# Ability of bacteria isolated from oil-contaminated soil to utilize lindane under aerobic and anaerobic conditions

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## Introduction

Lindane is an organochlorine compound (Figure 1.) that belongs to the group of hexachlorocyclohexane (HCH). Hexachlorocyclohexane is a group of compounds consisting of cyclohexane having one chlorine and one hydrogen attached to each carbon; their common chemical formula is $C_6H_6Cl_6$. Lindane is stable in nature so it is one of the persistent organic pollutants and its degradation has been the subject of many studies.

Persistent Organic Pollutants are organic compounds that, due to their persistence, pose a danger to humans and the living world. They are difficult to decompose chemically and biologically, so they retain their structure for a long time.

During the 1950s, the use of lindane began due to its tasteless, odorless and colorless properties. The estimated amount of lindane used globally in the period from 1950 to 2000 is about 600,000 tons (Vijgen et al., 2006).

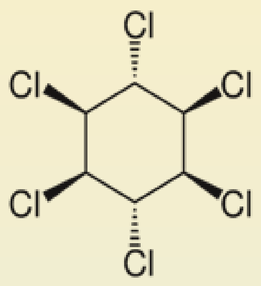


Figure 1. Chemical structure of lindane

The application of such a large amount of lindane led to the production of a large amount of HCH waste. Lindane has even been used as a medicine in humans, more precisely as an ingredient in a shampoo against scabies and pediculosis (Solomon et al., 1977).

Since the 1990s, research has begun on possible ways to remove lindane. In addition to more traditional methods such as storage or incineration, catalysts have been discovered that enable faster photodegradation such as $TiO_2$ or mixtures of $TiO_2$ with other metal oxides like $CeO_2$ (Radić et al., 2022). Meanwhile, the microbial breakdown of lindane contamination is becoming more and more recognized as an economical and environmentally friendly alternative to traditional treatment methods (Bhatt et al., 2019). Lindane can be biodegraded under both aerobic (Nagata et al., 2017) and anaerobic conditions (Bashir et al., 2018).

## Materials & Methods

For our experiment, we used bacterial strains isolated from oil-contaminated soil. Mineral medium supplemented with diesel D2 (as a source of hydrocarbons) was used for isolation microorganisms that decompose hydrocarbons (1 g $NH_4NO_3$, 0.25 g $CaHPO_4$, 50 mL soil extract, and 1 L $dH_2O$) (Löser et al., 1998). Diesel D2 (2 g $L^{-1}$) was added after sterilization at 121 °C, for 25 min. In the Erlenmeyer flask were added 100 mL of mineral medium and 1 g of soil and it was incubated at 28 °C, 150 rpm, for 7 days. 1 mL of suspension was transferred in the new mineral medium and incubated. After triple inoculation onto mineral medium, the consortium of microorganisms was transferred to an agar plate with same composition (16 g $L^{-1}$ agar was added) and with the same growing conditions.

We successfully isolated six bacterial strains: two strains of the genus *Pseudomonas* (PS1 and PS2), two strains of the genus *Acinetobacter* (ACB1 and ACB2), one strain of the genus *Achromobacter* (ACH1), and one strain from the genus *Citrobacter* (CB1). Because of their ability to survive in extreme conditions and use oil as a source of energy and carbon (Imron et al., 2020), we hypothesized that they could also use lindane.

The growth of microorganisms in the presence of lindane. A mineral salt medium of the following composition was made: 4.8 g $K_2HPO_4$, 1.2 g $KH_2PO_4$, 4 g $NaNO_3$, 0.6 g $MgSO_4x7H_2O$, 0.04 g $Ca(NO_3)_2x4H_2O$, 0.004 g $Fe_2(SO_4)_3$, and 1 L $dH_2O$; pH 7.00 (Sahoo and Chaudhuri, 2019). After sterilization glucose (5 g $L^{-1}$) and lindane (10 ppm) were added to the medium as a sources of hydrocarbons. Glucose was added by filtering through a syringe filter, while lindane was added by first dissolving in a minimal amount of acetone. After 7 days at 28 °C, 150 rpm it has been observed that at mineral salt medium with glucose and lindane microorganisms were grown. After that, microorganisms (1 mL) were transferred to a medium with same mineral composition supplemented only with lindane (10 ppm) as a source of hydrocarbons. After the microorganisms were grown for 7 days at 28 °C, aerobically (on a rotary shaker at 150 rpm) and anaerobically in parallel, the number of microorganisms was determined. The number of aerobic bacteria was determined on nutrient agar (15 g peptone I, 3 g meat extract, 5 g NaCl, 0.3 g $K_2HPO_4$, 18 g agar and 1 L deionized water; pH 7.30). The number of anaerobically grown bacteria was determined on the same agar supplemented with 0.5 % glucose.

## Results & Discussion

Six bacterial strains were successfully isolated and assessed for their ability to utilize lindane as the sole source of carbon. Table 1. shows us estimated number of cells of strains that were isolated.

The growth and ability of these bacteria to use lindane as the sole source of carbon and energy reflects the ability of these strains to biodegrade lindane in contaminated soil. Also the ability to degrade in both aerobic and anaerobic conditions shows the ability to biodegrade in different environmental conditions.

These findings contribute to the growing body of evidence supporting bacterial degradation as an eco-friendly way for lindane removal. Further research could explore kinetics of lindane removal by these bacterial strains.

Table 1. Estimated number of bacterial cells of each strain using only lindane as carbon source

| | Estimated number of bacterial cells (CFU $mL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Bacterial strain | PS1 | PS2 | ACB1 | ACB2 | ACH1 | CB1 |
| Aerobic conditions | $9.9 \times 10^7$ | $8.2 \times 10^6$ | $7.5 \times 10^6$ | $1.27 \times 10^7$ | $1.14 \times 10^8$ | / |
| Anaerobic conditions | / | / | / | / | / | $> 10^6$ |

## Conclusion

In this study we have explored the ability of bacteria isolated from oil-contaminated soil to utilize lindane under aerobic and anaerobic conditions. Six bacterial strains including *Pseudomonas*, *Acinetobacter*, *Achromobacter*, and *Citrobacter*, were successfully isolated and tested for their ability to utilize lindane as a sole source of carbon. The results indicated significant growth of these microorganisms in the presence of lindane.

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