Revista Facultad de Ingeniería

Title: **Gentle remediation options for DDT- and HCH-contaminated soils**

Authors: María Camila Portilla-Saldarriaga, Stiven Camilo Dueñas-García, Inés Hernández-Celi and Martha Jhoana Estévez-Gómez

DOI: **10.17533/udea.redin.20241143**

To appear in: *Revista Facultad de Ingeniería Universidad de Antioquia*

Received: March 30, 2023
Accepted: November 21, 2024
Available Online: November 22, 2024

Gentle remediation options for DDT- and HCH-contaminated soils

Opciones de remediación amigable para suelos contaminados con DDT y HCH

María Camila Portilla-Saldarriaga[1*] https://orcid.org/0000-0001-8641-9459, Stiven Camilo Dueñas-García[1] https://orcid.org/0000-0002-8031-0804, Inés Hernández-Celi[2] https://orcid.org/0000-0001-9517-5329, Martha Jhoana Estévez-Gómez[1] https://orcid.org/0000-0002-5089-5725

[1] Grupo de Investigación en Ingeniería Ambiental y Recursos Naturales (GINAM), Facultad de Ingeniería Ambiental, Universidad Santo Tomás. Carrera 27 # 180-395. C. P. 681003. Floridablanca, Colombia.

[2] Grupo de Investigación en Ciencias Básicas y Aplicadas (GICIBAYA), Departamento de Ciencias Básicas, Universidad Santo Tomás. Carrera 27 # 180-395, C. P. 681003. Floridablanca, Colombia.

Corresponding author: María Camila Portilla-Saldarriaga
E-mail: maria.portilla@ustabuca.edu.co

KEYWORDS
Aerobic conditions; anaerobic conditions; bioaugmentation, biostimulation; persistent organic pollutants (POPs); vermiremediation
Condiciones aerobias; condiciones anaerobias; bioaumentación; bioestimulación; contaminantes orgánicos persistentes (COP); vermirremediación

ABSTRACT: Gentle remediation options (GROs) such as vermiremediation have not been applied in Colombian soils with DDT and HCH yet, while bioaugmentation and biostimulation under anaerobic and/or aerobic conditions have been successfully implemented. However, it is important to determine under which of the latter conditions the assessed consortium performs better. This research evaluated vermiremediation and bioaugmentation under anaerobic and/or aerobic conditions, assisted with biostimulation, in a DDT- and HCH-contaminated soil. Bacteria were isolated from the contaminated soil and used for bioaugmentation. Two GROs were conducted for 60 days: 1) vermiremediation by *Eisenia foetida* without/with biostimulation with compost from organic waste; 2) bioaugmentation of a *Proteobacteria* and *Firmicutes* consortium under anaerobic and/or aerobic conditions and biostimulation with the compost. Finally, the removals determined the efficiency of each treatment. In the first GRO, all the earthworms died by intoxication after 48 h of experimentation. In the second GRO, the highest removals were obtained with the anaerobic treatment: 27% 4,4'-DDT, 52% 4,4'-DDE, 58% 4,4'-DDD, 72% α-HCH, 35% β-HCH, 92% γ-HCH, and 23% δ-HCH. The results indicate that vermiremediation is not feasible for restoring soils with these pollutants at the studied levels. On the contrary, although the synergy between bioaugmentation and biostimulation represents a promising alternative, it is crucial to conduct longer evaluations of the proposed treatments to better understand their effects on the decontamination of soils with DDT and HCH.

RESUMEN: Opciones de remediación amigable (ORA) como vermirremediación no se han aplicado en suelos colombianos con DDT y HCH todavía, mientras que bioaumentación y bioestimulación en

condiciones anaerobias y/o aerobias han sido implementadas exitosamente. Sin embargo, es importante determinar en cuál de las últimas condiciones el consorcio evaluado se desempeña mejor. Este estudio evaluó vermirremediación y bioaumentación en condiciones anaerobias y/o aerobias, asistidas de bioestimulación, en un suelo contaminado con DDT y HCH. Se aislaron bacterias del suelo contaminado y se utilizaron para bioaumentación. Dos ORA se efectuaron durante 60 días: 1) vermirremediación por *Eisenia foetida* sin/con bioestimulación con compost de residuos orgánicos; 2) bioaumentación de un consorcio de *Proteobacteria* y *Firmicutes* en condiciones anaerobias y/o aerobias y bioestimulación con el compost. Finalmente, las remociones determinaron la eficiencia de cada tratamiento. En el primer ORA, todas las lombrices murieron por intoxicación después de 48 h de experimentación. En el segundo ORA, se obtuvieron las mayores remociones con el tratamiento anaerobio: 27% 4,4'-DDT, 52% 4,4'-DDE, 58% 4,4'-DDD, 72% α-HCH, 35% β-HCH, 92% γ-HCH y 23% δ-HCH. Los resultados indican que la vermirremediación no es viable para restaurar suelos con estos contaminantes en los niveles estudiados. En cambio, aunque la sinergia entre bioaumentación y bioestimulación representa una alternativa prometedora, es crucial realizar evaluaciones más prolongadas de los tratamientos propuestos para comprender mejor sus efectos en la descontaminación de suelos con DDT y HCH.

1. Introduction

The term DDT, chemically known as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane, usually refers to a group of isomers: 4,4'-DDT and 2,4'-DDT, and its metabolites 4,4'-DDE, 4,4'-DDD, 2,4'-DDE, and 2,4'-DDD [1]. Similarly, HCH (1,2,3,4,5,6-hexachlorocyclohexane) has eight isomeric forms; α-, β-, γ-, and δ-HCH are the best known [2]. Of these two groups, only 4,4'-DDT and γ-HCH (also known as lindane) have insecticidal properties [1], [3].

These compounds were effectively used in agriculture and hygiene in the past [4], [5]. Nevertheless, due to their wide dispersal away from zones of production and use, high liposolubility (which favors bioaccumulation, bioconcentration, and biomagnification throughout the food chain), great chemical stability (remaining for many years in the environment), and toxicity in humans and other animals, some of them (4,4'-DDT, α-, β-, and γ-HCH) have been listed in the Stockholm Convention: a global treaty to protect human health and the environment from persistent organic pollutants (POPs) [6], [7].

Although DDT and HCH were banned decades ago in many countries, they are still detected in different places due to their persistent nature [8], [9]. For example, in Colombia, DDT and lindane were banned in 1993 and 1997, respectively [10]. However, 1983.5 m^3 of soil contaminated with pesticides POPs were estimated in Agustín Codazzi (Cesar) in 2006 [11]. Therefore, considering the adverse effects of DDT and HCH in different environmental compartments [12]–[15], the remediation of these soils is essential. Gentle remediation options (GROs) such as bioaugmentation, vermiremediation, and biostimulation have become popular in soil decontamination because they are environmentally friendly, cost-effective, and relatively efficient technologies [16], [17].

Bioaugmentation has been widely applied for restoring soils with DDT and HCH [8], [18]–[20]. This methodology has been implemented with individual strains or groups of bacteria (consortia). Consortia represent a better alternative because a heterogeneous bacterial group provides more catabolic pathways for the mineralization of pollutants. The use of autochthonous bacteria is usually more successful as the evolution of the enzymes involved in the catabolism of a compound makes the microorganisms more

efficient in its degradation [21]. Recently, the phyla *Proteobacteria* and *Firmicutes* have been reported as suitable candidates for the remediation of agricultural soils with a long record of pesticide usage [22]. Furthermore, the presence or absence of oxygen (O_2) is important in DDT and HCH biodegradation. The choice of any of these conditions is mainly influenced by the involved microorganisms and the number of chlorine (Cl) atoms in the molecules [21], [23].

Vermiremediation consists of the utilization of daily processes carried out by earthworms (burrowing, feeding, metabolism, secretion, and excretion) or the interrelation between these organisms and abiotic/biotic components to extract-accumulate and degrade pollutants [24]. *Eisenia foetida* is a great option for vermiremediation because it is easily cultured in the laboratory, grows rapidly, tolerates higher concentrations of pollutants than other earthworms, and its reproductive rate is high [25]. Likewise, the capacity of *E. foetida* for the remediation of soils with DDT and HCH has been evidenced [26]–[28]. However, vermiremediation is still an emerging technology in the treatment of soils with these compounds; in fact, it has not been applied in Colombia. The few studies realized in the country about the use of earthworms have been oriented to their application in soils degraded with mercury from mining [29], [30].

Although bioaugmentation and vermiremediation have been successfully implemented in soils with organochlorine pesticides, the synergy between each one of these techniques and biostimulation has reached better results [31], [32]. The incorporation of organic amendments is important during these processes as the DDT and HCH biodegradation is mainly cometabolic; in other words, it occurs fortuitously because organisms do not use the pollutant but a cosubstrate as carbon and energy source [23], [33]. Compost is normally used as an organic soil amendment. This material is a good choice as a biostimulant due to its nutritional richness, low or no economic cost, improvement of soil physical properties (e.g., porosity), and integration of microorganisms with the potential for restoring DDT- and HCH-contaminated soils [34], [35]. Additionally, the incorporation of organic materials in contaminated soils is a way of giving a second chance to waste that is usually disposed of in dumpsites, which is related to the circular economy.

Considering all the above, the aim of this research was to evaluate the efficiency of vermiremediation by *E. foetida* and bioaugmentation of a *Proteobacteria* and *Firmicutes* consortium under anaerobic and/or aerobic conditions, both processes assisted by biostimulation with compost from organic waste, in a soil long-term contaminated with DDT and HCH. Furthermore, taking into account that a large bacterial diversity has been identified in soils contaminated with these compounds [34], [36], and that their biodegradation may occur under anaerobic and/or aerobic conditions [21], [23]; it is important to determine under which condition the evaluated consortium performs better in the bioremediation of DDT- and HCH-contaminated soils.

2. Methodology

2.1. Collection and preparation of the soil and compost

The soil was collected from Agustín Codazzi, a municipality with a previous history of organochlorine pesticide contamination [11]. The soil was taken from three different points (10°1'15.452"N, 73°14'23.856"W; 10°1'12.569"N, 73°14'11.429"W; 10°1'11.960"N, 73°14'10.892"W) as shown in

Figure 1, and then the three soil samples were homogenized to form a composite sample. Additionally, organic waste compost was acquired from *Planta de Valorización de Residuos Orgánicos Biodegradables* of *Universidad Santo Tomás*, Floridablanca Campus (Santander, Colombia). After that, the soil and compost were ground and sieved through a 2 mm sieve. It is important to emphasize that the soil and compost were not dried at room temperature because they were perceived as dry.

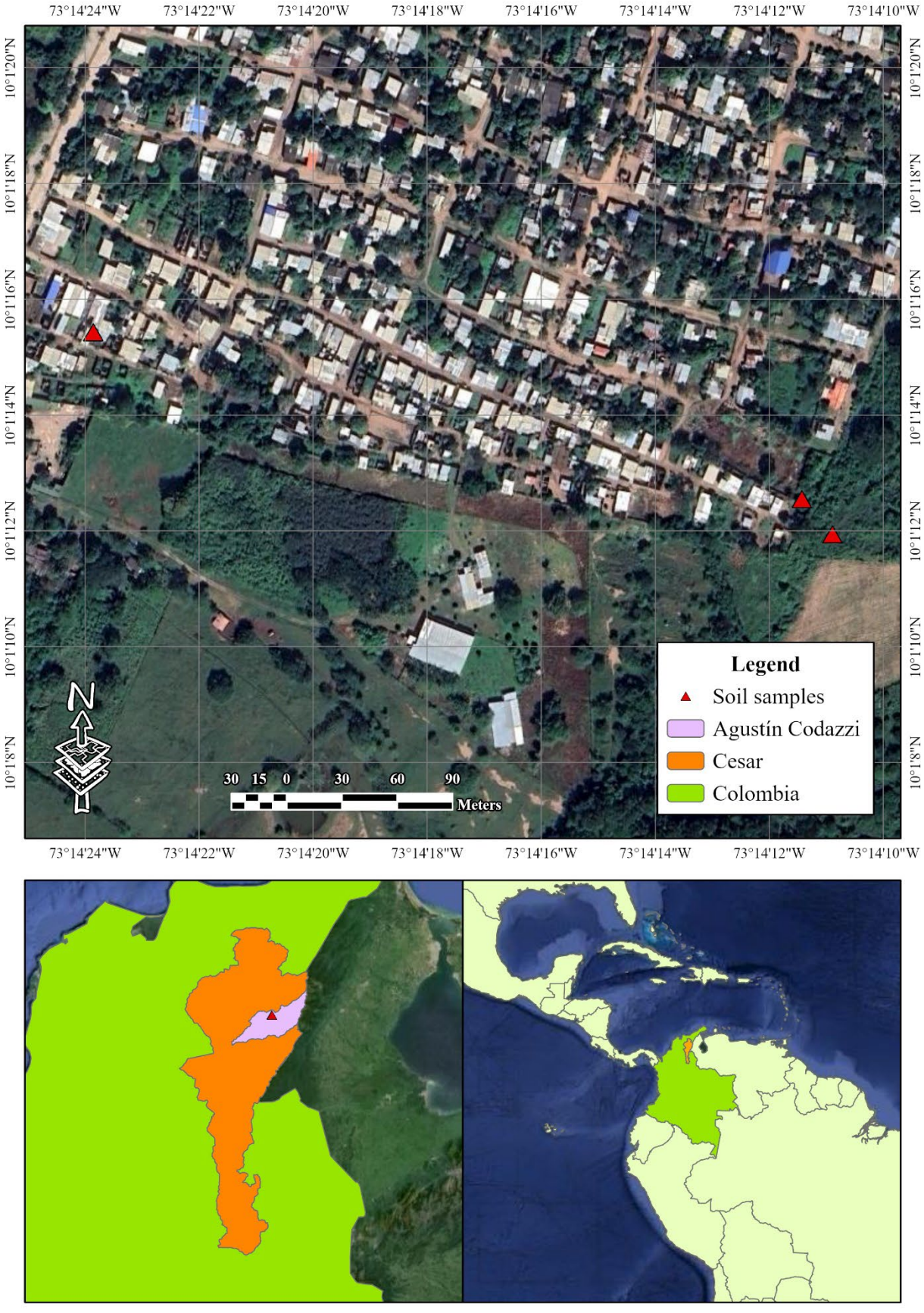
73°14'24"W
73°14'22"W
73°14'20"W
73°14'18"W
73°14'16"W
73°14'14"W
73°14'12"W
73°14'10"W
10°2'1"N
10°1'8"N
10°1'9"N
10°1'4"N
10°1'2"N
10°1'1"N
10°1'8"N
N
30 15 0 30 60 90
Meters
Legend
Soil samples
Agustín Codazzi
Cesar
Colombia

Figure 1 Location of the contaminated soils evaluated in this research

2.2. Physical, chemical, and microbiological characterization of the soil and compost

Physical, chemical, and microbiological properties of the soil and compost were measured using standard methods proposed by *Instituto Colombiano de Normas Técnicas y Certificación (ICONTEC)*, Canadian Society of Soil Science (CSSS), *Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT)* of Mexico, and employing procedures recommended in other sources.

The gravimetric method was applied to determine the water content [37]. The soil water content was measured after 72 h to determine the field capacity [38]. Throughout the experimentation, the water content was maintained as much as possible at 60 and 77% of the soil field capacity in the aerobic and anaerobic treatments, respectively [9], [20], [27], [28], [39]. The hydrometer method was used to determine the texture [40].

The electrical conductivity was measured in the filtrate of a 1:5 soil or compost/water suspension [41]. The pH was measured in 1:1 soil/water and 1:2 compost/water suspensions [42]. The oxidation with potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4) was used to determine the total organic carbon; the organic matter was calculated by the product of the total organic carbon value and the van Bemmelen factor (1.724); the total nitrogen was measured by the Kjeldahl method; and the colorimetric method with an extractant solution of hydrochloric acid (HCl) and ammonium fluoride (NH_4F) was applied to determine the total phosphorous [43].

2.2.1 Bacterial enrichment and isolation

Initially, 100 g of the contaminated soil and 5 mL of nystatin were added to 500 mL of an enrichment medium with 2.7 $(NH_4)_2HPO_4$, 4.3 Na_2HPO_4, 4.2 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$, and 0.06 $CaCl_2 \cdot 2H_2O$ g/L of distilled water. After that, the suspension was maintained at 110 rpm, room temperature, and aerobic conditions for 6 days. On day 6, inocula were taken, each one was grown by the streaking method on Petri dishes with nutrient and MacConkey agars (Oxoid™), and the dishes were incubated at room temperature and aerobiosis for 48 h. Finally, the bacteria were isolated based on their macroscopic characteristics. Each bacterial isolate was grown by the same method on Petri dishes with nutrient agar (Oxoid™) and incubated at the same conditions [19].

2.2.2 Bacterial identification

The bacteria were identified by phenotypic methods [44]. Gram stain (+), catalase activity (+), starch hydrolysis test (+) [45], and Schaeffer-Fulton or spore stain (+) [46] were carried out to identify the bacteria of the *Bacillus* genus, according to the Bergey's manual of determinative bacteriology [47]. On the other hand, Gram-negative and oxidase-negative bacteria were identified with the RapID™ ONE System (Remel™).

2.2.3 Quantification of bacterial populations

The spread plate method was applied to estimate the number of viable cells. Before the implementation of this procedure, serial dilutions up to 10^{-6} were carried out using sterile saline solution at 0.85%. Each dilution was grown on a Petri dish with nutrient agar (Oxoid™) and incubated for 24 h at room temperature and aerobic conditions. Lastly, the dish with a number of colonies between 30 and 300 was selected for counting [45], [46].

2.3. Organochlorine pesticide extraction and analysis

The matrix solid-phase dispersion (MSPD) was applied to extract organochlorine pesticides. Separation, detection, and quantification of the isolated substances were performed by gas chromatography-mass spectrometry, operated in selected ions monitoring mode (GC-MS/SIM). The certified mixture of organochlorine pesticides Part No. M-8081-SC (AccuStandard, Inc., 125 Market Street, New Haven, CT 06513) was used as a reference standard. Chromatographic analysis was carried out in an AT 6890 Series Plus gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), coupled to a mass selective detector (AT, MSD 5973). The column used in the analysis was DB-5MS of 5%-Ph-PDMS, 60 m × 0.25 mm × 0.25 μm. The injection was performed in splitless mode (V_{iny} = 1 μL).

2.4. Experimental design

2.4.1 First GRO, vermiremediation without/with biostimulation

The microcosms were carried out in plastic flowerpots. Three treatments were conducted: natural attenuation (NA, control), vermiremediation (V), and vermiremediation + biostimulation (V + B). There were three replicates per treatment.

NA and V contained 2.322 kg of organochlorine pesticides-contaminated soil, while V + B was filled with 1.786 kg of contaminated soil and 0.536 kg of compost thoroughly mixed (77:23 ratio) [48]. Furthermore, individuals of *E. foetida* were purchased from Abonos y Lombrices (Granja El Cortijo Lynn, Rionegro, Santander, Colombia). A total of 16 healthy and sexually mature earthworms per kg were added [31], resulting in the incorporation of 37 individuals in the treatments V and V + B.

Each flowerpot was covered at the top with a disposable polypropylene bouffant cap to allow oxygen transfer and prevent external interferences. The temperature of each treatment was verified to be in the range of 14-27 °C [49]. Manual turning was applied to maintain the temperature in the required interval and to aerate the medium [28].

After 60 days, a well-mixed composite sample of soil from each treatment (same amount of replicates one, two, and three) was used to quantify the organochlorine pesticides. The efficiency of each treatment was determined from the obtained removals, calculated using **Equation 1**.

$$\text{Removal} = \frac{\text{Initial concentration - Final concentration}}{\text{Initial concentration}} * 100 \quad \textbf{(1)}$$

2.4.2 Second GRO, bioaugmentation under anaerobic and/or aerobic conditions and biostimulation

The microcosms were carried out in plastic flowerpots. Four treatments were conducted: natural attenuation (NA, control), bioaugmentation + biostimulation under aerobic conditions (B + B_{Ae}), bioaugmentation + biostimulation under anaerobic and subsequent aerobic conditions (B + $B_{An\text{-}Ae}$), and bioaugmentation + biostimulation under anaerobic conditions (B + B_{An}). There were three replicates per treatment.

NA contained 2.322 kg of organochlorine pesticides-contaminated soil; while B + B_{Ae}, B + B_{An-Ae}, and B + B_{An} were filled with 1.786 kg of contaminated soil and 0.536 kg of compost thoroughly mixed (77:23 ratio) [48]. Additionally, for the bioaugmentation, all the bacterial strains from the contaminated soil were grown in a nutrient broth (Millipore®) until a total rate of 10^8 colony-forming units (CFU) per mL, and it was maintained at 110 rpm, room temperature, and aerobic conditions for 72 h. After that, 100 mL of this bacterial suspension was added on days 1 and 30 to the treatments B + B_{Ae}, B + B_{An-Ae}, and B + B_{An} [50].

The aerobic treatments were covered at the top with a disposable polypropylene bouffant cap, and the anaerobic treatments were completely wrapped with polyethylene and placed inside an airtight chamber along with a sachet of AnaeroGen™ 2.5 L (Oxoid™) to promote anaerobic conditions. Anaerobiosis and aerobiosis in the treatment B + B_{An-Ae} lasted 30 days each one, with the total experimental time divided equally between the two conditions (50:50) [51].

After 60 days, a well-mixed composite sample of soil from each treatment (same amount of replicates one, two, and three) was used to quantify the organochlorine pesticides. The efficiency of each treatment was determined from the obtained removals, calculated using **Equation 1**.

3. Results and discussion

3.1. Initial physical, chemical, and microbiological characterization of the soil and compost

The initial physical, chemical, and microbiological properties of the soil and compost are shown in **Table 1**. The soil from Agustín Codazzi was slightly moist [52]. Therefore, it was necessary to add water for the growth and stability of the organisms: bacteria and earthworms [49], [53]. It has been established that the adequate water content in the remediation by aerobic bacteria is between 50 and 70% of the field capacity. Furthermore, higher moisture levels cause the saturation of soil pores, which favors anoxic conditions, thus generating a jump from aerobic to anaerobic metabolism [39]. For this reason, the water content in the anaerobic treatments was maintained at 77% of the soil field capacity, to generate a low oxygen diffusion through the soil. On the other hand, the texture was classified as silt loam [52]. In that regard, it is a soil suitable for bioremediation and moderately appropriate for microbial colonization [38], [39].

The soil was categorized as non-saline and the compost as very slightly saline [52]. These electrical conductivities do not represent difficulties for the organisms, enzymes, and soil physical properties such as infiltration, permeability, and hydraulic conductivity [39], [54], [55]. Additionally, the soil was cataloged as slightly acidic and the compost as slightly alkaline [52]. These pHs are suitable for most microorganisms, enzymes, and *E. foetida* [39], [45], [49], [53].

At the beginning, the soil had low organic matter, medium total nitrogen, and low total phosphorus. After mixing the soil and compost, these parameters were medium, very high, and low, respectively [43], which significantly increased the nutritional content and probably promoted the cellular metabolism of the organisms [45], [46].

Table 1 Initial physical, chemical, and microbiological properties of the soil and compost

Property	Arithmetic mean ± standard deviation	
	Soil	**Compost**
Water content [%]	9.7 ± 0.4	
Field capacity [%]	25.8 ± 0.4	
Texture [%]	29.4 ± 1.0 sand 14.9 ± 0.2 clay 55.7 ± 1.1 silt	
Electrical conductivity [dS/m]	0.53 ± 0.02	2.18 ± 0.02
pH	6.47 ± 0.03	7.79 ± 0.04
Total organic carbon [%]	0.63	5.17
Organic matter [%]	1.09	8.87
Total nitrogen [%]	0.12	0.85
Total phosphorus [mg/kg]	0.17	0.23
Bacteria	$6.8 \times 10^4 \pm 7.8$ CFU/g	
	Kluyvera intermedia[*] *Klebsiella pneumoniae*[**] *Serratia plymuthica*[*] *Yersinia enterocolitica*[*] 2 *Bacillus* spp.	
Organochlorine pesticides [mg/kg]	1119 4,4'-DDT 105 4,4'-DDE 774 4,4'-DDD 344 α-HCH 86 β-HCH 76 γ-HCH 35 δ-HCH	

[*]99.9 and [**]96.09% probabilities of coincidence.

In the case of the microorganisms, the bacterial populations were low [39]. Furthermore, the bacterial isolates from the soil have demonstrated their potential to reduce the concentrations of organochlorine

pesticides, organophosphate pesticides, and heavy metals in other studies [34], [36], [56]–[60], as shown in **Table 2**.

Table 2 Bacterial isolates and their processes to reduce organochlorine pesticides and other pollutants

Bacterium	Process and pollutant	Source
K. pneumoniae	DDT degradation under anaerobic conditions	[58]
Bacillus thuringiensis	DDT degradation through anaerobic reductive dechlorination	[36]
Bacillus megaterium	DDT degradation through anaerobic reductive dechlorination	[60]
Bacillus vietnamensis	Degradation of DDT and isomers of HCH	[34]
K. intermedia	Association with sorghum to boost the phytoextraction of cadmium (Cd) and the phytostabilization of lead (Pb) and arsenic (As)	[57]
S. plymuthica	Phytostabilization of the pesticides Prosper, Cuivox, Fungastop, Nimbecidin, and Maneb	[56]
Y. enterocolitica	Degradation of the organophosphate pesticide tetrachlorvinphos	[59]

Finally, two groups of organochlorine pesticides were identified: 1) 4,4'-DDT and its principal metabolites 4,4'-DDE and 4,4'-DDD, and 2) the isomers α-, β-, γ-, and δ-HCH. The compounds 4,4'-DDT, α-, β-, and γ-HCH are listed in the Stockholm Convention [6]. It is important to note that, after extensive search, it was identified that the concentrations quantified in the present research are considerably high —possibly due to burial or dumping of these pesticides in the soil— and such amounts had not been detected in other Colombian soils. Additionally, it is relevant to point out that, to date, no other study has reported the simultaneous presence of DDT and HCH in the soils of the country. On the

other hand, according to the Cabinet Regulation No. 804 of 2005 of Latvia [61], the concentrations of the identified pollutants directly threaten human health and the environment. In this case, the situation is even more dangerous because there are human settlements very close to the sites where the soil samples were taken (as can be seen in **Figure 1**).

3.2. Bioremediation experiments

3.2.1 First GRO, vermiremediation without/with biostimulation

All the specimens of *E. foetida* died after 48 h of experimentation. The earthworms exhibited coiling, curling, extrusion of celomic fluid, segmental swelling, severe constriction, and slimming, as shown in **Figure 2**. These symptoms indicated intoxication and were also evidenced in other research, in which *E. foetida* was exposed to different concentrations of the pesticides trichlorfon, dimethoate, carbendazim, tebuconazole, and prochloraz [62], and cypermethrin, fenvalerate, carbaryl, and carbofuran [63].

1) The concentration of total DDT (DDT plus its metabolites) did not exceed 72.35 mg/kg in soils from the same study area of this research [19], [60]. Furthermore, 2) *E. foetida* survived in soil with 50 mg/kg of DDT for 42 days. Also, all the earthworms survived until day 28 when they were exposed to soil with 100 mg/kg of the same pesticide, from day 28 to 42 about 10% of the population died [64]. Considering these two premises, vermiremediation was a viable alternative in the current research. Nevertheless, the initial quantification of DDT and HCH and vermiremediation were carried out simultaneously, which did not allow foreseeing that the concentrations of these compounds were elevated and would cause the death of the earthworms.

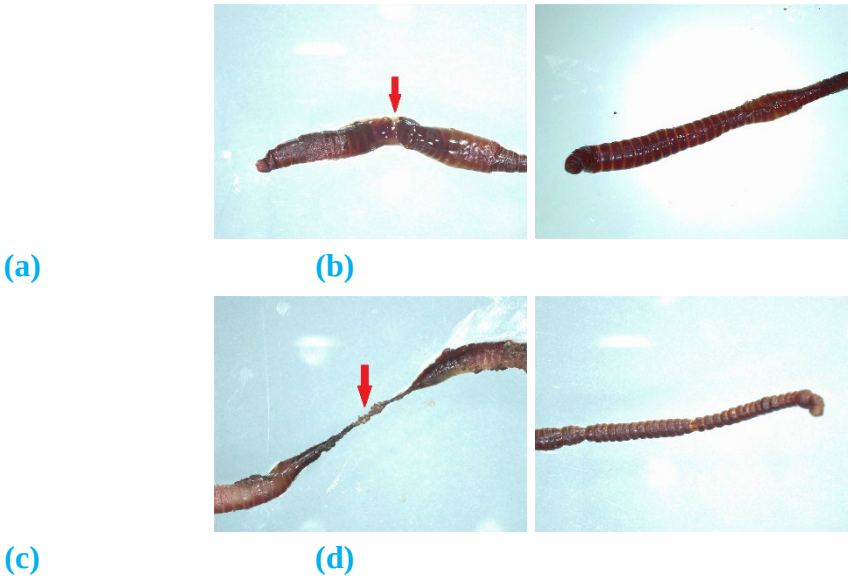

Figure 2 Symptoms in *E. foetida* after 48 h of exposure to the soil contaminated with DDT and HCH: **(a)** Extrusion of celomic fluid, **(b)** segmental swelling, **(c)** severe constriction, and **(d)** slimming

3.2.2 Second GRO, bioaugmentation under anaerobic and/or aerobic conditions and biostimulation

The content of 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in each treatment after bioremediation is shown in **Figure 3**. The concentration of 4,4'-DDT increased in NA, so the removal was negative, equivalent to -6%. Additionally, 2 and 32% were the removals of 4,4'-DDE and 4,4'-DDD in the same treatment, respectively. The introduction of energy (e.g., aeration) may accelerate sorption and desorption [65]. In this sense, after aerating the medium by manual turning for 60 days, the organochlorine pesticides were probably desorbed. Consequently, new molecules of the pollutants may have appeared, and thus, the final concentration was higher than the initial concentration.

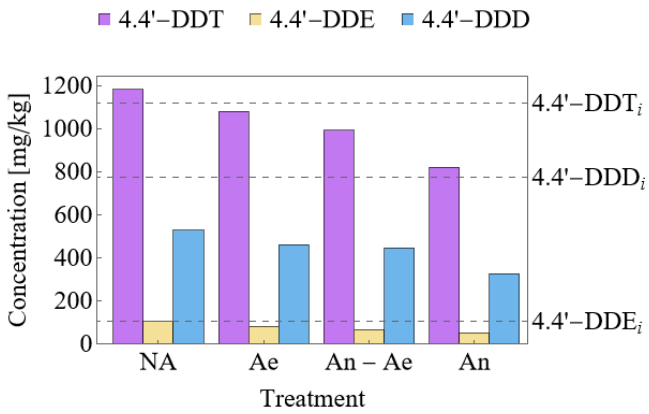

Figure 3 Content of 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in each treatment after bioremediation. The dashed lines represent the initial concentrations of the pollutants

The decreases of the metabolites in NA may be attributed to mechanisms that occur naturally in soil, such as volatilization, adsorption, chemical transformation, and biodegradation. However, natural attenuation is a passive process that requires more time than active processes to reach the same remaining concentrations [66]. For this reason, the treatments B + B achieved higher removals compared with NA. Aerobic, anaerobic-aerobic, and anaerobic conditions are the three pathways that have been used for the bioremediation of DDT- and HCH-contaminated soils [18], [19], [21], [60]. The implementation of any of these pathways is mainly influenced by two aspects: the involved microorganisms and the number of Cl atoms in the molecules [21], [23].

In a study, it was found that in four soils with a long history of pesticide use, the relative abundance of bacteria was 30-36% *Proteobacteria*, 15-20% *Actinobacteria*, 13-14% *Firmicutes*, and 7-13% *Bacteroidetes*. Likewise, some core bacterial genera and some of their genes involved in pesticide degradation were identified [22]. The bacteria isolated and identified in the present research belong to the phyla *Proteobacteria* and *Firmicutes* [45]. Additionally, these bacteria are facultative anaerobic according to the literature (those identified up to species level, i.e., *K. intermedia*, *K. pneumoniae*, *S. plymuthica*, and *Y. enterocolitica*) [58], [67]–[69]. Therefore, their growth and stability are feasible in anaerobiosis and aerobiosis [46]. Nevertheless, it is not clear under which of these two conditions the

bacteria are more efficient in the degradation of DDT and HCH yet, except for *K. pneumoniae* subsp., which has demonstrated the ability to initiate the anaerobic pathway for the degradation of 4,4'-DDT [70].

In the case of the second aspect, it has been proposed as a generality that low-chlorinated molecules (one to three Cl atoms) are more susceptible to aerobic biodegradation; whereas if the amount of Cl is higher, anaerobic conditions are more convenient [71], [72]. 4,4'-DDT has five Cl atoms, in consequence, its first steps of biodegradation occur under anaerobic conditions. However, it has been shown that one of the rings of this pesticide is cleaved in subsequent aerobiosis [23]. In this sense, higher removals were expected in B + B $_{An-Ae}$ because it is a more complete treatment; nevertheless, the bacteria achieved the highest reductions under anaerobic conditions after 60 days of experimentation. This result may have been due to the short duration and, therefore, the incomplete anaerobiosis in B + B $_{An-Ae}$, which meant that during the aerobic conditions the compound was still highly chlorinated, and the removals were not significant. The reductions of 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in B + B $_{An}$, B + B $_{An-Ae}$, and B + B $_{Ae}$ were equivalent to 27, 52, and 58%; 11, 37, and 43%; and 4, 24, and 41%; respectively.

On the other hand, the content of α-, β-, γ-, and δ-HCH in each treatment after bioremediation is shown in **Figure 4**. In general, high removals of γ- and α-HCH were obtained in comparison with β- and δ-HCH. The chemical stability and the biodegradation of the HCH isomers are influenced by the orientation of the Cl atoms in the molecules. The axial (a) and equatorial (e) positions of the Cl in α-, β-, γ-, and δ-HCH are aaaaee, eeeeee, aaaeee, and aeeeee, respectively. It is considered that axial Cl atoms facilitate spaces for enzymatic degradation. For this reason, α- and γ-HCH are more easily biodegraded than β- and δ-HCH [33].

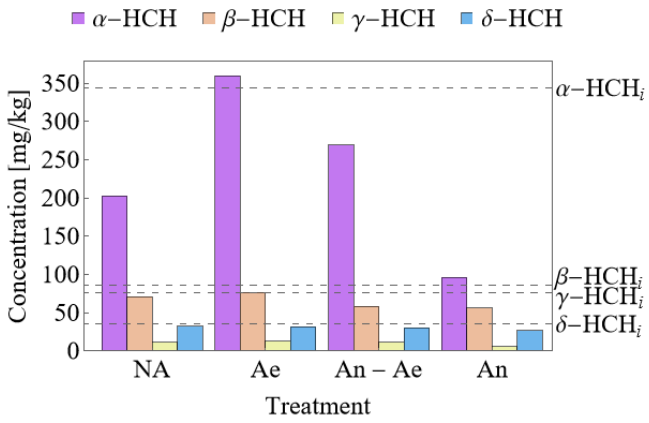

Figure 4 Content of α-, β-, γ-, and δ-HCH in each treatment after bioremediation. The dashed lines represent the initial concentrations of the pollutants

The reductions of α-, β-, γ-, and δ-HCH in NA were 41, 18, 85, and 7%, respectively. The significant decreases of γ- and α-HCH in this treatment may be attributed, besides their easy biodegradation due to the Cl atoms orientation in the molecules [33], to their use as sole carbon and energy sources under aerobic conditions by the bacteria, as evidenced by [73] with γ-HCH and the halophilic bacterium *Chromohalobacter* sp. LD2, isolated from an HCH dumpsite.

In B + B_{An}, B + B_{An-Ae}, and B + B_{Ae} the removals of α-, β-, γ-, and δ-HCH were equivalent to 72, 35, 92, and 23%; 22, 32, 85, and 13%; and -5, 11, 84, and 10%; respectively. In other words, the reductions in these treatments occurred in the order B + B_{An} > B + B_{An-Ae} > B + B_{Ae}. As with 4,4'-DDT in NA, the increase of α-HCH concentration in B + B_{Ae} may be attributed to the desorption of the pollutant [65].

The mineralization of HCH isomers is usually reached under aerobic conditions [33]. For example, *Sphingomonas paucimobilis* UT26 has shown the ability to completely degrade γ-HCH in the presence of O_2 as it possesses *lin* genes, which encode enzymes involved in the degradation of this compound. However, not all aerobic bacteria are successful in the mineralization of HCH [21].

In contrast, although anaerobic-aerobic treatments have been considered more efficient in the degradation of HCH isomers —because, having six Cl atoms, they are initially degraded under anaerobic conditions, and then their less chlorinated products are more easily degraded under aerobic conditions [21]—, in the current research, the bacteria removed the highest amounts of α-, β-, γ-, and δ-HCH in anaerobiosis. Similar to the DDT section, this result may have been strongly influenced by the duration of the studied conditions: anaerobiosis and aerobiosis.

4. Conclusions

Vermiremediation is not a feasible technology for the decontamination of soils with DDT and HCH at levels such as those determined in the present research. In the end, this GRO acted as an ecotoxicity assay, evidencing the negative impacts of these pollutants on organisms such as earthworms.

On the contrary, the synergy between bioaugmentation of the *Proteobacteria* and *Firmicutes* consortium formed by *K. intermedia*, *K. pneumoniae*, *S. plymuthica*, *Y. enterocolitica*, and two *Bacillus* spp. and biostimulation with compost from organic waste gave positive results. In general, higher removals were achieved in the simultaneously bioaugmented and biostimulated treatments compared to the natural attenuation (control). Likewise, after 60 days, it was determined that this consortium performed better in the degradation of DDT and HCH in anaerobiosis, which was the most efficient treatment. Nevertheless, it is important to evaluate the proposed treatments over a more extended period to better understand their effects on the decontamination of soils with these pollutants.

Declaration of competing interest

The authors declare that they have no significant competing interests including financial or non-financial, professional, or personal interests interfering with the full and objective presentation of the work described in this manuscript.

Acknowledgments

The authors thank *Universidad Santo Tomás* of Bucaramanga for funding this project and Dr. Modesto Eusebio Portilla Gamboa for his help in the final review of this paper.

Funding

This work was supported by Universidad Santo Tomás of Bucaramanga under the Project titled *Recuperación de suelos agrícolas degradados por plaguicidas organoclorados utilizando técnicas biológicas, a nivel de laboratorio* (No. 599, dated May 5, 2021).

Author contributions

María Camila Portilla-Saldarriaga: Conceptualization, methodology, formal analysis, investigation, writing - original draft, writing. Stiven Camilo Dueñas-García: Methodology, formal analysis, investigation, and writing - original draft. Inés Hernández-Celi: Conceptualization, methodology, investigation, resources, and writing. Martha Jhoana Estévez-Gómez: Conceptualization, methodology, investigation, resources.

Data availability statement

The authors confirm that the data supporting the findings of this research are available within the article.

References

[1] S. Miyagawa, T. Sato, and T. Iguchi, "Dichlorodiphenyltrichloroethane," in *Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research*, Y. Takei, H. Ando, and K. Tsutsui, Eds., Oxford, U.K.: Academic Press, 2016, pp. 579–580. [Online]. Available: https://doi.org/10.1016/B978-0-12-801028-0.00242-7
[2] G. Chen, "Hexachlorocyclohexanes including lindane," in *Encyclopedia of Toxicology*, vol. 2, 3rd ed. P. Wexler, Ed., London, U.K.: Academic Press, 2014, pp. 874–876. [Online]. Available: https://doi.org/10.1016/B978-0-12-386454-3.00152-4
[3] E. J. Mrema, C. Colosio, and F. M. Rubino, "Pesticide residues: Organochlorines," in *Encyclopedia of Food Safety*, vol. 3, Y. Motarjemi, Ed., San Diego, CA, USA: Academic Press, 2014, pp. 23–30. [Online]. Available: https://doi.org/10.1016/B978-0-12-378612-8.00238-9
[4] M. del P. Cabildo Miranda, M. del P. Cornago Ramírez, C. Escolástico León, S. Esteban Santos, C. López García, and D. Sanz del Castillo, *Bases Químicas del Medio Ambiente*. Madrid, Spain: Univ. Nac. Educ. Distancia, 2013.
[5] N. Garg, P. Lata, S. Jit, N. Sangwan, A. K. Singh, V. Dwivedi, et al., "Laboratory and field scale bioremediation of hexachlorocyclohexane (HCH) contaminated soils by means of bioaugmentation and biostimulation," *Biodegradation*, vol. 27, no. 2–3, pp. 179–193, Jun. 2016. [Online]. Available: https://doi.org/10.1007/s10532-016-9765-6
[6] Secretariat of the Stockholm Convention, *Stockholm Convention on Persistent Organic Pollutants (POPs)*. Geneva, Switzerland: United Nations, 2020. [Online]. Available: http://chm.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx
[7] D. W. Sparling, *Ecotoxicology Essentials: Environmental Contaminants and Their Biological Effects on Animals and Plants*. London, U.K.: Academic Press, 2016.
[8] X. Liu, H. Huang, D. Huang, K. Zhong, Q. Rong, X. Qin, et al., "Enhanced biodegradation of hexachlorocyclohexane in soil by application of exogenous amendments," *Water Air Soil Pollut.*, vol. 232, no. 8, Aug. 2021, Art. no. 325. [Online]. Available: https://doi.org/10.1007/s11270-021-05273-2
[9] I. A. Mirsal, *Soil Pollution: Origin, Monitoring & Remediation*, 2nd ed. Berlin, Germany: Springer-Verlag, 2008. [Online]. Available: https://doi.org/10.1007/978-3-540-70777-6
[10] Ministerio de Ambiente y Desarrollo Sostenible, "Plaguicidas COP: Aspectos básicos," Bogotá, D.C., Colombia, 2017. [Online]. Available: https://quimicos.minambiente.gov.co/wp-content/uploads/2021/05/Cartilla_Plaguicidas_COP_2017.pdf
[11] Ministerio de Ambiente, Vivienda y Desarrollo Territorial and Investigación y Desarrollo Agrícola Experimental - PERPAR, "Inventario nacional de existencias de plaguicidas COP, Colombia, 2006,"

Bogotá, D.C., Colombia, 2007. [Online]. Available: https://quimicos.minambiente.gov.co/wp-content/uploads/2021/05/Inventario_PlaguicidasCOP_2007.pdf
[12] L. Joseph, S. V. Paulose, N. Cyril, S. K. Santhosh, A. Varghese, A. B. Nelson, et al., "Organochlorine pesticides in the soils of Cardamom Hill Reserve (CHR), Kerala, India: Geo spatial distribution, ecological and human health risk assessment," *Environ. Chemistry Ecotoxicol.*, vol. 2, pp. 1–11, Jan. 2020. [Online]. Available: https://doi.org/10.1016/j.enceco.2020.01.001
[13] Y. Kang, R. Zhang, K. Yu, M. Han, Y. Wang, X. Huang, et al., "First report of organochlorine pesticides (OCPs) in coral tissues and the surrounding air-seawater system from the South China Sea: Distribution, source, and environmental fate," *Chemosphere*, vol. 286, no. 2, Jan. 2022, Art. no. 131711. [Online]. Available: https://doi.org/10.1016/j.chemosphere.2021.131711
[14] J. A. Racero-Casarrubia, J. Ballesteros Correa, J. Marrugo-Negrete, and J. J. Pinedo-Hernández, "Plaguicidas organoclorados en murciélagos (*Chiroptera*) asociados al bosque húmedo tropical en Córdoba, Colombia," *Caldasia*, vol. 43, no. 2, pp. 320–330, Aug. 2021. [Online]. Available: https://doi.org/10.15446/caldasia.v43n2.84862
[15] S. Tyagi, M. Siddarth, B. K. Mishra, B. D. Banerjee, A. J. Urfi, and S. V. Madhu, "High levels of organochlorine pesticides in drinking water as a risk factor for type 2 diabetes: A study in North India," *Environ. Pollut.*, vol. 271, Feb. 2021, Art. no. 116287. [Online]. Available: https://doi.org/10.1016/j.envpol.2020.116287
[16] A. K. Patel, R. R. Singhania, F. P. J. B. Albarico, A. Pandey, C.-W. Chen, and C.-D. Dong, "Organic wastes bioremediation and its changing prospects," *Sci. Total Environ.*, vol. 824, Jun. 2022, Art. no. 153889. [Online]. Available: https://doi.org/10.1016/j.scitotenv.2022.153889
[17] S. I. Singh, S. Singh, Bhawana, and A. P. Vig, "Earthworm-assisted bioremediation of agrochemicals," in *Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers*, M. N. V. Prasad, Ed., Oxford, U.K.: Butterworth-Heinemann, 2020, pp. 307–327. [Online]. Available: https://doi.org/10.1016/B978-0-08-103017-2.00013-1
[18] C. Abbes, A. Mansouri, N. Werfelli, and A. Landoulsi, "Aerobic biodegradation of DDT by *Advenella kashmirensis* and its potential use in soil bioremediation," *Soil Sediment Contam.: Int. J.*, vol. 27, no. 6, pp. 455–468, Aug. 2018. [Online]. Available: https://doi.org/10.1080/15320383.2018.1485629
[19] M. Kopytko, S. N. Correa-Torres, and M. J. Estévez-Gómez, "Biodegradación estimulada de los suelos contaminados con pesticidas organoclorados," *Rev. Invest. Agrar. Ambient.*, vol. 8, no. 1, pp. 119–130, Jun. 2017. [Online]. Available: https://doi.org/10.22490/21456453.1843
[20] E. E. Raimondo, J. D. Aparicio, A. L. Bigliardo, M. S. Fuentes, and C. S. Benimeli, "Enhanced bioremediation of lindane-contaminated soils through microbial bioaugmentation assisted by biostimulation with sugarcane filter cake," *Ecotoxicol. Environ. Saf.*, vol. 190, Mar. 2020, Art. no. 110143. [Online]. Available: https://doi.org/10.1016/j.ecoenv.2019.110143
[21] V. Srivastava, T. Srivastava, and M. Suresh Kumar, "Fate of the persistent organic pollutant (POP) hexachlorocyclohexane (HCH) and remediation challenges," *Int. Biodeterior. Biodegradation*, vol. 140, pp. 43–56, May 2019. [Online]. Available: https://doi.org/10.1016/j.ibiod.2019.03.004
[22] M. A. Malla, A. Dubey, A. Kumar, and S. Yadav, "Metagenomic analysis displays the potential predictive biodegradation pathways of the persistent pesticides in agricultural soil with a long record of pesticide usage," *Microbiol. Res.*, vol. 261, Aug. 2022, Art. no. 127081. [Online]. Available: https://doi.org/10.1016/j.micres.2022.127081
[23] J. Foght, T. April, K. Biggar, and J. Aislabie, "Bioremediation of DDT-contaminated soils: A review," *Bioremediation J.*, vol. 5, no. 3, pp. 225–246, Jul. 2001. [Online]. Available: https://doi.org/10.1080/20018891079302

[24] Z. Shi, J. Liu, Z. Tang, Y. Zhao, and C. Wang, "Vermiremediation of organically contaminated soils: Concepts, current status, and future perspectives," *Appl. Soil Ecol.*, vol. 147, Mar. 2020, Art. no. 103377. [Online]. Available: https://doi.org/10.1016/j.apsoil.2019.103377
[25] Z. Usmani, R. Rani, P. Gupta, and M. N. V. Prasad, "Vermiremediation of agrochemicals," in *Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers*, M. N. V. Prasad, Ed., Oxford, U.K.: Butterworth-Heinemann, 2020, pp. 329–367. [Online]. Available: https://doi.org/10.1016/B978-0-08-103017-2.00015-5
[26] J. D. Aparicio, R. G. Lacalle, U. Artetxe, E. Urionabarrenetxea, J. M. Becerril, M. A. Polti, et al., "Successful remediation of soils with mixed contamination of chromium and lindane: Integration of biological and physico-chemical strategies," *Environ. Res.*, vol. 194, Mar. 2021, Art. no. 110666. [Online]. Available: https://doi.org/10.1016/j.envres.2020.110666
[27] R. G. Lacalle, J. D. Aparicio, U. Artetxe, E. Urionabarrenetxea, M. A. Polti, M. Soto, et al., "Gentle remediation options for soil with mixed chromium (VI) and lindane pollution: Biostimulation, bioaugmentation, phytoremediation and vermiremediation," *Heliyon*, vol. 6, no. 8, Aug. 2020, Art. no. e04550. [Online]. Available: https://doi.org/10.1016/j.heliyon.2020.e04550
[28] Z. Lin, X.-M. Li, Y.-T. Li, D.-Y. Huang, J. Dong, and F.-B. Li, "Enhancement effect of two ecological earthworm species (*Eisenia foetida* and *Amynthas robustus* E. Perrier) on removal and degradation processes of soil DDT," *J. Environ. Monit.*, vol. 14, no. 6, pp. 1551–1558, Jun. 2012. [Online]. Available: https://doi.org/10.1039/c2em30160a
[29] T. Mosquera Córdoba, "Eficiencia del lombricompostaje en la biorremediación de suelos degradados por la minería a cielo abierto en el municipio de Unión Panamericana, departamento del Chocó," M.S. thesis, Univ. Manizales, Manizales, Colombia, 2016. [Online]. Available: https://ridum.umanizales.edu.co/xmlui/handle/20.500.12746/2929
[30] I. C. Zapata, L. Martínez, E. Posada, M. E. González, and J. F. Saldarriaga, "Efectos de la lombriz roja californiana (*Eisenia foetida*), sobre el crecimiento de microorganismos en suelos contaminados con mercurio de Segovia, Antoquia," *Cienc. Ing. Neogranadina*, vol. 27, no. 1, pp. 77–90, Jan. 2017. [Online]. Available: https://doi.org/10.18359/rcin.1911
[31] Z. Lin, J. Bai, Z. Zhen, S. Lao, W. Li, Z. Wu, et al., "Enhancing pentachlorophenol degradation by vermicomposting associated bioremediation," *Ecol. Eng.*, vol. 87, pp. 288–294, Feb. 2016. [Online]. Available: https://doi.org/10.1016/j.ecoleng.2015.12.004
[32] R. Werheni Ammeri, G. Di Rauso Simeone, Y. Hidri, M. S. Abassi, I. Mehri, S. Costa, et al., "Combined bioaugmentation and biostimulation techniques in bioremediation of pentachlorophenol contaminated forest soil," *Chemosphere*, vol. 290, Mar. 2022, Art. no. 133359. [Online]. Available: https://doi.org/10.1016/j.chemosphere.2021.133359
[33] T. M. Phillips, A. G. Seech, H. Lee, and J. T. Trevors, "Biodegradation of hexachlorocyclohexane (HCH) by microorganisms," *Biodegradation*, vol. 16, no. 4, pp. 363–392, Aug. 2005. [Online]. Available: https://doi.org/10.1007/s10532-004-2413-6
[34] Q. Liang, M. Lei, T. Chen, J. Yang, X. Wan, and S. Yang, "Application of sewage sludge and intermittent aeration strategy to the bioremediation of DDT- and HCH-contaminated soil," *J. Environ. Sci.*, vol. 26, no. 8, pp. 1673–1680, Aug. 2014. [Online]. Available: https://doi.org/10.1016/j.jes.2014.06.007
[35] M. Siedt, A. Schäffer, K. E. C. Smith, M. Nabel, M. Roß-Nickoll, and J. T. van Dongen, "Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides," *Sci. Total Environ.*, vol. 751, Jan. 2021, Art. no. 141607. [Online]. Available: https://doi.org/10.1016/j.scitotenv.2020.141607

[36] B. Betancur-Corredor, N. J. Pino, S. Cardona, and G. A. Peñuela, "Evaluation of biostimulation and Tween 80 addition for the bioremediation of long-term DDT-contaminated soil," *J. Environ. Sci.*, vol. 28, pp. 101–109, Feb. 2015. [Online]. Available: https://doi.org/10.1016/j.jes.2014.06.044
[37] J. W. Lanfranco, A. E. Pellegrini, and V. M. Cattani, *Contenidos de Edafología: Génesis, Evolución y Propiedades Físico Químicas del Suelo*. La Plata, Argentina: Editorial Univ. Nac. La Plata, 2014. [Online]. Available: http://sedici.unlp.edu.ar/handle/10915/37325
[38] L. M. Thompson and F. R. Troeh, *Los Suelos y su Fertilidad*, 4th ed. Barcelona, Spain: Editorial Reverté, 1982.
[39] R. M. Atlas and R. Bartha, *Ecología Microbiana y Microbiología Ambiental*, 4th ed. Madrid, Spain: Pearson Educación, 2002.
[40] D. Kroetsch and C. Wang, "Particle size distribution," in *Soil Sampling and Methods of Analysis*, 2nd ed. M. R. Carter and E. G. Gregorich, Eds., Boca Raton, FL, USA: CRC Press, 2008, pp. 713–725. [Online]. Available: https://doi.org/10.1201/9781420005271
[41] *Calidad del Suelo. Determinación de la Conductividad Eléctrica*, NTC 5596, Instituto Colombiano de Normas Técnicas y Certificación, Bogotá, D.C., Colombia, Mar. 2008.
[42] *Calidad del Suelo. Determinación del pH*, NTC 5264, Instituto Colombiano de Normas Técnicas y Certificación, Bogotá, D.C., Colombia, Mar. 2008.
[43] *Que Establece las Especificaciones de Fertilidad, Salinidad y Clasificación de Suelos. Estudios, Muestreo y Análisis*, NOM-021-RECNAT-2000, Secretaría de Medio Ambiente y Recursos Naturales, Ciudad de México, México, Dec. 2002. [Online]. Available: http://www.ordenjuridico.gob.mx/Documentos/Federal/wo69255.pdf
[44] G. Bou, A. Fernández-Olmos, C. García, J. A. Sáez-Nieto, and S. Valdezate, "Métodos de identificación bacteriana en el laboratorio de microbiología," *Enferm. Infec. Microbiol. Clin.*, vol. 29, no. 8, pp. 601–608, Oct. 2011. [Online]. Available: https://doi.org/10.1016/j.eimc.2011.03.012
[45] M. T. Madigan, J. M. Martinko, P. V. Dunlap, and D. P. Clark, *Brock: Biología de los Microorganismos*, 12th ed. Madrid, Spain: Pearson Educación, 2009.
[46] G. J. Tortora, B. R. Funke, and C. L. Case, *Introducción a la Microbiología*, 12th ed. Buenos Aires, Argentina: Editorial Med. Panamericana, 2017.
[47] J. G. Holt, Ed., *The Shorter Bergey's Manual of Determinative Bacteriology*, 8th ed. Baltimore, MD, USA: Williams & Wilkins, 1977.
[48] L. F. Durán Acosta and M. J. Ladera Hernández, "Biorremediación de suelos contaminados por organoclorados mediante la estimulación de microorganismos autóctonos, utilizando biosólidos," *Nexo Rev. Cient.*, vol. 29, no. 01, pp. 22–28, Jul. 2017. [Online]. Available: https://doi.org/10.5377/nexo.v29i01.4397
[49] M. Schuldt, *Lombricultura: Teoría y Práctica*. Madrid, Spain: Mundi-Prensa, 2006.
[50] M. C. Vásquez, J. T. Guerrero Figueroa, and A. del P. Quintero, "Biorremediación de lodos contaminados con aceites lubricantes usados," *Rev. Colomb. Biotecnol.*, vol. 12, no. 1, pp. 141–157, Jan. 2010. [Online]. Available: https://revistas.unal.edu.co/index.php/biotecnologia/article/view/15579
[51] A. Corona-Cruz, G. Gold-Bouchot, M. Gutierrez-Rojas, O. Monroy-Hermosillo, and E. Favela, "Anaerobic-aerobic biodegradation of DDT (dichlorodiphenyl trichloroethane) in soils," *Bull. Environ. Contam. Toxicol.*, vol. 63, no. 2, pp. 219–225, Aug. 1999. [Online]. Available: https://doi.org/10.1007/s001289900969
[52] Soil Science Division Staff, *Soil Survey Manual*. Washington, DC, USA: GPO, 2017. [Online]. Available: https://www.iec.cat/mapasols/DocuInteres/PDF/Llibre50.pdf
[53] J. B. Eweis, S. J. Ergas, D. P. Y. Chang, and E. D. Schroeder, *Principios de Biorrecuperación: Tratamientos Para la Descontaminación y Regeneración de Suelos y Aguas Subterráneas Mediante Procesos Biológicos y Físico-químicos*. Madrid, Spain: McGraw-Hill, 1999.

[54] G. Mamani-Mamani, F. Mamani-Pati, H. Sainz-Mendoza, and R. Villca-Huanaco, "Comportamiento de la lombriz roja (*Eisenia* spp.) en sistemas de vermicompostaje de residuos orgánicos," *J. Selva Andina Res. Soc.*, vol. 3, no. 1, pp. 44–54, Aug. 2012. [Online]. Available: https://doi.org/10.36610/j.jsars.2012.030100044
[55] G. M. Pierzynski, G. F. Vance, and T. J. Sims, *Soils and Environmental Quality*, 3rd ed. Boca Raton, FL, USA: CRC Press, 2005. [Online]. Available: https://doi.org/10.1201/b12786
[56] I. Aroua, G. Abid, F. Souissi, K. Mannai, H. Nebli, S. Hattab, et al., "Identification of two pesticide-tolerant bacteria isolated from *Medicago sativa* nodule useful for organic soil phytostabilization," *Int. Microbiol.*, vol. 22, no. 1, pp. 111–120, Mar. 2019. [Online]. Available: https://doi.org/10.1007/s10123-018-0033-y
[57] C. L. Boechat, F. S. Carlos, C. W. A. do Nascimento, P. D. de Quadros, E. L. S. de Sá, and F. A. de O. Camargo, "Bioaugmentation-assisted phytoremediation of As, Cd, and Pb using *Sorghum bicolor* in a contaminated soil of an abandoned gold ore processing plant," *Rev. Bras. Cienc. Solo*, vol. 44, Oct. 2020, Art. no. e0200081. [Online]. Available: https://doi.org/10.36783/18069657rbcs20200081
[58] P. K. Mohapatra, *Textbook of Environmental Microbiology*. New Delhi, India: I.K. International Publishing House, 2008.
[59] M. L. Ortíz-Hernández and E. Sánchez-Salinas, "Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México," *Rev. Int. Contam. Ambient.*, vol. 26, no. 1, pp. 27–38, Feb. 2010. [Online]. Available: https://www.redalyc.org/articulo.oa?id=37014382003
[60] M. A. Prada-Vásquez, S. A. Cardona-Gallo, and J. C. Loaiza-Usuga, "Anaerobic biodegradation of DDT in contaminated soil by biostimulation: Laboratory and pilot-scale studies," *Acta Univ. Carolinae Geogr.*, vol. 52, no. 2, pp. 199–207, Oct. 2017. [Online]. Available: https://doi.org/10.14712/23361980.2017.16
[61] Cabinet Regulation No. 804 of 2005. (2005, Oct. 25). *Regulations Regarding Quality Standards for Soil and Ground*. [Online]. Available: http://faolex.fao.org/docs/pdf/lat190875ENG.pdf
[62] P. N. Saxena, S. K. Gupta, and R. C. Murthy, "Comparative toxicity of carbaryl, carbofuran, cypermethrin and fenvalerate in *Metaphire posthuma* and *Eisenia fetida* —A possible mechanism," *Ecotoxicol. Environ. Saf.*, vol. 100, pp. 218–225, Feb. 2014. [Online]. Available: https://doi.org/10.1016/j.ecoenv.2013.11.006
[63] A. Rico, C. Sabater, and M.-Á. Castillo, "Lethal and sub-lethal effects of five pesticides used in rice farming on the earthworm *Eisenia fetida*," *Ecotoxicol. Environ. Saf.*, vol. 127, pp. 222–229, May 2016. [Online]. Available: https://doi.org/10.1016/j.ecoenv.2016.02.004
[64] Y. Shi, Q. Zhang, D. Huang, X. Zheng, and Y. Shi, "Survival, growth, detoxifying and antioxidative responses of earthworms (*Eisenia fetida*) exposed to soils with industrial DDT contamination," *Pestic. Biochem. Physiol.*, vol. 128, pp. 22–29, Mar. 2016. [Online]. Available: https://doi.org/10.1016/j.pestbp.2015.10.009
[65] M. Kästner and A. Miltner, "Application of compost for effective bioremediation of organic contaminants and pollutants in soil," *Appl. Microbiol. Biotechnol.*, vol. 100, no. 8, pp. 3433–3449, Apr. 2016. [Online]. Available: https://doi.org/10.1007/s00253-016-7378-y
[66] Bureau Veritas, *Manual Para la Formación en Medio Ambiente*. Madrid, Spain: Editorial Lex Nova, 2008.
[67] M. E. Pavan, R. J. Franco, J. M. Rodriguez, P. Gadaleta, S. L. Abbott, J. M. Janda, et al., "Phylogenetic relationships of the genus *Kluyvera*: transfer of *Enterobacter intermedius* Izard *et al.* 1980 to the genus *Kluyvera* as *Kluyvera intermedia* comb. nov. and reclassification of *Kluyvera cochleae* as a later synonym of *K. intermedia*," *Int. J. Syst. Evol. Microbiol.*, vol. 55, no. 1, pp. 437–442, Jan. 2005. [Online]. Available: https://doi.org/10.1099/ijs.0.63071-0

[68] M. F. Peruzy, M. Aponte, Y. T. R. Proroga, F. Capuano, D. Cristiano, E. Delibato, et al., "*Yersinia enterocolitica* detection in pork products: Evaluation of isolation protocols," *Food Microbiol.*, vol. 92, Dec. 2020, Art. no. 103593. [Online]. Available: https://doi.org/10.1016/j.fm.2020.103593
[69] N. Pradhan and A. O. Ingle, "Mineralization of phenol by a *Serratia plymuthica* strain GC isolated from sludge sample," *Int. Biodeterior. Biodegradation*, vol. 60, no. 2, pp. 103–108, Jan. 2007. [Online]. Available: https://doi.org/10.1016/j.ibiod.2007.01.001
[70] B. Betancur-Corredor, N. Pino, G. A. Peñuela, and S. Cardona-Gallo, "Biorremediación de suelo contaminado con pesticidas: Caso DDT," *Gestión Ambient.*, vol. 16, no. 3, 2013. [Online]. Available: https://revistas.unal.edu.co/index.php/gestion/article/view/33173
[71] F. Castillo Rodríguez, M. D. Roldán Ruiz, R. Blasco Plá, M. J. Huertas Romera, F. J. Caballero Domínguez, C. Moreno-Vivián, et al., *Biotecnología Ambiental*. Madrid, Spain: Editorial Tébar, 2005.
[72] B. E. Rittman and P. L. McCarty, *Biotecnología del Medio Ambiente: Principios y Aplicaciones*. Madrid, Spain: McGraw-Hill, 2001.
[73] S. Bajaj, S. Sagar, S. Khare, and D. K. Singh, "Biodegradation of γ-hexachlorocyclohexane (lindane) by halophilic bacterium *Chromohalobacter* sp. LD2 isolated from HCH dumpsite," *Int. Biodeterior. Biodegradation*, vol. 122, pp. 23–28, Aug. 2017. [Online]. Available: https://doi.org/10.1016/j.ibiod.2017.04.014