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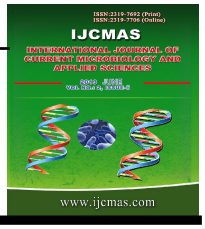


**Original Research Article**

# Degradation of Pentachlorophenol by a bacterial consortia and the effect of cured soil on *Phaseolus mungo* L.

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Biodegradation; Pentachlorophenol (PCP); Bacterial consortia; *Pseudomonas; Phaseolus mungo* L

**A B S T R A C T**

Chlorophenols are quite persistent in the environment causing serious pollution problems to surface and subsurface environments. In the present study, experiments were carried out in glass troughs with lower (0.075 %) and higher (0.15%) concentrations of pentachlorophenol (PCP). Biodegradation of PCP by the bacterial consortium in 50 days was evaluated. The treatment efficiency was measured in terms of reduction in PCP and phenolic carbon content. At low concentration of PCP (0.075 %), degradation was 62%, whereas, the degradation was found to be 43 and 42% for higher concentrations of PCP (0.15%) undergoing treatment in the presence and absence of nutrients. During the treatment period, the bacterial consortia utilized PCP as a substrate for growth, which resulted in the steady decrease of carbon content. Carbon utilization was found to be higher for low PCP (0.075%) than for higher concentration (0.15%). The treatment efficiency was measured in terms of the effect of treated samples on plant species *Phaseolus mungo*. The germination study shows that, the units treated by microbial consortium resulted in 100, 93 and 96% germination indicating the efficiency of the treatment.

## Introduction

Soil contamination due to the compounds of chlorophenols is very difficult to treat. Among the chlorophenols, pentachlorophenol (PCP) is quite persistent in the environment which causes serious pollution problem to the surface and subsurface environment (Berta *et al.*, 2007). PCP, an important class of compounds, is used in many industrial applications, and is found in the effluent industrial processes (Xie, 1986). PCP was formed during the main process of chlorine bleaching of paper and subsequently released into the environment (Parker *et al.*, 1993). Asian countries are the largest manufacturers of pesticides. Usage of chlorinated hydrocarbon pesticide contributes considerable release of PCP in the environment (Mathur, 1999). This

enormous application of pesticide contributes considerable amount of dichlorophenol contamination in the agricultural fields. The enzymatic chlorination of naturally occurring organic matter may also be responsible for chlorophenols occurrence in the environment (Holdin, 1991). Chlorophenols are hazardous to the environment and PCP has toxic effect on plants and embryos in animals even at lower concentration (BMZ, 1996). Hence, it is necessary to treat the pentachlorophenol before it reaches the environment and contaminate it. Among the many alternatives tried out to treat pentachlorophenol, remediation using microorganisms have shown to be an efficient and cost effective treatment method (Nakagawa *et al.,* 2006; Man-Jin *et al.,* 2007 and Gregorich and Carter, 2007). In the present study, the contaminated soil was treated using a bacterial consortium for a period of 50 days and the treatment efficiency was evaluated by its effect on the plant species *Phaseolus mungo* L.

## Materials and Methods

### Isolation of bacterial consortia

In the present study, 25 bacterial strains (pure culture) were isolated from various sites such as coal conversion, petroleum refineries and agriculture fields. They were screened for their ability to grow on pentachlorophenol as sole carbon source. Out of these, 6 strains were selected based on their growth in the PCP supplemented mineral medium. The selected strains were used as mixed culture for the experimental work. The soil for this study was collected from an agricultural land which was artificially contaminated with pentachlorophenol. The bacterial count was done by standard plate count method on nutrient agar and different bacterial colonies were identified.

### Experimental setup

A bench-scale biotreatability study to evaluate the efficiency of solid-phase bioremediation to treat pentachlorophenol-contaminated soil was done in circular glass troughs (size 30-cm diam 14-cm ht). For the experimental work, 0.75 g/kg (0.075%) and 1.5 g/kg (0.15%) of pentachlorophenol (PCP) were used. Nutrients amendment with ammonium nitrate (4.8g/1000g) and dipotassium hydrogen phosphate (0.86g/1000 g) was done to one set of treatment with higher concentrations of pentachlorophenol. In each trough, 1 kg of soil was taken. The experiments were carried out as per the details given in Table 1. Bacterial consortium at $10^6$ cells/g of soil was inoculated in all the troughs except in those marked as control. The experiment was carried out in duplicates for a period of 7 weeks. During the experimental period, the moisture level was maintained at 15-20%.

### Sampling and chemical analysis

Sampling was done once in a week and samples were analyzed for chloride release, pentachlorophenol content, phenolic carbon and microbial count. Chloride was determined by silver nitrate method. The pentachlorophenol was extracted from the soil and was estimated at 240 nm as reported by Mullar *et al.,* (1991). Hexane was used as blank. For the analysis of phenolic carbon (hexane fraction) in the soil, the pentachlorophenol extracted in the organic phase was re-extracted with 0.5 N $NH_4OH$ and then analysed using TOC analyser (Wang *et al.,*

2010). The soil nutrient status was analysed as per Trivedi and Goel (1986).

### Plant studies

About 20 seeds of *Phaseolus mungo* L. were grown on 250 gms of remediated soil to verify the efficiency of PCP degradation by bacterial consortium. Experiments were carried out for a period of 40 days. During the experiment, germination percentage of seeds in different concentration was calculated and after 40 days of experiment, plant biomass and leaf chlorophyll content were estimated by following the reports of Hiscox and Israelstam, (1979).

## Results and Discussion

### Pentachlorophenol degradation

Figure.1 depicts pentachlorophenol utilizing profile of bacterial consortia during the period of study. From figure 1, it is evident that the utilization of PCP was found to be higher in low concentration experimental setup than the high concentration experimental setup which undergoes biological degradation. PCP showed reduction at low concentration from 0.75g/kg to 0.28g/kg in 50 days. This reduction accounts for 64% from the initial value. At high concentration of PCP used the bacterial consortium showed reduction from 1.5 g/kg to 0.85 with nutrient and 0.87 g/kg without nutrients. This reduction of PCP accounts to 43 (with nutrients) and 42% (without nutrients). The PCP reduction for the control unit was found to be 27% for low concentration (0.017%) and 10% for high concentration (0.15%). From the results of PCP utilization, it was clear that the increase in concentration of PCP decreases the utilization by bacterial consortia (Fig. 1). Similar to our study, Rhadehaus and Schmidt (1992) have reported that the increase in concentration of chlorophenols resulted in the decrease in the rate of degradation. The bacterial consortium initially showed low degradation of PCP which may be attributed to the acclimatization of the consortium in soil to utilize PCP as sole carbon source. Middledrop *et al.,* (1990) have observed similar trend in mineralisation *by Rhodococcus chlorphenolicus* for low and high concentration of chlorophenol. When compared to the unit undergoing treatment, the control unit shows poor efficiency on degradation of PCP. It is due to the fact that, lack the specific group of efficient bacterial consortia responsible for the degradation of PCP in the control units. The nutrient addition has no significant impact on the utilization of pentachlorophenol.

### Bacterial growth pattern

Figure. 2 shows the growth pattern of bacterial consortium during the degradation of PCP contaminated soil. The slow initial increase of microbial population during the first week of degradation (lag phase) is observed which can be attributed to the acclimatization process. Then growth of bacterial consortia increased steadily and the trend continuos for a period of five weeks in all experimental setup inoculated with bacterial consortia. A decline in the growth of bacterial consortia was noticed after five weeks (Fig. 2). The declining phase of microbial growth leads to slow utilization of PCP during the final phase of degradation. The bacterial growth was inhibited in the control experimental unit. The microbial growth pattern of the control unit was found to be low which indicates the toxicity of PCP over the general group of bacterial consortia. It is

evident from the figure that, the microbial population was found to be higher for the low PCP concentration (0.075%) compared to high concentration of PCP (0.15%). From the data, it is clear that the higher concentration of PCP inhibits the growth of bacterial consortium. The reduction in microbial population at higher concentration of PCP (0.15%) was due to increasing level of toxicity. PCP is known for its inhibition on the growth of microorganisms Dwyer *et al.,* (1986). Short *et al.,* (1991) have observed that, the metabolite produced by genetically engineered microorganisms during 2,4-Dichlorophenoxy acetate degradation at 5000 ppm, 2,4 dichlorophenol completely inhibited the growth of fungi. In the present study, though a consortium of 6 strains was inoculated initially, the final analysis showed 4 strains only. From the isolated strains, 2 strains were belongs to *Pseudomonas* and the rest of strains belongs to *Bacillus* genus.

The soil was analyzed for the reduction in carbon content during the process of degradation of pentachlorophenol and the data was presented in figure. 3. It is evident that, the carbon utilization was found to be high for low PCP concentration (0.075%) compared to high concentration (0.15%). A decrease in phenolic carbon content could be due to the utilization of carbon present in the dichlorophenol as an energy source. For instance, in case of the experimental set up A1, utilization of carbon was from 202 to 78.9 mg/Kg. For high concentration of PCP, the utilization of carbon was from 405 mg/Kg soil to 222 (with nutrients-C) (B1and 231 mg/Kg soil (without nutrients-B1) respectively (Fig. 3).

During the utilization of dichlorophenol as the substrate for growth, there was a steady increase in the amount of chloride released in the soil (Fig. 4). It was evident that, the amount of chloride released from the experimental setup was corresponding to the degradation of PCP. The highest amount of chloride release for experimental set up A1 (0.075%) was 302-mg/Kg soil and the PCP degradation accounts for 62%. The lowest amount of chloride release was recorded (84 mg/kg soil) for the control experimental unit B1, where the PCP degradation was found to be low of 10%. Figure 3 and 4 are the indirect evidence for the degradation of pentachlorophenol. During PCP degradation, the bacterial consortium use PCP as an electron acceptor and degradation happens by intracellular enzyme. This process is called reductive dechlorination and it exposes PCP internal carbon for microbial utilization (Mohn and Kennedy, 1992; Nicholson *et al*., (1992) and Utkin *et al.,* (1995). As a result of dechlorination during biodegradation process, chloride is released. Among the experimental setup, the chloride release was found to be more in the experimental units with lower concentration of PCP (0.075%) indicating higher amount of PCP degradation. Similarly, the carbon utilization is also high for low concentration of PCP.

**Table.1** Experimental Details of Soil Contaminated with Pentachlorophenol

| **Sample Designation** | **Experimental condition** |
|---|---|
| A | 0.075% of PCP (control) |
| B | 0.15% of PCP (control) |
| A1 | 0.075% of PCP + bacterial consortium |
| B1 | 0.15% of PCP + bacterial consortium |
| C | 0.15% of PCP + bacterial consortium + nutrients |

**Figure 1.** Utilization of pentachlorophenol during bioremediation

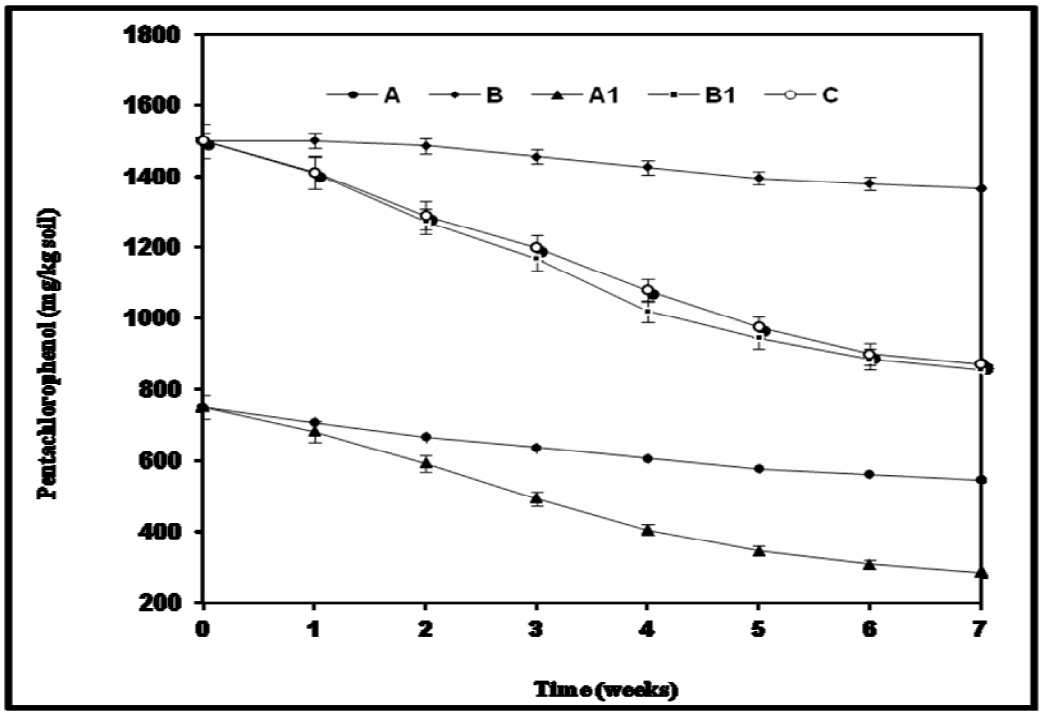


**Figure 2.** Growth of bacterial consortia with PCP as sole carbon source

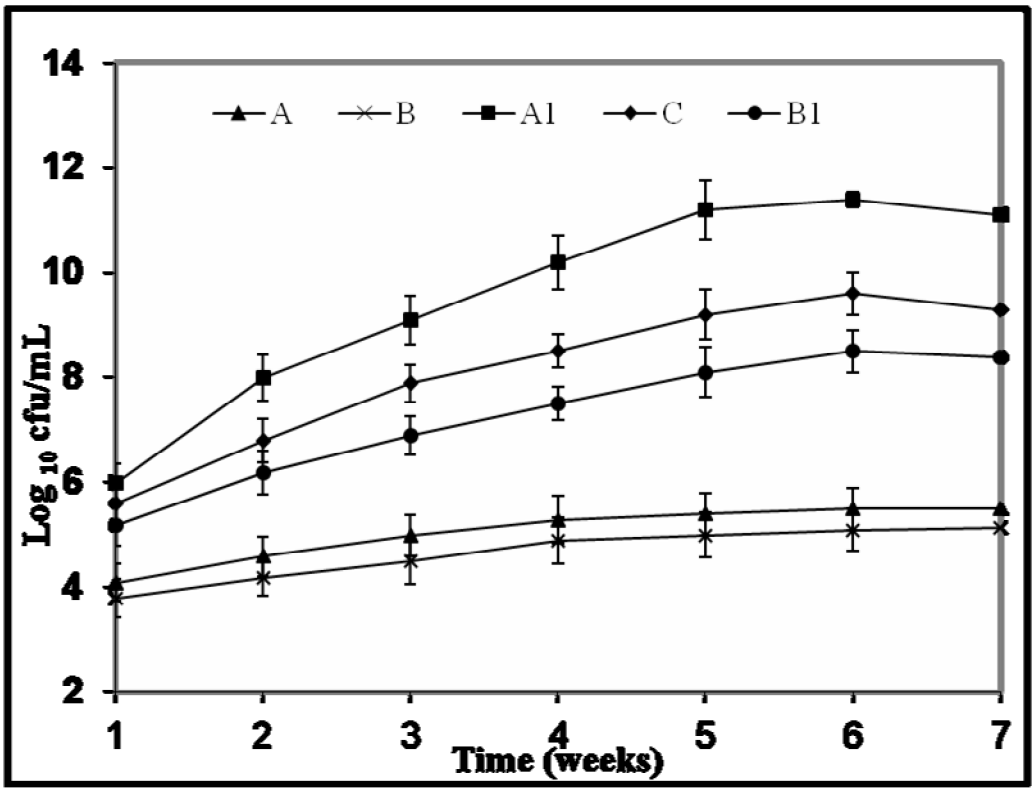

**Figure 3.** Utilization of phenolic carbon during bioremediation of PCP

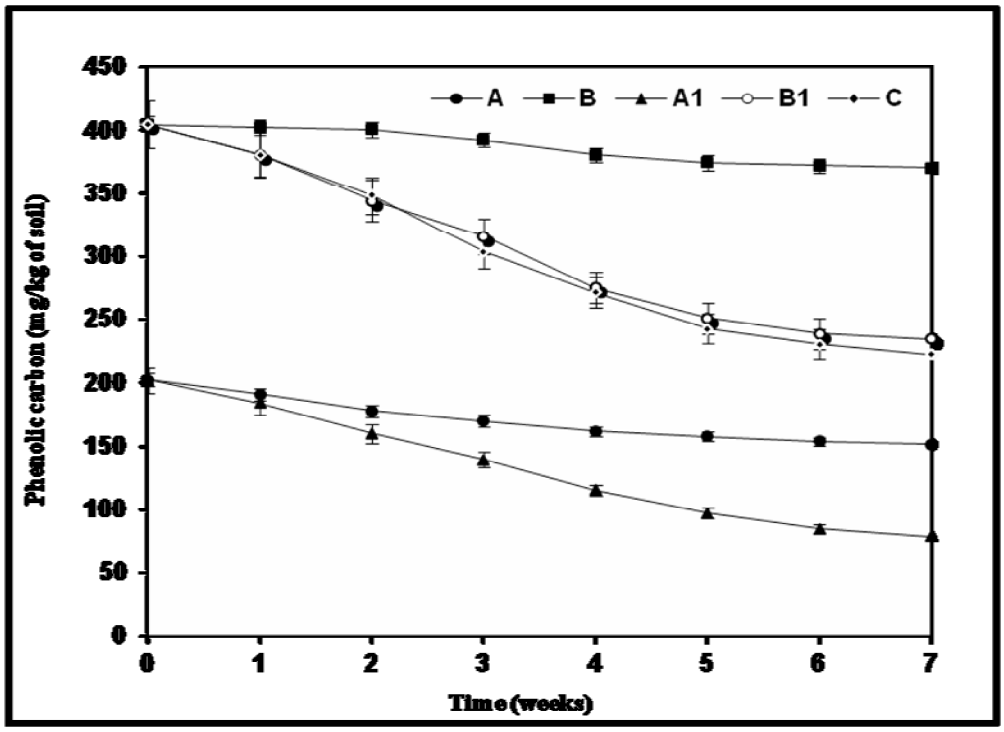


**Figure 4.** Chloride release during bioremediation of soil amended with PCP

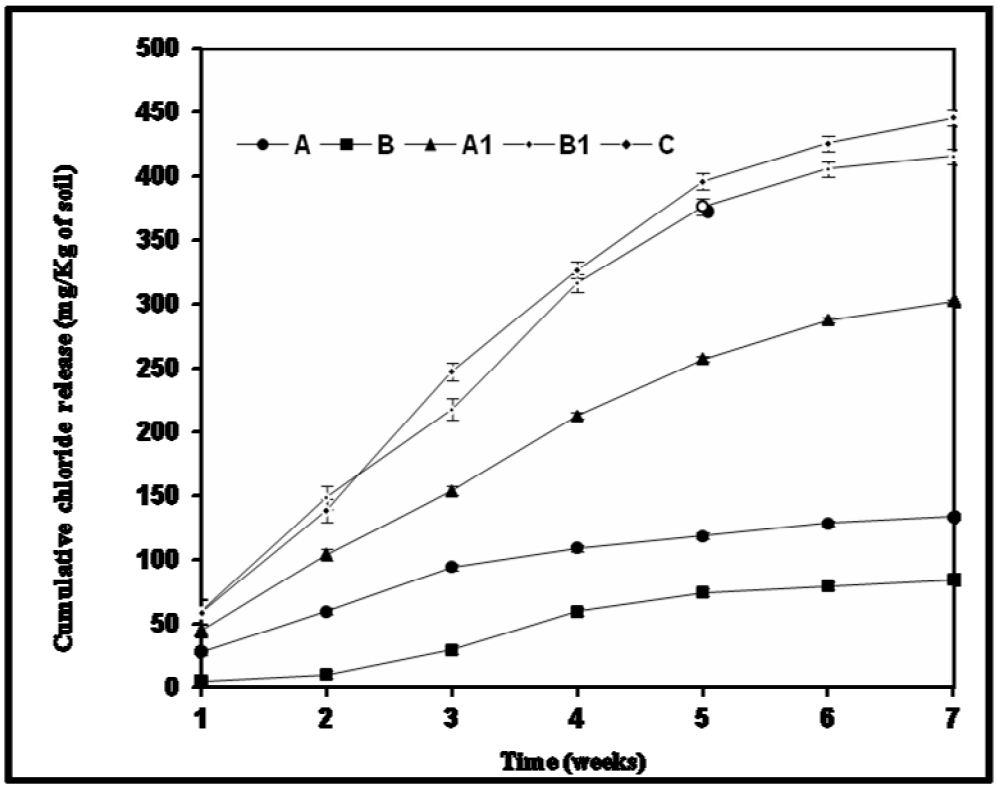

**Table.2** Soil nutrients status before and after treatment

| Sample | Before treatment | | After Treatment | |
|---|---|---|---|---|
| | Total Nitrogen (mg/kg) | Phosphorus (mg/Kg) | Total Nitrogen (mg/kg) | Phosphorus (mg/kg) |
| A | 276.40 | 0.0061 | 156.82 | 0.0060 |
| B | 277.10 | 0.0062 | 176.21 | 0.0062 |
| AI | 276.20 | 0.0061 | 30.10 | 0.0112 |
| BI | 275.10 | 0.0061 | 31.20 | 0.0114 |
| C | 394.00 | 0.0660 | 56.80 | 0.0300 |

**Table 3.** Plant growth analysis after bioremediation of soil

| Samples | Germination (%) | Chlorophyll (mg/g) | Fresh Plant Weight (g) |
|---|---|---|---|
| Plant Control Soil | 100 | 0.0052 | 0.0678 |
| PCP (0.075%) Control (A) | 0 | 0 | 0 |
| PCP (0.15%) Control (B) | 0 | 0 | 0 |
| PCP (0.075%) (AI) | 100 | 0.0034 | 0.0422 |
| PCP (0.15%) (BI) | 93.33 | 0.0028 | 0.0312 |
| PCP (0.15%) + Nutrients (C) | 96.60 | 0.0029 | 0.0386 |

Effect of bioremediation on soil nutrient status was shown in Table 2. It is evident from the table that the nitrogen content of the soil was decreased during the period of study. Among the experimental units, those inoculated with bacterial consortia (AI, BI and C) shows higher amount of decrease in nitrogen content when compared to those without microbial inoculation (A, B). In experimental set up A1 and B, nitrogen content decreases from 276mg/Kg to 28 and 45mg/Kg, and in case of A and B, the final content was found to be 156 and 176 mg/kg, respectively. The high utilization of nitrogen content in A1, B1 and C may be attributed to the higher colonization of microbial population. From the phosphorus data, it is clear that concentration was increased in all the treatment units except C, which decreases with increase in period of treatment.

The increase in phosphorus content in the soil after the study period could be attributed to the possible release of inorganic phosphates by the phosphorus solubilizing bacteria (Rajesh banu *et al*., 2005). The decrease in phosphorus content in nutrient amended soil treatment units C could be due to the ready availablity of nutrients form for the bacterial consortia.

After the treatment of soil with bacterial consortium, *P. mungo* L. was grown in the treated soil (Table. 3) which is used to find out the efficiency of treatment. Data from the table revealed that, in the uncontaminated soil the germination was 100% indicating there were no inhibitory substances. Whereas, in the control units A and B (0.075% and 0.15%) germination was not observed, which reflects the toxicity of PCP on *P.mungo* L. seeds. In experimental setup during degradation A1,

B1 and C, the germination was found to be 100, 93 and 96% respectively. The higher percentage of seed germination in the experimental units reflects in PCP toxicity reduction by the bacterial consortium. After 40 days period of study, the plant was analyzed for its weight and chlorophyll content. When compared to plant in control soil, a reduction in the chlorophyll content and plant biomass was observed in the experimental units A1, B1 and C. The study on the effect of cured soil on the plant species *Phaseolus mungo* L. clearly indicates the toxicity reduction by the bacterial consortia. When compared to plant control, even for the low concentration (0.075%) cured soil, the chlorophyll content and plant biomass weight was found to be low. This was due to the presence of residual PCP, which inhibits the plant growth.

From the foregoing, bioremediation appears to be a viable option for treating soil contaminated with chlorophenols. Bioremediation of pentachlorophenol contaminated soil could be enhanced with an additional mixed bacterial consortium to native soil. Addition of nutrient during bioremediation has no significant influence in the rate of bioremediation. Further studies focusing on the microbial dynamics, impacts of environmental factors and engineering aspects like reactor design would help to evaluate the process and its application which pave the way for pilot plant experiments.

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