Effect of nutrients on disappearance time of anthracene in a river bed soil under ambient conditions

Kaabi Abdul, Dihingia Niloy and Sarma Prahash Chandra*
Department of Chemistry, Cotton University, Guwahati-781001, Assam, INDIA
*prahash.sarma@cottonuniversity.ac.in

Abstract

In an experiment to find out the disappearance time for 50 percent in days i.e. DT-50 of the polycyclic aromatic hydrocarbon anthracene from a known applied amount of 500 ppm concentration, it has been found that the process of disappearance follows first order kinetics. In a sandy soil having slightly alkaline pH (7.21), the rate constant of the reaction is found to be 0.0109 per day and the DT-50 is found to be 64 days.

On the other hand, in presence of an equimolar mixture of NPK nutrients at a concentration of 11060 ppm, the process of disappearance of anthracene is accelerated and the first order rate constant becomes 0.0205 per day. As such, the DT-50 comes down to 34 days. The experiment was spread over 90 days in a humid climate region and at a room temperature in the range of 23.3 – 39.1^{0}C.

Keywords: Anthracene, DT-50, HPLC, NPK, Polycyclic Aromatic Hydrocarbons, Sandy Soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a major class of potentially carcinogenic organic substances consisting of two or more fused benzene or with pentacyclic rings in linear, cluster or angular structure[6,11]. PAHs are the key pollutants of soil, water and air. The process of oil spills from tankers and refineries. Exploration of oil and burning of fossil fuels releases these pollutants into the aquatic environment[14]. PAHs bind to the particles of soils and sediments due to their hydrophobic nature that makes them less available for biological uptake and hinders the biodegradation of heavier compounds[3,10]. PAHs are categorized by their stability in the environment which helps them to accumulate in the soil for a long span of time and degrade with difficulty.

However, their disintegration depends upon a large extent of soil properties and the scope of their harmful effect on soil organisms which is directly related to soil type[9,12]. PAHs and their derivatives are associated with the serious risk to human health since many of them are toxic, carcinogenic and mutagenic[5]. Degradation of PAHs in soils is extremely affected by various environmental factors such as pH, salinity, temperature, PAH bioavailability, water availability, oxygen level, nutrient requirements of microbes as well as adaptation of the microorganism's population[2]. Long exposure to PAHs causes severe symptoms such as vomiting, eye irritation and nausea. Also, high concentration of PAHs can form skin inflammation, kidney and liver damage, can suppress immune reactions and an embryo toxic influences during pregnancy. It can also show carcinogenic, genotoxic, teratogenic and mutagenic effect. Thus, it is important to degrade the PAHs from the environment[1].

Material and Methods

Sampling: The soil/sediment used in this study was collected from a depth of 0–10 cm from five different locations in the direction of water current of the bed of river Brahmaputra in Goalpara district of Assam, India ($26^0/6^/$ to$26^013^/$ N and $90^014^/$ to $90^058^/$E). Multiple samples were collected for each of the five different locations from different spots and then air dried, grinded and sieved by a 2mm mesh sieve.

The collected samples were mixed thoroughly and experimental sample of 2kg was collected by quartering process[13]. A total of 14 physico-chemical parameters of the soil was determined according to standard methods of soil analysis and recorded properly[7].

Mixing of PAH and NPK: The experimental PAH i.e. anthracene was applied to each container of soil samples A and B in the form of a solution in methanol (solubility 18g/kg at 19.5℃) as shown in table 1. The solution of anthracene in methanol was prepared by dissolving 0.25g of accurately measured anthracene in 50 mL of methanol of density 0.7914 g/cc. Each of the two containers of soils was thus contaminated. The washings (in 20mL water) of the containers of solutions were also added to the sample containers. The concentration of the PAH became 500 ppm excluding the mass of solvents which were treated as volatile substances.

An equimolar mixture of NPK fertilizers viz. urea [H_2NCONH_2], muriate of potash [KCl] and superphosphate of lime [$Ca(H_2PO_4)_2$] was prepared by mixing 0.90g of urea, 1.12g of muriate of potash and 3.51g of super phosphate of lime and the mixture was added to sample B in order to monitor if there was any influence of these nutrients on DT-50 of the PAH in soil or not.

The contents of both containers were mixed thoroughly and allowed to stand indoor in open glass containers. These constitute two closed thermodynamic systems. The samples were moisturized with distilled water regularly at an interval of 5 days and stirred thoroughly.

Table 1
Samples and Composition

Sample Code	Sample Location	Mass of Soil	Anthracene Added	Nutrient Added	Total Mass	PAH Concentration	NPK Concentration
		g	g	g	g	ppm	ppm
A	River Bed	499.75	0.25	0	500	500	0
B	River Bed	494.22	0.25	5.53	500	500	11060

Soxhlet extraction of PAH: The soil samples were subjected to Soxhlet extraction using petroleum ether and DCM mixture in the proportion of 7:3 after definite interval of time spread over 90 days. After withdrawal of accurately measured 20g of dry soil from the open containers, extraction of the organic materials was done by running Soxhlet extraction for 24 hours with a cycle gap of 12 hours during night. During this cycle gap, the soil of the thimble inside the instrumental set up was under the solvent.

HPLC analysis of the extracts: The residual concentration of anthracene was determined by HPLC analysis. For this purpose, the solvent of the Soxhlet extracts were distilled off. The contents of the distillation flask was then dissolved in 20mL fresh HPLC grade methanol and 2mL of it was transferred into a small vial. This was then subjected to HPLC analysis along with standard solution of anthracene in methanol for calibration. Standard solutions of anthracene in methanol having 400, 350, 300, 250 and 200 ppm concentrations were prepared.

For this purpose, 0.02g of anthracene was dissolved in 63.15 mL of methanol of density 0.7914g per cubic cm to get a stock solution of 400ppm. Thereafter, by adding 1.25, 2.50, 3.75 and 5.00 mL of methanol to 8.75, 7.50, 6.25 and 5.00 mL of stock solution respectively, standard solutions of 350, 300, 250 and 200 ppm were prepared. HPLC analysis was done for 20 minutes with specification DAD: Signal A, 254 nm/ Bw: 4 nm. AR grade anthracene, petroleum ether, dichloromethane, HPLC grade methanol were used for the experiment.

Determination of order of the degradation process-Method of integration: Initially rate constants of the reactions were calculated by taking the integrated rate equations for zero and first order reactions.

The expression for rate constant of a zero order reaction is given by: $k=\frac{[R]o-[R]}{t}$.
The expression for rate constant of a first order reaction is given by: $k=\frac{2.303}{t}\log\frac{[R]_o}{[R]}$

Here $[R]_o$ and [R] are the initial and final concentrations of the reactant i.e. anthracene. In this work, instead of molar concentrations, the concentrations in ppm obtained from HPLC reports are directly used since these are proportional to molar concentration. Moreover, in first order reaction expression, the two concentrations (initial and final) appear as a fraction in the rate equations and hence use of ppm or molar concentration is the same.

At the same time, the area or percent area of a peak is also proportional to concentration and hence these can also be used in the rate equation where there is a question of comparison or taking of a ratio. Integrated rate equation gives equal values of rate constants at different intervals of time. After ascertaining the order of the reaction, the half-life equation of the concerned order is used to calculate the half-life period or DT-50 i.e. disappearance time for 50 percent of the hydrocarbon in number of days.

Results and Discussion

The physicochemical parameters of the soil studied are recorded in table 2. In general, a soil suitable for adequate microbial activities is also suitable for agricultural purposes. Such a soil needs to possess Soil Organic Carbon (SOC) between 1-3%. But, the experimental soil sample has SOC 0.15% which is quite low. SOC and Soil Organic Matter (SOM) are related by a quantitative relationship of 1: 1.724.

Thus, the quantity of SOM in a soil sample is the quantity of SOC multiplied by 1.724. This is called Van-Bemmelen factor. Since the desired quantity of SOC for a good soil is 1-3%, the desired quantity of SOM is 1.7 to 5.2%. As such, the soil sample is also not good with respect to SOM content[15].

A soil sample of moderate texture i.e. loam is most suitable for agricultural purposes. It is due to superior retention of water and nutrient supplying capability. With respect to texture, the soil is not good for agricultural purposes since the texture is far different from loam. According to US Salinity report, a soil having electrical conductivity (EC) less than $2dSm^{-1}$ indicates a non-saline soil. The experimental soil sample is of very low conductivity.

The soil is slightly alkaline with respect to soil reaction. Since, most of the crops grow best in a soil having pH 6-7, the soil is suitable for growth of limited plants. Sodium content below 397ppm and nitrate content below 10ppm are considered low for a good soil. In the experimental soil sample, both were found to be very low than the cited values.

HPLC analysis of the Soxhlet extracts of sample A in methanol solvent was done after calibration of the instrument [Fig. 1]. Anthracene appears at a retention time between 8.707 – 8.713 minutes in calibration and between

8.700 – 8.740 minutes in the soil extracts for soil sample A [Fig. 2-7]. These extractions were done on 0, 10, 20, 30, 40 and 50 days after mixing the PAH with soil. The resulting concentrations of anthracene were found to be 431.60, 368.68, 349.10, 311.49, 299.96 and 250.29 ppm respectively. By considering the initial concentration of 431.60 ppm, the rate constants for the disappearance of anthracene from soil under ambient conditions are calculated for various days anticipating order of the reaction to be either zero or first [Table 3].

As per method of integration for determination of order of a reaction, the order of the reaction was found to be one since the rate constants for different intervals of time in first order are almost same, mostly varying between 0.0106 to 0.0109, although there are two values beyond this range i.e. 0.0091 and 0.0158 per day. The highlighted values 0.0106, 0.0109 and 0.0109 per day stand for the three closest values, out of which 0.0109 is the concordant value. As such, 0.0109 is taken as the actual rate constant for the disappearance process. The disappearance process is largely biodegradation and biodegradation is a process of oxidation[4].

Thus the rate constant for oxidation of anthracene in ambient condition is found to be 0.0109 per day. Now, since the DT-50 is the half-life period of the PAH in days by using the expression $T_{1/2} = 0.693/ k$, the DT-50 of anthracene is found to be 63.58 i.e. 64 days. The experiment was performed in the city of Guwahati, Assam, India having a humid climate during June- August where the ambient temperature during the experimental period was in between 23.3^0C and 39.1^0C. This ambient temperature is suitable for microbial activities in a particular place[8].

Similarly, HPLC analysis of the Soxhlet extracts of sample B in methanol solvent was done after calibration of the instrument. Anthracene appears at a retention time of 8.847 minutes in the soil extracts. These extractions were done on 20, 80 and 90 days following the indoor placement of samples. The resulting concentrations of anthracene are found to be 283.87, 83.12 and 67.10 ppm respectively in a gap of 20, 80 and 90 days [Fig. 8-10].

Table 2
Physico-chemical Parameters of the soil sample

S.N.	Parameters (Unit)	Values		S.N.	Parameters (Unit)	Values
1	Clay %	2.12		8	SOC (%)	0.15
2	Silt%	2.65		9	SOM (%)	0.26
3	Sand%	95.23		10	Na(mg/kg)	39.52
4	Texture	Sandy		11	K(mg/kg)	20.17
5	Porosity (%)	44		12	Ca(mg/kg)	8.76
6	pH	7.21		13	NO_3^-(mg/kg)	3.89
7	EC (dSm^{-1})	0.18		14	Cl^- (mg/kg)	90.63

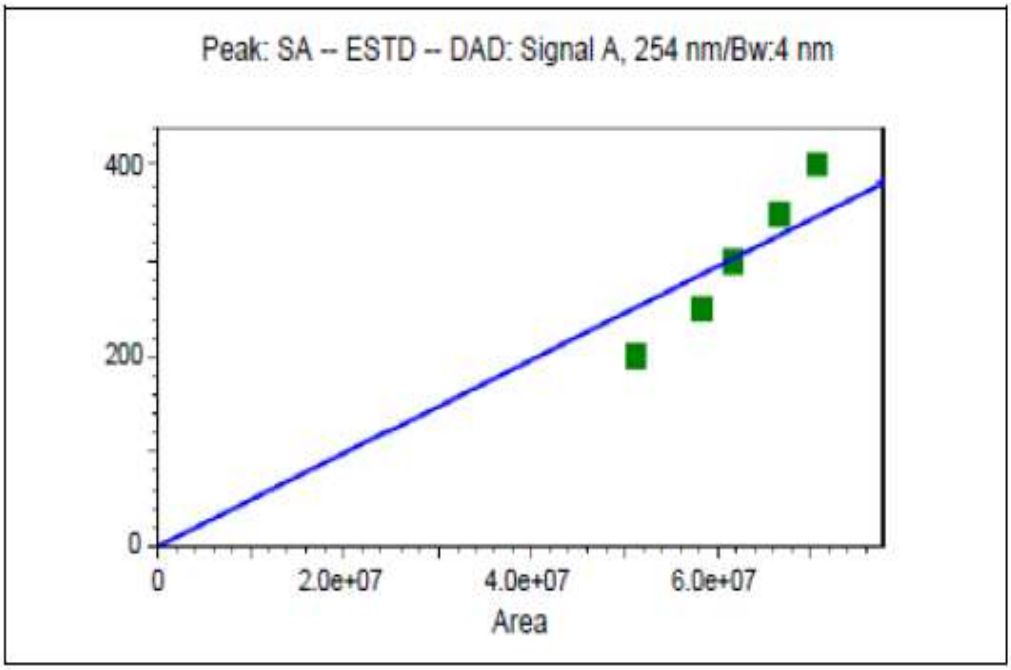
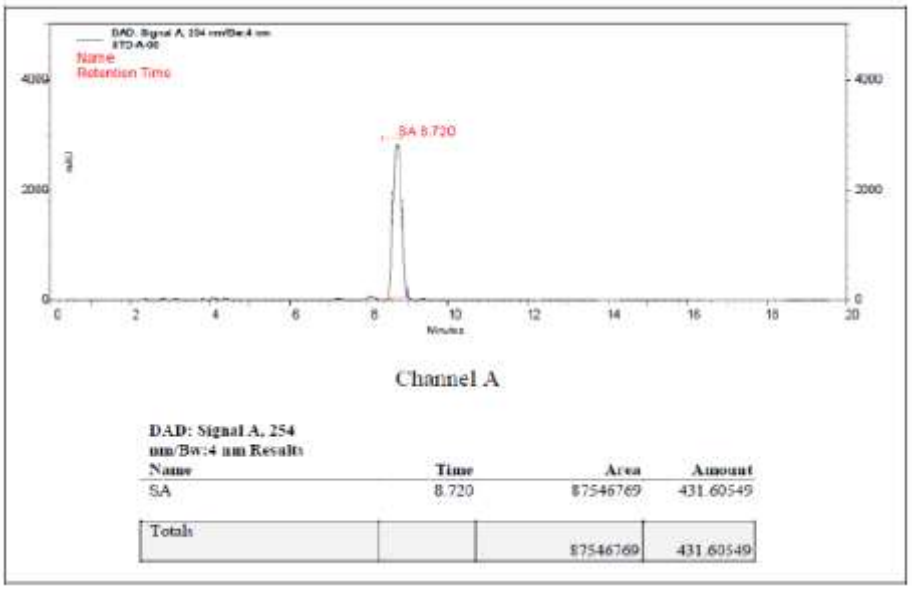


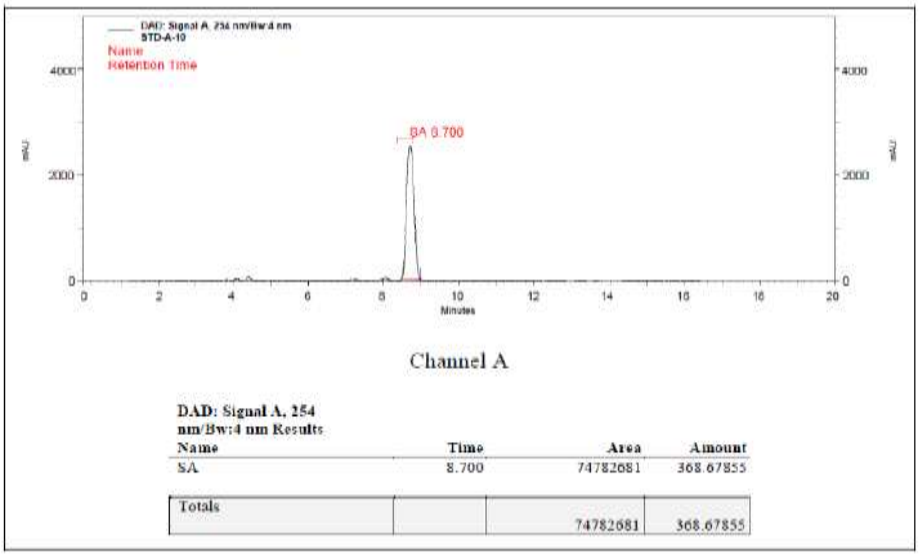
Fig. 1: HPLC Calibration curve of Anthracene.



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.720	87546769	431.60549
Totals		87546769	431.60549

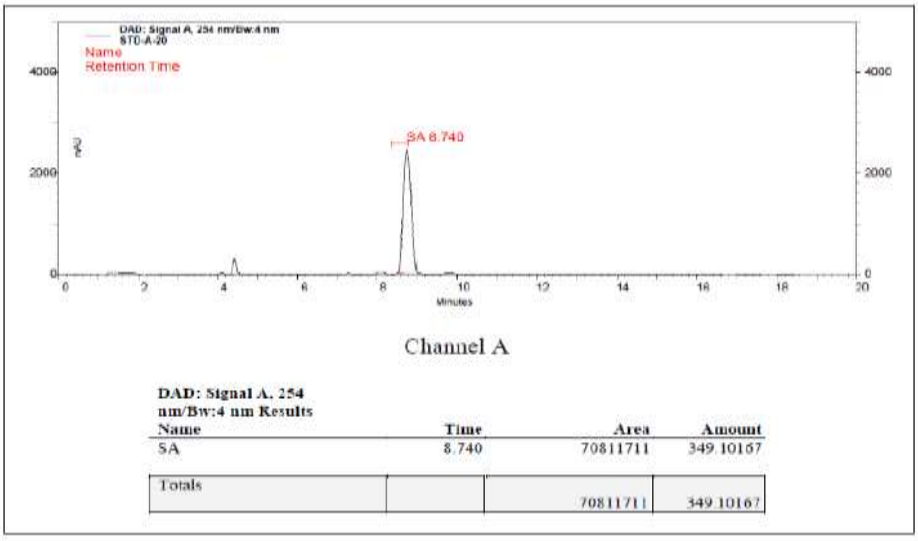
Fig. 2: HPLC data of Anthracene at 0 day for Sample A



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.700	74782681	368.67855
Totals		74782681	368.67855

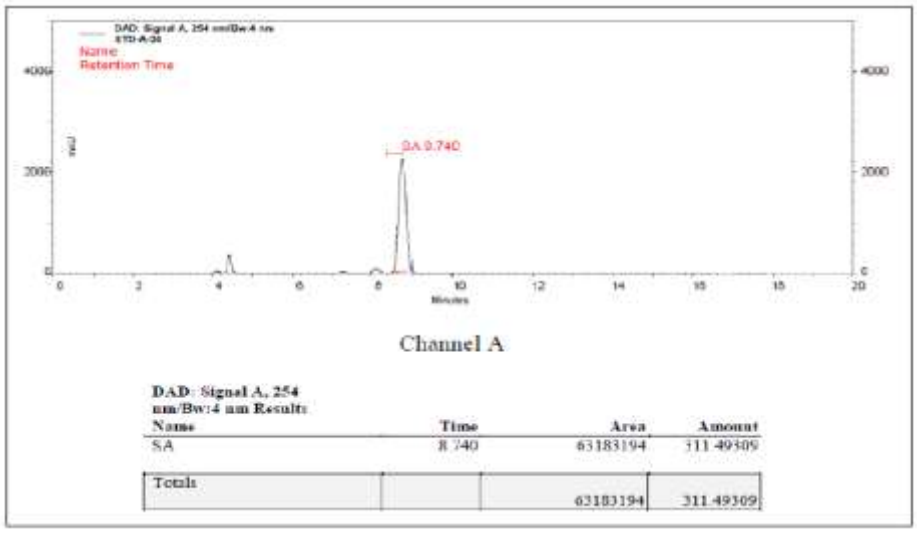
Fig. 3: HPLC data of Anthracene at 10 days for Sample A



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.740	70811711	349.10167
Totals		70811711	349.10167

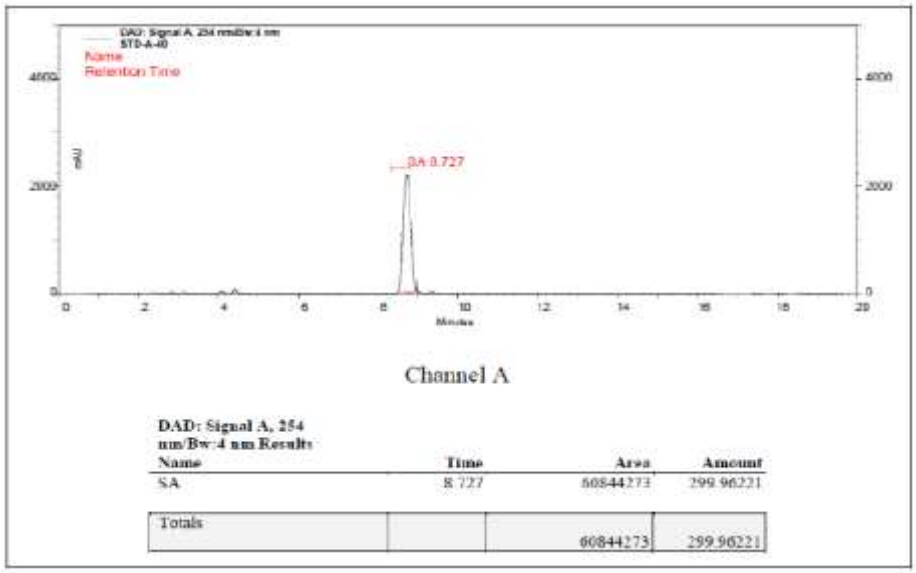
Fig. 4: HPLC data of Anthracene at 20 days for Sample A



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.740	63183194	311.49309
Totals		63183194	311.49309

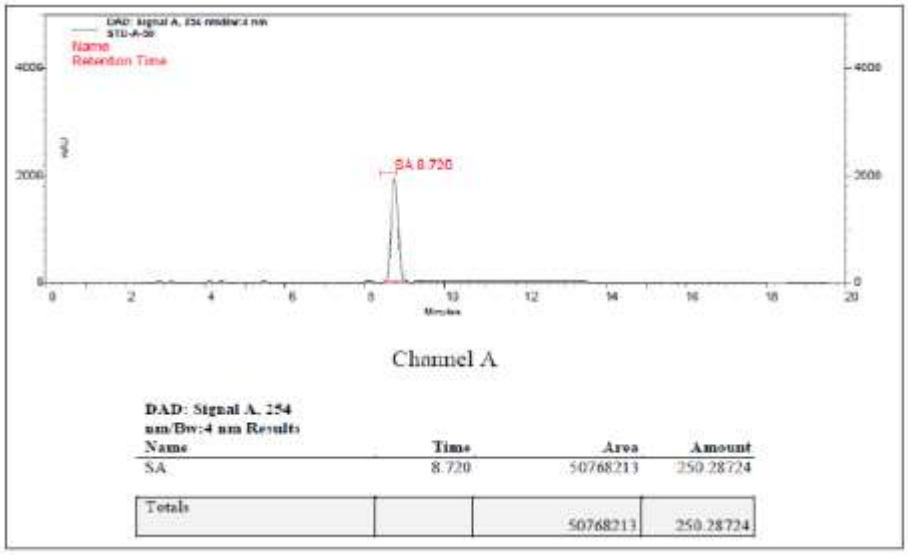
Fig. 5: HPLC data of Anthracene at 30 days for Sample A



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.727	60844273	299.96221
Totals		60844273	299.96221

Fig. 6: HPLC data of Anthracene at 40 days for Sample A



DAD: Signal A, 254 nm/Bw:4 nm Results

Name	Time	Area	Amount
SA	8.720	50768213	250.28724
Totals		50768213	250.28724

Fig. 7: HPLC data of Anthracene at 50 days for Sample A

Table 3
Calculation of rate constants for disappearance of anthracene in soil sample A as per zero and first order integrated rate equations

S.N.	Initial Conc.$[R]_0$	Final Conc.[R]	Time Interval (t)	Rate Constants (k) for order	
	ppm	ppm	days	Zero (ppm day^{-1})	First (day^{-1})
1	431.60	368.68	10	6.292	0.0158
2	431.60	349.10	20	4.125	**0.0106**
3	431.60	311.49	30	4.003	**0.0109**
4	431.60	299.96	40	3.291	0.0091
5	431.60	250.29	50	3.626	**0.0109**

Table 4
Calculation of rate constants for disappearance of anthracene in soil sample B as per zero and first order integrated rate equations

S.N.	Initial Conc.$[R]_0$	Final Conc.[R]	Time Interval (t)	Rate Constants (k) for order	
	ppm	ppm	days	Zero (ppm day^{-1})	First (day^{-1})
1	431.60	283.87	20	7.386	0.0210
2	431.60	83.12	80	4.356	**0.0206**
3	431.60	67.10	90	4.050	0.0207
4	283.87	83.12	60	3.345	0.0205
5	283.87	67.10	70	3.096	**0.0206**

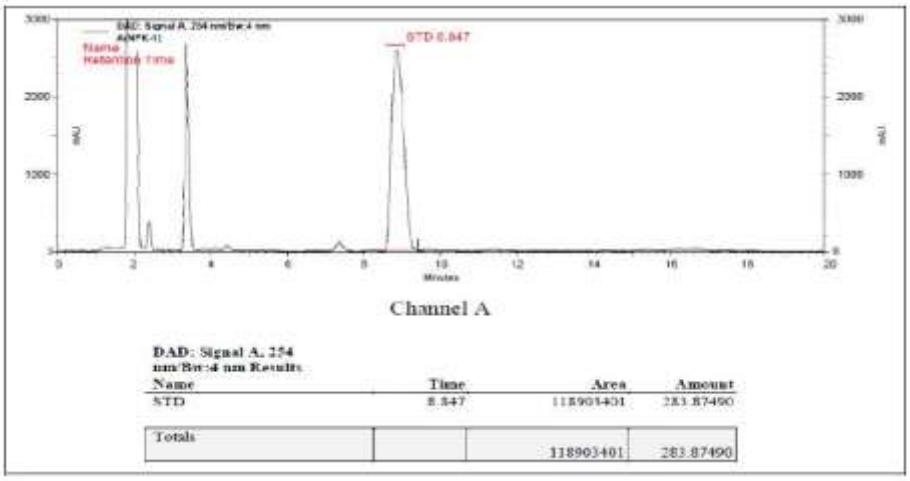


Fig. 8: HPLC data of Anthracene at 20 days for Sample B

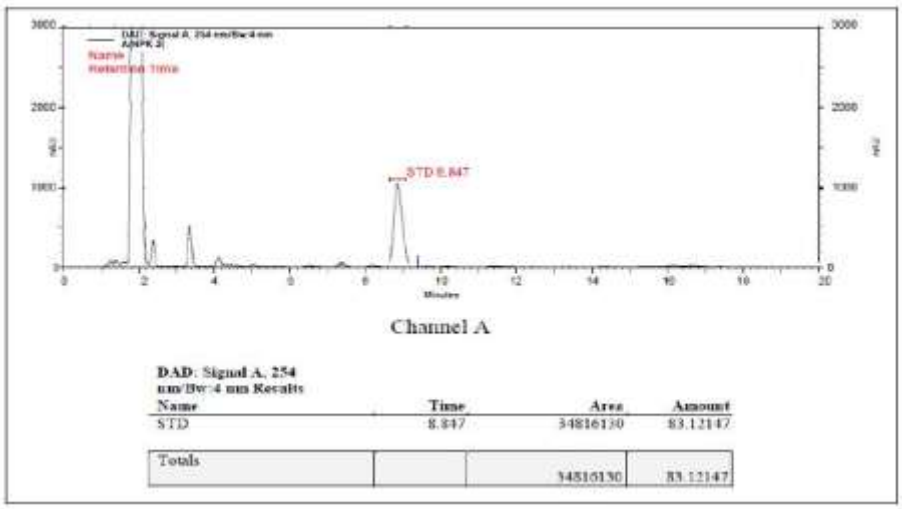


Fig. 9: HPLC data of Anthracene at 80 daysfor Sample B

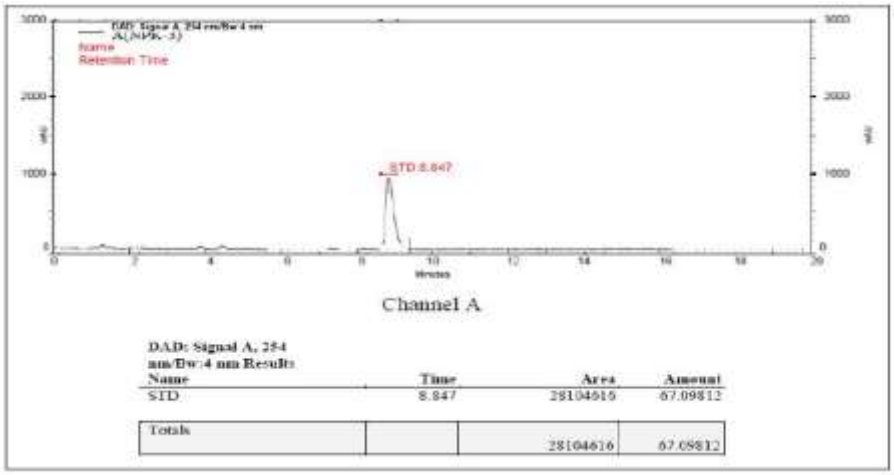


Fig. 10: HPLC data of Anthracene at 90 days for Sample B

By taking the initial concentration of 431.60 ppm and 283.87 ppm, the rate constants for the disappearance of anthracene from soil under ambient conditions are calculated for various days anticipating order of the reaction to be either zero or first [Table 4]. As per method of integration for determination of order of a reaction; the order of the reaction is found to be one, since the rate constants for different intervals of time in first order are almost same, varying between 0.0205 to 0.0207. The value beyond this range is 0.0210.

The highlighted value 0.0206 per day stands for the two occasions and this concordant value of 0.0206 is taken as the actual rate constant for the disappearance process. Now, half-life period of the PAH in days by using the expression $T_{1/2}$ =0.693/k, the DT-50 of anthracene in presence of 11060 ppm of NPK is found to be 33.64 i.e. 34 days. Thus presence of NPK nutrients of about 11000ppm in the soil has lowered the DT-50 value by 47%. It indicates that application of NPK nutrients enhances the rate of degradation of anthracene in soil.

Conclusion

Many investigations indicate that anthracene is not carcinogenic, but a good number of anthracene derivatives are carcinogenic. Besides this, the crude sample of anthracene is generally contaminated with other PAHs which might be carcinogenic. It has been reported that anthracene is readily biodegraded in soil. This degradation is accelerated by the presence of light. The present experiment shows that the degradation of anthracene in a sandy soil follows first order kinetics.

In spite of high degradability of anthracene in soil, its DT-50 is 64 days. The rate of degradation is accelerated by use of NPK nutrients. There is possibility that a PAH which is not readily degradable in soil may have higher DT-50 compared to anthracene and as such, opportunity to enter food chain will remain open for those hydrocarbons which will be a matter of great concern.

Acknowledgement

The authors are thankful to Guwahati Biotech Park Incubation Centre, IIT Complex, Guwahati for HPLC support.

References

1. Agrawal N., Verma P. and Shahi S.K., Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi Ganodermalucidum isolated from the hardwood stump, *Bioresources and Bioprocessing*, **5(11),** 1-9 **(2018)**

2. Balba A.M., Elshibiny G. and Elkhatib E.S., Effect of Lead Increments on the Yield and Lead Content of Tomato Plants, *Water Air and Soil Pollution*, **57(8)**, 93-99 **(1991)**

3. Boonchan S., Britz M.L. and Stanley G.A., Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures, *Appl. Environ. Microbiol.*, **66,** 1007–1019 **(2000)**

4. Devi U. and Sarma P.C., Gas Chromatographic analysis of Persistant Hydrocarbon Components of Crude Oil and their Oxygenated Derivatives in Soil, *Res. J. Chem. Environ.*, **13(3)**, 66-68 **(2009)**

5. Enell A., Reichenberg F. and Ewald G., Desorption kinetics studies on PAH-contaminated soil under varying temperatures, *Chemosphere*, **61**, 1529–1538 **(2005)**

6. Fengmei L., Shuhai G. and Niels H., Electrokinetics-enhanced biodegradation of heavy polycyclic aromatic hydrocarbons in soil around iron and steel industries, *Electrochimica Acta*, **85**, 228–234 **(2012)**

7. Gupta P.K., Methods in Environmental Analysis, Agrobois, India, 167-309 **(2009)**

8. Khan Z. and Anjaneyulu Y., Review on application of Bioremediation Methods for Decontamination of Soils, *Res. J. Chem. Environ.*, **9(2),** 75-79 **(2005)**

9. Maliszewka-Kordybach B. and Smreczak B., Phytotoxic activity of PAH in soils of different properties, *Roczn. Glebozn*., **1**, 15 **(1999)**

10. Moretto L.M., Silvestri S., Ugo P., Zorzi G., Abbondanzi F., Baiocchi C. and Iacondini A., Polycyclic aromatic hydrocarbons degradation by composting in a soot-contaminated alkaline soil, *Journal of Hazardous Materials*, **126,** 141–148 **(2005)**

11. Muckian L., Grant R., Doyle E. and Clipson N., Bacterial community structure in soils contaminated by polycyclic aromatic hydrocarbons, *Chemosphere*, **68(8),** 1535- 1541 **(2007)**

12. Oleszczuk P. and Baran S., Degradation of Individual Polycyclic Aromatic Hydrocarbons (PAHs) in Soil Polluted with Aircraft Fuel, *Polish Journal of Environmental Studies*, **12(4),** 431-437 **(2003)**

13. Singh D., Chhonkar P.K. and Pandey R.N., Soil Plant Water analysis, ICAR, 1-46 **(2001)**

14. Viecelli N.C., Lovatel E.R., Cardoso E.M. and Filho I.N., Degradation of polycyclic aromatic hydrocarbons in soil, *Environmental Science and Biological Engineering*, **156**, 295-301 **(2014)**

15. Wild A., Soils and the environment, Cambridge University Press, 32 **(1996)**.

(Received 09th May 2020, accepted 15th June 2020)