperature, sunlight, etc.). Therefore, although very promising, the application of microorganisms for environmental remediation demands a previous effort to completely understand the biochemical mechanisms, environmental constraints and the byproducts of these mechanisms, in order not to generate a worst unpredictable problem. For instance, Duke et al. (2000) and Brito et al. (2009) independently selected hydrocarbonoclastic bacterial communities and strains that were very efficient under laboratory conditions; but, when applied directly in situ, on mangrove ecosystems, had their oil bioremediation ability clearly reduced. These authors suggested that the oil mitigation stimulated by microorganisms is significantly influenced by the natural conditions of the impacted environment, such as the availability of other carbon sources that are more easily degraded than the hydrocarbons.

Our goal in the present work was to characterize contamination status of different stations in the Lerma River, chronically contaminated by hydrocarbons as well as by other industrial and urban discharges. We also investigated the influence of these contaminations in the sediment bacterial communities by using a TRFLP (Terminal Restriction Fragment Length Polimorfism) approach. The capacity for PAH biodegradation of the indigenous bacteria population was finally investigated through isolation and biochemical tests.

2. Material and methods

2.1. Description of the sampling sites and sample collection

In order to cover the river stretch most heavily impacted by Salamanca's (population 260,769 according to 2010 census; INEGI, 2011) urban and industrial areas, six sampling points were selected (Fig. 1). The location of these points is given in Table 1. Two of these points, 0La and 0Le, are located before Salamanca city, at the confluence of the Laja River (another important river of Guanajuato State) with the Lerma River. Point 1Le is just after the outflow of a Thermoelectric Power Plant. Sampling site 2Le is located after the main petrochemical discharges of the Oil Refinery. Sample 3Le was collected in the middle of Salamanca city, close to downtown, whereas the last sampling point, 4Le, was situated where the river starts to leave the city. The sampling was done in March 2008.

The surface sediments (0–2 cm) from all sampling sites were collected to suit all different analyses. For the metal analyses nearly 100 g of sediments were taken (with plastic spoons) and the samples were transferred to the laboratory in ziploc® bags, lyophilized and crashed with mortar. For the hydrocarbon analyses also 100 g of samples were collected using metal spoons, transferred to the laboratory in aluminum films, dried at room temperature, crushed with mortar, and stored in glass bottles. All tools were previously cleaned, rinsed with acetone and pyrolyzed. Both sets of samples were sent to *Eduardo Penna Franca* Radioisotope Laboratory (LREPF-UFRJ, Rio de Janeiro, Brazil) for analysis. For the microbiological analysis, sterile spoons were used and samples (nearly 50 g) were stored in sterile falcon™ tubes for their transport to the laboratory. Enrichment cultures were immediately prepared and the subsamples were stored at 4 °C for microbial isolation experiments and at −20 °C for molecular analyses.

2.2. Chemical characterization

The physico-chemical parameters, such as pH, conductivity, dissolved oxygen and temperature, were measured in situ by using specific probes (Conductronic PC 18 pH meter, and Hach® SensION 6).

Metal content was determined with the method described by Malm et al. (1989). Briefly, samples were digested with concentrated HNO_3 and HF (Merck P.A.), for 18 h at 120 °C and, afterwards evaporated, and resuspended by adding 10 mL of 0.1 N HCl. They were analyzed using a Varian spectrophotometer (AA240FS, Fast Sequential Atomic Absorption Spectrometer, USA). Hg analysis was performed by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System 400, Perkin Elmer USA (Bastos et al., 1998). Blanks were run throughout the analyses to check for any contamination. Triplicate measurements were done for each heavy metal determination. The detection limits (all in mg·kg^{-1}) were as follows: 0.0001 for Hg, 0.262 for Zn, 0.560 for Mn, 0.010 for Cr, 0.003 for Ni, 0.216 for Pb, 0.005 for Cu, 0.002 for Cd, and 0.017 for Fe. Detection limits were calculated using the formula: $(3 \times S_b)/X_b$ where S_b is the standard deviation of 6 measurements of the blank and X_b is the mean of the angular coefficient of the calibration curve (Silva and Alves, 2006). Certified analytical grade reference samples were used for calibration.

For organic compounds (PCBs and PAHs), 4–6 g of dried sediment samples was submitted to a sonication extraction (at 90 °C during 20 min) with 12 mL of hexane:acetone solution. The extraction was done three times with hexane:acetone ratios of 1:4 (v/v), 1:1 (v/v) and 4:1 (v/v). The resulting 36 mL of merged extracts were then concentrated (Meire, 2006). The organic extract was submitted to a clean-up process, which used Na_2SO_3, NaOH and Al_2SO_4 (at 11% H_2O w/w) in a chromatographic column eluted with hexane in order to remove humic material and elemental sulfur (Japenga et al., 1988). This organic solution was divided in two fractions, one for PAHs and the other for PCB analysis. The PAHs were separated by column chromatography using 3 g of silica gel 60 (70–230 mesh ASTM) and eluted with 35 mL hexane:ethyl ether (3:1). For the PCBs fraction, the same chromatographic column was

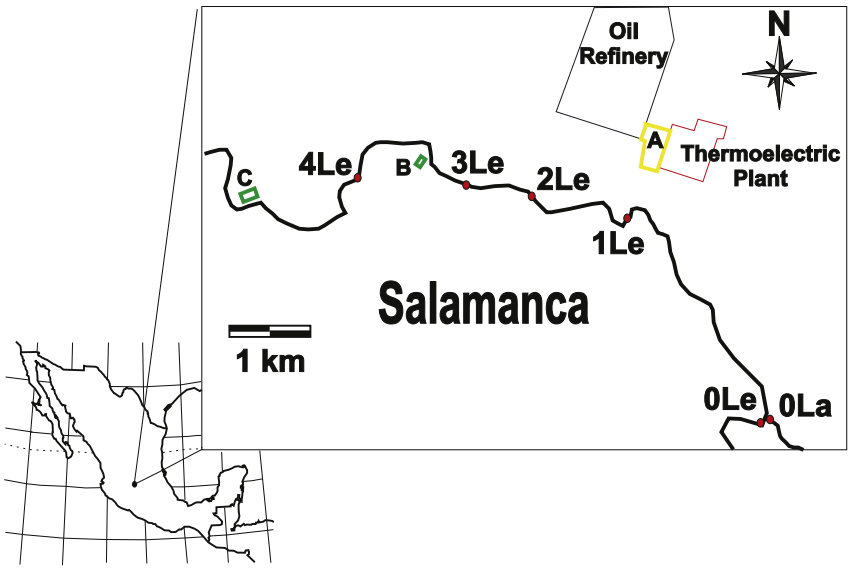

Fig. 1. Location of Lerma River stretch passing though Salamanca city. Points 0La and 0Le are upstream outside the urban area, while the others are inside it (see Table 1). Wastewater treatment plants are marked by letters: (A) at Thermoelectric Plant (160 $L \cdot s^{-1}$) and Oil Refinery (255 $L \cdot s^{-1}$); (B) Didactic Treatment Plant at *Ecoparque* (25 $L \cdot s^{-1}$, open on February 2010); and (C) City Treatment Plant (200 $L \cdot s^{-1}$, open on August 2013).

used and the sample was eluted with 15 mL hexane to extract PCBs CB-28, CB-52, CB-101, CB-118, CB-138, CB-153 and CB-180.

The 16 studied PAHs were: acenaphthene (Ace), naphthalene (Nap), fluorene (Flu), acenaphthylene (Acy), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (IP), benzo[g,h,i]perylene (B[ghi]P) and dibenz[a,h]anthracene (D[ah]A). They were determined by gas chromatography/mass spectrometry (GCMS-QP2010 Plus Ion Source Series Shimadzu, Japan) Electronic Ionization, equipped with a split–splitless injector, a split liner and capillar column 5MS, 30 m long and 0.25 mm in diameter, 0.25 μm in film. Helium was used as the carrier gas, the oven temperature program was: 60 °C for 1 min, 60 to 200 °C at 20 $°C \cdot min^{-1}$, 200 to 250 °C at 10 $°C \cdot min^{-1}$, 250 to 300 °C at 20 $°C \cdot min^{-1}$ and 300 °C for 15 min. Pyrene was quantified by HPLC (Thermoseparation products — spectra System TSP U/6000LP — USA), equipped with the Supercosil column (LC-PAH 25 cm with 46 mm) using isocratic condition of 80% acetonitrile (v/v) at flow rate (4.5 $mL \cdot min^{-1}$).

The PCB determination was performed by gas chromatography coupled to an electron capture detector (ECD-GC, Shimadzu GC-14B with autosampler AOC-17) with capillary columns (Shimadzu CBP1 and CBP5). The carrier gas was hydrogen. The injector and detector temperatures were 300 and 310 °C, respectively. The oven temperature program starts at 110 (for 1 min), rising to 170 (at 20 °C per min) and subsequently to 290 °C (at 7.5 °C per min), where it remained for 12 min.

2.3. In situ microbial communities

Total DNA from 1 g of sediment was extracted with UltraClean® Soil DNA Isolation Kit (Mo Bio Laboratories) following the manufacturer's procedure. DNA quality was verified by electrophoresis in a 1% agarose gel in Tris-Acetate-EDTA buffer (TAE). DNA extracts were stored at −20 °C for further analysis.

The total DNA from sediments was used to amplify the 16S rDNA by PCR using primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′; Lane, 1991) fluorescently labeled with carboxifluorescein (6-FAM) and unlabeled 907R (5′-GCCCCCGTCAATTCMTTTRAGTTT-3′; Lane et al., 1985). PCR conditions, PCR product purification and digestion with restriction enzymes (*Hae*III or *Hin*PI1) and TRFLP condition were as described in Brito et al. (2006).

Data sets were normalized and TRFs representing less than 1% of total fluorescence were not taken into account for the analysis (Hewson and Fuhrman, 2006). Sets were constructed considering the peaks whose fluorescence was higher than 100 units for at least one sample (Bordenave et al., 2004). Statistical analyses were carried out with a hierarchical cluster analysis, based on Jaccard coefficient by the unweighted-pair group method using arithmetic averages (UPGMA). Statistical analyses were carried out with MVSP software (Multi-Variate Statistical Package 3.1, Kovach Computing Services, UK).

2.4. Hydrocarbon-degrading bacteria

Two culture media were used: the sediment extract media (SEM) and a mineral media (MM). The SEM was prepared by sterilization of a sediment suspension prepared from 300 g of Lerma sediments and 700 mL of destilated water, which was sterilized 3 times (during 2 h) at 24 h intervals. On the other hand, a strict chemical composition was used for the MM, which contained (per liter): 3.2 g K_2HPO_4, 0.1 g NaH_2PO_4, 5 g Na_2SO_4, 0.001 g Ca_2SO_4, 0.29 g $(NH_4)_6Mo_7O_{24}{}^{\circ}4H_2O$, 0.066 g $CaCl_2$, 1.8×10^{-5} g FeSO4.7H2O, and 1.8×10^{-7} g $MgSO_4 \cdot 7H_2O$. For both media, after the pH was adjusted to 7.3, 1 mL of trace

Table 1
Sampling points (S.P.) along the stretch of Lerma River passing through Salamanca city.

S.P.	Latitude	Longitude	Distance along stream (km)	Reference
0La	20°32′19.22″N	101°09′46.53″W	0.0	Laja River, just before confluence
0Le	20°32′18.89″N	101°09′48.30″W	0.0	Lerma River, just before confluence
1Le	20°33′40.64″N	101°10′40.11″W	3.2	Thermoelectric + Refinery
2Le	20°33′50.98″N	101°11′25.75″W	4.9	Close to “Negro” bridge
3Le	20°33′53.32″N	101°11′55.55″W	5.8	btw “Obregón” and “Molinito” bridges
4Le	20°33′56.96″N	101°12′42.28″W	7.9	Close to “Cazadora” bridge

Table 2
Physico-chemical analysis.

Parameter[a]	0La	0Le	1Le	2Le	3Le	4Le	EPA[b]
Temperature (°C)[c]	25.8	25.5	28.5	29.9	29.7	29.2	8.3
Conductivity (mS)[c]	672	693	614	2800	2330	2340	29.4
DO (mg/L)[c]	5.9	3.7	8.4	3.4	3.0	1.3	>5
(% saturation)[c]	(70%)	(43%)	(105%)	(42%)	(38%)	(15%)	–
pH[c]	5.5	7.5	5.1	5.0	5.2	4.4	–
Cd	1.2	1.2	0.6	1.8	2.5	9.6	1.2
Cr	50	41	51	27	52	29	81
Cu	246	20	37	58	55	40	34
Fe	9.4×10^4	8.7×10^4	8.4×10^4	6.9×10^4	1.1×10^5	5.6×10^4	–
Hg	0.050	0.015	0.036	0.222	ND	0.075	0.150
Mn	288.23	ND	288.07	61.71	55.57	122.97	–
Ni	23	18	20	20	40	14	21
Pb	78	55	56	107	87	109	47
Zn	66	59	71	201	222	85	150
Nap	2720	4910	3400	12,970	ND	3195	–
Acy	<DL	<DL	<DL	<DL	ND	<DL	–
Ace	<DL	<DL	<DL	<DL	ND	<DL	–
Flu	20	<DL	<DL	390	ND	90	–
Phe	60	130	60	2270	ND	200	–
Ant	35	45	115	815	ND	155	–
Flt	<DL	330	55	1480	ND	90	–
Pyr	<DL	150	20	3940	ND	125	–
Chr + B[a]A	<DL	<DL	30	>1000	ND	410	–
B[b]F + B[k]F	100	140	100	190	ND	270	–
B[a]P	<DL	90	<DL	2730	ND	90	–
IP	<DL	<DL	<DL	280	ND	45	–
D[ah]A	<DL	<DL	<DL	550	ND	55	–
B[ghi]P	<DL	<DL	<DL	2815	ND	235	–
Total PAH	2935	5795	3780	28,430	ND	4960	–
CB-8	12.9	12.3	13.6	<DL	ND	0.9	–
CB-18	14.3	13.0	8.1	<DL	ND	7.1	–
CB-31	17.3	14.3	10.1	<DL	ND	7.9	–
CB-28	16.9	17.4	9.5	<DL	ND	9.0	–
CB-33	15.7	17.5	10.2	<DL	ND	9.3	–
CB-52	17.3	16.0	9.2	<DL	ND	2.0	–
CB-49	15.7	17.4	8.3	<DL	ND	9.7	–
CB-44	12.9	16.0	13.6	<DL	ND	8.0	–
Total PCB	123.2	123.6	82.5	<DL	ND	54.0	30.0

[a] ND: not determined or DL: below the detection limit. Metal (Hg, Zn, Mn, Cr, Ni, Pb, Cu, Cd and Fe) concentrations are expressed in $mg \cdot kg^{-1}$, while the Hydrocarbon Polycyclic Aromatics and the Polychlorinated Biphenyl (PCB) concentrations are expressed in $\mu g \cdot kg^{-1}$. The determined Polycyclic Aromatics Hydrocarbons were: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), chrysene (Chr), benz(a)anthracene (B[a]A), benzo(b)fluoranthene (B[b]F), benzo(k)fluoranthene (B[k]F), benzo(a)pyrene (B[a]P), indeno(1,2,3,-cd)pyrene (IP), dibenzo(a,h)anthracene (D[ah]A), and benzo(g,h,i)perylene (B[ghi]P). The determined PCBs were: 2,4′-Dichlorobiphenyl (CB-8); 2,2′,4-trichlorobiphenyl (CB-18); 2,4′,5′-trichlorobiphenyl (CB-31); 2,4′,4′-trichlorobiphenyl (CB-28); 2′,3,4-trichlorobiphenyl (CB-33); 2,2′,5,5′-tetrachlorobiphenyl (CB-52); 2,2′,4,5′-tetrachlorobiphenyl (CB-49); and 2,2′,3,5′-tetrachlorobiphenyl (CB-44).
[b] Parameter measured in water.
[c] Standard upper limits suggested by the US-EPA; the metal concentration values are based on the level that causes negative effects on 10% the exposed fauna and flora.

elements solution and 1 mL vitamin solution were added (Overmann et al., 1992). The trace element solution contained (per liter): 2.5 g EDTA, 10 mg $ZnSO_4 \cdot 7H_2O$, 1.5 g $MnSO_4 \cdot H_2O$, 5.0 g $FeSO_4 \cdot 7H_2O$, 390 mg $CuSO_4 \cdot 5H_2O$, 240 mg $CoNO_3 \cdot 6H_2O$, and 177 mg $Na_2B_4O_7 \cdot 10H_2O$. The vitamin solution contained (per liter): 0.2 mg biotin, 10 mg acid nicotinic, 50 mg thiamine, 50 mg riboflavin, and 50 mg inositol. For solid media, 15 $g \cdot L^{-1}$ of bacteriological agar was added.

Culture media were further supplemented with model hydrocarbon compounds as the only carbon and energy source (Nap, at 100 $mg \cdot L^{-1}$, or Pyr, at 50 $mg \cdot L^{-1}$). A hydrocarbon stock solution at high concentration (500 $mg \cdot L^{-1}$, dissolved in acetone) was prepared just before use. An alicote of less than 1 mL was used as carbon source. This solution was poured as a thin layer over the agar media or to the empty penicillin bottles, respectively for the solid and liquid cultures. 20 mL of media was used for both cultures. The latter was added only after the acetone had been evaporated. To obtain the hydrocarbonoclastic bacterial consortia, enrichments were conducted in liquid medium incubated at 30 °C and under continuous shaking (100 rpm). After two subcultures of about 20 days each, an aliquot served as inoculum for bacterial isolation on agar plates. They were incubated at room temperature (20–27 °C) until colonies were visible. They were collected and transferred to liquid medium for further characterization including microscopic observations and identification through phenotypic (API-20NE kit) or phylogenetic analysis. The isolated strains were stored at −20 and −80 °C in culture medium containing 20% (v/v) glycerol.

The total DNA of bacterial isolates, obtained following Brito et al. (2013), adapted from Tsai and Olson (1991), was used to amplify the 16S rDNA gene by PCR using unlabeled primers 8F and 1 489R (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-TACCTTGTTACGACTTCA-3′ respectively). The amplicons were sequenced using the BigDye sequencing kit (Applied Biosystem®). The obtained sequences were checked for chimeras and compared to those in the database of the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) using BLAST. The sequences, together with close reference ones, were aligned with the ClustalW® program and phylogenetic trees were obtained with the Mega software using the neighbor-joining method. The confidence of the phylogenetic trees was assessed by bootstrap using 1000 resampling. The sequences determined in this study have been submitted to GenBank® database and assigned accession numbers KP161867 to KP161874.

Degradation capacities for all the isolated strains were evaluated in liquid medium containing model hydrocarbon compounds provided at 50, 100 and 200 $mg \cdot L^{-1}$ for Nap, and at 50 and 100 $mg \cdot L^{-1}$ for Pyr and Flt. After 21 days of incubation, hydrocarbons were extracted with 5 mL dichloromethane, and quantified by gas chromatography or

HPLC as described above. The percentage of degradation was calculated using as control inactivated strains.

3. Results

3.1. Chemical characterization of sample sites

Basic physico-chemical parameters, measured in the overlaying waters, and the concentration of metals, PCBs and PAHs, analyzed in the sediments, are presented in Table 2. For accessing the significance of these measured values, they were compared with the United States Environmental Protection Agency (EPA, U.S., 1986) suggested values for natural waters. These data revealed that, even before the Salamanca industrial park, the Lerma River was outside the acceptable range for maintaining life (like fishes) or for recreational use by humans. The conductivity is 20 times above the standard, while the dissolved oxygen (DO) levels are only acceptable when the river enters the city (points 0 and 1). The most critical situation is clearly the one presented by the river after receiving the main industrial and urban discharges (points 2, 3 and 4): The conductivity reaches a level 80 times above the standard and the DO is dangerously below the recommended value. Station 4 exhibited hypoxic conditions with oxygen saturation of 15%.

Similar results were found by the analysis of the heavy metal contents in the sediments. Again the three downstream points showed metal concentrations above the acceptable limits. These three sampling points exhibited high values for Pb and Cd (up to about 8 times the reference); points 2 and 3 presented also high values of Zn and Cu; point 2 presented the highest value for Hg and point 3 the highest value of Ni and Fe. Another point that exhibited metal concentrations above the reference, specifically Pb and Cu (the last more than 7 times the reference), is the point 0La. This is due to other sources of contamination along the Laja River, before the confluence, probably caused by dye discharges from handcraft factories.

The PCB concentrations found in Lerma River sediment samples showed only small variations with total values ranging from 54 to 124 $\mu g \cdot kg^{-1}$. These sediments may be considered as contaminated according to EPA references values. These data were higher than the ones observed in other studies for similar ecosystems. Uncontaminated sediments usually contain concentrations as low as 3 $\mu g \cdot kg^{-1}$ (Funil Reservoir — Rio de Janeiro, Brazil), 2–19 $\mu g \cdot kg^{-1}$ (Santana River — also Rio de Janeiro, Brazil; Brito et al., 2005), 10 $\mu g \cdot kg^{-1}$ (Yangtze River Estuary — China; Yang et al., 2012) and 21 $\mu g \cdot kg^{-1}$ (Catalonia Basin — Spain; Peré-Trepat et al., 2006). Moderately and highly contaminated sediments may contain PCBs with concentrations ranging from 48–486 $\mu g \cdot kg^{-1}$, as found in the Zhujiang River, to 3 396 $\mu g \cdot kg^{-1}$,

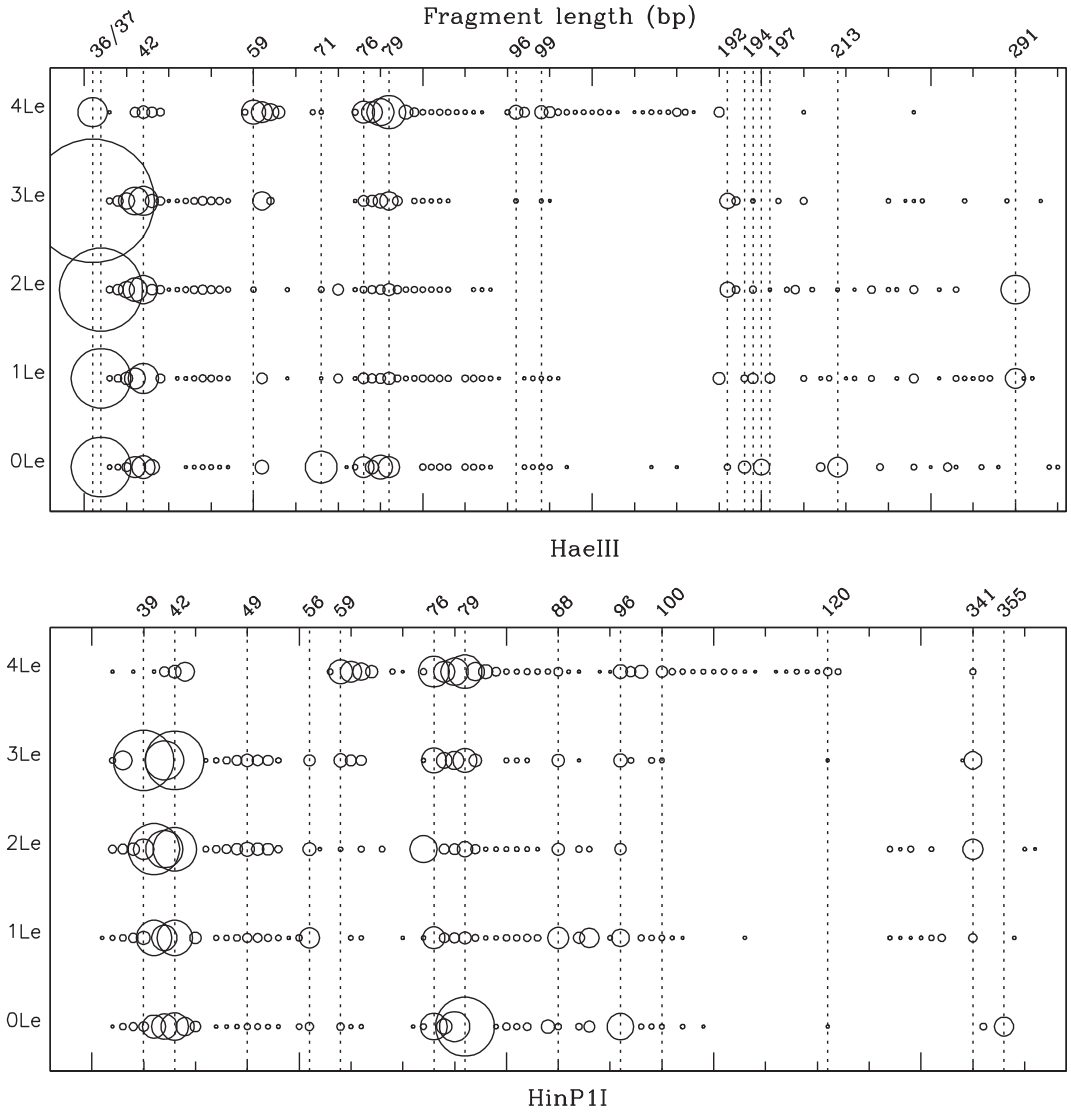

Fig. 2. TRFLP analysis with *Hae*III and *Hin*P1I enzyme restrictions. Each circle is one population while its size represents its relative abundance inside the community.

measured in Macao harbor, the last ones in China (Fu et al., 2003), respectively.

We determined, in our sediment samples, the 16 priority PAHs according to the US-EPA (Table 2). The overall concentrations are relatively low except for point 2Le, which showed a total PAH concentration of 28 430 $\mu g \cdot kg^{-1}$ (6 times higher than the previous ones). This point is just after the pipeline of a petroleum refinery and thermoelectric plant. This disparity is clear and consistent in all PAH that could be measured, the only exception being benzo(b)fluoranthene and benzo(k)fluoranthene that showed a slightly higher value at one station (4Le). Individually, the PAH that showed the highest concentrations was the naphthalene (between 2 720 and 4 910 $\mu g \cdot kg^{-1}$, in points 0, 1 and 4; and 12 970 $\mu g \cdot kg^{-1}$ in point 2). Moderately contaminated samples show PAH concentrations like 25–275 $\mu g \cdot kg^{-1}$ (South Sea — China; Yang, 2000), while samples with 330–1 093 $\mu g \cdot kg^{-1}$ (Danube Estuarine Coast — Romania; Tsymbalyuk et al., 2011) and 22–2 792 $\mu g \cdot kg^{-1}$ (Pearl River Delta — China; He et al., 2008) are considered polluted to highly polluted. Extremely large values have also been reported: 90,000 $\mu g \cdot kg^{-1}$ (Fraser River — Canada; Yunker et al., 2002) and 250,000 $\mu g \cdot kg^{-1}$ (Black River U.S.A.; Gu et al., 2003). Thus, Lerma sediment samples may be considered at least highly contaminated with HPAs.

3.2. Bacterial characterization of sample sites

The TRFLP fingerprints of each sample (except of 0La), obtained with the two restriction enzymes, are shown in Fig. 2. This figure shows a relatively high bacterial diversity with average numbers of TRFs of 52 and 45, respectively for *Hae*III and *Hin*P1I enzymes. A slightly better performance was obtained with *Hae*III (50, 61, 51, 44 and 52 TRFs, for 0La, 1Le, 2Le, 3Le and 4Le, respectively) as compared to *Hin*P1I (44, 53, 42, 35 and 52 TRFs, respectively). Only one dominant population (above 10% relative abundance) was observed with *Hae*III-restriction enzyme (TRF 36–37 in samples 0Le, 1Le, 2Le and 3Le). For *Hin*P1I-restriction enzyme, 3 dominant populations, represented by TRFs 39–40, 42 and 79, were observed in samples 2Le, 3Le and 0La. It is important to note that the results obtained with only one enzyme are not enough for completely defining the profile of the population because: i) the range of covered base pairs is limited (very small and very large fragments are lost); ii) very small populations usually cannot be detected by this technique and iii) fragments of different OTUs but with the same size may occur (e.g., Caretta and Brito, 2011). This is why more than one enzyme is usually used. Concerning to our results, the dominant population detected with *Hae*III is probably composed by at least 3 dominant populations found with *Hin*P1I. The intermediate most abundant populations (between 2 and 10%) corresponded to 35% of the populations for *Hae*III-restriction enzyme and 44% for *Hin*P1I-restriction enzyme, while the poorly represented ones (less than 2%) represented 63% and 54% respectively.

The dendogram from cluster analysis, taking into account the abundance data (data not shown), revealed that the waste output from Salamanca urban area noticeably affects these populations, the most distant samples being 3Le and 4Le. The Principal Component Analysis (PCA), on the other hand (Fig. 3), revealed that sample 4Le was clearly separated from all the others along the *x* axis explaining 41.8% of total variation between samples. The *y* axis explaining 22.6% of the total variation separated the upstream station (0Le) from the 3 remaining sampling points.

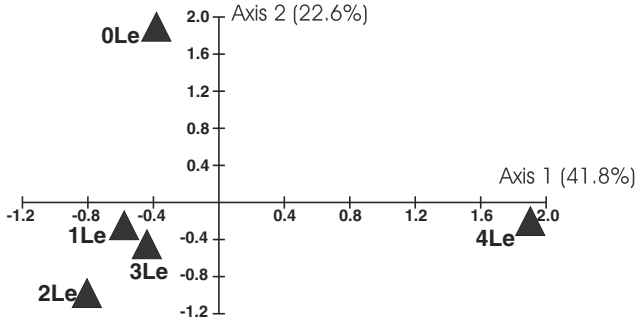

Fig. 3. PCA analysis for TRFLP (*Hae*III and *Hin*P1I digested data).

3.3. Hydrocarbonoclastic bacteria isolation

In order to carry out the bioprospection of hydrocarbonoclastic bacteria from the studied river samples, we initially used two culture media: one with a well defined chemical composition (mineral media, MM) and another prepared by sterilizing Lerma River sediments (sediment extract media, SEM). For both, we also tested two distinct unique carbon sources: naphthalene (Nap, 100 $mg \cdot L^{-1}$) and pyrene (Pyr, 50 $mg \cdot L^{-1}$). After two enrichments in liquid media and isolation in solid media, 19 morphologically distinct colonies were obtained (Table 3), most of them from the less contaminated sites. Eight strains were actually characterized and identified (Table 4), 4 from pyrene enrichments and 4 from naphthalene enrichments. Three of these strains, gram negative and oxydase positive, were unequivocally identified as *Pseudomonas* spp. based on API20NE system (see Table 4 for API codes).

As shown in Fig. 4, the comparison of the 16S rRNA gene sequences of the eight strains to the NCBI library (http://blast.ncbi.nlm.nih.gov) suggested high similarity with representatives of the genera *Pseudomonas*, *Aeromonas* and *Mycobacterium*, within gammaproteobacteria and actinobacteria classes. Two remaining cultures (consortia) were dominated by *Pseudomonas* related bacteria as revealed by both microscopic observation and API 20NE identification.

3.4. Hydrocarbonoclastic potential of the isolates and consortia

The ability of the isolates and consortia to use different hydrocarbons as their only carbon and energy sources was tested with two different concentrations of pyrene and fluoranthene (100 and 50 $mg \cdot L^{-1}$), and three of naphthalene (200, 100 and 50 $mg \cdot L^{-1}$). The incubation conditions were similar to the ones used on the isolation tests, i.e., 21 days at room temperature, neither with light or agitation. Hydrocarbon concentration measurements were done at the beginning and the end of the incubation period. Controls (medium without inocula) were used as reference to 0% of stimulated biodegradation.

All strains and consortia were able to degrade completely or almost completely the Nap (degradation above 87% at 50 $mg \cdot L^{-1}$, above 92% at 100 $mg \cdot L^{-1}$ and above 95% at 200 $mg \cdot L^{-1}$; Fig. 5).

These results might be expected, since the isolated strains were similar to bacterial species known to be hydrocarbonoclastic and, secondly, because it is usual that the hydrocarbonoclastic bacteria are able to degrade and use Nap as carbon source because this is the less complex PAH (Brito et al., 2005). Similarly, all the strains and consortia were able to degrade Pyr, more efficiently at the highest concentration (degradation's between 15 and 72%, with a median of 37%). Interestingly, the consortia exhibited the highest degradation potentials on pyrene (Fig. 5).

4. Discussion

The Lerma River basin is a very important source for freshwater to west-central Mexico. The Lerma River itself is 965 km long, beginning on the Mexican Plateau at an altitude over 3000 m above sea level, 24 km southeast away from Toluca City (Mexico State), and emptying into Chapala Lake (Jalisco State). It drains waters from many agricultural areas, urban regions (e.g., Irapuato and several other Guanajuato State cities) and industrialized regions such as Queretaro and Salamanca. When it crosses Salamanca city (also in Guanajuato State) it receives discharges from a Thermoelectric Power Plant and a Petrochemical

Table 3
Characteristics of hydrocarbon resistant bacterial colonies.

	Observed colonies					Isolated strains		
Carbon source[a]	Nap		Pyr		Total	Nap	Pyr	Total
Media culture[b]	MM	SEM	MM	SEM				
Sampling Site								
0Le	2	1	2	0	5	–	0Le-2P, 0Le-3P	2
0La	2	0	0	0	2	0La-3N	–	1
1Le	3	2	2	1	8	1Le-1N[c], 1Le-2N, 1Le-3N, 1Le-4N[c]	1Le-3P	3
2Le	0	0	1	0	1	–	–	0
4Le	1	0	2	0	3	4Le-4N	4Le-3P	2
Total	8	3	7	1	19	4 strains	4 strains	8

[a] N: naphthalene; P: pyrene.
[b] MM: minimum media; SEM: sediment extract media.
[c] Probable bacterial consortia (3 in total with naphthalene).

Refinery, both installed in 1950, but also inputs of untreated domestic wastewater.

Despite the great economical and ecological importance of the Lerma River, the results of this study suggest that even before the Salamanca industrial park, the Lerma River is already chronically contaminated by metals and organic compounds, and among them PCBs. The possible sources of the latter are associated to inadequate storage of contaminated waste and obsolete equipment. The PCBs were widely used during the 1930s and 1940s, especially as a dielectric fluid in capacitors and transformers (Borja et al., 2005). Due to their great environmental persistence and carcinogenicity, the production and use of PCBs were banished since the 80s. However their contamination is currently still of concern, especially from electric and electronic wastes (Yang et al., 2012). Salamanca city is a highly industrialized city, and was included in the United Nation Development Program (UNDP) plan for PCB management and destruction. In fact, Guanajuato state has about 56,000 electrical transformers to be eliminated (UNDP-PCBs, 2009). The results indicate that point 0La is already impacted by discharges of remnant PCBs, with concentrations of 123 $\mu g \cdot kg^{-1}$. This value is 6 times larger than the highest values observed for uncontaminated sites (see Section 3.1). Noticeably PCB concentrations, within the studied stretch, decreased along the river flow, indicating that the sources for PCBs to Lerma River were probably not the industrial and urban discharges of Salamanca city.

As for PCBs, the average PAH concentration in the sediments were very high, ranging, with exception of sampling point 2Le, from 3000 to 5000 $\mu g \cdot kg^{-1}$ (also more than one order of magnitude larger than the highest values for uncontaminated sites, see also Section 3.1). As could be expected from macroscopic observations, sampling point 2Le seemed largely affected by the industrial discharges with a strong increase in total PAH concentrations (28,430 $\mu g \cdot kg^{-1}$) and high amount of phenanthrene, pyrene, benzo[a]pyrene, benzo[g,h,i]perylene. Although the industries have their own water treatment plants, their sewer to the river (downstream 1Le) showed clear sensitive signs of contamination (very high turbidity and fetid smell). In all sampling points after 1Le, oil stains were observed in situ over the water and trapped into the sediments. The bacterial communities seem not to be notably influenced by this discharge spot in 2Le since bacterial communities did not vary much between points 1Le and 3Le (Fig. 3). On the other hand, the impact of organic material discharges from Salamanca city and their associated nutrients becomes visible through the decrease of dissolved oxygen and pH observed between station 1Le and 4Le. Thus, these parameters are probably the main environmental factors affecting bacterial community diversity as demonstrated in station 4Le where oxygen saturation decreased to 15%. Since the recollection and treatment of urban wastewaster of Salamanca city has improved in the last years, the impact caused by organic material sewages from urban discharges can be more easily mitigated.

The treatment of PAH contamination, in contrast, must be largely improved given the very high PAH concentrations detected in station 2Le. This treatment may include biological processes, which must be developed and applied.

Since heavy metals are common constituents of crude oil and derivatives, it is usually expected to find high levels of them in sites chronically contaminated by petroleum (Máthé et al., 2012). This co-occurrence may affect in a particular way the microbial diversity and activity due to the extra stress caused by the metallic ions.

Considering the ability of bacterial communities to adapt themselves to chronically contaminated conditions, i.e., oil contamination, some sites of Lerma River were used to perform bioprospection studies for potentially hydrocarbonoclastic microorganisms. Three bacterial consortia (0La-1N, 1Le-1N and 1Le-4N) and 8 isolates (Table 4) were obtained from this site. Although the attempts to isolate the microorganisms of these consortia were not successful, they showed a high ability to degrade hydrocarbons. In fact, it is known that some bacterial species can cooperate within each other to better survive stressing environmental conditions. This cooperation is usual in the presence of toxic contaminants like petroleum compounds, where one specie can act on the first stage of oil degradation, yielding a less toxic (or even not toxic at all) product, whereas the second species may use it as carbon source (Luan et al., 2006). All the isolated strains showed similarity with bacteria known for their hydrocarbonoclastic capacities (Brito et al., 2006, 2009), being their PAHs degradation mechanisms already described (Atlas, 1981; Juhasz and Naidu, 2000; Smith, 1990; Cerniglia, 1992; Lu et al., 2011).

The results of the experiment for determining the hydrocarbonoclastic potential showed, for all the isolated strains, that naphthalene was completely degraded under all the tested conditions, i.e., only carbon source, while fluoranthene and pyrene were partially degraded. For these last two PAHs, the three most efficient strains, 0La-3N, 0Le-1N and 1Le-2N, could diminish both pyrene (55, 72 and 57%, respectively)

Table 4
Accession number and API20NE code for the bacterial isolates and colonies.

Strain	Accession numbers	API code	Most similar type strain
0Le-2P	KP161873	–	*Aeromonas veronii* (99%)
0Le-3P[a]	KP161871	0,354,575	*Pseudomonas aeruginosa* (100%)
0La-3N	KP161868	–	*Mycobacterium smegmatis* (99%)
1Le-3P[a]	KP161872	1,354,777	*Pseudomonas aeruginosa* (100%)
1Le-1N[b]	–	0,472,763	–
1Le-2N	KP161867	–	*Mycobacterium smegmatis* (99%)
1Le-3N[a]	KP161874	0,140,457	*Pseudomonas lundensis* (99%)
1Le-4N[b]	–	0,440,757	–
4Le-3P	KP161869	–	*Mycobacterium smegmatis* (99%)
4Le-4N	KP161870	–	*Mycobacterium smegmatis* (99%)

[a] Identification match of API20NE code.
[b] Probable bacterial consortia.

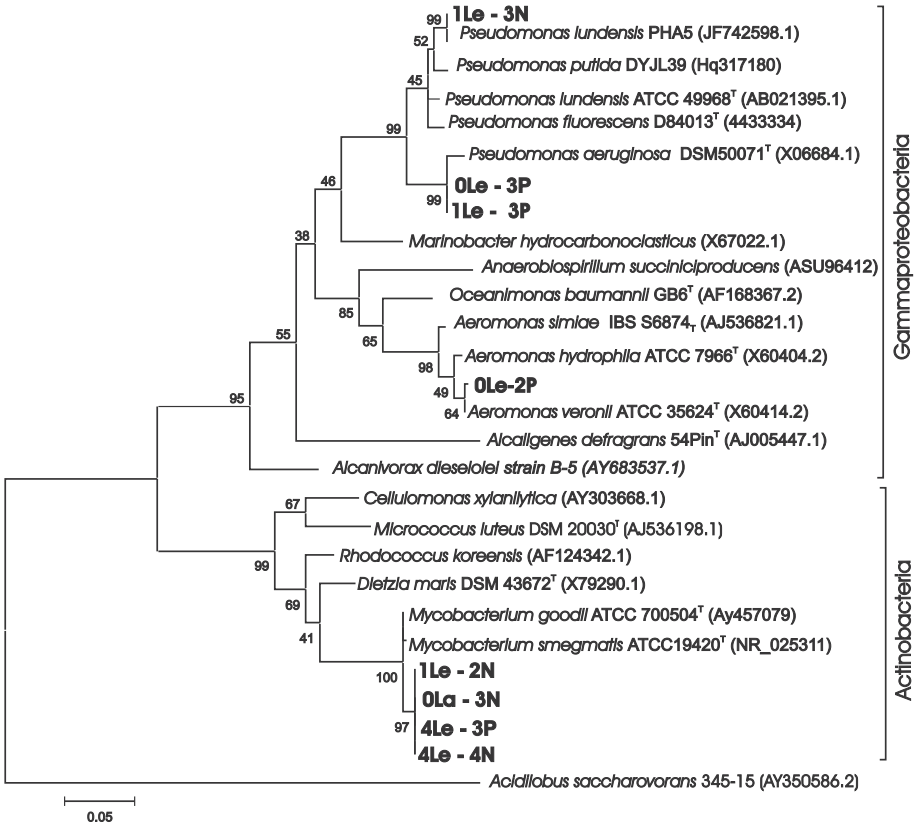

Fig. 4. Phylogenetic tree based on 16S rRNA encode gene, showing the position of isolated strains within the radius of members of representative groups (gammaproteobacteria or actinobacteria). The tree was generated using maximum parsimony and neighbor-joining analyses. All accession numbers of type strains are indicated in parenthesis.

and fluoranthene (50, 39 and 35%), while strains 1Le-1N and 0Le-3P could degrade, respectively, pyrene (63%) and fluoranthene (52%).

0La-3N and 1Le-2N strains were similar to *Mycobacterium goodii* (as well as strains 4Le-3P and 4Le-4N), which was found on environmental soil samples and is considered human pathogenic (Friedman and Sexton, 2001; Toda et al., 2006). The ability of this strain to degrade oil was described by (Li et al., 2003). The *Mycobacterium* sp. is, in fact, one of the first bacteria known to degrade hard PAHs like pyrene (Heitkamp et al., 1988; Cerniglia, 1992). Since then, considerable work has been applied for describing the degradation pathway used by some species of this genus for mineralizing such organic compounds (Berekaa and Steinbüchel, 2000; Stingley et al., 2004; Kim et al., 2007). Our results show that *M. goodii* is able to degrade pyrene at a rate of about $4\%\cdot day^{-1}$ and fluoranthene at $3\%\cdot day^{-1}$ (both considering a constant degradation rate along our experiment). The degradation rate, q, is calculated by the equation: $q = 1-\sqrt[21]{\frac{C_f}{C_i}} = 1-\sqrt[21]{\frac{100-D}{100}}$, where C_i and C_f are, respectively, initial and final PAH concentrations and D is the degraded percentage shown in Fig. 5. The last of the three most efficient "isolates", 0Le-1N, is a consortium, for which the API20NE test was not applied because it revealed previously to be Gram positive. Since *Mycobacterium* sp. are Gram positive strains, and half of the sequenced isolates were similar to them, 0Le-1N is probably a community containing this population. This consortium was able to degrade up to a rate of about $6\%\cdot day^{-1}$ of pyrene.

Another consortium that revealed to be efficient on degrading pyrene was 1Le-1N ($5\%\cdot day^{-1}$), identified by API20NE test as a *Pseudomonas* sp., suggesting that this population is probably the most abundant in the community. For the third consortium obtained, 1Le-4N, the test also revealed a probable dominance of *Pseudomonas* sp. Furthermore, 0Le-3P, which was also efficient on degrading fluoranthene ($3\%\cdot day^{-1}$), is a strain similar to *Pseudomonas aeruginosa* (as well as is 1Le-3P). Another Pseudomonas isolated in our samples was 1Le-3N, similar to *Pseudomonas lundensis*, a bacterial strain isolated from natural mineral water (Elomari et al., 1996). The usually pathogenic Pseudomonas are also known to possess great plasticity for consuming distinct contaminants, including soft and hard PAHs.

Finally, strain 0Le-2P revealed to be similar to *Aeromonas veronii*, another pathogenic bacteria that, although recognized to be capable of growing in naphtalene and phenanthrene (Kiyohara et al., 1976), did not have its ability to consume the PAHs studied here previously verified.

Hydrocarbonoclastic bacterial communities are usually found in oil impacted environments; thus these microorganisms can effectively use the petroleum hydrocarbons as carbon source (Brito et al., 2006; Ben Said et al., 2008; Zhong et al., 2011). In fact, it has been shown that some microorganisms have the ability to transfer the hydrocarbon degradation capability to other members of the bacterial community by horizontal transfer (Habe and Omori, 2003). In this context, the bioprospection and study of hydrocarbonoclastic organisms disclose a high potential for the development of bioremediation technologies. Newly discovered microorganisms and innovative bioprocesses, capable of using totally or partially the petroleum hydrocarbons, may allow minimization of the impact caused by oil spills.

5. Conclusions

The presented results warn us that the industrial and urban discharges of Salamanca city into the Lerma River are poorly treated. They reveal the lack of local environmental policies to protect the river water, directly affecting both the fauna and flora, in addition to human health. Interestingly, this contamination seems not to be altering, considerably, the studied hydrocarbonoclastic bacterial community. Eight isolated strains and three bacterial consortia, with capacity to degrade different classes of hydrocarbons, were isolated and identified. They showed an ability to

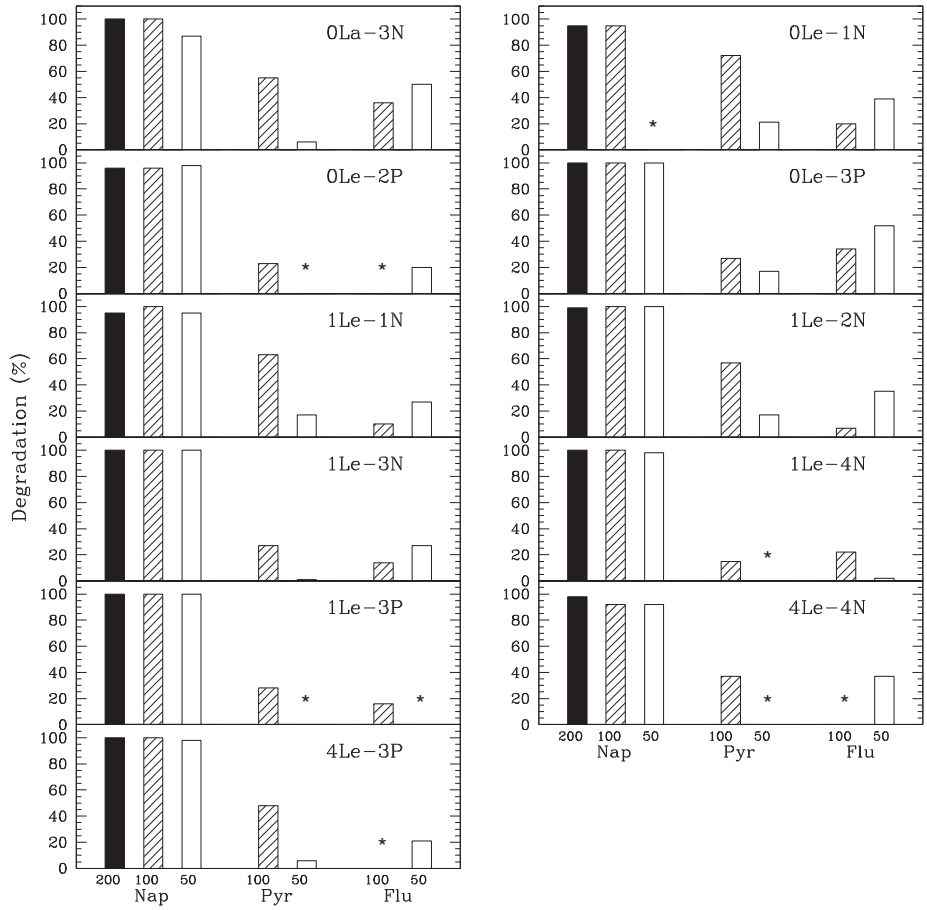

Fig. 5. Hydrocarbon biodegradation with naphthalene (Nap), pyrene (Pyr) or fluoranthene (Flu) as only carbon source. The (*) symbol means that no degradation was detected.

use hydrocarbons that are difficult to be degraded, like pyrene and fluoranthene. These experiments revealed that known hydrocarbonoclastic bacteria as *Mycobacterium* sp., *Pseudomonas* sp. and *Aeromonas* sp. can be found in environments also impacted by PCBs and heavy metals. It is clear that the ability of a microorganism to degrade a certain pollutent is not intrinsic, but dependent on the environmental conditions and other stress levels this microorganism is subjected to. This is why the studies "in situ" usually do not reproduce the results observed in the laboratory. Many contaminated sites present different levels of contamination of distinct pollutants, like in the case for the stretch of Salamanca River we have studied — this co-occurrence may determine a specific behavior of the microorganisms and their ability to survive and degrade the contaminants. The present work is intent to go on this direction: investigate the ability of native microorganisms to degrade distinct contaminants that co-occur in a certain polluted site. For obvious reasons, there is a special interest on microorganisms that are indigenous and endemic of the contaminated site, as is the case of the strains and consortia from Lerma River sediments identified in the present work. Also, the simple use of these sediments as bacterial hydrocarbonoclastic inocula is possible, for example, for applying on biopilas systems in order to stimulate oil biodegradation. Nevertheless, it is pivotal to advance on isolating, studying and comprehending the mechanisms used by these organisms to make the oil biodegradation as effective as possible.

Conflict of interest

This study was done under international agreements comprehending researchers from Brazil, France and Mexico, which received support from ECOS-NORD/SEP/CONACyT/ANUIES and BIOMETAL: ANR/CONACyT (both between France and Mexico), CNPq-CONACyT (between Brazil and Mexico), Aquitaine Regional Government Council (France), and SEP-PROMEP and DAIP-UG (Mexico). We have no conflict of interest in this study.

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References

Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 450 (1), 180–209.

Baboshin, M., Golovleva, L., 2012. Aerobic bacterial degradation of polycyclic aromatic hydrocarbons (PAHs) and its kinetic aspects. Microbiology 810 (6), 639–650.

Bartha, R., Atlas, R., 1977. The microbiology of aquatic oil spills. In: Perlman, D. (Ed.), Advances in Applied Microbiology vol. 22. Academic Press, pp. 225–266. http://dx.doi.org/10.1016/S0065-2164(08)70164-3 (URL http://www.sciencedirect.com/science/article/pii/S0065216408701643).

Bastos, W., Malm, O., Pfeiffer, W., Cleary, D., 1998. Establishment and analytical quality control of laboratories for hg determination in biological and geological samples in the amazon, brazil. Cienc. Cult. 500 (7), 255–260.

Ben Said, O., Goñi-Urriza, M., El Bour, M., Dellali, Aissa, P., Duran, R., 2008. Characterization of aerobic polycyclic aromatic hydrocarbon-degrading bacteria from Bizerte lagoon sediments, Tunisia. J. Appl. Microbiol. 104, 987–997 (ISSN 1364–5072).

Berekaa, M.M., Steinbüchel, A., 2000. Microbial degradation of the multiply branched alkane 2, 6, 10, 15, 19, 23-hexamethyltetracosane (squalane) bymycobacterium fortuitum and mycobacterium ratisbonense. Appl. Environ. Microbiol. 660 (10), 4462–4467.

Bordenave, S., Jézéquel, R., Fourçans, A., Budzinski, H., Merlin, F.X., Fourel, T., Goñi-urriza, M., Guyoneaud, R., Grimaud, R., Caumette, P., Duran, R., 2004. Degradation of the erika oil. Aquat. Living Resour. 17, 261–267. http://dx.doi.org/10.1051/alr:2004027.

Borja, J., Taleon, D.M., Auresenia, J., Gallardo, S., 2005. Polychlorinated biphenyls and their biodegradation. Process Biochem. 400 (6), 1999–2013. http://dx.doi.org/10.1016/j.procbio.2004.08.006 (ISSN 1359–5113, URL http://www.sciencedirect.com/science/article/pii/S0032959204003620).

Brito, E.M.S., Reis Vieira, E.D., Machado Torres, J.P., Malm, O., 2005. Persistent organic pollutants in two reservoirs along the Paraiba do Sul-Guandu river system, Rio de Janeiro, Brazil. Quim. Nova 280 (6), 941–946. http://dx.doi.org/10.1016/j.marpolbul.2008.12.008 (ISSN 0025-326X, URL http://www.sciencedirect.com/science/article/pii/S0025326X08005663).

Brito, E.M.S., Guyoneaud, R., Goñi-Urriza, M., Ranchou-Peyruse, A., Verbaere, A., Crapez, M.A., Wasserman, J.C.A., Duran, R., 2006. Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Res. Microbiol. 1570 (8), 752–762. http://dx.doi.org/10.1016/j.resmic.2006.03.005 (ISSN 0923–2508, URL http://www.sciencedirect.com/science/article/pii/S0923250806000830).

Brito, E.M.S., Duran, R., Guyoneaud, R., Goñi-Urriza, M., de Oteyza, T.G., Crapez, M.A., Aleluia, I., Wasserman, J.C., 2009. A case study of in situ oil contamination in a mangrove swamp (Rio de Janeiro, Brazil). Mar. Pollut. Bull. 580 (3), 418–423. http://dx.doi.org/10.1016/j.marpolbul.2008.12.008 (ISSN 0025-326X, URL http://www.sciencedirect.com/science/article/pii/S0025326X080056).

Brito, E.M.S., Piñón-Castillo, H.A., Guyoneaud, R., Caretta, C.A., Gutiérrez-Corona, J.F., Duran, R., Reyna-López, G.E., Nevárez-Moorillón, G.V., Fahy, A., Goñi-Urriza, M., 2013. Bacterial biodiversity from anthropogenic extreme environments: a hyper-alkaline and hyper-saline industrial residue contaminated by chromium and iron. Appl. Microbiol. Biotechnol. 970 (1), 369–378.

Caretta, C.A., Brito, E.M.S., 2011. *In silico* restriction for identifying microbial communities in T-RFLP fingerprints. J. Comput. Interdiscip. Sci. 20 (2), 123–129.

Cerniglia, C.E., 1992. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3, 351–368. http://dx.doi.org/10.1007/BF00129093 (ISSN 0923–9820).

Duke, N.C., Burns, K.A., Swannell, R.P., Dalhaus, O., Rupp, R.J., 2000. Dispersant use and a bioremediation strategy as alternate means of reducing impacts of large oil spills on mangroves: the Gladstone field trials. Mar. Pollut. Bull. 410 (712), 403–412. http://dx.doi.org/10.1016/S0025-326X(00)00133-8 (ISSN 0025-326X, URL http://www.sciencedirect.com/science/article/pii/S0025326X00001338).

Elomari, M., Coroler, L., Host, B., Gillis, M., Izard, D., Leclerc, H., 1996. DNA relatedness among pseudomonas strains isolated from natural mineral waters and proposal of *pseudomonas veronii* sp. nov. Int. J. Syst. Bacteriol. 460 (4), 1138–1144. http://dx.doi.org/10.1099/00207713-46-4-1138 (URL http://ijs.sgmjournals.org/content/46/4/1138.abstract).

Friedman, N., Sexton, D., 2001. Bursitis due to *mycobacterium goodii*, a recently described, rapidly growing *mycobacterium*. J. Clin. Microbiol. 390 (1), 404–405.

Fu, J., Mai, B., Sheng, G., Zhang, G., Wang, X., Peng, P., Xiao, X., Ran, R., Cheng, F., Peng, X., Wang, Z., Tang, W., 2003. Persistent organic pollutants in environment of the Pearl River delta, China: an overview. Chemosphere 520 (9), 1411–1422.

Gu, S.-H., Kralovec, A.C., Christensen, E.R., Van Camp, R.P., 2003. Source apportionment of PAHs in dated sediments from the black river, Ohio. Water Res. 370 (9), 2149–2161. http://dx.doi.org/10.1016/S0043-1354(02)00584-5.

Habe, H., Omori, T., 2003. Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci. Biotechnol. Biochem. 670 (2), 225–243.

He, L., Song, J., Peng, P., 2008. Characterization of extractable and non-extractable polycyclic aromatic hydrocarbons in soils and sediments from the Pearl River delta, China. Environ. Pollut. 1560 (3), 769–774. http://dx.doi.org/10.1016/j.envpol.2008.06.003.

Heitkamp, M.A., Freeman, J.P., Miller, D.W., Cerniglia, C., 1988. Pyrene degradation by a mycobacterium sp.: identification of ring oxidation and ring fission products. Appl. Environ. Microbiol. 540 (10), 2556–2565.

Hewson, I., Fuhrman, J., 2006. Improved strategy for comparing microbial assemblage fingerprints. Microb. Ecol. 510 (2), 147–153.

INEGI, 2011. Censo de población y vivienda 2010. Instituto Nacional de Estadística y Geografía (INEGI) (page 189 pp., URL http://www.inegi.org.mx).

Japenga, J., Wagenaar, W., Salomons, W., Lacerda, L., Patchineelam, S., Filho, C., 1988. Organic micropollutants in the Rio de Janeiro coastal region, Brazil. Sci. Total Environ. 750 (23), 249–259. http://dx.doi.org/10.1016/0048-9697(88)90038-1 (ISSN 0048–9697, URL http://www.sciencedirect.com/science/article/pii/00489697889003).

Juhasz, A.L., Naidu, R., 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int. Biodeterior. Biodegrad. 450 (1–2), 57–88. http://dx.doi.org/10.1016/S0964-8305(00)00052-4 (ISSN 0964–8305, URL http://www.sciencedirect.com/science/article/pii/S0964830500000524).

Kim, S.-J., Kweon, O., Jones, R.C., Freeman, J.P., Edmondson, R.D., Cerniglia, C.E., 2007. Complete and integrated pyrene degradation pathway in mycobacterium Vanbaalenii pyr-1 based on systems biology. J. Bacteriol. 1890 (2), 464–472.

Kiyohara, H., Nagao, K., Nomi, R., 1976. Degradation of phenanthrene through o-phthalate by an aeromonas sp. Agric. Biol. Chem. 400 (6), 1075–1082.

Kostka, J.E., Prakash, O., Overholt, W.A., Green, S.J., Freyer, G., Canion, A., Delgardio, J., Norton, N., Hazen, T.C., Huettel, M., 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deep-water horizon oil spill. Appl. Environ. Microbiol. 770 (22), 7962–7974. http://dx.doi.org/10.1128/AEM. 05402-11.

Lane, D., 1991. 16 s/23s rRNA sequencing. In: Stackebrandt, G., Goodfellow, M. (Eds.), Nucleic Acid Techniques Systematics, 1st edition John Wiley & Sons, Chinchester.

Lane, D.J., Olsen, G.J., Stahl, D., Sogin, M.L., 1985. Rapid determination of 16 s ribosomal RNA sequences for phylogenetic analysis. Proc. Natl. Acad. Sci. U. S. A. 82, 6955–6959.

Li, F.L., Xu, P., Ma, C.Q., Luo, L.L., Wang, X.S., 2003. Deep desulfurization of hydrodesulfurization-treated diesel oil by a facultative thermophilic bacterium mycobacterium sp. x7b. FEMS Microbiol. Lett. 2230 (2), 301–307.

Lu, X.-Y., Zhang, T., Fang, H.-P., 2011. Bacteria-mediated PAH degradation in soil and sediment. Appl. Microbiol. Biotechnol. 890 (5), 1357–1371. http://dx.doi.org/10.1007/s00253-010-3072-7 (ISSN 0175–7598, URL http://dx.doi.org/10.1007/s00253-010-3072-7).

Luan, T.G., Yu, K.S.H., Zhong, Y., Zhou, H.W., Lan, C.Y., Tam, N.F.Y., 2006. Study of metabolites from the degradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial consortium enriched from mangrove sediments. Chemosphere 650 (11), 2289–2296. http://dx.doi.org/10.1016/j.chemosphere.2006.05.013 (Dec, ISSN 0045–6535).

Malm, O., Pfeiffer, W., Fiszman, M., Azcue, J., 1989. Heavy metal concentrations and availability in the bottom sediments of the Paraiba do Sulguandu river system, RJ, Brazil. Environ. Technol. Lett. 100 (7), 675–680. http://dx.doi.org/10.1080/09593338909384785 (URL http://www.tandfonline.com/doi/abs/10.1080/09593338909384785).

Máthé, I., Benedek, T., Táncsics, A., Palatinszky, M., Lányi, S., Márialigeti, K., 2012. Diversity, activity, antibiotic and heavy metal resistance of bacteria from petroleum hydrocarbon contaminated soils located in Harghita County (Romania). Int. Biodeter. Biodegr. 73, 41–49.

Meire, R.O., 2006. Avaliação de Hidrocarbonetos Policclicos Aromáticos (HPAs) em áreas de proteção permanente no sudeste brasileiro. (PhD thesis). Universidade Federal do Rio de Janeiro Rio de Janeiro.

Overmann, J., Fischer, U., Pfennig, N., 1992. A new purple sulfur bacterium from saline littoral sediments, *Thiorhodovibrio winogradskyi* gen. nov. and sp. nov. Arch. Microbiol. 1570 (4), 329–335.

Peré-Trepat, E., Olivella, L., Ginebreda, A., Caixach, J., Tauler, R., 2006. Chemometrics modelling of organic contaminants in fish and sediment river samples. Sci. Total Environ. 3710 (1), 223–237.

Silva, A., Alves, M., 2006. Como iniciar a validação de métodos analíticos. Congresso e Feira da Qualidade em Metrologia Rede Metrológica do Estado de São Paulo (REMESP), ENQUALAB, São Paulo.

Smith, M.R., 1990. The biodegradation of aromatic hydrocarbons by bacteria. Biodegradation 1, 191–206. http://dx.doi.org/10.1007/BF00058836 (ISSN 0923–9820).

Stingley, R.L., Brezna, B., Khan, A.A., Cerniglia, C.E., 2004. Novel organization of genes in a phthalate degradation operon of mycobacterium vanbaalenii pyr-1. Microbiology 1500 (11), 3749–3761.

Toda, H., Sato, K., Iimori, M., Yamazumi, T., Furuta, I., Satoh, A., Katsukawa, C., 2006. A case of *mycobacterium goodii* infection wifh isolation from blood and a pacemaker lead. Kansenshogaku Zasshi 800 (3), 262–266.

Tsai, Y.L., Olson, B.H., 1991. Rapid method for direct extraction of dna from soil and sediments. Appl. Environ. Microbiol. 570 (4), 1070–1074 (URL http://aem.asm.org/content/57/4/1070.abstract).

Tsymbalyuk, K., Denga, Y., Berlinsky, N., Antonovich, V., 2011. Determination of 16 priority polycyclic aromatic hydrocarbons in bottom sediments of the danube estuarine coast by gc/ms. Geo-Eco-Marina 17, 67–72.

U.S. Environmental Protection Agency, 1986. The Gold Book in Quality Criteria for Water: EPA 440/5–86–001. (URL www.epa.gov/scitech/swguidance/standards/criteria/aqlife).

Uhlmann, D., 2011. After the spill is gone: the Gulf of Mexico, environmental crime and the criminal law. Mich. Law Rev. 109, 1413–1462.

UNDP-PCBs, 2009. Project Document: Environmental Sound Management and Destruction of PCBs in Mexico. United Nations Development Programme UNDP-PCBs.

Yang, G.-P., 2000. Polycyclic aromatic hydrocarbons in the sediments of the South China Sea. Environ. Pollut. 1080 (2), 163–171.

Yang, H., Zhuo, S., Xue, B., Zhang, C., Liu, W., 2012. Distribution, historical trends and inventories of polychlorinated biphenyls in sediments from yangtze river estuary and adjacent east china sea. Environ. Pollut. 1690 (11), 20–26.

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. Pahs in the fraser river basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 330 (4), 489–515. http://dx.doi.org/10.1016/S0269-7491(99)00245-6.

Zhong, Y., Luan, T., Lin, L., Liu, H., Tam, N.F., 2011. Production of metabolites in the biodegradation of phenanthrene, fluoranthene and pyrene by the mixed culture of *mycobacterium* sp. and *sphingomonas* sp. Bioresour. Technol. 1020 (3), 2965–2972. http://dx.doi.org/10.1016/j.biortech.2010.09.113 (ISN 0960–8524, URL http://www.sciencedirect.com/science/article/pii/S09608524100164).