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# Microbial Fuel Cell Bio-Remediation of Lambda Cyhalothrin, Malathion and

Chlorpyrifos on Loam Soil Inoculated with Bio-Slurry

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# Article Information

# ABSTRACT

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In microbial fuel cell technology, the substrate is consumed by microbes in anaerobic conversion of substrate to electricity. Bio-remediation of pollutants involves microbial environmental cleanup using green approach. The primary problems with pesticides are linked to the non-negligible proportion of the sprayed active ingredient that does not reach its intended target thereby contaminating environmental compartments persistently. The primary objective of this study was to assess the potential of microbial fuel cell technology in bio-remediation of lambda cyahlothrin, chlorpyrifos and malathion in Limuru loam soil. H-shaped double chamber microbial fuel cell was fabricated where the anodic chamber was loaded with 750 mL loam soil inoculated with 750 mL bio-slurry doped with 10 mL of 10 ppm lambda cyhalothrin, chlorpyrifos and malathion pesticide solutions. The cathodic chamber was loaded with 1500 mL distilled water. The setup was incubated for a 90 days retention time where voltage and current were recorded daily using a multi-meter. The degradation level was assessed using a GC-MS after sample extraction using standard QuEChERs method. The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhalothrin and malathion and from day 0 to day 20 in chlorpyrifos and pesticide mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and pesticide mix (MCL) respectively. The bioremediation levels for chlorpyrifos and malathion were 65.80 % and 71.32 %, respectively while no detectable, lambda cyhalothrin was observed after day 60 of the study. This study concludes that bioremediation of lambda cyhalothrin, chlorpyrifos and malathion in Limuru loam soil can be achieved using microbial fuel cells.

# **INTRODUCTION**

Agricultural pesticides, petroleum hydrocarbons, and heavy metals (HMs) have drastically degraded the quality of soils, thereby presenting serious danger to health and environment (Rodríguez-Eugenio et al., 2018). Urgent efforts are needed to treat the contaminated soils to minimize further damage. One of the sustainable and environment-friendly approaches is the use of microbial bio-surfactants that can deliver an economically feasible bio-remediation technique to restore polluted areas (Fatima et al., 2022).

Pesticides are chemical or biological substances that are used to kill or destroy pests that interfere with crop production (Gilden, 2010). These pesticides are applied to prevent diseases, suppress weeds as well as kill pests. In the soil, they can transform to complex metabolites (Doolotkeldieva et al., 2018). Some of the pesticides commonly used in "container gardens" are lambda cyhalothrin, malathion and chlorpyrifos (Mbugua et al., 2015). Pesticide degradation refers to transformation of complex parental pesticide molecule into simpler by-products which may be non-toxic or still toxic as the original molecule from which they were derived from (WHO, 2007). There are number of processes that break down pesticides in the environment, these include photo-degradation and hydrolysis just to mention a few. Microbes such as fungi and bacteria (Vargas, 1975) have

also been reported to aid pesticides degradation. For example, insecticides such as parathion (O, O-diethyl-O-P-nitrophenylphosphorothiate) is extensively used and undergoes enzymatic hydrolysis to produce p-nitro phenol, which further hydrolyses to produce nitrous acid and hydroquinone which is a metabolic intermediate. 2, 4-dichlorophenoxyacetic acid (2, 4-D) bio-degrades to produce phenolic compounds such as 2, 4-dichlorophenol and 4-chloro-2-hydroxyphenol (Sánchez-González et al., 2018). Chemical pollutants can adversely affect human and environmental health. In sediments, pollutants such as polycyclic aromatic hydrocarbons (PAHs), heavy metals and pesticides have the potential to exert an array of toxic effects on susceptible organisms. Certain chemicals including dichlorodiphenyltrichloroethane (DDT), various pharmaceuticals and endocrine disrupting agents (ex. nonylphenol) are recalcitrant in sediments, complicating removal (Rodríguez-Eugenio et al., 2018). Agents such as dioxins bio-accumulate in plant and animal tissues used for human consumption (Pisciotta and Dolceamore, 2016). Microbial-bioremediation process utilizes the indigenous microbial communities to clean up the environmental contamination. The rate at which the contaminants are detoxified depends on a number of factors such as the composition of the indigenous microbial communities, nature, and extent of the pollutant and environmental conditions (Ghosal et al.,

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2016). Microorganisms transformation of toxic pollutants into less or more-toxic forms or total mineralization yielding water and either carbon dioxide or methane can be identified as biodegradation (Hutchinson et al., 2001; Singh and Ward, 2004). As far as the mechanism of microbial degradation of contaminants like pesticides is concerned, most of the microorganisms consume the contaminants as their energy or nutrient sources. They degrade some pollutants in order to gain nutrients or energy released during the breaking down of chemical bonds. Optimization and control of bioremediation is a complex procedure driven by many factors (Das and Dash, 2014; Laurent et al., 2021). The efficiency of the bio-remediation of pollutants is highly influenced by some factors. For example, the presence of microbial population with bioremediation ability is the major factors (Das and Dash, 2014) and the availability of sufficient concentration of pollutant or toxic compounds that can be utilized by microorganisms for their nutrients or energy needs is one of the most important factor that affects bio-remediation potential (Boopathy, 2000; Singh and Ward, 2004). "Temperature, pH, availability of oxygen" or any other electron acceptors (Boopathy, 2000; Singh and Ward, 2004; Das and Dash, 2014), redox potential (Eh), salinity (Aislabie & Lloyd-Jones, 1995; Boopathy, 2000), and moisture content (Aislabie & Lloyd-Jones, 1995; Singh et al., 2013; Das and Dash, 2014) are major environmental factors that directly affect the bioremediation potential. In addition to the major limitations that occurred due to inappropriate bioavailability, substrate and environmental factors, some other limiting factors can also be identified. Among them, the Cost-benefit ratio may also be a limitation for the bioremediation process (Varshney, 2019). Moreover, environmental disruptions may also become a limitation. For instance, when microorganisms are introduced to a natural land for the purpose of bioremediation, this can be disruptive to some other beneficial organisms due to the competition for nutrients or any other interactions among them (Randika et al., 2022). Therefore, in this study we assess the potential of microbial fuel cell technology in bio-remediation of lambda cyhalothrin, chlorpyrifos and malathion in Limuru loam soil.

# METHODOLOGY

The procedure used to carry out loam soil analysis and bioremediation of the pesticide residues is explained in this section.

### Loam Soil Analysis

# Available nutrient elements (P, K, Na, Ca, Mg and Mn): Mehlich Double Acid Method

### (Tran & Simard, 1993, Mehlich, A. 1953)

The oven-dry soil samples were extracted in 1:5 ratios (w/v) with a mixture of 0.1 N HCl from Kobian distributors and 0.025 N H2SO4 (Tran & Simard, 1993). A flame photometer was used in determination of K, Ca and Na while calorimetrically was used in determination of P, Mg and Mn (Mehlich, 1953).

# Total organic carbon: Calorimetric method (Gislason & Craig, 2005)

All organic C in the soil sample is oxidized by acidified dichromate at 1500C for 30minutes to ensure complete oxidation. Barium chloride was added to the cool digests. After mixing thoroughly, digests are allowed to stand overnight. The concentration is read on the spectrophotometer at 600 nm.

**Total nitrogen: Kjeldahl method (Persson et al., 2008)** Soil samples were digested with concentrated sulphuric acid containing potassium sulfate, selenium and copper sulfate hydrated at approximately 350 0C. Total N is determined by distillation, followed by titration with H2SO4.

### Soil pH (1:1 soil-water)

Soil pH was determined in a 1:1 (w/v) soil-water suspension using a high precision pen type Gray348697 pH meter with 0.01pH resolution, 0-80 0C operating temperature and 2 by 1.5 V power supply.

Available trace elements (Fe, Zn & Cu) Extraction with 0.1 M HCl

The oven-dry soil samples are extracted in a 1:10 ratio (w/v) with 0.1 M HCl. The elements are determined using an atomic absorption flame emission spectrophotometer (AAS).

# Cation Exchange Capacity (CEC) pH 7.0 and Exchangeable Ca, Mg, K and Na

The soil sample was leached with 1N ammonium acetate buffered at pH 7. The leachate was analyzed for exchangeable Ca, Mg, K and Na. The sample was further leached with 1N KCl, and the leachate is used for the determination of the CEC. Elements such as Na and K were being determined with a flame photometer and Ca and Mg with AAS (atomic absorption spectrophotometer). CEC is determined by distillation, followed by titration with 0.01M HCl (Turner & Clark, 1966).

# Microbial Fuel Cells Construction

Two 1.2 liter containers were prepared as anode and cathode chambers. Two small holes were made on the caps of the containers to insert the wire through. One end of the copper wire was attached to 5.7 cm long and 0.7 cm diameter graphite rod electrodes. A salt bridge was prepared using 2.5 litres of 1M NaCl, 3% agarose solution and lamp wicks. The wicks were boiled in NaCl and 3% agarose solution for 10 minutes after which it was kept in the freezer at -4°C for solidification. The solidified salt bridge was passed through PVC pipes and attached to the chambers using an adhesive, which makes them leakproof. The electrodes used in this study were spent battery carbon rods stuck together using a zero-resistance copper wire as shown in figure 1. The carbon rods were obtained from batteries after which they we thoroughly cleaned using water and later scrub using a sand paper. They were then socked in concentrated Sulphuric acid for 24 hours before stacking them together. The electrodes had a 0.00399 m2 operating surface area. The assembly of the H-shaped MFC was done, as shown in figure 1 as earlier described by Kamau et al., 2018. A digital voltmeter was



attached to the copper wires from the cathodic and anodic chambers, and the voltage and current were monitored daily (Mbugua et al., 2022; Kinyua et al., 2022b).



Figure 1. Set-up of H-shaped microbial fuel cells with a multi-meter

### **Bio-remediation studies**

The microbial bio-remediation study involved investigation of efficiency of microbial fuel cells in degradation of lambda cyhalothrin, malathion and chlorpyrifos pesticide residues. The anodic chamber was fed with 750 g loam soil (previously analysed) inoculated with 750 mL bio-slurry from a running biogas digester

spiked with 10 mL, of 100 ppm lambda cyhalothrin, malathion and Chlorpyrifos and a mixture solution of lambda cyhalothrin, malathion and Chlorpyrifos.(Mbugua et al., 2022; Kinyua et al., 2022a). The degradation levels were determined by measuring the concentration of the pesticide after every 5 days for 90 days. The Voltage and current generated were recorded on daily basis. The degradation levels were determined by measuring the concentration of the pesticide after every 5 days for 90 days. The pesticides after degradation were extracted using the standard QuEChERS method (Anastassiades et al., 2003). The sample extracts were placed onto a tray for automated GC/MS analysis as described by (Amirahmadi et al., 2013). The Voltage and current generated were recorded on daily basis. The control experiment was run by loading the loam soil into the anodic chamber and reading the daily voltage and current for 90 days.

# RESULTS AND DISCUSSIONS Loam soil properties

The macro and micro properties of the loam soil used in this study is shown in table 1. From the analysis, the soil pH was in the range of  $6.5 - 6.8 \pm 0.51$  while the electrical conductivity of this soil was  $0.03\pm 0.01$  ms/cm.

Profile	Properties	Profile	Properties
Soil depth cm	Тор	Calcimn milli-equivalent%	44.4±2.11
Soil pH-H20 (1:2.5)	6.5±0.51	Magnesium me%	3.1±0.09
Elect . Cond. ms/cm	0.3±0.01	Potassium me%	1.5±0.66
Carbon %	2.7±0.32	Sodium me%	3.6±1.11
Sand %	40±3.56	Sum me%	52.6±3.44
Silt %	40±4.55	Base %	100+
Clay %	20±2.88	ESP	14.4±6.74
Textm·e Class	Loam	Total nitrogen %	0.25±0.08
Cat. Exch. Capacity. me%	24.8±2.67	Phosphorns ppm	44± 5.00
Zinc ppm	62.9±10.22	Iron ppm	96.2± 12.90
Copper ppm	1.22±0.11	me is milli- equivalent	

Table 1: The properties of the loam soil

The loam soil was top soil collected about 1-2 cm deep. The organic matter was removed from the surface before sampling. The cation exchange capacity of the loam soil was  $24.8\pm2.67$  while carbon levels were  $2.70\pm0.32$  %. The microbes use carbon as source of nutrient and energy and therefore soil carbon is a very important parameter in soil analysis. Soil quality does not depend just on the physical, physico-chemical and chemical properties of soil but closely linked to the soil microbiological properties (Elliot et al., 1996). Microorganisms are vital for soil fertility and for the degradation of organic matter and pollutants in soils. Some of the important biosurfactant-synthesizing bacterial species include Pseudomonas sp., Bacillus

sp., Acinetobacter sp., Stenotrophomonas sp., and Burkholderia sp. The bacterial biosurfactants increase the rate of biodegradation of hydrophobic (insoluble) organic pollutants such as pesticides and petroleum in the soil or enhance the removal of heavy metals through a series of modes of action such as increasing their mobility, micelle formation, and increasing bioavailability to bacteriadegrading microorganisms (Fatima et al., 2022) Microbial biomass in soil is considered as an important attribute of soil quality (Doran and Parkin, 1994). It serves as a measure of potential biological activity and its dynamic changes help in understanding the processes involved in nutrient cycling and ecosystem functioning (Rath et



al., 1998). Pal et al., (2006) reviewed of the information available in the literature highlighting the various soil properties, which influence the degradation of pesticides. The extent of biodegradability depends upon the chemical structure of the pesticides and the soil physicochemical properties. They also noted that soil microbial components largely govern pesticide degradation in soil. The graph obtained from plots of current and voltage of the control (loam soil) data is shown in figure 2. The daily voltage showed a slow upward trend for the first twenty days with steep increase of voltage for eight days before it started to drop. Similar results were obtained for current since both current and voltage are relates proportionally according to Ohms law.



Figure 2: Plots of voltage and current generated from the loam soil

Current generation means that the soil micro-organisms are breaking down the carbon in the soil thereby generation an electron (current). The slow increase in current and voltage is explained by the fact that the microbes were in aerobic conditions before sampling and therefore takes time to adapt to the anaerobic setup in the anodic chamber (Mbugua et al., 2022). On full adaptation (day 20), the rate of electron increases subsequently increasing the voltage and current generation. On depletion of the available carbon in the soil, the microbes start dying and therefore a voltage and current drop is observed. Similar results had been obtained using market wastes like avocado and tomato by Kamau et al., 2018. In other studies, by Kinyua et al., 2022b and and Imwene et al., 2021 using tomato and cabbages as substrates, the voltage and current increased as observed in this study. The voltage and current means were used calculations of power, current and power density using equations 1 to 3, respectively The plots of power and current density are



shown in figure 3. Similar to plots of current and voltage, the power density increased with retention time. The current and power density shows the current and power per electrode surface area and mostly used to show the efficiency of microbial fuel cell in electricity generation.



Figure 3: Plots of power and current density with retention time

The bio-remediation levels for chlorpyrifos and malathion were 65.80 % and 71.32 %, respectively while no detectable, lambda cyhalothrin was observed after day 60 of the study (figure 4). The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhalothrin and malathion and from day 0 to day 20 in chlorpyrifos and MCL mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and MCL respectively. A clearer illustration is shown using 3D plots of voltage generated against degradation levels with retention time for the three pesticides. The 3D plot of pesticides Concentration, Voltage and Retention Time in loam soil is shown by figures 5, 6 and 7. The observed





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3D plot of chlorpyrifos Concentration, Voltage and Retention Time



**Figure 5:** A 3D plot of chlorpyrifos Concentration, Voltage and Retention Time in loam soil



Figure 6: A 3D plot of lambda cyhalothrin Concentration, Voltage and Retention Time in loam soil



**Figure 7:** A 3D plot of malathion Concentration, Voltage and Retention Time in loam soil

degradation levels were 79.32 %, 99.90 % and 78.20 % in chlorpyrifos, lambda cyhaolthrin, malathion, respectively as shown in figure 5-7, respectively.

# DISCUSSIONS

Current and voltage generation is an indication of

substrate breakdown by microbes in anaerobic anodic chamber of microbial fuel cells. The rate of current generation shows the rate at which microbes are degrading the substrate/carbon releasing electrons. (Kamau et al., 2018). The current generated in this study as per figure showed a slow increase from day 1 to day 27 as microbes are adapting to the experimental environment. As carbon matter in pesticide and soil depletes, the current and voltage generation start to decrease as microbes reaches the death phase. This trend is similar to what had been observed by Kinyua et al., 2022b and Imwene et al., 2021 in vegetable and fruit substrates.

In bio-remediation of pollutants, the pesticide molecule serves as a carbon sources and therefore it's broken down by micro-organisms in soil (Cycoń et al., 2009). The microbial activity is highly influenced by pesticide properties and environmental factors (Chowdhury et al., 2008). From the initial pesticide concentrations of 10 ppm, the observed degradation levels were 79.32 %, 99.90 % and 78.20 % in chlorpyrifos, lambda cyhalothrin, malathion, respectively as shown in figure 5-7, respectively. This means that the microbes feeds on the pesticide molecule and soil organic carbon for their growth and energy. These result are similar to what was previous observed by Kinyua et al., 2022a and Mbugua et al., 2022 on bio-remediation of chlorpyrifos, lambda cyhaolthrin, malathion on on loam soil, cabbage and tomato inoculated with microbe rich rumen waste and by anaerobic digestion bio-slurry, respectively. Similarly, on bio-remediation of chlorpyrifos, lambda cyhaolthrin and malathion inoculated with rumen fluid, 0.312V, 0.572V, 0.364V were recorded in tomato, loam soil and cabbage, respectively (Kinyua et al., 2022a). The bioremediation levels were 79.32 %, 99.90 % and 78.20 % in chlorpyrifos, lambda cyhalothrin, malathion, respectively in loam soil, 65.80 % and 71.32 % for chlorpyrifos and malathion respectively in cabbage. In tomato setup, 75.60% and 80.10 % chlorpyrifos and malathion levels were observed, respectively with undetectable levels of lambda cyhalothrin (Kinyua et al., 2022b). In the study by Mbugua et al., 2022, the observed maximum voltage on doping the biogas bio-slurry with the chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533V respectively with bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90th day of incubation.

Malathion is degraded by carboxyesterase enzyme and it is detected in several fungi like Aspergillus sp., Penicillum sp. and Rhizoctonia sp. (Mostafa et al., 1972). Omar (1998) and Hasan (1999) also demonstrated the same type of fungal utilization and degradation of Malathion. Adhikari, 2010 suggested bio-remediation of malathion from the environment as a pollution control measure. Similarly, to the current study, the bio-remediation of malathion on contaminated sterile and non-sterile soil showed a degradation levels of 84.81% and 74.11% of malathion, respectively, from malathion concentration of



1.5% kg-1 soil degraded by strain PU after 7 days (Singh et al., 2013).

In bioremediation study of chlorpyrifos by Jaiswal et al., 2017, the potential degradative microorganisms possess opd (organophosphate degrading) gene which hydrolyses the chlorpyrifos and utilizes it as a sole carbon source. A fungal strain Verticillium capable of utilizing chlorpyrifos as sole carbon and energy sources from soil and degradation of chlorpyrifos in pure cultures and on vegetables has been reported. The 3,5,6-trichloro-2pyridinol (TCP) and diethylthiophosphate (DETP) as primary products are made when chlorpyrifos is degraded by soil microorganisms which further break into nontoxic metabolites as CO2, H2O, and NH3 (Gilani et al., 2016). Pseudomonas is a diversified genus possessing a series of catabolic pathways and enzymes involved in pesticide degradation. Pseudomonas putida MAS-1 is reported to be more efficient in chlorpyrifos degradation by a rate of 90% in 24 h among Pseudomonas genus (Gilani et al., 2016). A bacterial strain C2A1 isolated from soil was found highly effective in degrading chlorpyrifos and its first hydrolysis metabolite 3,5,6-trichloro-2-pyridinol (TCP) (Anwar et al., 2009).

Bacillus thuringiensis ZS-19 has been reported to completely degraded cyhalothrin in minimal medium within 72 h. The bacterium transformed cyhalothrin by cleavage of both the ester linkage and diaryl bond to yield six intermediate products (Chen et al., 2015). Furthermore, strain ZS-19 participated in efficient degradation of a wide range of pyrethroids including cyhalothrin, fenpropathrinn, deltamethrin, betacypermethrin, cyfluthrin and bifenthrin. In a study by Kumar and Jahangir, 2018, the strain Rhodococcus erythropolis was proven suitable for the efficient and rapid bioremediation of Lambda cyhalothrin pesticide contaminated environment.

In other pesticides bio-remediation, it has been found that after 21 days 85% carbaryl has been degraded from soil treated with nitrogen source (Naqvi et al., 2011). Pal et al., 2006 investigated the factors influencing the degradation of pesticides in soil, impacts of pesticides on soil microbial biomass, soil ergosterol content, soil respiration, fluorescein diacetate hydrolyzing activity, ecophysiological parameters and the correlation between pesticide transformation and above microbial parameters. Pesticides, which enter the soil environment, are subject to a variety of degradative processes. The overall degradation of a pesticide from soil results from a combination of mechanisms such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and surface runoff. The degree to which each mechanism will contribute to the overall degradation of the pesticide is in turn dependent on the physicochemical properties of the pesticide (e.g., water solubility, sorptive affinity), characteristics of the soil (e.g., pH, organic matter content, microbial biomass, redox status), environmental conditions (e.g., temperature, moisture) and management practices (e.g., application rate, formulation type). (Pal

et al., 2006). The kinetics and pathways of degradation depend on abiotic and biotic factors (Beigel et al., 1999), which are specific to a particular pesticide and therefore find preference. Adverse effect of pesticidal chemicals on soil microorganisms (Araujo et al., 2003), may affect soil fertility (Schuster and Schroder, 1990) becomes a foreign chemicals major issue. Soil microorganisms show an early warning about soil disturbances by foreign chemicals than any other parameters.

The fate and behavior of these chemicals in soil ecosystem is very important since they are degraded by various factors and have the potential to be in the soil, water etc. So it is indispensable to monitor the persistence, degradation of pesticides in soil and is also necessary to study the effect of pesticide on the soil quality or soil health by in depth studies on soil microbial activity (Chowdhury et al., 2008). Previous studies have on fipronil bio-remediation in the non-sterile clay loam soil, which resulted in the formation of metabolite, MB45950. The degradation of fipronil in non-sterile clay loam soil was mainly influenced by the soil microbes (Zhu et al., 2004). The half-lives in nonsterile clay loam soil were 9.72 and 8.78 d at 25 and 35 °C, respectively compared to 33.51 and 32.07 d at 25 and 35 °C, respectively in the sterile soil. The microbial viability test showed that non-sterile clay loam soil had viable microorganisms throughout the experiment. Fipronil did not adversely affect the microbes once soil microbes adapted to the presence of fipronil in the clay loam soil (Zhu et al., 2004).

# CONCLUSIONS

The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhalothrin and malathion and from day 0 to day 20 in chlorpyrifos and pesticide mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and pesticide mix (MCL) respectively. The bioremediation levels for chlorpyrifos and malathion were 65.80 % and 71.32 %, respectively while no detectable, lambda cyhalothrin was observed after day 60 of the study. Therefore this study concludes that microbial fuel cells technology is an Eco-friendly technique which should be applied in bio-remediation of different classes of pesticides in loam soils. Further studies are suggested to investigate lambda cyhalothrin, chlorpyrifos and malathion bio-remediation mechanisms and degradation derivatives.

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