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RESEARCH ARTICLE

A CONTEST OF EFFECTIVENESS BETWEEN CHEMICAL OXIDATION BASED REMEDIATION AND BIOREMEDIATION BY ENVIRO-FRIENDLY PAH-BIODEGRADER BACTERIA; AND STUDY THE EFFECT OF BOTH ON DEGRADATION OF PAH IN SOIL

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ABSTRACT

Polycyclic Aromatic hydrocarbons(PAHs) being a toxic xenobiotic aromatic compounds generated as a result of incomplete combustion of organic matter pose a direct threat to human health due to their mutagenic and carcinogenic nature. PAHs being non-polar have low solubility in water and hence are difficult to be eradicated by any means. A constant struggle of scientific community and incessant research has espied various methodologies to increase the rate of degradation of PAHs by modification of environmental factors. One of the most successful amongst the technologies is the process of Chemical Oxidation (specially by potassium permanganate which is also the device of this research) which has recently gained much recently recognition by the scientific community. The aim of this research is to find out whether Bioremediation of PAHs by ecofriendly PAH degrading Bacteria can be the new solution to the PAH problem. Also the study tries to find out the comparative testing of effectiveness of Bioremediation by PAH- Biodegrading bacteria over chemical oxidation.

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INTRODUCTION

Chemical oxidation is a rapid and commonly used soil and groundwater remediation technology and has proven to be effective for removal of many contaminants such as polycyclic aromatic hydrocarbons (Chen *et al*., 2009; Seol *et al*., 2003). Fenton's reagent (hydrogen peroxide and ferrous iron), ozone, persulfate ($S_2O_8^{2+}$) and permanganate (MnO_4^-), are the most commonly used oxidants (Chen *et al*., 2009; Doğan *et al*., 2013; Seol *et al*., 2003; Silva *et al*., 2009a; Sirguey *et al*., 2008). The investigations of Ma *et al*. (2013) showed that potassium permanganate acts as the most effective remediation oxidant compared to others such as hydrogen peroxide, Fenton's reagent, modified Fenton's reagent, activated sodium persulfate. Potassium permanganate was used in this study as an oxidising agent. Potassium permanganate (Mn^{7+}) reduces to manganese dioxide (MnO_2) and (Mn^{4+}), which precipitates out of solution (Chen *et al*., 2009). The hypothesis for this chapter was that potassium permanganate oxidation of PAH would be as efficient as microbial breakdown of PAH. Therefore, the main aim of Experiment (i), oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate was to examine the effect of potassium permanganate on the oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in sterile soil treated with and without potassium permanganate solution at pHs 5.0, 6.0, 7.0 and 8.0 in the J. Arthur Bower's top soil. The main aim of Experiment (ii), oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil was to compare the effect of potassium permanganate at pH 7.5 on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil inoculated with the roadside soil (Treatment A), microbial degradation in the soil inoculated with the roadside soil (Treatment B) and potassium permanganate oxidation in the sterile soil (Treatment C). See Table 1 for the objectives of each experiment.

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MATERIALS AND METHODS

As the research is divided into 2 experiments the section of paper is divided into two parts:

Table 1. Objectives of chapter 5

Oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate	To study the effect of permanganate (0.09 M) on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (250 $mg.kg^{-1}$) in the sterile soil in comparison to degradation of PAH in the sterile soil without permanganate at the four pHs (5.0, 6.0, 7.0, and 8.0)
	To investigate the effect of pH on permanganate (0.09 M) oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (250 $mg.kg^{-1}$) in the sterile soil at the four pHs (5.0, 6.0, 7.0 and 8.0)
Oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil	To monitor the effect of permanganate (0.09 M) on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (91 $mg.kg^{-1}$) in the soil inoculated with roadside soil at pH 7.5 (Treatment A)
	To investigate the biodegradation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (91 $mg.kg^{-1}$) in the soil inoculated with roadside soil but without permanganate at pH 7.5 (Treatment B) To study the effect of permanganate (0.09 M) on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (91 $mg.kg^{-1}$) in the sterile soil without inoculation of roadside soil at pH 7.5 (Treatment C) To monitor the bacterial growth during permanganate (0.09 M) oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (91 $mg.kg^{-1}$) at pH 7.5 (Treatment A) To examine the bacterial growth during biodegradation of the four PAHs (91 $mg.kg^{-1}$) in the soil at pH 7.5 (Treatment B)

Section 1: Experiment (i)

See Table 2 for the experimental layout.

Table 2– Experimental (i) layout

1. Measuring pH/WHC of experimental soil
2. Drying (90 ºC), sieving (2 mm) and sterilising (15 min, 15 psi, 121 ºC) soil
3. Filling a beaker with the prepared and sterilised Arthur Bower's top soil
4. Contaminating the prepared soil using PAH solution
5. Evaporating the n-hexane under a fume hood (48 hours)
6. Checking weight of a beaker containing the soil
7. Inoculating the soil with roadside soil
8. Transferring the soil from beaker into centrifuge tubes
9. Adjusting the soil water content to 30 % of the WHC
10. Adjusting pH of the soil using HCl or Na2CO3
11. Adding potassium permanganate solution
12. Incubating the centrifuge tubes (20 ºC for 144 hours)
13. Checking moisture content of all the treatments
14. Adding sodium bisulfite solution to stop the reaction at each time point
15. Sampling for HPLC (every 48 hours) and for bacterial enumerating (every week)
16. Extracting of PAH and enumerating of bacteria from the soil

Experiment (i), oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate: The effect of potassium permanganate on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the sterile soil in comparison to degradation of PAH in the sterile soil without permanganate at the four pHs (5.0, 6.0, 7.0, and 8.0) was studied. The pH and percentage water-holding capacity of the soil were measured as described below. (Ashish *et al*., 2016b)

Measurement of pH of the soil

The pH of J. Arthur Bower's top soil was confirmed by taking 5.0 g of the soil diluted with 10 cm^3 distilled water in a 50 cm^3 centrifuge tube. The suspension was vortexed for two minutes and left at 20^0C room temperature for 30 minutes. The pH of the supernatant was measured with pH probe and pH 7.0 was recorded (Kissel *et al*., 2010).

Measurement of Percentage water-holding capacity of the soil

100 g of the J. Arthur Bower's top soil was taken and then saturated with Milli-Q water. The soil was filtered, using 25 cm Whatman filter paper (no. 6) in a funnel. The wet filter paper was weighed first and after 15 minutes, the soil and filter paper were weighed again and the weight of a wet filter paper was subtracted. The water-holding capacity was then calculated as described below (Table 3) (Hagood *et al*., 2012).

Table 3. Determination of water-holding capacity in the J. Arthur Bower's top soil

- Mass of hilgard soil cup + Mass of filter paper + Mass of dried soil = 75.01 g
- Mass of hilgard soil cup + Mass of filter paper = 65.93 g
- Mass of dried soil = A-B = 09.08 g
- Mass of hilgard soil cup + Mass of filter paper + Mass of saturated soil = 81.52 g
- Mass of hilgard soil cup + Mass of filter paper = 65.93 g
- Mass of saturated soil= D-E = 15.59 g
- Mass of water content in saturated soil = F-C = 6.51 g
- Percentage of water-holding capacity = G/F*100 = 41.75 %

The soil moisture content for all experiments was then adjusted to 30 % of the water-holding capacity by adding

different volumes of Milli-Q (100g of Soil has 41.75 cm^3 of Milli-Q water for a 100% Moisture content hence for 30% Moisture content for 100 g of Soil Milli-Q water added must be 12.52 cm^3 respectively) The pH of the soil and water-holding capacity were 7.0 and 41.75 %, A beaker was filled with 200 g of the prepared soil as described below. (Ashish *et al*., 2016b)

Preparation of stock solution containing the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and method of contaminating the soil

The four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) used for soil contamination were phenanthrene, anthracene, fluoranthene and pyrene all purchased from Sigma, Steinheim, Germany. The PAH solutions were prepared by adding 50 mg of each phenanthrene, anthracene, fluoranthene and pyrene to a volumetric flask and then made up to 500 cm^3 with n-hexane HPLC grade (Sigma). This produced a stock solution of four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) with a concentration each of 100 mg. dm^{-3}. This solution was used to contaminate the soil. The experimental containers for the soil and PAH were mixed manually for 20 minutes to ensure equal distribution of the PAH in the soil. The soil container was weighed and placed under a fume hood for 48 hours to allow n-hexane to evaporate (Sirguey *et al*., 2008). The container weight was checked frequently until it reached pre-contamination level. The same method but different concentrations and volumes were used during each experiment. The soil was contaminated with 200 cm^3 of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) stock solution. Therefore, the final concentration of individual PAH in each beaker was 250 $mg.kg^{-1}$ (Table 4). The sterile soil contaminated with the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a)- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) was left under the fume hood for 48 hours to allow n-hexane to evaporate and the weight of beaker was checked to ensure the original weight was achieved.

The sterile soil contaminated with the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) was divided into 32 sterile 50 cm^3centrifuge tubes each containing 5 g of the soil. The pH of the sterile soil was adjusted individually in each tube as described in section 2.4 by adding different volumes of hydrogen chloride and sodium carbonate (Table 5). 0.09 M potassium permanganate solution (Brown *et al*., 2003) was prepared (Table 6). 10 cm^3 of the prepared potassium permanganate solution was added to each treated sample; whilst 10 cm^3 of sterile deionised water was added to the each control samples. The samples were incubated in a shaking incubator (70 rev/min) at 20 ºC until required for sampling. Moisture content was monitored every three days and water loss was compensated by addition of sterile Milli-Q water. One sample for each pH, with and without permanganate was removed from the incubator at 0, 48, 96 and 144 hours and 10 cm^3 of sodium bisulfite (Table 7) was added to stop the reaction; whilst 10 cm^3 of sterile deionised water was added to the controls and mixed well with a sterile spatula. The samples were centrifuged at 6,000 rpm for five minutes and the supernatant discarded. The soil was transferred into a weighing boat and air-dried in a fume hood for 48 hours. This experiment was replicated four times. PAH remaining in the soil samples were extracted from the soil by adding 1.5 cm^3 of acetonitrile solution containing 200 $mg.dm^{-3}$ of carbozole as an internal standard to 0.5 g of soil in Micro Centrifuge tubes (Table 8).

HPLC analysis of the samples in experiment (i)

Micro Centrifuge tubes were vortexed using a round table vortex for 15 minutes and then centrifuged for another 15 minutes. The solids in the Micro Centrifuge tubes were allowed to sediment prior to HPLC analysis. The standard solutions of PAH plus carbozole and experimental samples respectively, were injected into the HPLC machine (Fig. 1 – 7). Experiment (i) (oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate), HPLC analysis (standard chromatograms and standard curves) Standard HPLC chromatograms and standard curves were obtained of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) used at six different concentrations of 1, 20, 40, 60, 80 and 90 $mg.dm^{-3}$.

Table 4. Experiment (i) (oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate), the amount, volume and concentration of chemicals used for the soil contamination

Chemical	Chemical added in solution (mg)	n-hexane volume in solution (cm^3)	Chemical concentration in solution ($mg.dm^{-3}$)	Soil (g)	Chemical final concentration in soil ($mg.kg^{-1}$)
Phenanthrene	250	1,000	250	200	250
Anthracene	250		250		250
Fluoranthene	250		250		250
Pyrene	250		250		250

Table 5. Experiment (i) (oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate), pH adjustment of the soil

Soil (g)	0.09 M Potassium permanganate solution (cm^3)	1 M HCl (µl)	0.1 M Na2CO3 (µl)	pH
5.0	9.6	400	-	5.0
5.0	9.8	200	-	6.0
5.0	10.0	-	-	7.0
5.0	9.7	-	250	8.0

Table 6. Preparation of the 0.09 M potassium permanganate solution

Potassium permanganate (g)	Final volume of solution diluted with sterile deionised water (cm^3)	Potassium permanganate (M)
158.00	1000	1.00
14.22	1000	0.09
5.68	400.00	0.09

*5.68 g of potassium permanganate powder was weighed and added into 500 cm^3 Duran glass bottle and then mixed with 400 cm^3 distilled water to make a solution with the concentration of 0.09 M.

Table 7. Preparation of the 0.09 M sodium bisulfite solution

Sodium bisulfite (g)	Final volume of solution diluted with sterile deionised water (cm^3)	Sodium bisulfite (M)
104.06	1000	1.00
9.36	1000	0.09
3.74	400.00	0.09

*3.74 g of sodium bisulfite powder was weighed and added into 500 cm^3 Duran glass bottle and then mixed with 400 cm^3 distilled water to make a solution with the concentration of 0.09 M.

Table 8. Experiment (i) (oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate), preparation of standard solutions at the concentrations of 1, 20, 40, 60, 80 and 90 $mg.dm^{-3}$

Standard solution concentration ($mg.dm^{-3}$)	PAH standard stock solution with con. of 250 $mg.dm^{-3}$ (µl)	Carbozole stock solution with con. of 200 $mg.dm^{-3}$ (µl)	Acetonitrile (cm^3)
1	80	4500	Appropriate volume to make the solution up to 20 cm^3
20	1600	4500	
40	3200	4500	
60	4800	4500	
80	6400	4500	
90	7200	4500	

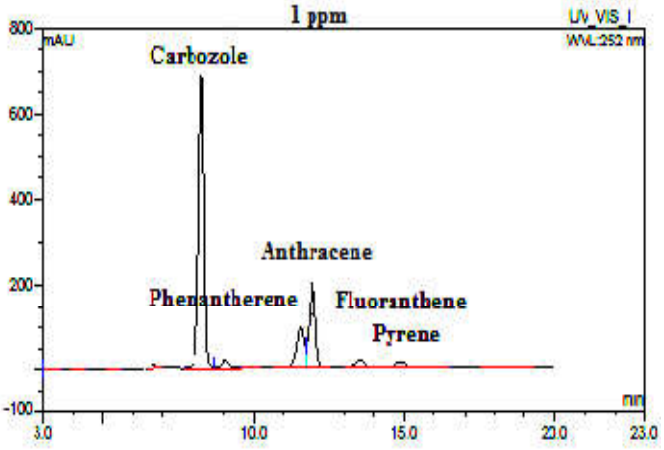

Figure 1. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 1 and 45 $mg.dm^{-3}$, respectively

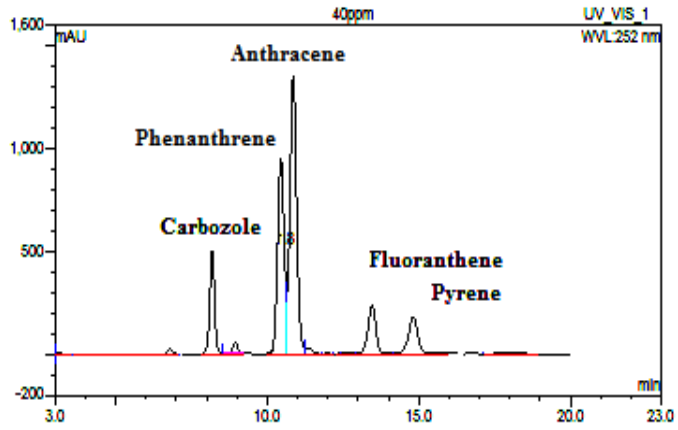

Figure 3. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 40 and 45 $mg.dm^{-3}$, respectively

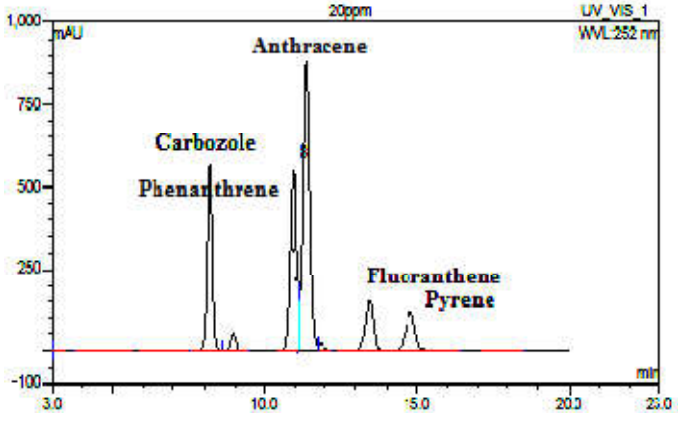

Figure 2 - HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 20 and 45 $mg.dm^{-3}$, respectively

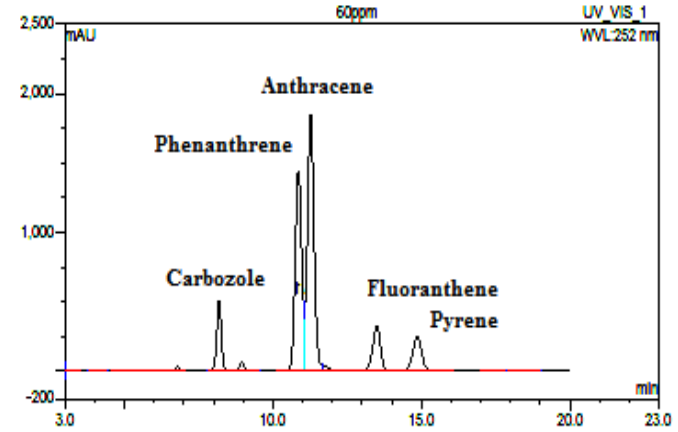

Figure 4. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 60 and 45 $mg.dm^{-3}$, respectively

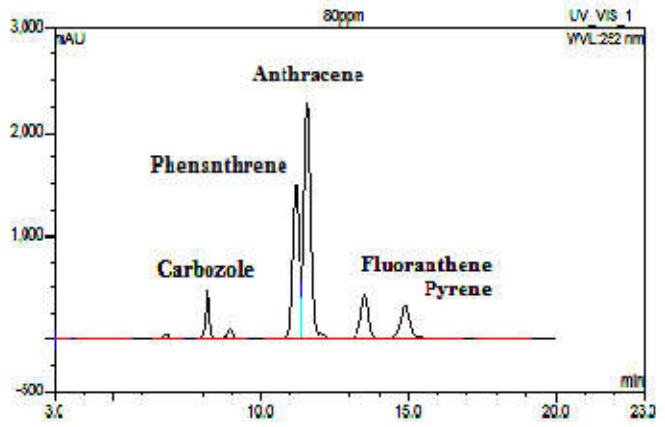

Figure 5. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 80 and 45 mg.dm^{-3}, respectively

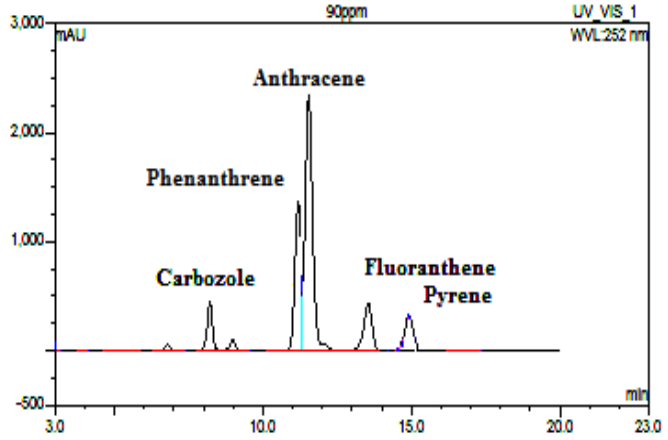

Figure 6. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 90 and 45 mg.dm^{-3}, respectively

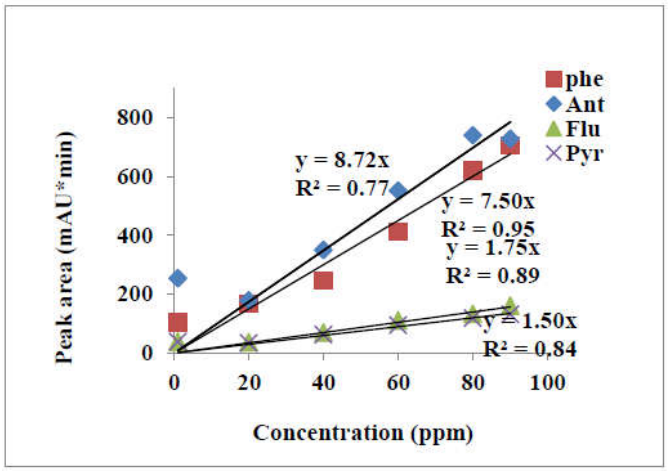

Figure 7. HPLC standard curves for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) showing peak area against concentration

Section 2: Experiment (ii)

See Table 9 for the experimental layout.

Table 9- Experimental layout

- Measuring pH/WHC of experimental soil
- Drying (90 ºC), sieving (2 mm) and sterilising (15 min, 15 psi, 121 ºC) the soil
- Filling a beaker with the prepared and sterilised Arthur Bower's top soil
- Contaminating the prepared soil using PAH solution
- Evaporating the n-hexane
- Checking weight of a beaker containing the soil
- Preparing 3 sets of treatments in 3 beakers of sterile prepared soil:
  - A - Potassium permanganate inoculated with roadside soil
  - B - Inoculated with roadside soil only
  - C - Potassium permanganate only
- Transferring the soil from beakers into centrifuge tubes
- Adjusting the soil pH and water content to 30 % of the WHC
- Incubating the centrifuge tubes (20 ºC for 35 days)
- Checking moisture content of all the treatments
- Adding sodium bisulfite solution to stop the reaction at each time point to treatments A and C
- Sampling for HPLC and bacterial enumerating (every week)
- Extracting of PAH and enumerating of bacteria from the soil

Experiment (ii), oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil: The effect of potassium permanganate on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil inoculated with the roadside soil (Treatment A), microbial degradation in the soil inoculated with the roadside soil (Treatment B) and potassium permanganate oxidation only (Treatment C) at pH 7.5 in the J. Arthur Bower's top soil was compared. The pH and percentage water-holding capacity of the soil were 7.0 and 49.67 %, respectively (Table 10).

Measurement of Percentage water-holding capacity of the soil

Table 10. Experiment (ii) (oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil), determination of water-holding capacity in the J. Arthur Bower's top soil

- Mass of hilgard soil cup + Mass of filter paper + Mass of dried soil= 76.29 g
- Mass of hilgard soil cup + Mass of filter paper= 67.13 g
- Mass of dried soil= A-B= 09.16 g
- Mass of hilgard soil cup + Mass of filter paper + Mass of saturated soil= 85.33 g
- Mass of hilgard soil cup + Mass of filter paper= 67.13 g
- Mass of saturated soil= D-E= 18.2 g
- Mass of water content in saturated soil=F-C= 09.4 g
- Percentage of water-holding capacity= G/F*100= 49.67 %

A glass beaker was filled with 250g of the dried and prepared soil as described above. The soil was contaminated with 227.5 cm^3 of the four PAH stock solution (Table 11).

Table 11. Experiment (ii) (oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil), the amount, volume and concentration of chemicals used for soil contamination

Chemical	Chemical added in solution (mg)	n-hexane volume in solution (cm^3)	Chemical concentration in solution ($mg.dm^{-3}$)	Soil (g)	Chemical final concentration in soil ($mg.kg^{-1}$)
Phenanthrene	50	500	100	2500	91
Anthracene	50		100		91
Fluoranthene	50		100		91
Pyrene	50		100		91

Table 12. Experiment (ii) (oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil), preparation of standard solution with the concentrations of 1, 5, 10, 15, 20, 25, 30 and 35 $mg.dm^{-3}$

Standard solution concentration ($mg.dm^{-3}$)	Volume taken from PAH standard stock solution with con. of 100 $mg.dm^{-3}$ (µl)	Volume taken from carbozole stock solution with con. of 100 $mg.dm^{-3}$ (µl)	Volume of acetonitrile (cm^3)
1	200	4000	Appropriate volume to make the solution up to 20 cm^3
5	1000	4000	
10	2000	4000	
15	3000	4000	
20	4000	4000	
25	5000	4000	
30	6000	4000	
35	7000	4000	

Therefore, the final concentration of individual PAH in a beaker was 91 $mg.kg^{-1}$. The same method, which is detailed in the Experiment (i), oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate was performed to evaporate the n-hexane. After n-hexane evaporation, 120 g of the soil was placed into two new and sterile beakers (Treatment A and C). 0.5 g of the roadside soil as inoculums was added only to treatment A to ensure the presence of oil degrading microorganisms for the degradation process. The prepared soils were transferred into 18 sterile 50 cm^3 centrifuge tubes each of which containing 5 g of soil (i.e. six time points plus three replicates equal to 18 tubes). Treatment B was set up with the same method as treatments A and C but without potassium permanganate. The pH of the sterile dried soil was adjusted to 7.5 by adding different volumes of hydrogen chloride and sodium carbonate. In Experiment (ii) (oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil), the volume of liquid which needs to be added into the soil to reach the purposed moisture content to 30 % of water-holding capacity and pH 7.5 was calculated as follows: 745 µl sterile distilled water – (30 µl Na2CO3 + 200 µl potassium permanganate) = 515 µl sterile distilled water.pH 7.5 was selected as an appropriated pH for the potassium permanganate oxidation according to previous studies in this thesis. Varying volumes of potassium permanganate solution, sodium bisulfite solution and sterile distilled water were calculated and added into each centrifuge tube individually for the both treatments A and C, respectively to provide the liquid content; whilst the same volume of sterile distilled water was added to the treatment B. All samples were incubated in the dark at 20 ºC. Moisture content was monitored every three days and water loss was compensated by addition of sterile Milli-Q water. Three samples were taken from each treatment every seven days for 35 days. The weights were checked to ensure the original weight before sampling was achieved. The reaction was stopped at each time point by adding 200 µl of sodium bisulfate to the treatments A and C; whilst 200 µl of sterile distilled water was added to the treatment B, which was not treated with potassium permanganate. Samples were mixed well with a sterile spatula to make a homogenised mixture. This experiment was replicated three times. PAH remaining in the soil samples were extracted from the soil by adding 1.5 cm^3 of acetonitrile solution containing 100 $mg.dm^{-3}$carbozole as an internal standard to 0.5 g of soil in Micro Centrifuge tubes. Micro Centrifuge tubes were vortexed using round table vortex for 15 minutes and then centrifuged for another 15 minutes. The solid in the Micro Centrifuge tubes were allowed to sediment prior to HPLC analysis. The standards and experimental samples were respectively injected into the HPLC machine. See Table 12 for the preparation of standard solutions.

HPLC analysis of soil according to Experiment (ii)

The mean values were calculated for all four replicates of samples and standard deviation quantified. See Figures 8 to 16 for the graphs of the HPLC standards and chromatograms.

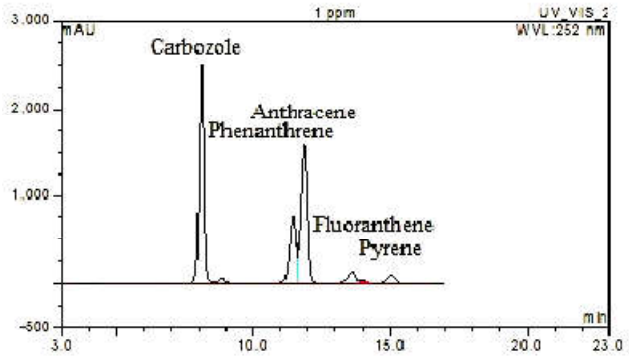

Figure 8. HPLC chromatogram for the four PAHs-Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al.*, 2016a) and carbozole at the concentrations of 1 and 20 $mg.dm^{-3}$, respectively

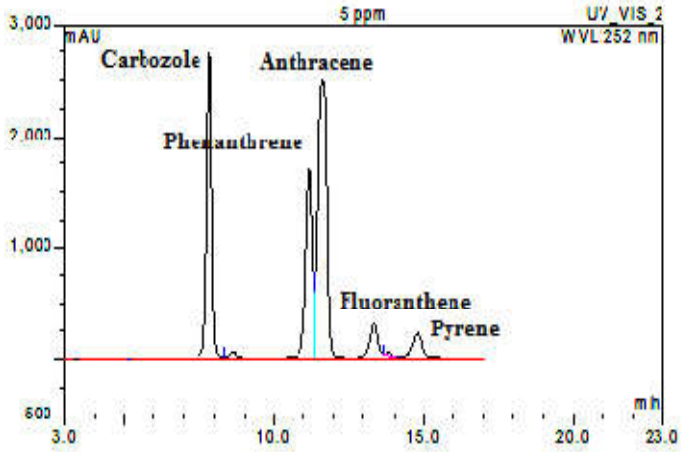

Figure 9. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 5 and 20 mg.dm^{-3}, respectively

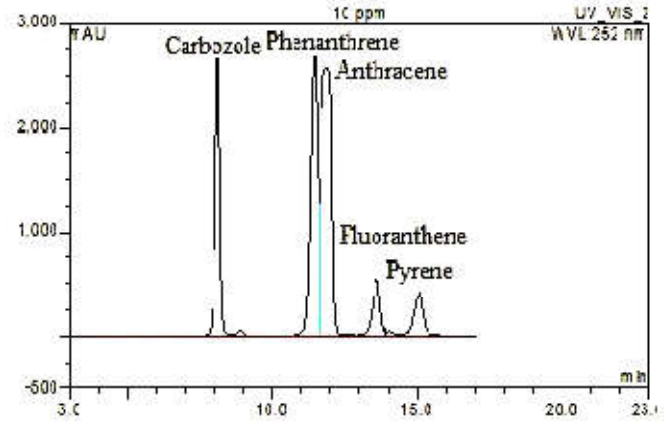

Figure 10. HPLC chromatogram for four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 10 and 20 mg.dm^{-3}, respectively

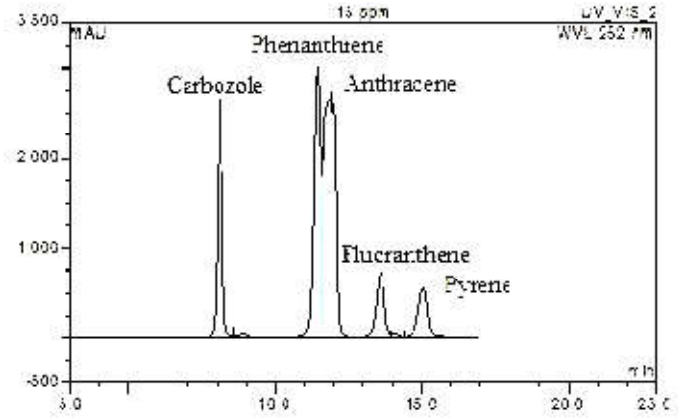

Figure 11. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 15 and 20 mg.dm^{-3}, respectively

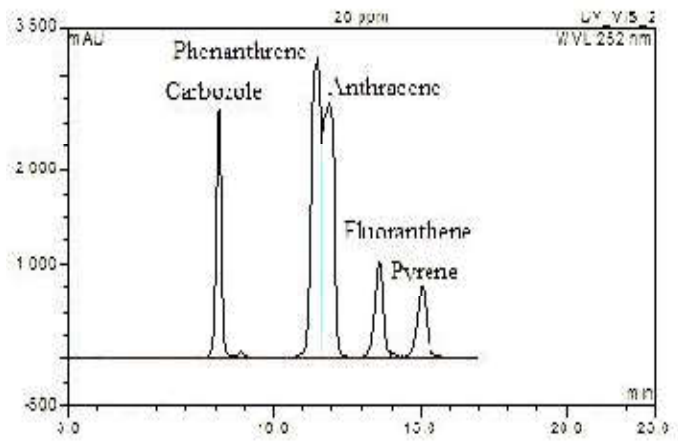

Figure 12. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 20 and 20 mg.dm^{-3}, respectively

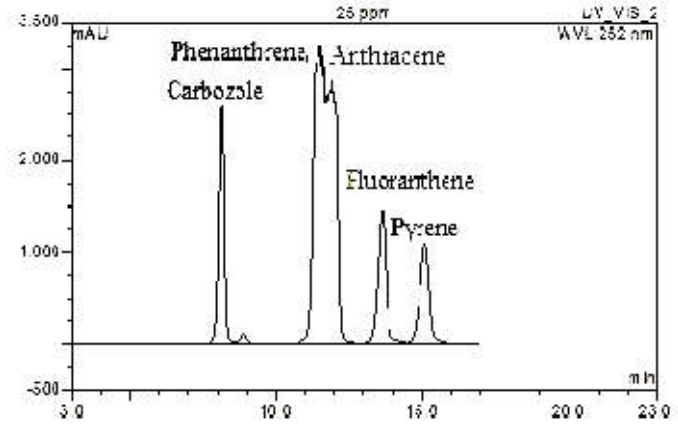

Figure 13. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 25 and 20 mg.dm^{-3}, respectively

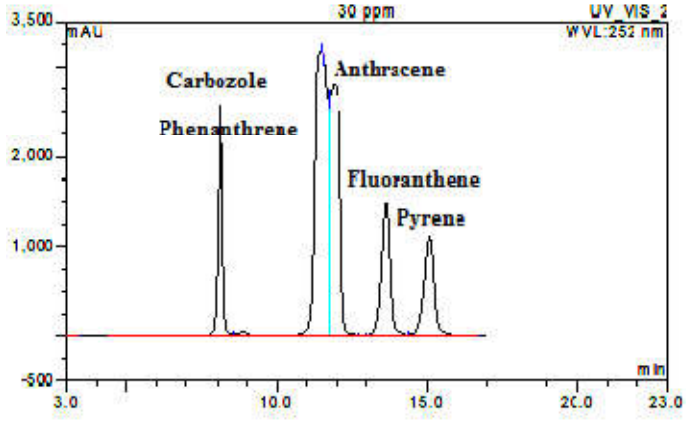

Figure 14. HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 30 and 20 mg.dm^{-3}, respectively

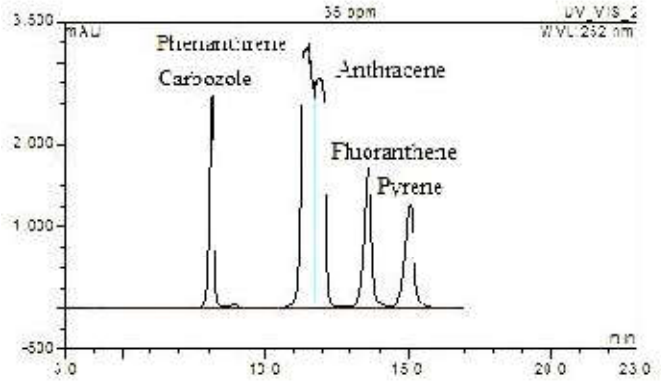

Figure 15 - HPLC chromatogram for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) and carbozole at the concentrations of 35 and 20 mg.dm^{-3}, respectively

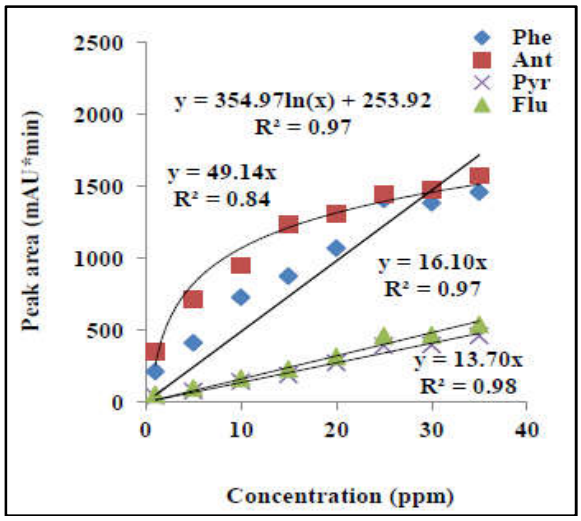

Figure 16. HPLC standard curves for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) showing peak area against concentration

Experiment (ii) (oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil), HPLC analysis (standard chromatograms and standard curves) Standard HPLC chromatograms and standard curves were obtained of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) used at eight different concentrations of 1, 5, 10, 15, 20, 25, 30 and 35 mg.dm^{-3}.

Bacterial Population Enumeration

Simultaneously, the bacterial populations were enumerated as described below.

The total bacteria extracted from the soil were enumerated via dilution series at varying time points to monitor the bacterial populations during degradation or oxidation process. Ringer's solution (Oxoid) was prepared according to the manufacturer's instructions. Universal bottles containing 9 cm^3 of Ringer's solution were prepared and sterilised by autoclaving. 1 g of the soil was taken by sterile spatula and added to 9.0 cm^3 of sterile Ringer's solution. Dilutions of 10-1, 10-2, 10-3, 10-4 and 10-5 were made up by adding 1 cm^3 of 10-n dilution and add to 9 cm^3 of autoclaved (15 min, 15 psi, 121 ºC) Ringer's solution to make up 10-(n+1) dilution. Nutrient agar (Oxoid) was prepared according to manufactures instructions (Table 13).

Table 13. Preparation of nutrient agar culture and its calculation

Table 13.1 Preparation of the nutrient agar culture	
Nutrient agar (11.2 g) powder was weighed and added into a 500 cm^3 glass bottle and then distilled water added to make up 400 cm^3 suspension. The bottle was autoclaved (15 min, 15 psi, 121 ºC). After allowing the bottle to get cool 20 mg of mycostatin/nystatin was measured and added to suspension and mixed well. The suspension was then poured into plastic Petri dishes and allowed to solidify.	
Table 13.2 - Calculation for preparation of the nutrient agar culture	
N.A (g)	Suspension (cm^3)
28	1000
11.3	400

Spread plates of dilutions 10-4 and 10-5 were prepared by adding 100 µl of each dilution to the Petri dish and spreading with a sterile glass spreader. Plates were incubated for 48 hours at 24 ºC and all colonies were counted after 48 hours (Asakawa and Hayano, 1995).

RESULTS

Experiment (i), oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate: The effect of permanganate (0.09 M) on the oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (250 mg.kg^{-1}) in the sterile soil in comparison to degradation of untreated control without permanganate in the sterile soil at the four pHs (5.0, 6.0, 7.0, and 8.0) was studied. Moreover, the effect of pH on permanganate (0.09 M) oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (250 mg.kg^{-1}) in the sterile soil at the four pHs (5.0, 6.0, 7.0 and 8.0) was investigated. Figures 17 to 20 indicated the percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil at varying pHs over 144 hours. Figure 17a shows that potassium permanganate caused some breakdown of phenanthrene; whilst as Figure 17b shows very little breakdown of phenanthrene in sterile soil.

Table 14. pH with the greatest and lowest degradation for the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in 0.09 M potassium permanganate treated samples and untreated controls. * Indicates significant difference (P<0.05)

Chemical	Potassium permanganate treated samples		Untreated controls	
	pH with the greatest degradation	pH with the lowest degradation	pH with the greatest degradation	pH with the lowest degradation
Phenanthrene	8 .0 *	5 .0	8 .0	5 .0
Anthracene	7 .0 *	5 .0	8 .0	5 .0
Fluoranthene	8 .0 *	5 .0	8 .0	6 .0
Pyrene	8 .0 *	5 .0	8 .0	5 .0

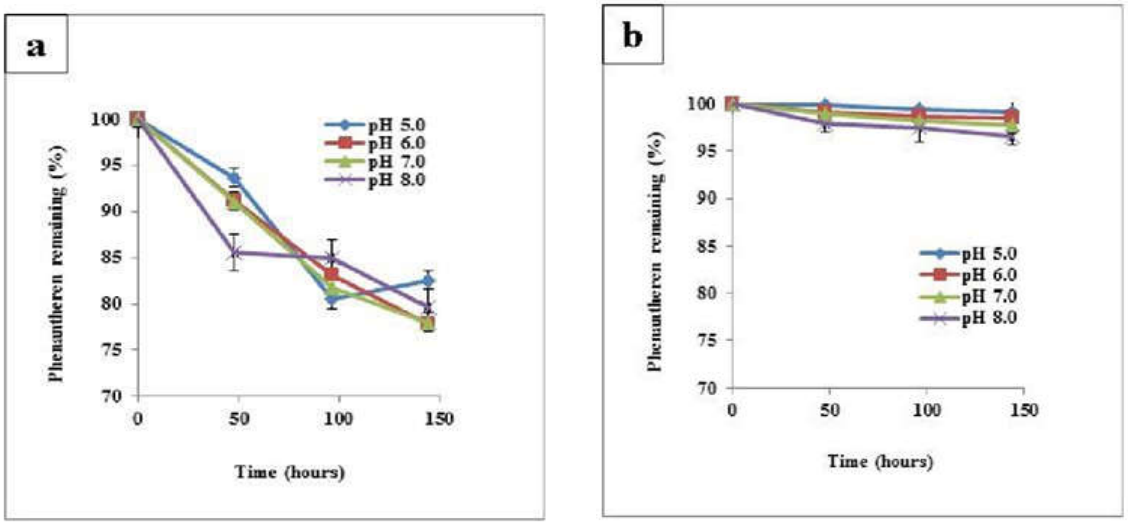

Figure 17. Percentage remaining of phenanthrene at varying pHs over time in the sterile soil. (a) Treated with 0.09 M potassium permanganate solution and (b) Untreated control (n=4 ± SD)

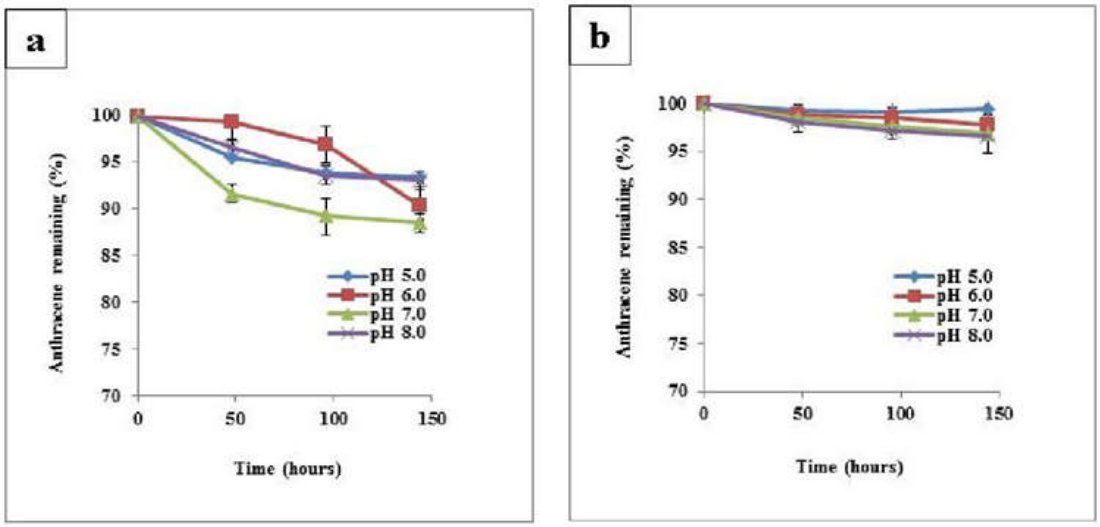

Figure 18. Percentage remaining of anthracene at varying pHs over time in the sterile soil. (a) Treated with 0.09 M potassium permanganate solution and (b) Untreated control (n=4 ± SD)

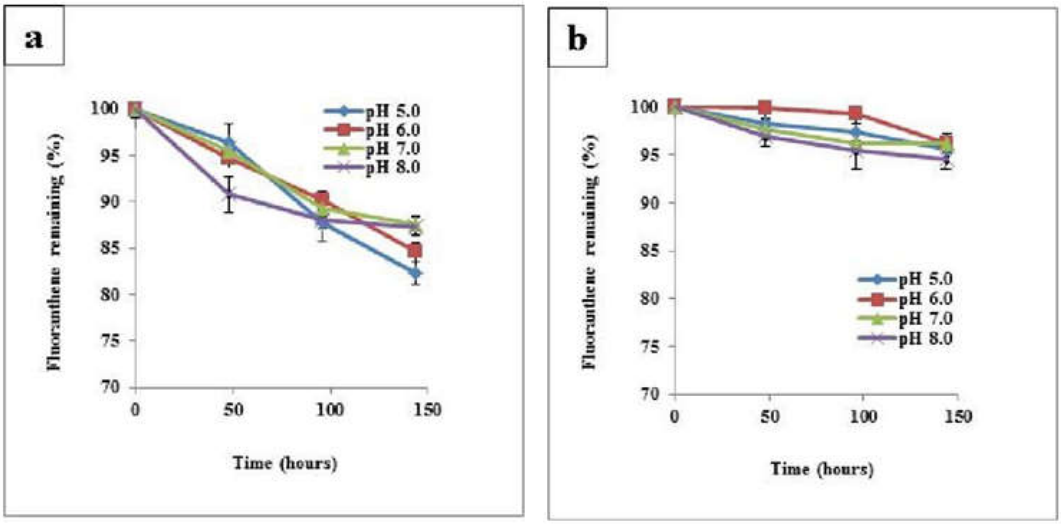

Figure 19. Percentage remaining of fluoranthene at varying pHs over time in the sterile soil. (a) Treated with 0.09 M potassium permanganate solution and (b) Untreated control (n=4 ± SD)

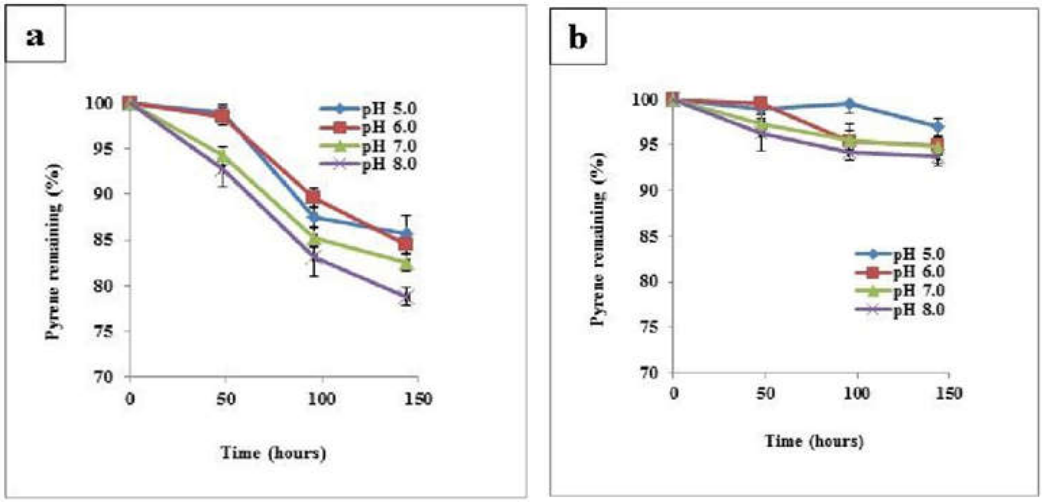

Figure 20. Percentage remaining of pyrene at varying pHs over time in the sterile soil. (a) Treated with 0.09 M potassium permanganate solution and (b) Untreated control (n=4 ± SD)

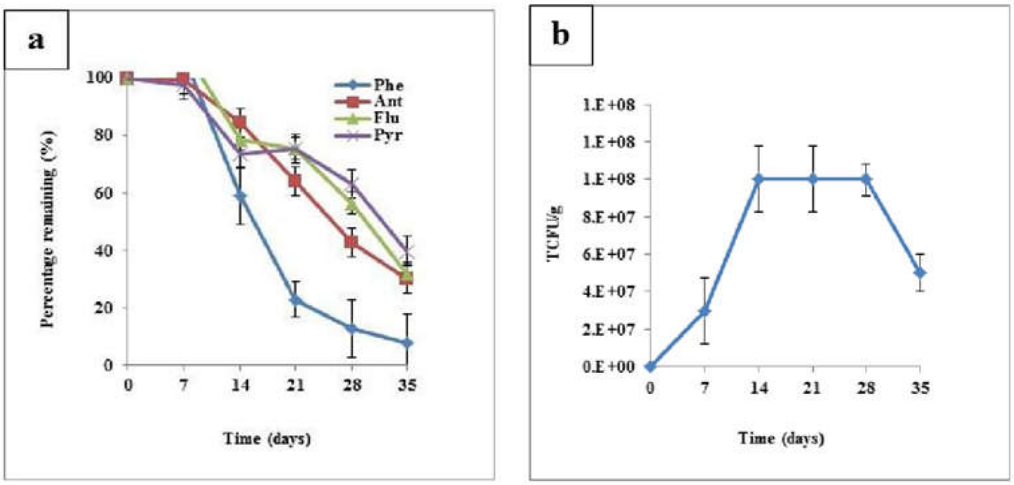

Figure 21. (a) Percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al.*, 2016a) over time in the soil inoculated with the roadside soil and treated with potassium permanganate at pH 7.5 against time (n=3 ± SD). (b) Total colony forming units of bacteria in the soil (TCFU/g) inoculated with the roadside soil and treated with potassium permanganate at pH 7.5 against time (n=3 ± SD)

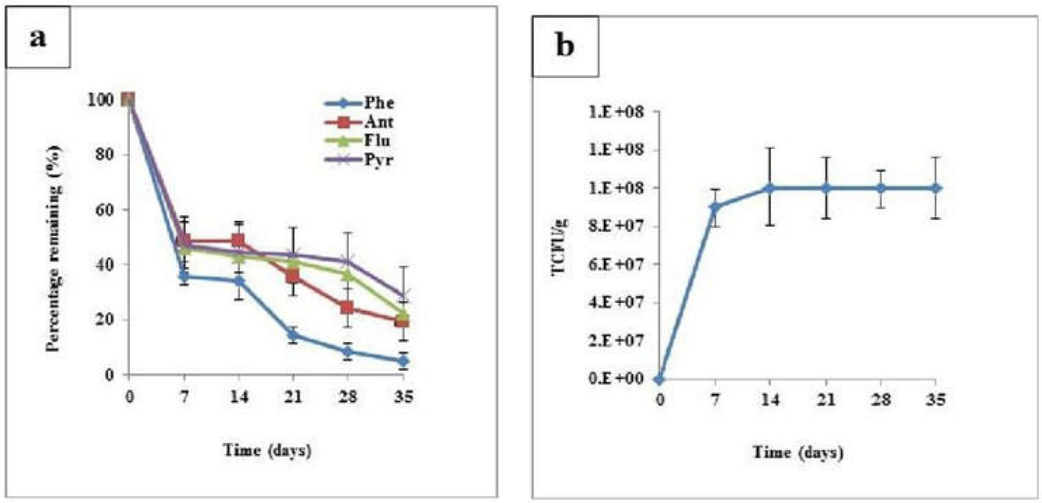

Figure 22. (a) Percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al.*, 2016a) over time in the soil inoculated with the roadside soil but without potassium permanganate at pH 7.5 against time (n=3 ± SD). (b) Total colony forming units of bacteria in the soil (TCFU/g) inoculated with the roadside soil but without potassium permanganate at pH 7.5 against time (n=3 ± SD)

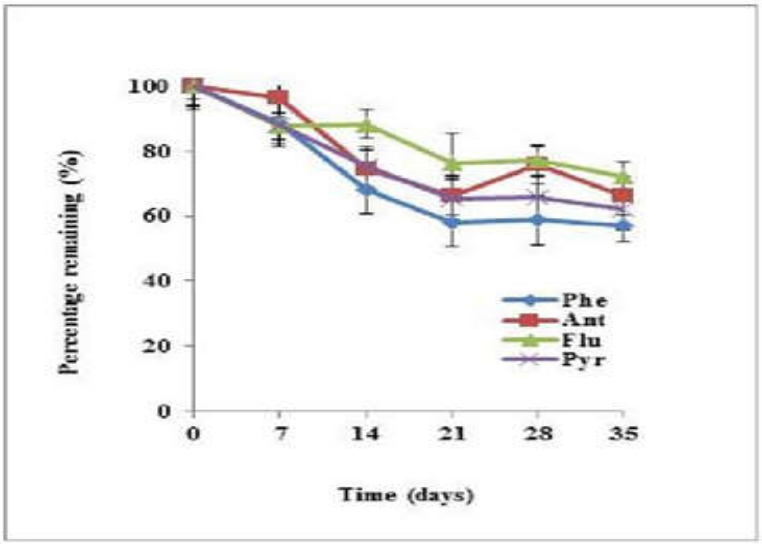

Figure 23. Percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) over time in the sterile soil without roadside soil inoculation, but treated with potassium permanganate at pH 7.5 against time (n=3 ± SD)

Table 15. Percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil on day 35 (n=3 ± SD). * Indicates significant difference (P<0.05) between the threetreatments

Chemical	Treatment A: Treated with both roadside soil and potassium permanganate (% remaining)	Treatment B: Treated withroadside soil but no potassium permanganate (% remaining)	Treatment C: Treated with potassium permanganate but no Roadside soil (% remaining)
Phenanthrene	7.58 *	5.05 *	57.26*
Anthracene	30.00*	19.23*	66.20*
Fluoranthene	31.96*	22.24*	72.30*
Pyrene	39.68	28.79*	61.85*

Experiment (ii), oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil: The effect of potassium permanganate on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil inoculated with the roadside soil (Treatment A), microbial degradation in the soil inoculated with the roadside soil (Treatment B) and potassium permanganate oxidation in the sterile soil (Treatment C) at pH 7.5 were compared. Figures 21a, 22 and 23a indicate the percentage remaining of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil at pH 7.5 over 35 days. Moreover, the effect of permanganate (0.09 M) and biodegradation on bacterial populations during oxidation and degradation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) (91 $mg.kg^{-1}$) at pH 7.5 was monitored in Figures 21b and 22b, respectively. Figure 21a, shows that there was a little degradation for the first seven days in presence of potassium permanganate. However, interestingly in Figure 22a there was a fast rate of degradation in the first seven days in the absence of potassium permanganate.

Figure 21a shows that phenanthrene was the most significantly ($p<0.05$) degraded PAH and pyrene was the lowest degraded PAH after 35 days. The figure shows there was a little degradation in the first seven days. However, the degradation of phenanthrene increased between days 7 to 14. The degradation process was continued to day 28. There was a little degradation between days 28 to 35. Statistical analysis (outsourced for statistical calculations) showed that there was a significant difference between the degradation of phenanthrene between treatments A and B on day 7. Interestingly, the degradation of PAH mirrored the bacterial number. Figure 21b shows that there was a buildup of bacteria in the first seven days, compared with Figure 22b, whereas there was a faster increase in bacterial number. Figure 21b shows that bacterial populations had reached up to 3.E+07 on day 7. The bacterial populations reached up to 1.E+08 on day 14. The bacterial populations were constant between days 14 to 28 and it was decreased to 5.E+07 on day 35. Figure 22a shows that phenanthrene was the most significantly ($p<0.05$) degraded PAH and pyrene had the least degradation after 35 days. Figure 22a shows that all the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) had a great degradation in the first seven days. However, phenanthrene had a slow degradation between days 7 to 14. The degradation process was continued to day 35.

Figure 22b shows that bacterial populations had reached up to 9.E+07 on day 7. The bacterial populations reached up to 1.E+08 on day 14. The bacterial populations were constant between days 14 to 28 and it was decreased to 1.E+08 on day 35 (Figure 22b). Figure 23 shows that the PAH had a little oxidation in the sterile soil without the roadside soil but treated with potassium permanganate at pH 7.5 after 35 days.

DISCUSSION AND CONCLUSION

Experiment (i), oxidation of PAH at different pHs in sterile soil in the presence or absence of potassium permanganate: Potassium permanganate oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the sterile soil at the four pHs were studied. Statistical analysis (outsourced for statistical calculations) showed that the treatment, which contained potassium permanganate had a significantly ($p<0.05$) greatest oxidation compared to controls without potassium permanganate. This indicated that oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the sterile soil was more effective in the presence of potassium permanganate compared to the sterile soil without potassium permanganate. Moreover, the greatest permanganate oxidation was obtained at higher pHs (7.0 and 8.0); whilst the lowest permanganate oxidation was found at lower pHs (5.0 and 6.0). This indicated that potassium permanganate oxidation has a greater effect on PAH oxidation at higher pHs rather than lower pHs. Investigations indicated that phenanthrene (Figure 17a) had the greatest degradation compared to the rest of PAH only in the presence of potassium permanganate. Experiment (ii), oxidation of PAH at pH 7.5 in sterile soil treated with potassium permanganate in the presence or absence of roadside soil: The effect of potassium permanganate on oxidation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil contaminated with the roadside soil (Treatment A), microbial degradation in the soil contaminated with the roadside soil and without potassium permanganate (Treatment B) and potassium permanganate oxidation in the sterile soil (Treatment C) at pH 7.5 was compared. Moreover, the effect of potassium permanganate (0.09 M) on oxidation and biodegradation of the four PAHs- Phenanthrene, Anthracene, Fluoranthene and Pyrene (Ashish *et al*., 2016a) in the soil was compared. Statistical analysis indicated that the treatment B had significantly ($p<0.05$) greatest degradation between the above three treatments on days 7 and 14. This part of the studies showed that the greatest degradation was found in the treatment B, inoculated with only the roadside soil microorganisms and without potassium permanganate (Figure 19). This suggested that potassium permanganate oxidation is not as effective as microbial degradation. Silva *et al*. (2009a) showed that potassium permanganate reduced PAH concentration in contaminated soil. Chemical reactions were studied as a rapid and commonly used soil or groundwater remediation technology (Silva *et al*., 2009a). Most PAH contaminated sites have a significant number of PAH degrading microorganisms. The bacterial populations are often limited by abiotic factors such as lack of aeration, bioavailability problems, and inadequate nutrients (Straube *et al*., 2003). Hence, though chemical oxidation was effective for removal of PAH it resulted in breakdown of soil organic matter and inhibited the bacterial populations (Chen *et al*., 2009; Silva *et al*., 2009a). Potassium permanganate had either inhibitory effect (direct oxidation of cell material or specific enzyme destruction) on microorganisms or it oxidised soil organic matter (oxidising agent) and therefore there were less microorganisms capable of growing and degrading PAH (Chen *et al*., 2009). The greatest degradation was found for the lowest molecular weight PAH phenanthrene and anthracene (Chapter 5). This indicated that the lowest molecular weight PAH degrades faster than the higher molecular weight ones due to higher solubility and greater bioavailability (Straube *et al*., 2003). The most degraded PAH was phenanthrene with percentage remaining of 7.58, 5.05 and 57.26 for the treatments A, B and C, respectively after 35 days (Table 15). The lowest biodegradation was found for the highest molecular weight PAH fluoranthene and pyrene. This might be related to the number of rings in PAH structure and their molecular weight. This may be due to stronger interactions between more hydrophobic and higher molecular weight PAH molecules and soil particles (Straube *et al*., 2003). The least degraded PAH was pyrene with the percentage remaining of 39.68, 28.79 and 61.85 for the treatments A, B and C at time 35, respectively (Table 15).

Through the in situ chemical oxidation, the best system to distribute the oxidants (e.g., potassium permanganate, hydrogen peroxide, and Fenton's reagent) is injecting/withdrawing it into a contaminated area (Seol *et al*., 2003). Moreover, a successful in situ oxidation is highly dependent on the heterogeneous distribution of oxidant. Seol *et al*. (2003) suggested that the best system for using chemical oxidation in situ is to use an injection/withdrawal system in the contaminated area. Oxidation reduces the PAH in contaminated soils but it may also have an effect on the soil quality. The impact of permanganate and Fenton oxidation on soil quality was investigated. Soil quality is restricted here to the potential for plant growth. Soil samples were collected from an agricultural field (S1) and a former coking plant (S4). Agricultural soil was spiked with phenanthrene and pyrene at two concentrations (S2: 700 mg phenanthrene/kg^{-1}, S3: 700 mg phenanthrene/kg^{-1} and 2100 mg pyrene/kg^{-1}). Soils were treated with both oxidation processes, and analysed for PAH. A plant germination and growth test was run with rye-grass on treated soils. Results showed that both treatments produced the expected reduction of PAH concentration (from 64% to 97%). Besides, a significant loss of organic C and N, and strong changes in available nutrients were observed. Permanganate treatment increased the specific surface area and the cation exchange capacity in relation to manganese dioxide precipitation. Plant growth was negatively affected by permanganate, related to lower soil permeability and aeration. Both treatments had an effect on soil properties (Sirguey *et al*., 2008).

The hypothesis for this chapter was that potassium permanganate oxidation of PAH would be as efficient as microbial breakdown of PAH. The results of this chapter disprove the hypothesis as microbial degradation of the PAH is shown to be significantly more effective than chemical oxidation with potassium permanganate.

REFERENCES

Asakawa, S. and Hayano, K. 1995. Populations of methanogenic bacteria in paddy field soil under double cropping conditions (rice-wheat): biology and fertility of soils, 20 (2), 113-117.

Ashish Pandey, Dr. Fanish Pandey "The Correlation of Shift of Ph Values on Bioremediation as Shown by (Polycyclic

Aromatic Hydrocarbon) PAH-Biodegrader Bacteria Isolated from Soil" *International Journal of Trend in Research and Development (IJTRD)*, ISSN:2394-9333, Volume-3 | Issue-2, URL: http://www.ijtrd.com/papers/ IJTRD3913.pdf(2016b)

Ashish Pandey, Dr. Fanish Pandey 2016. "A Study on Isolation, Characterization and Preparation of the Best Suited Microbial Formulation for Ecofriendly Bioremediation and Biodegradation of Polycyclic Aromatic Hyrdocarbons (Pahs) from Soil as Inoculum" *International Journal of Trend in Research and Development (IJTRD)*, ISSN:2394-9333, Volume-3 | Issue-1, URL: http://www.ijtrd.com/papers/IJTRD3898.pdf(2016a)

Brown, G. S., Bartonb, L. L. and Thomsonc, B. M. 2003. Permanganate oxidation of sorbed polycyclic aromatic hydrocarbons: Waste Management, 23, 737-740.

Chen, W., Hou, L., Luo, X. and Zhu, L. 2009. Effects of chemical oxidation on sorption and desorption of PAHs in typical Chinese soils: Environmental pollution, 157, 1894-1903.

Doğan, E., Accoe, F., Boon, N., Bastiaens, L. and Dejonghe, W. 2013. Impact of Chemical Oxidation on the Heavy Metals and the Microbial Population in Sediments. Water Air Soil Pollut, 224, 1386.

Hagood, M. A., Miller, D. E. and Larsen, E. C. 2012. Water holding capacities of Columbia basin irrigation project soils. Retrieved July 2, 2012, from http://ebookbrowse.com /water-holding-capacities-columbia-basin-irrigation-project-soils-pdf-d196987944

Kissel, D. and Miller, R. O. 2010. Cmparision of Soil pH Methods on Soils of North America: Nutrient Management & Soil & Plant Analysis, 74 (1), 310-316.

Ma, W. F., Guo, H., Ye, J. D., Han, D. M. and Ma, X. W. 2013. Removal Efficiency and Distribution Characteristics of PAHs in Coking Plant Contaminated Soils by In Situ Chemical Oxidation Remediation: Advanced Materials Research, 690-693, 1490-1497.

Seol, Y., Zhang, H. and Schwartz, F. W. 2003. A Review of In Situ Chemical Oxidation and Heterogeneity: Environmental & Engineering Geoscience, 9, 37-49.

Silva, P., Santos, E. C, Menezes, C. R., Faria, A. F., Franciscon, E., Grossman, A. and Durrant, L.R. 2009a. Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia: Bioresource Technology, 100, 4669-4675.

Sirguey, C., Silva, P., Schwartz, C. and Simonnot, M. 2008. Impact of chemical oxidation on soil quality: Chemosphere, 72, 282-289.

Straube, W. L., Nestler, C. C., Hansen, L. D., Ringleberg, D., Pritchard, P.H. and Jones-Meehan, J. 2003. Remediation of Polyaromatic Hydrocarbons (PAHs) through Landfarming with Biostimulation and Bioaugmentation: Acta. Biotechnol., 23, 179-196.

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