

Bio-Remediation of Lambda Cyhalothrin, Malathion and Chlorpyrifos Using Microbial Fuel Cells

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ABSTRACT

The common pollutants present in the environment are heavy metals, polycyclic aromatic hydrocarbons (PAHs), or pesticides. Bioremediation is one approach employed in degradation of these persistent organic pollutants. Bio-electrochemical approaches offer a simple, effective, and environmentally friendly solution to pollutant remediation. In the current study, microbial fuel cell technology was employed in bio-remediation of lambda cyhalothrin, malathion and chlorpyrifos on loam soil, cabbage and tomato inoculated with microbe rich rumen waste. The anodic chamber was loaded with 1.5 liters of loam soil, tomato and cabbage blend and doped with 10 ml 10 ppm pesticides while the cathodic chamber was loaded with distilled water. The two chambers were connected using a salt bridge made of 3% NaCl agarose. Carbon rods connected to copper wires were used as electrode. Concentration of the pesticides was determined using GC-MS while daily voltage was done using a digital multimeter. The voltage recorded in the control experiments (without the pesticides) showed 0.580V, 0.473V, 0.271V and 0.568V in un-inoculated tomato, cabbage, loam soil and rumen fluid, respectively. On inoculating the wastes with rumen fluid 0.312V, 0.572V, 0.364V were recorded in tomato, loam soil and cabbage, respectively.

Keywords : Bio-Remediation, Lambda Cyhalothrin, Malathion, Chlorpyrifos, Voltage

I. INTRODUCTION

The common pollutants present in the soil are heavy metals, polycyclic aromatic hydrocarbons (PAHs), or pesticides (Mirsal, 2008). Pesticides contamination result from farming activities and careless disposal of empty pesticides containers (Kim

et al., 2017). Pesticide's persistence in the environment is of particular concern as its residues are toxic and negatively impact on the environment and human health. Bioremediation which involves the use of naturally occurring microorganisms is an environmental clean-up technique that can be used in a wide range of normal biological breakdown or

biodegradation processes (Yadav and Devi, 2017). Aerobic-anaerobic combined conditions are employed in an air cathode H-shaped double chamber to form a microbial fuel cell (MFC) (Uqab et al., 2016; Finley et al., 2010). Microbial fuel cells technology is one of the bioremediation techniques that can be employed for complete or partial breakdown of chlorpyrifos (CP), lambda cyhalothrin and malathion. The rate at which bio-degradation of these pesticide residues take place is influenced by conditions that affect microbial activities (Gavrilescu, 2005; Hussain *et al.*, 2009). Figures 1 shows the structural formula of malathion, lambda Cyhalothrin, chlorpyrifos respectively.

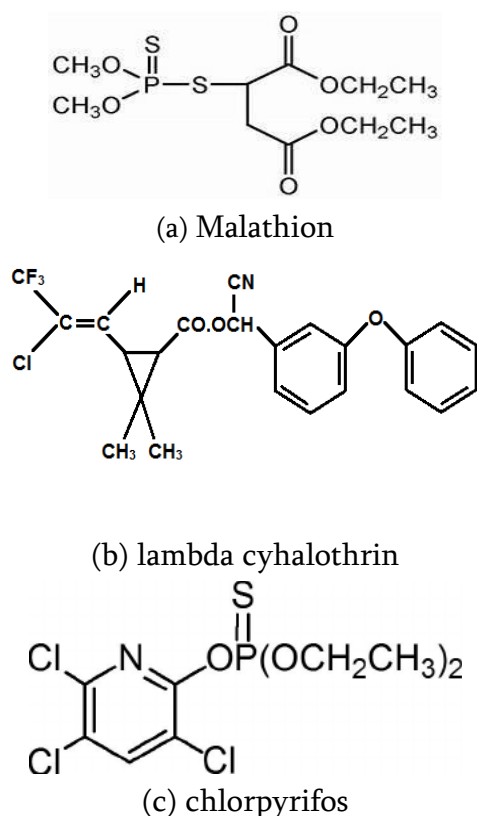


Figure 1.1 : Structural formula for malathion, lambda Cyhalothrin and chlorpyrifos

Microorganisms (bacteria and fungi) use the pesticide as an energy source for growth (Shanahan P (2004). Bacteria species that degrade the pesticides belongs to genera *Flavobacterium*, *Arthobacter*, *Azotobacter*, *Burkholderia*, and *pseudomonas* (Glazer and Nikaido,

2007). Recently *Bacterium raoultella spp.* is also found to degrade pesticide. Bacterial bioremediation seeks to break down environmental contaminants using aerobic and/ or anaerobic bacteria. Techniques include: bio stimulation through selective enrichment of autochthonous soil bacteria populations; bio augmentation through addition of specific bacterial strains; bioaccumulation with storage of contaminants inside live cells; biofilm bio sorption, which involves removal of contaminants through surface adsorption (Srivastava *et al.*, 2014). An advantage of bacterial bioremediation is that bacteria are often resistant to the presence of specific contaminants. Bacteria utilize their surroundings and interaction with organisms to obtain carbon, energy and nutrients needed for growth (Srivastava *et al.*, 2014). Consequently, the availability of carbon, energy and or nutrients can be a limiting factor for microbial growth, reducing the rates of pollutant degradation (El-Bestawy *et al.*, 2014). In accordance with Liebig's Law of the Minimum, the major limiting factor isn't the sum of the total resources available, but rather the scarcest nutrient available relative to the microbial species' demands. Identifying and delivering the limiting resource[s] can thus optimize bioremediation (Benyahia and Embaby, 2016). Ideally, the pollutant being catabolized may serve as a source of both energy and carbon in the case of heterotrophic species. Autotrophic bacteria, such as cyanobacteria, can be useful for bioremediation of pollutants that lack organic carbon, like certain metals (Mota *et al.*, 2016). Chemolithoautotrophic bacteria such as *Thiobacillus sp.* have the ability to bioremediate heavy metals in soil or sediment by producing metal-leaching sulfuric acid (Lloyd, 2004). These metals can then be removed as insoluble sulfides using sulfate-reducing bacteria (Lloyd, 2004). The complete biodegradation of the pesticide involves the oxidation of the parent compound resulting into carbon dioxide and water, this provides energy to microbes (Uqab *et al.*, 2016). The soil where innate microbial population cannot be able to manage pesticides, the external addition of pesticide degrading

micro flora is recommended. Degradation of pesticides by microbes not only depends on the enzyme system but also the conditions like temperature, pH and nutrients (Uqab *et al.*, 2016). Some of the pesticides are easily degraded however some are recalcitrant because of presence of anionic species in the compound. Besides organophosphorus compounds, the Neonicotinoids are degraded by the

Pseudomonas species. The minor structural changes that fungi do to degrade pesticides and render them into nontoxic substances and release them into soil where it is susceptible to further degradation. The various fungi and enzyme which have shown ability to degrade pesticides are given in Table 1.

Table 1: Fungi and enzyme with ability to degrade residues

Species of fungi	Potential for degrading pesticide	Reference
<i>Flammulina velupites</i> , <i>Stereum hirsutum</i> , <i>Coriolus versicolor</i> , <i>Dichomitus squalens</i> , <i>Hypholoma fasciculare</i> , <i>Auricularia auricula</i> , <i>Pleurotus ostreatus</i> , <i>Avatha discolor</i> and <i>Agrocybe semiorbicularis</i>	Triazine, Phenylurea, Dicarboximid, Chlorinated Organophosphorus Compounds	Watanabe <i>et al.</i> ,(2001)
White-rot fungi	Heptachlor atrazine, terbuthylazine, lindane, metalaxyl, chlordane mirex, gammahexachlorocyclohexane (g-HCH), dieldrin, diuron, aldrin, DDT, etc.,	Watanabe <i>et al.</i> ,(2001)
Enzyme	Source	Degradation
Organophosphorus acid anhydrolase (OPAA)	<i>Alteromonas undina</i> and <i>Alteromonas haloplanktis</i>	Xenobiotics compounds
Organophosphorus hydrolase (OPH)	<i>B.diminuta</i> and <i>Flavobacterium</i> sp.	Xenobiotics compounds
Arly acylamidase	<i>Bacillus sphaericus</i>	Herbicide and fungicide

II. Methodology

In the current study, the potential of various microbes in rumen fluid to degrade malathion, lambda Cyhalothrin and chlorpyrifos generating voltage in loam soil tomatoes and cabbages is investigated and discussed.

Reagents and chemicals

All pesticide standards were supplied from Dr. Ehrenstorfer GmbH Co. (Germany). The selected pesticides were 15 pesticides pronounced permissible in cultivation of tomato by the "Iranian National Standards Organization" (14). The stock solution of these compounds were prepared in acetonitrile at a concentration of 1000 mg/L and stored at -18 °C.

Acetonitrile and toluene (all analytical reagent grades) were purchased from Romil (Italy). Anhydrous sodium sulphate and sodium chloride (extra pure), were obtained from Merck(Germany). PSA bonded silica (Primary secondary amine) used in sample clean-up step were purchased from Supelco (USA). Tomato and cabbage samples were purchased from Kangemi/Nairobi fruit and vegetable markets.

1) Microbial Fuel Cells Construction

Two 1.2liter 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 5.7cm long and 0.7cm 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 Araldite adhesive, which makes them leak-proof. The electrodes used in this study were spent battery carbon rods stuck together using a zero-resistance copper wire as shown in figure. The carbo rods were obtained from batteries after which they were thoroughly cleaned using water and later scrub using a sand paper. They were then soaked in concentrated Sulphuric acid for 24 hours before stacking them together. The was 000cm^2 operating electrodes surface area. The assembly of the H-shaped MFC was done, as shown in figure 3.33 as earlier described by Mbugua *et al.*, 2020. A digital voltmeter was attached to the copper wires from the cathodic and anodic chambers, and the voltage and current were monitored daily.



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

Bioremediation studies

The 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 750g tomato, cabbage and loam soil inoculated with 750ml rumen wastes spiked with 10ml, of 100ppm lambda cyhalothrin, malathion and Chlorpyrifos and a mixture solution of lambda cyhalothrin, malathion and Chlorpyrifos. 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.

Bio-remediation Pesticide Analysis

Sample preparation

A modified version of the QuEChERS method for sample preparation of vegetables was used (Anastassiades *et al.*, 2003). Cabbage and tomatoes were blended in a warring blender to become homogenized. 10 g of the homogenized tomato sample was weighted in a 50 ml centrifuge tube. Then 10 μL TPM (5 mg/ml) was added as the internal standard and 10 ml acetonitrile was added afterwards. In this stage, the centrifuge tube was shaken for 1 min on the vortex at the full speed. Then 1 g of sodium chloride was added. Tubes were shaken for another 1 minute and then were centrifuged at 4500 rpm for 5 min at -5°C . 7 ml of the upper phase was transferred to a 10 mL centrifuge tube containing 2 g anhydrous magnesium

sulfate and 0.35 g PSA and was shaken for 60 sec. Then tubes were centrifuged for 5 min at 4500 rpm at -5 °C. 4 ml aliquot was transferred to dark vials and its solvent was evaporated under nitrogen. 1ml toluene was added to vials and then was shaken for 3 sec. Extracts were transferred into auto sampler vials. The samples were placed onto a tray for automated GC/MS analysis (Amirahmadi *et al.*, 2013, Jahanmard *et al.*, 2016).

Method validation

Linearity of the calibration curves

All pesticides showed linearity in the SIM mode. Linear spiked calibration curves for all of the pesticides under study were obtained with correlation factors >0.99 (Table 2). Limits of detection and limits of quantification. The quantification limits (LOQs) and detection limits (LODs) were calculated based on the standard deviations of the intercept and calibration curve parameters (Miller and Miller, 2005). Good LODs were achieved under the optimized experimental conditions, ranging between 1.63-10.5 mg/kg. Good LOQs were also obtained for tomato samples, ranging between 5.43-35mg/kg

Table 1: Linearity and correlation factors of spiked calibration curves

Pesticide	Equation of regression	Correlation coefficient (R ²)	LOD (ppb)
Chlorpyrifos	Y=0.0002x - 0.0028	0.9974	6.37
Malathion	Y=0.0006x- 0.0013	0.9905	8.04
Lambda cyhalothrin	Y=0.0002x- 0.0028	0.9967	7.36

1) Recovery

The recovery and repeatability validation experiments were conducted in tomato matrix at three spiking levels for each pesticide. The recovery of pesticides at 3 concentration levels triplicates was in the range of 83.84-119.73%. In terms of repeatability, the majority of the pesticides gave a relative standard deviation (RSD) <20.54%.

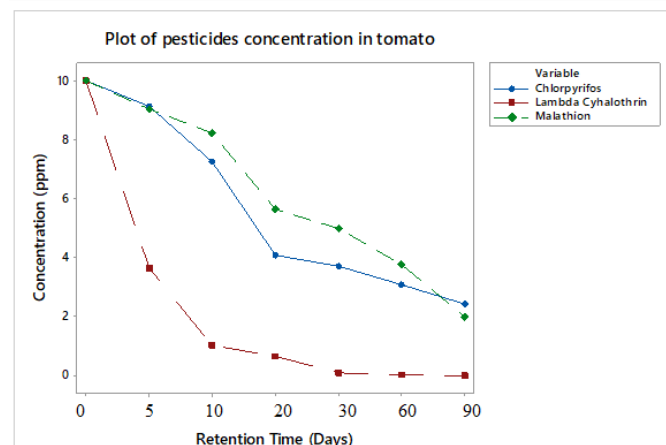
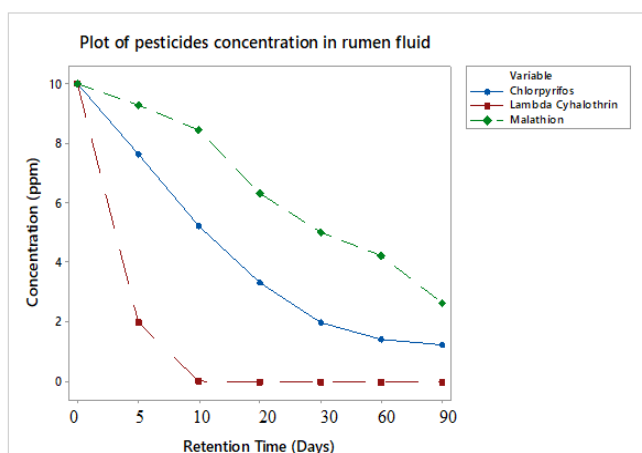
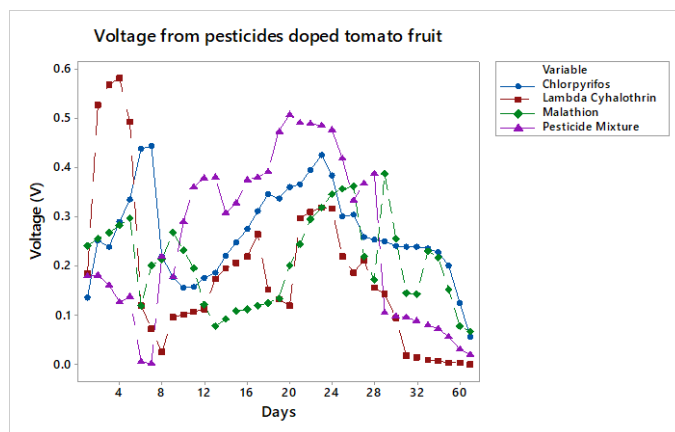
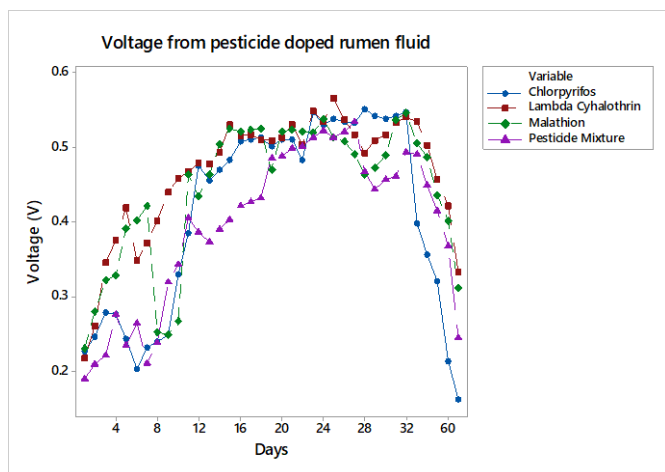
2) Data collection

The voltage and current generated were recorded from the digital multi-meter at 24 hours for the indicated number of 90 days. The experiments were done in triplicate and mean ± STD was used to plot the graphs

III. Results and Discussions

The voltage recorded in the control experiments (without the pesticides) showed 0.580V, 0.473V, 0.271V and 0.568V in un-inoculated tomato, cabbage, loam soil and rumen fluid, respectively. On inoculating the wastes with rumen fluid 0.312V, 0.572V, 0.364V were recorded in tomato, loam soil and cabbage, respectively.

On doping cabbages, tomato, loam soil and rumen fluid with chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM), the daily voltage generated and the degradation levels are discussed. The maximum generated voltage on doping the rumen fluid with the chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533 V respectively. The voltage generated increased steadily from the initial setup from day 0 to day 17 with low increasing rate up to day 31 where a downward voltage generation was observed (figure 2). the bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90th day of this study.



Figures 2 : Daily voltage and degradation levels from pesticides doped rumen fluid

As shown in figures 3, the voltage generation trend showed a step increase from day 0 to day five for all the pesticides apart from the pesticide mixture. A downward trend was then observed for three days after which voltage increase was observed until day 31. The maximum generated voltage was 0.436 V, 0.582 V, 0.363 V and 0.509 V for chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) respectively while the observed degradation levels were 75.60% and 80.10 % for chlorpyrifos and malathion respectively with undetectable levels of lambda cyhalothrin as shown by figure 3.

Figure 3 : Daily voltage and degradation levels from pesticides doped tomato fruits

Similarly, to tomato doped with pesticide residues, the voltage generated from the dual chamber MFC (figure 4a) increase from day 0 to day five for all the pesticides including the pesticide mixture. A downward trend was observed henceforth up to day 15 after which an upward trend was observed for five days after which a constant voltage was observed.

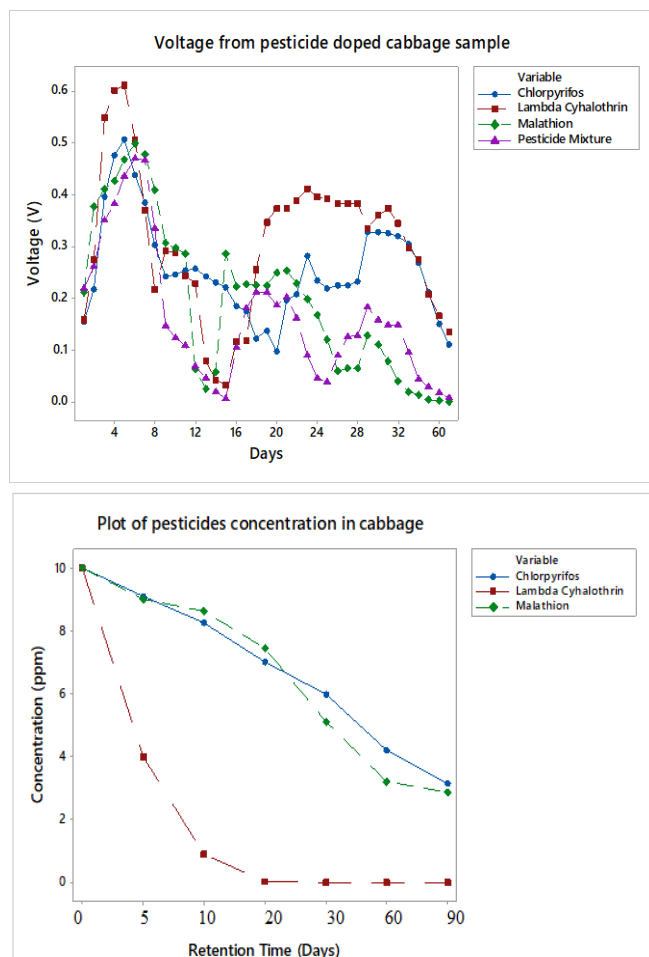


Figure 4: Daily voltage and degradation levels from pesticides doped cabbage

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 (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.

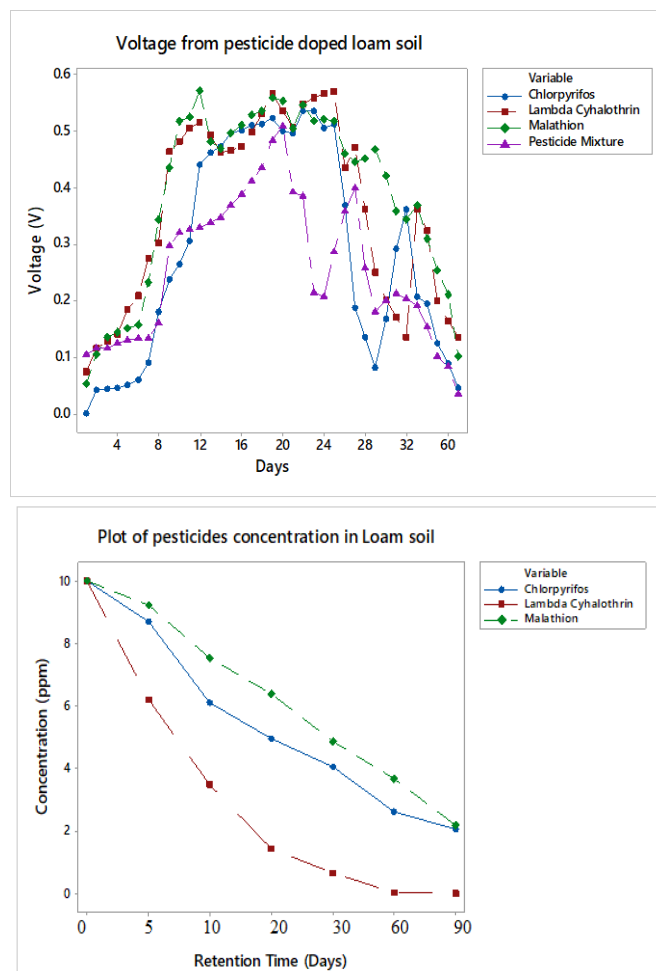


Figure 5: Daily voltage and degradation levels from pesticides doped loam soil

The observed degradation levels were 79.32 %, 99.90 % and 78.20 % in chlorpyrifos, lambda cyhalothrin, malathion, respectively as shown in figure 5.

IV. Discussions

The results of voltage obtained in this study relates with what was observed by Mbugua *et al.*, (2020) who reported highest voltage of 0.702 V on day 20 from tomato waste in an investigation of voltage generation from market wastes and 0.396 V from cabbage wastes inoculated with rumen fluid. This study concludes that proximate properties of substrates influence the current and voltage generation in a microbial fuel cell. Biodegradation of CP is carried out both aerobically and anaerobically by the microbes through two major degradation pathways, i.e., catabolism and co-

metabolism. The catabolic process involves the complete breakdown of complex organic compounds or their fragments, whereas co-metabolic process leads to partial degradation of organic compounds, without serving any benefit to the organism (Yadav *et al.*, 2016). Under aerobic conditions, the bacteria tend to transform CP into highly electrophilic by-products, i.e., CP-Oxon (diethyl 3,5,6 trichloropyridin-2-yl phosphate) or TCP (3,5,6-trichloro-2-pyridinol) and DETP (Diethyl thiophosphate) through oxidative desulfuration or dearylation respectively, with TCP as the most common metabolite. CP-Oxon is further hydrolyzed to DEP (diethyl phosphate) and TCP (Tiwari and Guha, 2014). Sardar and Kole (2005) stated that CP is broken down into TCP through the process of hydroxylation in the soil, which was further degraded into TMP (3,5,6-trichloro-2-methoxy-pyridine). Singh and Walker (2006), reported that DETP undergoes hydrolysis with the production of ethanol and phosphorothioic acid, which were utilized by the CP degrading microbes as the source of carbon, phosphorus, and sulfur. They also reported the formation of 2,3-dihydroxypyridine from TMP by the process of de-chlorination, which further undergoes hydroxylation to give rise the production of 2,5,6-trihydroxy pyridine. The metabolites produced during the reaction system undergo further oxidation that leads to the production of carbon fragments, aliphatic amines and inorganic phosphates (Singh *et al.*, 2006). According to Reddy *et al.* (2012), 2,3-dihydroxypyridine is broken down into maleamic acid, which intern undergoes oxidation and leads to the production of pyruvic acid. These products then make their entry into the Krebs cycle of CP degrading microbes. Tiwari and Guha (2014) carried out the biodegradation study of CP under anaerobic conditions and they reported the production of TCP and DETP during CP degradation, which indicates that under anaerobic conditions CP gets directly hydrolyzed to TCP and releases DETP. They also reported that detection of TCP and DETP from the experiments during CP degradation was

evident, but TMP and other by-products (degradation products) were not detected, which indicated their degradation into smaller water-soluble products or complete breakdown (mineralization) of CP (Tiwari and Guha, 2014).

Foster *et al.* (2004) studied the aerobic degradation of Ethion by mesophilic bacteria isolated from contaminated soils surrounding disused cattle dip sites. Two isolates, identified as *Pseudomonas* and *Azospirillum* species, were capable of biodegrading Ethion when cultivated in minimal salts medium. The abiotic hydrolytic degradation products of Ethion such as Ethion Dioxon and O, O diethylthiosphosphate were not detected. In another study, Istiqomah *et al.* (2021) observed four isolate which could survive the highest concentration of pesticides (90 ppm) in the range 105 Cfu (colony forming unit). Bacterial adaptation towards pesticides provoke pesticide resistance. The mechanisms can be biofilm formation, induced mutations, and horizontal or vertical gene transfer through plasmids or transposons, as well as through the increased expression of certain hydrolytic enzymes (Rangsamy *et al.*, 2018).

V. CONCLUSIONS

In conclusion, MFC is a promising technology for simultaneous pollutant reduction and electricity generation. The maximum voltage generated from doped loam soil was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and MCL respectively. While the maximum generated voltage on doping the rumen fluid with the chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533 V respectively. The bio-remediation 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.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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