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Bioremediation Potential of Plant-Bacterial Consortia for Chlorpyrifos Removal Using Constructed Wetland

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The extensive and unchecked application of chlorpyrifos against crop insects has caused contamination of various ecosystems, such as soil, sediments, and water, posing harm to plants, animals, useful arthropods, and humans. The present study aimed at evaluating the ability of proto-type constructed wetland to biodegrade chlorpyrifos and its major metabolites especially 2-hydroxy-3, 5, 6-trichloropyridine/ol (TCP) using chlorpyrifos-degrading indigenous bacterial strains, namely, *Acinetobacter baumanni* and *Bacillus cibi* with *Canna* spps. and indigenous *Mentha* spps. as a bacterial–plant consortium. Soil and plant samples were collected at regular time intervals for 12 weeks; analytes were extracted using the toluene method and evaluated through gas chromatography–mass spectrometry (GC-MS). In case of wetland vegetation with *Canna* and *Mentha*, 2-hydroxy-3, 5, 6-trichloropyridine (TCP, m/z = 198) and 2- hydroxypyridine (m/z = 97) with deprotonated molecular ions at m/z = 69 $(M\text{-}H)^-$were detected as the intermediate metabolites, while in the bacterial–plant consortium, instead of TCP, 3, 5, 6-trichloro-2-methoxypyridine (TMP, m/z = 212) was formed along with di-ethylthiophosphate (DETP, m/z = 169). Based on the metabolite analysis using GC-MS, the biodegradation pathway for chlorpyrifos degradation through bacterial–plant consortia is predicted. The constructed wetland with the bacterial–plant consortium showed its potential to either bypass TCP generation, or TCP may have been immediately biodegraded by the plant part of the consortium. The designed constructed wetland provided a novel remedial measure to biodegrade chlorpyrifos without producing harmful metabolites.

Keywords: chlorpyrifos, bioremediation, crop insects, constructed wetland, biodegradation pathway

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1 INTRODUCTION

Chlorpyrifos is a broad-spectrum pesticide, extensively used against pests in agricultural (cotton, grains, and fruits) and urban (lawns, commercial, and domestic buildings) settings. It belongs to the organo-phosphate class, under the chemical name O, O-diethyl O-(3, 5, 6-trichloro-2- pyridinyl)-phosphorothioate ($C_9H_{11}C_{l3}NO_3PS$) having low water solubility (2 mg/L) but soluble in organic solvents (Tariq et al., 2007).

Less than 1% of chlorpyrifos (CP) is applied to the target organisms, and most of the remaining chlorpyrifos ends up contaminating the atmosphere, soil, and water (Shi et al., 2019). Therefore,

long-term and irregular applications of chlorpyrifos have resulted in large-scale pollution of soil, groundwater, sediment, and air. It eradicates non-targeted organisms along with the targeted ones including fish, useful arthropods, plants, animals, and humans. Its exposure leads to acetylcholine accumulation leading to high irritation and nerve compression (Gilani et al., 2016). This nerve compression leads to seizures and finally death of insects and mammals. Furthermore, CP and its metabolites are associated with endocrine disruption (Ur-Rehman et al., 2021; Ramos et al., 2019). This non-targeted biocidal activity on these organisms may also be responsible for the loss of biodiversity and overall environmental quality deterioration (Eskenazi et al., 1999; Matthews, 2006; Benachour et al., 2007).

The half-life of CP in water is 50 days (Dores & De-Lamonica-Freire, 2001), while in soil it varies from 60 to 120 days, although it can deviate from 2 weeks to over 1 year, depending on the soil type, climate, and other environmental conditions (Uniyal et al., 2021). Therefore, it has been detected as the second most common pesticide in food and water (John and Shaika, 2015). In aquatic environments, its metabolites, for e.g., 3, 5, 6-trichloropyridinol (TCP) and diethyl chlorpyrifos (DEC) are found, among which TCP has been documented as more toxic, persistent, and mobile than its parent compound CP, by the US-EPA with a half-life ranging from 65 to 360 days in soil (El-Hellow et al., 2013; Sud et al., 2020). The long half-life and antimicrobial nature pose a hurdle for the complete remediation of CP through microorganisms, leading to the accumulation of TCP which results in the loss of soil biodiversity.

There are numerous methods available for detoxification of chlorpyrifos including chemical treatment (Rayment and Higginson, 1992), photodecomposition, volatilization, and incineration, but most of them are not applicable for complete removal of contamination at low concentration due to their inefficiency, expensive, and environmentally unfriendly nature (Abraham et al., 2013). In the past few years, physicochemical (advanced oxidation process) and biological treatment approaches have been widely employed for pesticide removal. Being a cost-effective and eco-friendly method (Walkley and Black, 1934), bioremediation (microbial and phyto-degradation) of environmental pollutants, especially pesticides have been a focus to improve environmental quality in general and soil quality in particular (Nandhini et al., 2021). Chlorpyrifos, previously shown to be resistant to enhanced degradation, has now been proved to undergo enhanced microbe-mediated decay (John and Shaike, 2015). Several bacterial genera, especially *Bacillus* and *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, *Sphingomonas*, and *Agrobacterium* sp. have been reported to biodegrade CP (Alizadeh et al., 2018; Anwar et al., 2009; Pino and Peñuela, 2011; Maya et al., 2011; Fulekar & Geetha, 2008; Yang et al., 2006). Among these reported bacterial species, *Acinetobacter* is reported as an efficient biodegrader of various organophosphates and is able to use those organophosphates as a sole carbon and energy source (Sabit et al., 2011). Therefore, microbial degradation is proven as a major factor determining the fate of organophosphate pesticides in the environment. In addition to microbial degradation, indigenous vegetation is reported to be involved in the degradation of CP. Plants may serve as a means to enhance the bioremediation process of contaminated soils as they can absorb and accumulate a variety of xenobiotics and metals from polluted soils and even degrade them, but the uptake process of organic pollutants and metals by plant roots is affected by several factors (Chandra et al., 2021). The herbaceous plants derived from local wetland species showed good growth when used to biodegrade chlorinated perchloro-ethylene (PCE) or its by-products (Avsar et al., 2007). Sometimes, the extended root system of plants in the soil apparently sustains microbial communities which are responsible for both anaerobic and aerobic biodegradative activity against such contaminants (Amon et al., 2007). These plants enhance the bioremediation process by release of exudates and enzymes such as carbohydrates, carboxylic acid, and amino acids that stimulate both microbial and biochemical activity in surrounding soil and mineralization of pollutants in rhizosphere soil (Tarla et al., 2020). The constant supply of carbon compounds from plant roots to rhizosphere microbes acts as fuels for complex interactions among rhizosphere organisms including those between microorganisms and plants (Daane et al., 2001). This consortium when exists as a land transition between terrestrial and aquatic systems, termed as wetland, plays an important role in the environment rehabilitation through natural decomposition or degradation (Terry and Bañuelos, 1999; Mejáre and Bülow, 2001). In addition to natural wetlands, lab-scale constructed wetland offers an option for *ex situ* bioremediation of contaminants, displaying a considerable potential to mitigate pesticide load including CP (Schulz and Peall, 2001). Plants in constructed wetlands also serve to stabilize the bed surface, increases porosity throughout the wetland volume for aerobic bacteria thriving in the soil, thus contributing toward the biodegradation process. Approximately, 92% removal of different pesticides from wastewater has been reported by Cooper et al. (2016) when a three-stage bio-bed was used as wetland. Retention capability was assessed by Schulz and Peall (2001) when no pesticide was detected in the outlet of a constructed wetland, while Gregoire et al. (2009) mentioned almost 80% removal efficiency for pesticide flux. Tang et al. (2019) reported a 98% CP removal with *Cyperus alternifolius*, *Canna indica*, *Iris pseudacorus*, *Juncus effusus*, and *Typha orientalis* in recirculating vertical flow constructed wetland systems. In a similar study, a constructed wetland (CW) cultivated with *Polygonum punctatum*, *Cynodon* spp., and *Mentha aquatic* showed approximately 98% removal efficiency of CP by Souza et al. (2017).

It has been shown that multiple bacterial species co-exist, not as isolated pockets of pure cultures, but as complex communities known as biofilms, which are capable of maximizing nutrient utilization and redox environments, etc., for example, some members of a community may convert plant exudates into a form available to other member of the community (Nottingham and Messer, 2021). Bacteria were isolated from the rhizosphere of *Phragmites australis* growing in the distillery effluent contaminated site mostly present in the lower region of roots, capable for the bioremediation of distillery wastewater contaminated sites (Chandra & Chaturvedi, 2002).

Taking into account the CP biodegradation process, the major CP metabolite, TCP, being antimicrobial and having a higher

water-soluble nature, makes it more mobile in various environmental matrices (Bose et al., 2021). Furthermore, the produced TCP, as a result of microbial biodegradation, in turn proves lethal for the biodegrading microorganisms resulting not only in decline in the biodegradation efficiency but also in the loss of diversity of soil microbial fauna (Abraham et al., 2013). In addition, studies concerning its fate and degradation in the soil are very limited. So, its further degradation is crucial to alleviate its concentration to prevent its magnification in the environment, thus mitigating the pollution and toxicity posed by TCP in particular and other metabolites of CP in general.

The CP biodegradation efficiency in a proto-type CW is influenced by many independent environmental factors such as bacterial species, nature of the pesticide, hydraulic retention time (HRT), and the plant species (deMatos et al., 2009; Tu et al., 2018). As reported by Romos et al. (2019) for pesticides with short aquatic half-lives, wetland systems require to exhibit much longer residence times (RTs). So, in the present study, the bacterial–plant consortium was used to 1) observe the potential of constructed wetland for bioremediation of CP and its major metabolites, especially TCP using the indigenous plant–bacterial consortium; 2) compare the biodegradation of chlorpyrifos (CP) and its metabolites using wetland vegetation alone and plant–bacterial consortium for possible biodegradation pathway analysis.

2 MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical-grade chemicals and reagents were purchased from Sigma-Aldrich, while commercial chlorpyrifos (48% w/v) was purchased from standard commercial suppliers. Minimal salt medium (MSM; pH 6.8–7.0) was prepared using the chemicals as described: dextrose, 1.0 g/L; K_2HPO_4, 7.0 g/L; KH_2PO_4, 2.0 g/L; sodium citrate, 0.5 g/L; $MgSO_4.7H_2O$, 0.1 g/L; and $(NH_4)_2SO_4$, 1.0 g/L (Parmar et al., 2014).

2.1.1 Soil Parameter Analysis

Different parameters of soil such as pH, soil nutrients, soil texture, and soil nitrogen contents were analyzed as described in the following sections.

2.1.2 pH Measurement

In a 100-ml bottle, about 10 g of air-dried soil was weighed, and 25 ml of distilled water was added and shaken for about 1 h. After shaking, the glass electrode was dipped in soil suspension and pH of soil was recorded (Ohtsu et al., 1994).

2.1.3 Organic Matter Determination

To the air-dried and sieved 1 g soil, 10 ml of $k_2Cr_2O_7$ solution (1 N) was added. Then, 20 ml of concentrated H_2SO_4 was added and mixed well, and the mixture was allowed to stand for about half an hour. On the other hand, 25–30 drops of diphenylamine indicator and 0.2 g of sodium fluoride were added to distilled water, and the solution was titrated with ferrous ammonium sulfate solution. A color change from dull green to brilliant green was noticed. A blank sample (without soil) was also run in the same way (Walkely–Black method).

2.1.4 Total Nitrogen

One gram of dried soil was shifted to a digestion tube along with 10 ml of H_2SO_4 and 5 g of catalyst mixture and heated to 100°C with an increase in temperature up to 400°C. The sample was noted, and the cooled sample was shifted into a distillation unit with 40 ml NaOH (40%) and 20 ml boric acid (4%) as an indicator, and any color change was noted. The distillate was titrated with sulfuric acid (0.02 N). A blank sample was also run using the same method (AOAC, 1995).

2.1.5 Total Phosphorous

Dried soil (2.5 g) was added to 0.5 g activated charcoal. To this mixture, 50 ml $NaHCO_3$ (0.5 M) solution was added and shaken for almost half an hour and filtered through the Whatman filter paper. The filtrate was acidified by adding H_2SO_4 (5N) and ascorbic acid with distilled water as an added solvent. A blank sample was also run with the similar conditions and compared with the test sample (Koenig & Johnson, 1942).

2.1.6 Determination of Potassium

Five gram of soil was weighed with 25 ml of ammonium acetate (NH_4OAc) solution, then shaken for five minutes, and filtered through the Whatman filter paper. Potassium extract was measured (Black, 1965).

2.2 Screening and Isolation of Chlorpyrifos-Degrading Bacteria

To isolate the potential bacterial strains, soil samples from selected chlorpyrifos-infested sites were collected from Kotli, Azad Jammu, and Kashmir-Pakistan (33.2896 oN 73.7414 oE). This particular field of choice has been exposed to continuous applications of chlorpyrifos for a considerable period of time. The soil samples were obtained from 5 to 10 cm layers below soil surface as described by Wang et al. (2021). One gram soil sample was mixed in distilled water (10 ml), and 1 ml of the solution was spread on the nutrient agar media through the spread plate method. The plates were incubated at 37°C for 24 h. Morphologically distinct colonies were selected and purified through the streak plate method.

2.2.1 Enrichment of the Bacterial Strains

Isolated strains were selected by subjecting them to the increasing concentrations of chlorpyrifos (250, 500, 750, and 1,000 ug/L). For this purpose, the isolated strains were grown in MSM medium (pH 7.0) on a shaker (IRMECO, I 3000, Germany) at 200 rpm for a period of 72 h as described by Abraham et al. (2013) with some modification. CP degradation was observed in the first set of experiments by bacterial isolates in minimal salt media (MSM) in different concentrations and combinations with CP as the sole carbon source along with positive and negative controls.

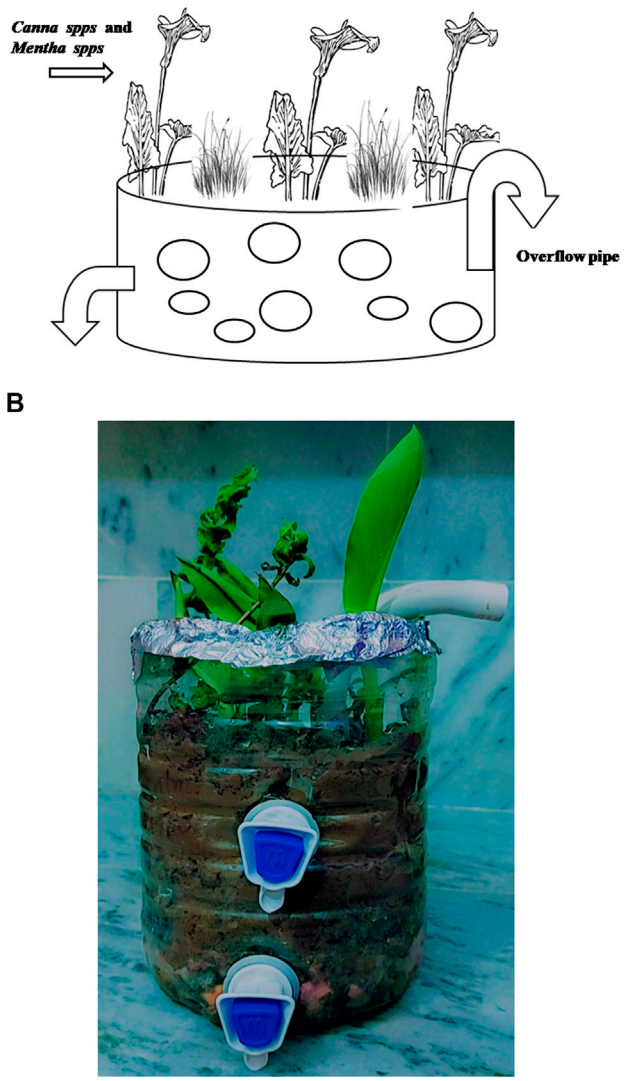

FIGURE 1 | (A) Proto-type constructed wetland. **(B)**. Execution of the prototype constructed wetland.

2.3 Proto-Type Constructed Wetland

A prototype wetland was constructed using pots filled with coarse and fine gravel, coarse gravel (20–30 mm diameter), fine gravel (2–10 mm diameter), sand, and soil from a specific site (**Figures 1A,B**). Prior to hand milling, soil was air-dried and screened through a sieve (2 mm pore size). The local plants, namely, *Canna* spps. and *Mentha* spps. (indigenous mint), were selected for constructed wetland (CW) establishment due to their ubiquitous abundance in the region, especially on the sampling site and along the river. The use of indigenous species is preferred as they do not pose any negative impact on micro-flora and has better survival chance (Farhan et al., 2021). A total of twelve (12) CW arrangements were maintained under different experimental conditions, namely, control, with isolated bacterial strains in soil alone, with plant and soil alone, and with a mix bacterial–plant consortium, and spiked with predefined CP concentrations.

2.3.1 Extraction of Chlorpyrifos From Soil

After a set retention time, the remaining chlorpyrifos was extracted from the CW soils using the toluene method as described by Reddy et al. (2013). A measured quantity of soil (12.5 g) was added to 20 ml toluene in 50-ml Teflon centrifuge tubes and kept on a horizontal shaker (IRMECO, OS 10, Germany) at 150 rpm for 4 h at 25°C. The tubes were then centrifuged at 4000 rpm for 10 min (Reddy et al., 2013). The resultant extract was filtered through anhydrous sodium sulfate (Na_2SO_4). For GC-MS analysis, 1 ml of toluene was added to the filtrate.

2.3.2 GC-MS Analysis for Chlorpyrifos Biodegradation

Degradation of chlorpyrifos to its metabolites was confirmed by GC-MS (Perkin Elmer, MS Claurus SQ 8S, GC Claurus 590, USA) equipped with a HP-5MS capillary column (30 m, 0.025 mm i.d) in helium carrier gas (1 ml per min) and with a splitless injection system. Initially, the column was maintained for 5 min at 90°C and then increased to 290°C at a rate of 8°C per min and held at 290°C for 5 min (Reddy et al., 2013). The injector and interface temperature were kept at 280°C and the source temperature at 250°C. A mass spectrum was obtained by the electron impact (EI) at 70 eV.

3 RESULTS AND DISCUSSION

3.1 Soil Analysis

Soil used in the constructed wetland was analyzed for different parameters such as saturation, pH, texture, organic matter, nitrogen content, phosphorous, and potassium. Soil saturation was 51% with basic pH (8.12) with a texture of clayey loam with 0.059%, 16, and 131 ppm of nitrogen, phosphorous, and potassium contents, respectively.

3.2 Isolation and Identification of Pesticide-Degrading Bacteria

In the present study, a bioremediation system was developed by isolating CP-degrading indigenous bacterial cultures, and their subsequent application in the indigenous plants using constructed wetland (CW). A total of two morphologically distinct CP-tolerant bacterial strains MBT035 and MBT037 were isolated and identified through ribotyping (16s rRNA) by Macrogen (Macrogen Inc., South Korea) as *Acinetobacter baumanni* and *Bacillus cibi*, respectively.

The CW establishment was applied in two combinations: a) soil and plants only and b) soil, plant, and bacterial consortium, to observe the effect of the bacterial isolate alone, plants alone, and in consortium. The CP removal efficiency in constructed wetland was analyzed by GC-MS, in which MS spectra of the extracted samples showed an effective biodegradation using constructed wetland when cultivated with indigenous *Canna* spps. and

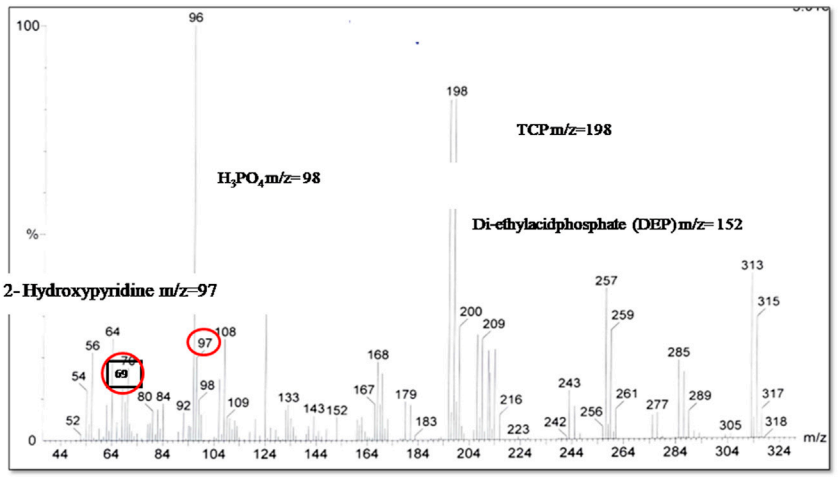

FIGURE 2 | GC-MS chromatograph for wetland with indigenous *Canna* spps. and *Mentha* spps.

Mentha spps. and bioaugmented with *Acinetobacter baumanni* and *Bacillus cibi* (CP-degrading bacterial isolates). By observing the chromatograph, it is evident that chlorpyrifos was metabolized to produce various intermediates of different m/z in various combinations of CWs, that is, plant alone and in the bacterial–plant consortium. The results are described in the following sections:

3.2.1 Wetland With Indigenous Plants

The wetland vegetation, *Cannas* spps. and *Mentha* spps., in a CW was used to monitor the biodegradation of CP through the phyto-degradation process. Several characteristic peaks of different metabolites of chlorpyrifos's biodegradation were observed in the GC-MS chromatogram. Among them, the most documented intermediate and degradation product, TCP (3, 5, 6-tricloro-2-piridinol) was detected, which was confirmed by the m/z peak of m/z = 198 (**Figure 2**).This product has already been documented in case of bacterial biodegradation by various researchers (Abraham et al., 2013; Zhu et al., 2019). But, it is also reported as a more toxic pollutant than the parent compound CP in the environment, especially for the microbes in the soil (antimicrobial agent). This may be a possible reason for resistance to enhanced microbial biodegradation for CP biodegradation. In the current study, TCP was also reported when indigenous plant vegetation was used. But, a good growth response to this toxic TCP was shown by the wetland vegetation. This toxic antimicrobial compound seemed either non-toxic to the indigenous plants or the plants might have coped with the toxicity posed by this particular intermediate of CP biodegradation. The study endorses the hypothesis of plant degradation potential for TCP.

Further degradative product, diethyl acid phosphate (DEP) with a molecular ion at m/z 152.98 $[M - 1]^-$ was also detected which was further metabolized into a molecule of m/z = 98, identified as H_3PO_4. These results were found in accordance with Shi et al. (2019).

TCP was further degraded to form 2-hydroxypyridine (m/z = 97) with deprotonated molecular ions at m/z = 69 (M-H)− as described by Uniyal et al. (2021).The results were found in accordance with Tang et al. (2019) who reported a decrease in the half-life of TCP when *Canna indica* was used as wetland vegetation with approximately 33 percent removal. These results showed efficient biodegradation of CP and its metabolites, especially TCP in proto-type constructed wetland when *Canna* and *Mentha* spps. were used.

3.2.2 Soil, Plant, and Bacterial Consortium

In the second phase of the experimentation setup of the plant–bacterial consortium, when wetland vegetation, i.e., *Canna* spps. and *Mint* spps., was used along with the indigenous bacterial strains, a CP metabolite with m/z = 212 was detected corresponding to TMP (3, 5, 6-trichloro-2-methoxypyridine). In addition to TMP, another metabolite (m/z = 169) was also detected and found out to be diethyl-thio-phosphate (DETP) a CP hydrolysis product mentioned by Bicker et al. (2005) in his studies (**Figure 3**). As described by Chen et al. (2012), the degrading microorganisms tend to metabolize chlorpyrifos by hydrolysis to form diethyl-thio-phosphoric acid (DETP) along with TCP. On the other hand, the bacterial–plant consortium showed good growth performance, depicting a good consortium establishment between plants and isolated strains (**Figure 4**). When metabolites of the biodegradation in both the experimental designs, i.e., wetland vegetation alone and plant–bacterial consortium, were compared, this TCP was not detected in plant–bacterial association rather TMP was detected. It is already described by Shi et al. (2019) that TCP can further generate TMP. The transient appearance of TCP and its subsequent degradation to TMP may be attributed to the mutual interaction and biodegradation ability of the plant–bacterial consortium. It may further be inferred from the GC-MS chromatogram that the formation of TCP may be either bypassed, or it was synthesized for a very short period of time and immediately biodegraded by the plant part of the

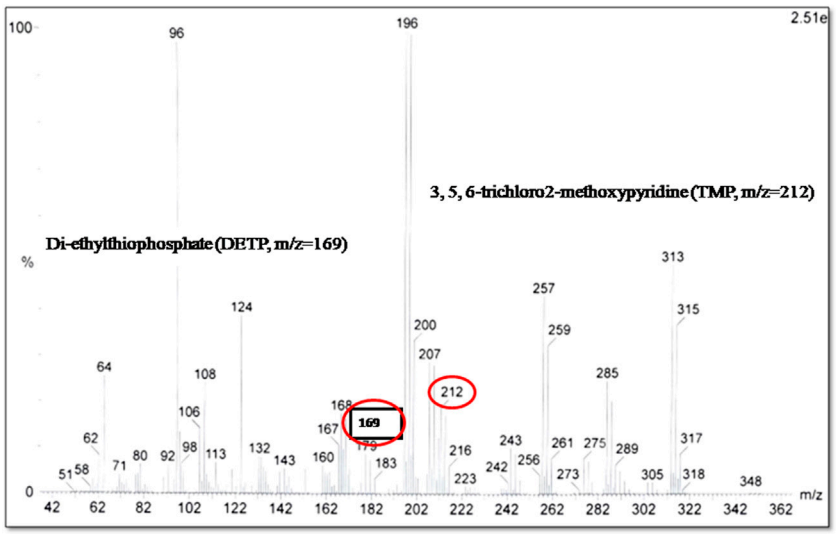

FIGURE 3 | GC chromatogram of the chlorpyrifos metabolites formed in the microbial–plant consortium.

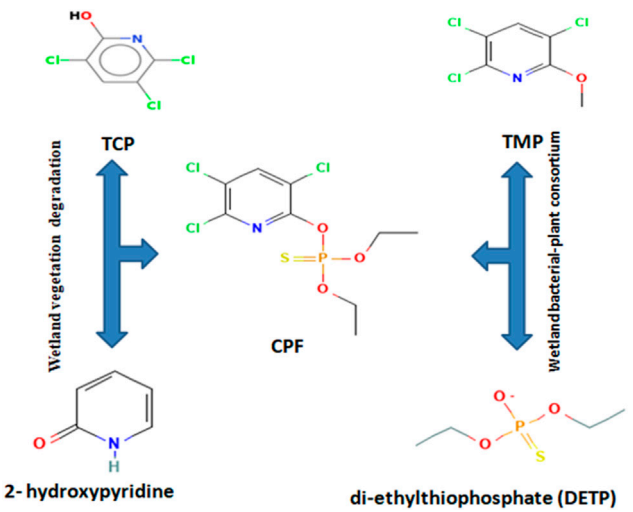

FIGURE 4 | Proto-type constructed wetland with indigenous bacterial strains and *Canna* and *Mentha* spps.

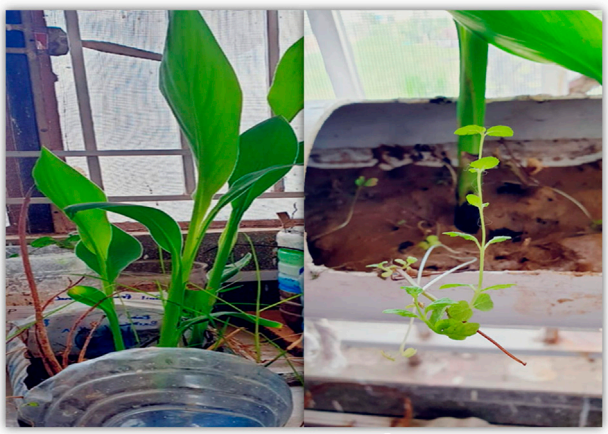

FIGURE 5 | Proposed biodegradative pathway for chlorpyrifos in wetland vegetation and bacterial–plant association.

consortium. The synthesis of TMP in place of TCP was found against the findings of Chen et al. (2012) as in most of the cases reported to date, the bacterial isolates tended to transform chlorpyrifos to yield TCP, which in turn accumulated in the batch cultures or soils and posed toxic effects on the microbial culture. So, the enhanced microbial degradation could not occur owing to its antimicrobial properties (Chen et al., 2012).

During the study, a good adaptation of the bacterial isolates to the applied CP was observed in the plant–bacterial consortium, since the bacterial number increased with increasing concentration of the pesticide to the soil. This could be reasoned to the absence of TCP in the bacterial–plant consortium which may be the reason for prolonged survival and good growth of the bacterial–plant consortium. An overall 96% of CP removal was observed in the bacterial–plant consortium. These results were found against the findings of Chen et al. (2012) who reported formation of TCP along with DETP.

Thus, the bacterial augmentation in the experimental soil with wetland vegetation eventually caused higher degradation, suggesting the compatibility of the augmented culture with the indigenous wetland vegetation. The dominant removal process was reported to occur through microbial degradation in wetland technology as described by Liu et al. (2019). In a similar study, Singh et al. (2003) observed maximum biodegradation of CP using *Pseudomonas putida* after 90 days in basic soils. These

results were also found in accordance with Souza et al. (2017) and Uniyal et al. (2021).

3.3 Proposed Biodegradation Pathway for CP

The insecticide CPF mostly undergoes hydrolysis to 3, 5, 6-trichloro-2-pyridinol (TCP), diethyl-thio-phosphoric acid (DETP), and negligible amounts of other intermediate products (Das and Adhya, 2015).TCP is a major metabolite of CP, and under all pathways, it is observed to undergo ring cleavage to form smaller organic and inorganic molecules as described by Sud et al. (2020). The degradation pathway of chlorpyrifos begins with cleavage of the phosphorus ester bond to yield 3, 5, 6-trichloro-2-pyridinol (TCP) (Lee et al., 2012). Based on the GC-MS analysis, the possible degradation pathway in case of wetland vegetation is proposed via hydrolysis as: CP (m/z = 358 > TCP (m/z = 198) >2- hydroxypyridine (m/z = **198)** > 69. While in case of bacterial–plant association, CP (m/z = 358)>TMP (m/z = 212>TCP-2H (m/z = 196) > DETP (m/z = 169) is suggested (**Figure 5**).

4 CONCLUSION

The present study was designed to observe the potential of constructed wetland using indigenous bacterial–plant association for the chlorpyrifos biodegradation. Therefore, a bioremediation system was developed by isolating CP-degrading indigenous bacterial cultures and their subsequent application in couple with indigenous plants using constructed wetland.

1. Two morphologically distinct CP-biodegrading bacterial strains *Acinetobacter baumanni* (MBT035) and *Bacillus cibi* (MBT037) were isolated and purified.
2. A pro-type constructed wetland (CW) with indigenous *Canna* spps. and *Mentha* spps. and bioaugmented with *Acinetobacter baumanni* and *Bacillus cibi* showed enhanced biodegradation of chlorpyrifos (CP) up to 96%.
3. TCP (3, 5, 6-tricloro-2-piridinol, m/z = 198) was observed when indigenous plant vegetation was used in CW which was further degraded to form 2-hydroxypyridine (m/z = 97) with de-protonated molecular ions at m/z = 69 (M-H)–.
4. In case of the bacterial–plant consortium, instead of TCP, a 3, 5, 6-trichloro-2-methoxypyridine (TMP, m/z = 212) was detected along with diethyl-thio-phosphate (DETP, m/z = 169).
5. The results of the present study showed good potential constructed wetland with *Canna* spps. and *Mentha* spps. and bioaugmented with *Acinetobacter baumanni* and *Bacillus cibi.*

5 FUTURE PROSPECTS

The current study promotes further research on plant–microbe joint combined remediation and examines the different behaviors. Following future prospects may be considered:

1. The fate of the other metabolites of CP and their toxicity may be monitored in the plants and animal species thriving in soil or environment.
2. Attempts could be made to optimize the bacterial–plant consortium for maximum and efficient bioremediation of the CP as well other environmental contaminants.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TA performed the experiments and wrote the manuscript. SR designed the experiments, administered the project, and wrote the manuscript. AS conceived the idea and designed the experiments. HN assisted in metabolite analysis. AF assisted in manuscript writing and editing. AJ assisted in the analysis of the results and review of the manuscript. SJ reviewed the manuscript.

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