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Evaluation of hydrocarbons and organochlorine pesticides and their tolerant microorganisms from an agricultural soil to define its bioremediation feasibility

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The concentrations of hydrocarbons and organochlorine pesticides (OCPs), nutrients and tolerant microorganisms in an agricultural soil from a locality in Tepeaca, Puebla, Mexico, were determined to define its feasibility for bioremediation. The OCPs detected were heptachlor, aldrin, trans-chlordane, endosulfán I, endosulfán II, 1,1,1-bis-(4-chlorophenyl)-2,2-trichloroethane (4,4′-DDT), 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (4,4′-DDE) and endrin aldehyde, with values of 0.69–30.81 ng g^{-1}. The concentration of hydrocarbons in the soil of Middle Hydrocarbons Fraction (MHF), C_{10} to C_{28}, was 4608–27,748 mg kg^{-1} and 1117–19,610 mg kg^{-1} for Heavy Hydrocarbons Fraction (HHF), C_{28} to C_{35}, due to an oil spill from the rupture of a pipeline. The soil was deficient in nitrogen (0.03–0.07%) and phosphorus (0 ppm), and therefore it was advisable to fertilize to bio-stimulate the native microorganisms of soil. In the soil samples, hydrocarbonoclast fungi 3.72×10^2 to 44.6×10^2 CFU g^{-1} d.s. and hydrocarbonoclast bacteria (0.17×10^5 to 8.60×10^5 CFU g^{-1} d.s.) were detected, with a tolerance of 30,000 mg kg^{-1} of diesel. Moreover, pesticideclast fungi (5.13×10^2 to 42.2×10^2 CFU g^{-1} d.s.) and pesticideclast bacteria (0.15×10^5 to 9.68×10^5 CFU g^{-1} d.s.) were determined with tolerance to 20 mg kg^{-1} of OCPs. Fungi and bacteria tolerant to both pollutants were also quantified. Therefore, native microorganisms had potential to be stimulated to degrade hydrocarbons and pesticides or both pollutants. The concentration of pollutants and the microbial activity analyzed indicated that bioremediation of the soil contaminated with hydrocarbons and pesticides using bio-stimulation of native microorganisms was feasible.

Keywords: Hydrocarbons, organochlorine pesticides, hydrocarbonoclasts, pesticideclasts, bioremediation.

Introduction

The production of the hydrocarbons arises from the necessity for a series of oil by-products which are necessary for society. This activity has increased with demand and is the source of contamination in ecosystems. Hydrocarbon pollution can be due to the drilling of oil wells, liquid extraction, refining and petrochemical production. Moreover, spills during transportation, can impact agricultural soils, because oil-transport pipelines are generally adjacent or below growing areas. In Mexico, the presence of hydrocarbons in soil is due to leaks from corroded pipes, and is the second most important source of contamination by petroleum hydrocarbons. The Mexican Official Norm[1] establishes the maximum permissible limits of hydrocarbons in soils and specifications for characterization and remediation, for agricultural, residential and industrial soils, of light (LHF), medium (MHF) and heavy (HHF) hydrocarbon fractions, Benzene, Toluene, Ethylbenzene and Xylene (BTEX) and Polycyclic Aromatic Hydrocarbons (PAHs).

Addition of pesticides to agricultural soils results in a high accumulation, since about 60% of these products are lost to the soil; additionally, persistent pesticides can affect human health and the environment.[2] Approximately 95% of pests, diseases and weeds are controlled by application of pesticides, and high yielding agriculture is inconceivable without the use of synthetic organic pesticides.[3] In Mexico, the majority of pesticides are used in agriculture and health campaigns, and a minor proportion in industrial activities, gardening and domestic use.[4]

In Mexico, there is no normativity for the maximum levels of pesticides for soil remediation, there is only an

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Official Catalog of Pesticides[5] which aims to control the import, production, marketing, transportation and use. This catalog allows the use of 306 pesticides, with 20 pesticides strictly prohibited (including aldrin, cyanophos, chloranil, dialifor, dieldrin and endrin). The DDT is used exclusively for health campaigns. In general, organochlorine pesticides (OCPs) are banned or restricted for agricultural use, except for dienochlor and endosulfan, for which use is permissible.

Bioremediation is a process for soil cleanup, which aims to decrease the total concentration of pollutants in soil, through degradation of organic compounds by native microorganisms, which are stimulated to use the pollutants as a nutrient and energy source.[6] The chemical and environmental factors that influence the remediation of contaminants in soils are the solubility of the compound, temperature, pH, oxygen, soil texture, organic matter (OM), moisture, nitrogen (N), phosphorus (P) and micronutrients.[7] The process of bio-stimulation introduces additional nutrients in the form of organic and/or inorganic fertilizers into a contaminated system, which promotes the growth conditions that increase and stimulate the population of indigenous microorganisms.[8] Therefore, for effective bioremediation it is necessary to perform physicochemical characterization of contaminated soil and determine if the native microbiota are capable of degrading contaminants by biostimulation.

In this paper, the levels of hydrocarbons and OCPs from an agricultural soil were determined, as well as the physicochemical parameters and the presence of contaminant degraders necessary for subsequent bioremediation by biostimulation was investigated.

Materials and methods

Study area

The soil samples were collected in the Tepeaca region, in state of Puebla, Mexico (97°54′29.52–30.78″W and 19°0′49.77–50.91″N) in a plot containing agricultural soil contaminated with pesticides and hydrocarbons. Alfalfa, maize, cabbage and cauliflower are produced in this area. The pesticides used for these crops, according to the Official Catalog of Pesticides,[5] are carbamates, organophosphates and organochlorine endosulfan. The presence of hydrocarbons in this agricultural soil is due to an oil spill in October 2010, caused by a rupture in a pipeline that passes beneath the agriculture area. This site has not had any remediation – the contaminated soil was transported to the side of the affected parcel, and a liner of high-density polyethylene (HDPE) placed to prevent contaminant migration to unaffected soil. The soil was distributed in mounds of about 1.0 m high, in a rectangular area of 1776 m^2.

Sampling

To characterize the contamination of agricultural soil, sampling was conducted in June 2011 to determine the concentration of pesticides and hydrocarbons and their physicochemical and microbiological properties. A total of four points and a total of eight samples of contaminated soil were collected, four in the surface layer (P1S, P2S, P3S and P4S) and four in the lower layer (P1L, P2L, P3L and P4L) at 0.5 m deep. The sampling points were distributed as four equidistant points within the rectangular area of 1776 m^2 containing the contaminated soil. Samples were collected using a sampler hand auger and placed in glass jars. The four sampling points were chosen considering the Mexican Official Norm,[1] which states that four sampling points must be in a contaminated area of 1000–1900 m^2.

Determination of physicochemical parameters

The first step to properly perform any remediation is to obtain a thorough understanding of the characteristics of the soil to be treated and its physicochemical and microbiological properties. Due to this, physicochemical and biological parameters were determined: N, P, OM, pH by pH meter HANNA model HI 98129 (Sigma-Aldrich, Federal District, Mexico), texture, moisture by moisture analyzer KERN model MLB 50-3 (Sigma-Aldrich, Federal District, Mexico) and field capacity (FC).

Analytical method for hydrocarbons and pesticides

The MHF extraction was performed by method EPA 3546[9] in a microwave oven CEM MARS-Xpress with turntable (Falcon, Federal District, Mexico). The analysis utilized method EPA 8015C,[10] using a gas chromatograph with flame ionization detector (GC/FID) – a Thermo Scientific GC Focus Series model chromatograph (Falcon, Federal District, Mexico) was used, equipped with GRACE capillary column (30 m length, 0.32 mm internal diameter and 0.25 μm of thickness of cap AT^{TM}-5), nitrogen as carrier gas at a flow of 1.3 mL min^{-1} and hydrogen and air as auxiliary gases. GC conditions: injector 250°C (split flow: 39 mL min^{-1}), detector 250°C and column 50–270°C (ramp at 17.67 min). Samples were concentrated to a volume of 2 mL, and 1 μL was injected into the GC. Limits of detection (LOD): 0.01 mg kg^{-1}. The standard used was a mixture of hydrocarbons, n-alkanes alkanes (C_{10}–C_{28}) Resteck DRO Mix on Rtx®-5 (Tecrom, Federal District, Mexico). The retention times and the sum of areas under the curve, calculated out by an integrator of areas of chromatograms, were used to determine concentrations of samples.

The HHF analysis was carried out by gravimetric determination using the method EPA 1664A,[11] EPA 3546,[9] and EPA 9071B[12] methods were used for extraction, using a CEM MARS-Xpress microwave.

Analysis of OCPs in soil samples was performed with method EPA 8081B,[13] by GC with electron capture

detector (ECD). Extraction was performed using method EPA 3546[9] and CEM MARS-Xpress microwave, and cleanliness of the extracts was realized with Florisil adsorption columns (Supelclean™ ENVI™ Florisil® SPE Tubes 6 mL 1 g, Sigma-Aldrich, Federal District, Mexico). GC Varian Model CP-3380 (Agilent, Federal District, Mexico), power source with 63Ni, with Varian capillary column (15 m long, 0.25 mm internal diameter and 0.25 μm of thickness of cap CP-Sil 5CB) and nitrogen as carrier gas was used at a constant pressure of 17 psi. GC conditions: injector 200°C, detector 300°C and column 100–230°C (35 min ramp). Samples were concentrated to a volume of 2 mL, and 1 μL was injected into the GC; retention time and the area under the curve measured by an area integrator. LOD was 0.01 ng g^{-1}. The standard mixture used was 20 OCPs, Restek Mix AB # 3 (Tecrom, Federal District, Mexico): α-BHC, β-BHC, γ-BHC (lindane), δ-BHC, heptachlor, heptachlor epoxide, trans-chlordane, cis-chlordane, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, methoxychlor, endosulfan I, endosulfan II and endosulfan sulfate.

QA/QC

Quantification of OCPs was determined from the external standard comparing peak area. The correlation coefficients (r) of calibration curves of OCPs were all higher than 0.971. The spiked recoveries of OCPs ranged from 83.0% to 120.7% and the relative standard deviation (RSD) was less than 25%. Laboratory blanks, solvent blanks and field blanks were analyzed using the same procedure as that used for real samples. No contaminants of OCPs were found in these blanks. Solvent blanks were run first and every six samples, using hexane as that used for real samples. All chemicals used in the study were analytical grade. The glassware was washed perfectly with detergent phosphate-free, rinsed with acetone and dried at 80°C for 24 h before use.

Microbiological analysis

To determine the microbial count of viable microorganisms in soil, colony forming units/ gram dry soil (CFU/g d.s.), the bacterial and fungal counts were determined by plate dilution methodology.

Hydrocarbonoclast bacteria and fungi are those capable of growing in the presence of hydrocarbons and use these as a source of carbon and energy.[14] The presence of these microorganisms is an indicator of the biodegradation potential of a soil. Determination was performed by plate dilution technique, but without a carbon source added to the Noble agar medium; essential nutrients for cell growth (Ca, Cu, Fe, K, Mg, N, Na and Zn) were added. Once the gelled medium was dispersed 1 mL of a solution of 30,000 mg kg^{-1} of diesel in acetone and the Petri dishes were inoculated The differences in culture media for bacteria and fungi were pH and addition of antibiotic streptomycin in the fungi medium.[14]

Quantification of bacteria and fungi tolerant to OCPs, named in this paper as "pesticideclasts", utilized the same technique and methodology used to assess hydrocarbonoclasts. The difference in this method was the addition of 1 mL of a mixture of endosulfan, heptachlor and DDT at a concentration of 20 mg kg^{-1}. These compounds were used because they were detected in agricultural soil samples. This quantification indicates the feasibility of OCPs degradation by native organisms in the problem soil.

A mixture of diesel and OCPs was added to determine whether the microorganisms were able to tolerate both these pollutants. This was performed by adding 1 mL of the mixture of both pollutants in Petri dishes inoculated at the above concentrations. Quantification and methodology were as used for hydrocarbonoclasts and pesticideclasts.

Statistical analysis

At each sampling point a correlation, and a comparison for significant differences, was performed between surface points and their respective point 0.5 m deep. This was by simple Pearson's correlations and Least Significant Difference (LSD) mean comparisons (α: 0.05) using SAS 9.1.

Results and discussion

Texture

The sampled soil generally had a loamy fine sand texture (78% sand, 4% clay and 18% silt) according to the texture triangle. A sandy soil, along with the amount of OM and the texturizing materials, can increase porosity and allow better diffusion of oxygen, which enhances microbial activity, stability and structure by water infiltration and gas exchange.[15] Therefore, this agricultural soil texture is adequate for the bioremediation process.

pH

Most samples had moderately acid pH (Table 1). The pH affects reactivity of the soil, influences the activities and abundance of different groups of organisms in the soil. Most microorganisms and plants grow at near neutral pH of 6–7, due to availability of nutrients at these pH values.[16] Extreme pH can limit the degradation of pollutants by microbial action,[17] therefore, it is important to know this parameter to determine the conditions under which they will be degrading contaminants.

Moisture

Soil moisture is essential for microbial growth and activity, because water in molecular form is involved in several

Table 1. Physicochemical and microbiological parameters of samples of agricultural soil in Tepeaca.

Sample	*pH*	*Moisture (%)*	*FC (%)*	*OM (%)*	*N (%)*	*Bacteria × 10^5 (CFU/g d. s.)*	*Fungi × 10^2 (CFU/g d. s.)*
P1S	5.82 ± 0.01	3.60 ± 0.25	74.99 ± 2.47	3.16 ± 0.20	0.04 ± 0.01	25.9 ± 0.45	24.9 ± 8.10
P1L	5.64 ± 0.01	7.37 ± 0.22	66.27 ± 3.18	3.08 ± 0.16	0.04 ± 0.01	26.6 ± 0.12	1.08 ± 0.00
P2S	5.70 ± 0.03	2.48 ± 0.11	59.84 ± 3.14	5.70 ± 0.10	0.05 ± 0.00	1.9 ± 0.01	2.39 ± 1.57
P2L	5.45 ± 0.02	9.71 ± 0.10	76.26 ± 6.53	4.66 ± 0.11	0.05 ± 0.00	50.6 ± 0.33	6.65 ± 5.75
P3S	5.44 ± 0.02	8.33 ± 0.09	76.33 ± 0.86	7.83 ± 0.12	0.07 ± 0.02	18.7 ± 0.11	1.82 ± 1.26
P3L	5.34 ± 0.04	10.46 ± 0.21	79.35 ± 0.07	7.61 ± 0.07	0.07 ± 0.00	17.0 ± 0.24	1.12 ± 0.00
P4S	5.52 ± 0.05	8.31 ± 0.14	58.1 ± 0.08	4.15 ± 0.13	0.03 ± 0.00	4.4 ± 0.27	7.63 ± 0.00
P4L	5.99 ± 0.05	7.65 ± 0.18	73.34 ± 3.31	4.61 ± 0.14	0.03 ± 0.00	15.2 ± 0.39	30.3 ± 16.0

Sampling surface layer = P1S, P2S, P3S, P4S; Sampling lower layer (0.5 m) = P1L, P2L, P3L, P4L.

cellular metabolic reactions, serving as a solvent and a carrier for soil nutrients.[14] The samples had moisture values in the range of 2.4–10.61% (Table 1), showing that there was at least moisture at 0.5 m depth.

Field capacity

The samples indicated that the soil had moderate water uptake and retention capacity (Table 1). The highest FC values, 79.3 and 76.33%, were in the P2L and P3S samples, respectively; and the lowest values for P2S and P4S with 59.84 and 58.1%, respectively.

Organic matter

The soil was moderately rich in OM (Table 1). The samples of agricultural soil in Tepeaca region had higher OM values compared to other agricultural soils, with values <2%.[18] This can be explained by the physicochemical and environmental characteristics of each site; however, these values may also be attributable to the pollution of this agricultural soil with hydrocarbons unlike other soils.

Nitrogen

The samples showed that the soil had a low amount of *N* (Table 1). The values obtained were lower compared to other agricultural soils,[18] which could be attributed to several factors including climatic variations, rapid degradation and assimilation by organisms and/or physicochemical characteristics of the soil, but might also indicate that this agricultural soil was not over-fertilized.

Phosphorous

There was no P detected in the soil samples, which could be because P is easily digestible as a nutrient by microorganisms, but could also be due to soil characteristics such as texture and pH.[19]

Analyses of OM, P and N are essential to control the amount of nutrients, fertilizers and organic material to be added to the contaminated soil to determine the nutritional conditions for carrying out bioremediation. The amounts of OM, N and P were determined by the ratio C:N:P (100:10:1). The determination of the appropriate amounts of nutrients will prevent them be limiting factors for development of microorganisms involved in bioremediation, because microorganisms require these chemical constituents for assimilation and cell synthesis.[20]

Bacterial and fungal viable counts

The quantification of total bacteria will define the number of heterotrophic soil bacteria that can grow in a culture medium (or viable cultural),[14] and this agricultural soil from Tepeaca region had a moderate microbial load (Table 1). The P2L and P1L samples showed the highest bacterial concentrations with values of 50.6×10^5 and 26.6×10^5 CFU g^{-1} d.s., respectively. The P2S sample had the lowest value of 1.9×10^5 CFU g^{-1} d.s., as well as the lowest moisture and FC values. Microorganisms require minimum moisture conditions for growth, and water serves as a transport medium for organic compounds so that nutrients are mobilized inside cells to allow their development.[21] Correlations between physicochemical parameters and bacterial counts were not significant.

In soil samples, fungal counts were most prominent in P4L (30.3×10^2 CFU g^{-1} d.s.) and P1S samples (24.9×10^2 CFU g^{-1} d.s.). The sample with the lowest amount of fungal colonies (1.08×10^2 CFU g^{-1} d.s.) was P1L (Table 1). There were low amounts of fungi in comparison to bacteria in samples, as also previously reported in contaminated soils and substrates.[22,23] There was no significant correlation between fungi counts and physicochemical parameters in the agricultural soil.

Hydrocarbons

According to the maximum allowable limits for MHF in the Mexican Official Norm, [1] the samples exceeded the limits for the use of agricultural soil (Tables 2 and 3)[24–27]. The P3S and P4L samples showed the highest

Table 2. Concentration of MHF and HHF in agricultural soil in Tepeaca.

Sample	*MHF (mg/kg)*	*HHF (mg/kg)*
P1S	5202.5 ± 233.25	1815.4 ± 366.78
P1L	4608.1 ± 710.50	1117.1 ± 381.70
P2S	7519.9 ± 78.27	8203.4 ± 725.09
P2L	5937.8 ± 191.55	3322.6 ± 783.15
P3S	27,747.9 ± 1510.25	19,609.9 ± 771.40
P3L	8392.7 ± 2134.25	5584.1 ± 1579.42
P4S	8265.3 ± 256.99	9270.3 ± 2313.58
P4L	21,640.1 ± 904.94	15,430.4 ± 382.84

Sampling surface layer = P1S, P2S, P3S, P4S; Sampling lower layer (0.5m) = P1L, P2L, P3L, P4L.

concentration of MHF with 27,747.9 and 21,640.1 mg kg^{-1}, respectively, while the P1L sample showed the lowest value of 4608.1 mg kg^{-1}. Most of the sampling points of HHF exceeded the limit established for agricultural soil in the same norm (Tables 2 and 3), with the highest concentrations in P4L and P3S of 19,609.9 and 15,430.4 mg kg^{-1}, respectively. There was a significant difference between surface contamination and 0.5 m deep – points P2S and P3S showed higher MHF and HHF concentrations respectively compared to P2L and P3L. The P4L sample showed a higher MHF contamination than its surface point P4S but no significant difference for HHF; and finally P1S and P1L showed no respective significant differences for MHF and HHF. Thus, we can conclude that there was no trend in distribution of soil contamination.

The hydrocarbon fractions analyzed, MHF and HHF, correspond to Diesel Range Organics (DRO): C_{10} to C_{28}) and Oil Range Organics (ORO): C_{28} to C_{35}, included in the determination of Total Petroleum Hydrocarbons (TPH): C_6 to C_{35}, EPA 418.1, commonly used in other characterization studies.[28]

The hydrocarbon concentrations obtained for these two fractions were higher than the cleanup levels established in regulations of other countries for soil of residential and industrial use (Table 3), although only in Canada and Mexico are considered maximum levels for agricultural soils.

The sum of the maximum values of MHF and HHF was used to obtain an approximate value of total hydrocarbons (~STPH), which was 47357.8 mg kg^{-1}. The concentrations in soil were higher than in north-central Mexico, where concentrations of TPH (EPA 418.1) of 47–21,093 mg kg^{-1} were determined in soil near an oil station.[29] They were lower than maximum values found in northern Mexico adjacent to oil pumping stations with TPH concentrations of 200–50,000 mg kg^{-1}[30] and in southeast Mexico near a pumping station with 13–101759 mg kg^{-1} in soil due to spills.[31]

The concentrations of the present study were higher than in an agricultural soil near an oil complex in south China with TPH concentrations of 1179.3–6354.9 mg kg^{-1},[32] a soil near an oil field in northeast China with 0.59–2.20 mg kg^{-1}[33] and soil near a refinery in eastern China where 9.45–652 mg kg^{-1} was found.[34] However, soil concentrations in the present study were lower compared to ground near a refinery in Australia with TPH concentrations of 16,453–68,856 mg kg^{-1}.[35]

Organochloride pesticides

The results showed that only eight of the 20 standard OCPs were detected in soil samples: heptachlor, aldrin, trans-chlordane, 4,4'-DDE, endosulfan I, endosulfan II, 4,4′-DDT and endrin aldehyde – the first four were detected in all samples (Table 4). Maximum concentrations in all sampled points were for trans-chlordane with values of 7.98–75.78 ng g^{-1}. The maximum value of the trans-chlordane was 75.78 ng g^{-1} detected in sample P3L, while the lowest was for endosulfan II with 1.27 ng g^{-1} in sample P1S. In most sampling points, there was no significant difference between surface points and points at 0.5 m depth, except that sample P2S had a higher concentration of heptachlor, trans-chlordane and aldrin compared to sample P2L. Similarly to hydrocarbons, there was no trend in the distribution of contamination of soil.

According to the Official Catalog of Pesticides,[5] agricultural use of OCPs lindane, methoxychlor, DDT, heptachlor, chlordane, aldrin, dieldrin and endrin is prohibited in Mexico. DDT use is restricted and can only be used by the Secretary of Health in health campaigns against malaria. Endosulfan is the only OCP allowed for growing of crops including sugar cane, snuff, husk tomato, wheat and watermelon. Currently, detection of these compounds

Table 3. Maximum permissible levels (mg kg^{-1}) for hydrocarbon fractions in different soil uses.

	TPH						
	Diesel Range Organics (DRO/MFH C_{10} a C_{28})			*Oil Range Organics (ORO/HHF C_{28} a C_{35})*			
	Agricultural	*Residential*	*Industrial*	*Agricultural*	*Residential*	*Industrial*	*Reference*
Canada	150	150	260	1300	1300	2500	[24]
Australia	—	5600	28,000	—	—	—	[25]
EUA	—	2300	6200	–	2300	6200	[26,27]
Mexico	1200	1200	5000	3000	3000	6000	[1]

Table 4. OCPs (ng g^{-1}) of samples of agricultural soil in Tepeaca.

Samples	*Heptachlor*	*Aldrin*	*trans-Chlordane*	*4,4′-DDE*	*Endosulfan I*	*Endosulfan II*	*4,4′-DDT*	*Endrin aldehyde*
P1S	6.09 ± 3.93	6.48 ± 0.00	7.98 ± 5.62	5.75 ± 4.36	2.33 ± 2.35	1.27 ± 0.52	4.38 ± 3.70	1.36 ± 0.96
P1L	20.42 ± 11.13	9.87 ± 5.28	30.61 ± 17.64	22.78 ± 9.21	10.54 ± 3.25	3.14 ± 3.01	11.06 ± 3.85	3.75 ± 2.32
P2S	16.51 ± 0.88	12.51 ± 1.57	30.81 ± 4.73	21.49 ± 3.33	—	—	—	—
P2L	8.61 ± 2.93	4.86 ± 1.63	17.06 ± 6.75	12.73 ± 5.19	—	—	—	—
P3S	15.90 ± 3.56	10.07 ± 2.14	28.29 ± 8.88	17.67 ± 3.67	—	—	—	—
P3L	19.41 ± 5.38	13.57 ± 3.27	75.78 ± 48.41	27.98 ± 7.48	—	—	- -	—
P4S	10.80 ± 3.81	7.02 ± 4.84	23.41 ± 5.87	13.86 ± 7.07	—	—	—	—
P4L	12.68 ± 1.64	10.10 ± 1.03	23.63 ± 3.96	14.05 ± 1.88	—	—	—	—

Sampling surface layer = P1S, P2S, P3S, P4S; Sampling lower layer (0.5 m) = P1L, P2L, P3L, P4L.

in agricultural soils indicates recent use or slow degradation in agricultural environments.

Pesticides such as aldrin, dieldrin, chlordane and heptachlor provided economical control of pests for many years,[36] and were widely used before a total ban in the early 1990s. The most persistent organochlorines are DDT (>10 *y*), endrin (10 *y*), chlordane (8 *y*), dieldrin (7 *y*), aldrin (5 *y*), heptachlor (4 *y*), γ-HCH or lindane (2 *y*), endosulfan II (2 *y*) and endosulfan I (3.5 months).[37]

The presence of heptachlor in the environment may be due to its direct application in fields or by the used of chlordane because heptachlor was used for the formulation of technical chlordane;[38] both pesticides are currently banned in most countries. In the agricultural soil, chlordane concentrations of 7.98–75.78 ng g^{-1} and heptachlor of 6.09–20.42 ng g^{-1} were detected (Table 4).

Aldrin and dieldrin are two organochlorines that were formulated and applied separately in agriculture, but the biotransformation of aldrin, through epoxidation, can generate dieldrin, which can be metabolized by organisms to endrin aldehyde and endrin ketone.[38] In the present study, aldrin was detected at all sampling points, with values within 6.48–13.57 ng g^{-1}, and endrin aldehyde in only two sampling points at 1.36–3.75 ng g^{-1}.

Endosulfan is an OCP still widely used, and has low persistence compared to other OCPs. In this agricultural soil, endosulfan was only detected in two sample points, indicating that it had possibly already been degraded, although its metabolite endosulfan sulfate was not detected, and neither were other metabolites (e.g. alcohol and ether). In soil, endosulfan I and II are primarily converted to endosulfan sulfate that is generated by oxidation due to microbial enzyme activity – it is highly persistent but less toxic than its precursors.[39]

Through reduction (dehydrochlorination) produced by microorganisms, DDT is slowly degraded in the environment to products such as DDE and DDD, which are also very persistent.[38] DDD has biocidal properties, but DDE has only slight biocidal activity.[37,40] In the present study, 4,4′-DDT was found at only two sampling points, but its metabolite 4,4′-DDE was detected in all samples with values within 5.75–27.98 ng g^{-1}.

The concentrations determined were lower than those determined in other soils of Mexico, as in the case of the agricultural area of Yaqui Valley in Sonora,[41] where average concentrations of OCPs were 1600–17,900 ng g^{-1}, because southern Sonora is an area of high agricultural activity (Table 5). However, concentrations in the present study were higher than in rural soils of southern Chiapas,[42] where average values of 0.006–8.5 ng g^{-1} were observed (Table 5). The concentrations obtained in the present work were lower than found in some other countries, such as case studies of agricultural and rural soils in Chile, Vietnam, China and Uganda;[43–45] but higher than in agricultural soils of Argentina and South Korea[46,47] (Table 5).

In Mexico, there are no regulations that specify maximum levels of OCPs according to type of soil use to prevent damage to the environment and human health. Therefore, to determine whether the concentrations detected in the present study are indicative or not of contamination of soil, a comparison with the limits established in other countries was made. According to the Chinese Environmental Quality Standard for Soils, soil quality is classified as Low (values <50 ng g^{-1}), Light (50–500 ng g^{-1}), Moderate (500–1000 ng g^{-1}) and High (>1000 ng g^{-1}) Pollution.[48] According to these values, only P3L had a value of 75.78 ng g^{-1} for trans-chlordane and was classified as Light Pollution. Unfortunately international standards do not consider the sum of all OCPs, although there can be synergistic effects of these chlorinated compounds in the environment and many of these pesticides share toxic properties.[49,50]

Compared with the General Reference Levels for OCPs to Protect Human Health, stipulated in Spain,[51] the results obtained in some samples may represent a risk to human health for soils used for food purposes, such as agricultural soil – because trans-chlordane and aldrin exceeded 10 ng g^{-1} in most samples. The concentrations of 4,4'-DDE, 4,4'-DDT and endosulfan I and II did not exceed the prescribed levels of 600, 200 and 600 ng g^{-1}, respectively. There are no reference levels in Spain for heptachlor and endrin aldehyde.

Compared to the General Reference Levels for the Protection of Ecosystems, stipulated in Spain,[51] some

Table 5. Mean concentrations of OCPs (ng g^{-1}) in agricultural areas from Mexico and other countries.

	Sonora, Mexico	*Chiapas, Mexico*	*Buenos Aires, Argentina*	*Chillán, Chile*	*Bacninha, Vietnam*	*Kihiihi, Uganda*	*Chulla, South Korea*	*Puebla, Mexico*
Heptachlor	NQ	NQ	2.71	NQ	NQ	NQ	1.76	13.80
Aldrin	1600	NQ	ND	100	NQ	NQ	0.56	9.31
trans-Clordane	NQ	0.006	0.36	NQ	NQ	NQ	NQ	29.70
4,4′-DDE	11200	8.5	0.81	600	50.22	33	0.34	17.04
Endosulfan I	6700	0.045	ND	NQ	NQ	13	0.64	6.43
Endosulfan II		ND	0.48	NQ	NQ	17	0.89	1.91
4,4′-DDT	17,900	3.5	15.23	16,900	24.70	46	ND	7.72
Endrín aldehyde	NQ	NQ	NQ	NQ	NQ	NQ	ND	2.55
Reference	[41]	[42]	[47]	[43]	[44]	[45]	[46]	This study

NQ = No quantified; ND = No detected.

samples exceeded the limit for effect on soil organisms: four samples of >10 ng g^{-1} for aldrin, one sample of 40 ng g^{-1} for trans-chlordane and one of 10 ng g^{-1} for endosulfan I. Several exceeded 10 ng g^{-1} for effects on aquatic organisms and terrestrial vertebrates, as was the case of aldrin, trans-chlordane and 4,4'-DDE (Table 4).

Hydrocarbonoclast microorganisms

In all sampling points, bacteria and fungi used diesel as a carbon source were detected, indicating that this soil with appropriate nutrients had native microorganisms with potential to degrade hydrocarbons. In all samples, populations of hydrocarbonoclast bacteria were higher than of fungi. The P1S, P2L and P3L samples showed greater amounts of hydrocarbonoclast bacteria, while P1S, P4S and P4L had more hydrocarbonoclast fungi (Table 6).

The tolerant bacteria detected ranged from 0.17×10^5 to 8.60×10^5 CFU g^{-1} d.s. The bacterial counts are higher compared to other studies, such as concentrations of 5×10^2 CFU g^{-1} d.s. in a contaminated soil from a refinery,[52] but lower compared to 1×10^6 CFU g^{-1} d.s. in a soil contaminated with hydrocarbon oil[53] and soil contaminated with engine oil with 1×10^6 CFU g^{-1} d.s. of degrading bacteria.[54] The hydrocarbonoclastic activity of native microorganisms of a soil is an indicator of the potential of native microorganisms to remove hydrocarbons.[55,56]

The values of tolerant fungi quantified, 3.72×10^2 to 44.6×10^2 CFU g^{-1} d.s., were lower compared to a soil contaminated by fuel with 1.62×10^4 CFU g^{-1} d.s.[22] The values in the present study were lower than concentrations of fungi determined in samples of tarballs with 0.13×10^2 to 5×10^2 CFU g^{-1} d.s.[23]

These results were consistent with those of other reports, showing that soil contaminated with hydrocarbons hosts microorganisms with hydrocarbonoclastic capacity.[57,58]

The correlations between ~STPH and the number of hydrocarbonoclastic microorganisms of each station were not significant. Thus, the presence of hydrocarbon tolerant microorganisms was not related to the amount of the pollutant (Fig. 1a).

Pesticideclast microorganisms

In the agricultural soil analyzed, tolerant bacteria and fungi were detected with potential to degrade OCPs at concentrations of 20 mg kg^{-1}. These concentrations were higher than those in soil samples of 0.69–75.78 ng g^{-1}, so the concentration of these

Table 6. Tolerant microorganisms to hydrocarbons and pesticides from agricultural soil in Tepeaca, Puebla Mexico.

	Hydrocarbonoclast		*Pesticideclast*		*Hydrocarbonclast+Pesticideclast*	
Sample	*Bacteria × 10^5 (CFU g^{-1} d.s.)*	*Fungi × 10^2 (CFU g^{-1} d.s.)*	*Bacteria × 10^5 (CFU g^{-1} d.s.)*	*Fungi × 10^2 (CFU g^{-1} d.s.)*	*Bacteria × 10^5 (CFU g^{-1} d.s.)*	*Fungi × 10^2 (CFU g^{-1} d.s.)*
P1S	7.43 ± 0.26	44.60 ± 3.74	5.26 ± 0.53	42.18 ± 2.61	7.33 ± 0.53	47.72 ± 4.15
P1L	4.61 ± 0.86	7.20 ± 1.25	4.71 ± 0.76	11.15 ± 1.65	4.28 ± 0.37	9.36 ± 0.62
P2S	0.17 ± 0.05	6.49 ± 0.59	0.15 ± 0.07	5.13 ± 2.71	0.27 ± 0.05	6.15 ± 1.03
P2L	8.12 ± 2.37	4.80 ± 1.28	8.71 ± 0.55	5.17 ± 1.69	9.38 ± 0.27	1.85 ± 0.63
P3S	3.85 ± 1.05	5.45 ± 1.09	2.65 ± 0.44	9.45 ± 0.63	3.64 ± 0.88	5.45 ± 1.89
P3L	8.60 ± 0.48	3.72 ± 3.59	9.68 ± 0.42	6.33 ± 0.64	9.83 ± 0.40	5.96 ± 3.41
P4S	2.33 ± 0.33	24.72 ± 6.01	3.24 ± 0.27	26.90 ± 4.41	3.60 ± 0.21	26.90 ± 7.66
P4L	7.36 ± 0.54	16.24 ± 4.72	9.06 ± 0.51	23.82 ± 4.33	9.10 ± 0.21	24.90 ± 6.76

Sampling surface layer = P1S, P2S, P3S, P4S; Sampling lower layer (0.5 m) = P1L, P2L, P3L, P4L.

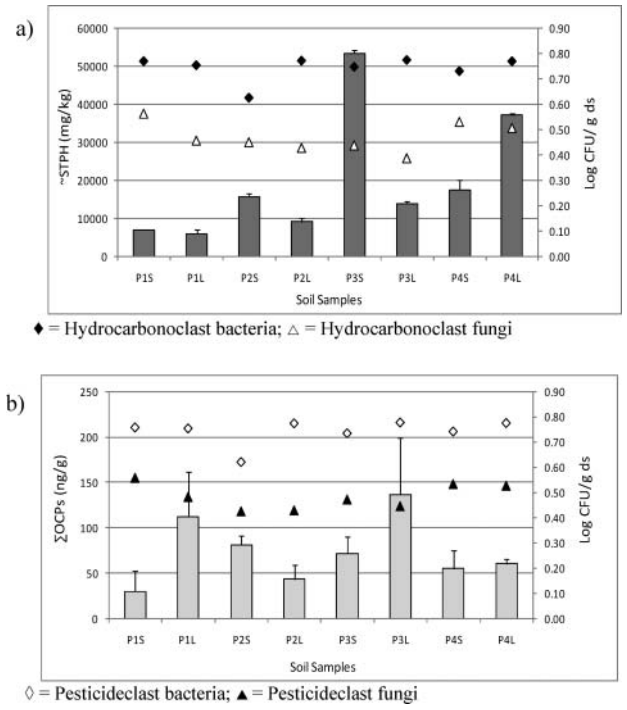

Fig. 1. (a) Total hydrocarbons (~STPH mg kg^{-1}) and hydrocarbonoclast microorganisms (Log CFU/g d.s.); (b) Sum of organochlorine pesticides (ΣOCPs ng g^{-1}) and pesticideclast microorganisms (Log CFU g^{-1} d.s.) of agricultural soil in Tepeaca. Sampling surface layer = P1S, P2S, P3S, P4S; Sampling lower layer (0.5m) = P1L, P2L, P3L, P4L.

compounds in the problem soil will not limit growth of microorganisms.

The P2L, P3L and P4L samples showed the highest amounts of pesticideclast bacteria, while samples with greater populations of pesticideclast fungi were P1S, P4S and P4L (Table 6). There are no reports of analysis of pesticideclast bacteria and fungi counts in polluted soils, and this determination is important to establish the feasibility of these microorganisms for degradation of halogenated pollutants.

To determine the sample with the highest OCP contamination a sum of all OCPs (∑OCPs) was calculated (Fig. 1b). P3L and P1L had the highest contamination levels with 136.74 and 112.18 ng g^{-1}, respectively. There was no significant correlation between pesticideclast microorganisms and ∑OCPs (Fig. 1b).

Hydrocarbonoclast and pesticideclast microorganisms

The P1S and P3L samples showed more hydrocarbonoclast and pesticideclast microorganisms than other samples, and also had higher microbial populations in medium containing the mixture of hydrocarbons and pesticides (Table 6). This study demonstrated that the presence of both pollutants was not a limiting factor for growth of the bacteria and fungi.

The microbiological results showed that bacteria had a higher population density than fungi, indicating that bacteria may be the main degrading agent. However, the power of branching and penetration of fungal hyphae (even at low fungal counts) in a contaminated soil allow some mobility and advantage over bacteria, which could mean a lot of activity in the removal of pollutants.[59,60] However, a microbial consortium of bacteria and fungi can mean a high feasibility for contaminant removal.[61–63] Because fungal hyphae can function as transport for degrading bacteria, this can allow bacteria to enter new niches and substrates to reach pollutants that otherwise would be unreachable.[62,64]

Conclusions

Agricultural soil of Tepeaca region had high concentrations of hydrocarbons at cleanup levels established in different normativities. Because of this, it is appropriate to perform a remediation program to mitigate this contamination. Moreover, this agricultural soil had slight contamination by OCPs, according to reference levels of other countries. However, the detection of prohibited pesticides indicates that possibly they were still being used or that there was slow degradation in the environment.

The general soil physicochemical and biological characteristics indicated that effective bioremediation of pollutants would require stimulation of the native microorganisms, because there were deficiencies of nutrients, e.g. N (0.03–0.07%) and P (0 ppm), for appropriate microbial development. Thus, addition of fertilizers is recommended for an adequate balance of C:N:P = 100:10:1 to achieve microbial biostimulation for suitable bioremediation.

All samples of agricultural soil showed a count of hydrocarbonoclast fungi and bacteria, tolerant to a concentration of 30,000 mg kg^{-1} of diesel. Furthermore, the presence of pesticideclast bacteria and fungi was determined with potential to degrade OCPs at concentrations of 20 mg kg^{-1}; and tolerant bacteria and fungi were also quantified in the presence of both pollutants. Therefore, it is expected that the presence and concentration of both pollutants would not limit the growth of microorganisms and indicates their potential to affect the bioremediation process. An analysis of the hydrocarbonoclast and pesticideclast microorganisms and nutrients could be an indicator of the potential for degradation by native species in contaminated soil, and also an exploratory initial test for bioremediation processes with bio-stimulation.

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