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SOME INTERMEDIATE BIO-TRANSFORMANTS DURING BIODEGRADATION OF HIGH MOLECULAR WEIGHT PHENANTHRENE AND FLUORANTHENE BY CYANOBACTERIAL SPECIES – *AULOSIRA FERTILISSIMA* GHOSE.

Nirmal Kumar, J.I.[1]*, Megha Barot[1] and Shamiyan R Khan[1]

[1*]P.G. Department of Environmental Science and Technology, Institute of Science and Technology for Advanced Studies and Research (ISTAR), Vallabh Vidya Nagar- 388 120, Gujarat, India.

[1*]Corresponding author email:istares2005@yahoo.com, Tel.: (+91) 9825968242 ; Fax: (+91) (02692) 234955

Abstract

The PAHs compounds are known to be carcinogenic, teratogenic, mutagenic and toxic to all living organism. Handful of literature is available on biodegradation of these compounds by bacteria and fungi, however, scanty work is done by using microalgae on biodegradation of these two PAHs. In this investigation, the efficiency of *Aulosira fertilissima* Ghose to remove fluoranthene ($0.001gm.ml^{-1}$), phenanthrene ($0.001gm.ml^{-1}$) and a mixture of both (each at concentration of $0.0005gm.ml^{-1}$) were evaluated for intermediate bio-transformants during biodegradation by using GCMS. The result showed that the efficiency of *Aulosira fertilissima* for removal and biodegradation of phenanthrene was higher than fluoranthene, indicate fluoranthene was more stable and recalcitrant. PAHs uptake after 7-days of treatment was 80% and 66% of these phenanthrene and fluoranthene ,respectively by the cyanobacteria .The synergetic effect of fluoranthene on phenanthrene was observed, presence of fluoranthene stimulate the degradation of phenanthrene due to which phenanthrene produce more bio-transformants . Some intermediates were observed like Methyl linoleate, 4-(2,2- dimethyl-6-methylenecyclohexylidene)-3-methyl-,(Z)- etc. for phenanthrene biodegradation process while 2,3-dihydrofluoranthene, (1R,5R)-2-isopropyl-5-methylcyclohexanol, for fluoranthene degradation. Moreover, 3-isopropylidene-2,2-dimethyl-6-phenyl-1,4-oxathiane, 7- phenyltridecane, diphenylacetylene, for mixture of two PAHs applied.

Keywords: Fluoranthene, Phenanthrene, *Aulosira fertilissima* Ghose, Polycyclic Aromatic Hydrocarbon (PAH), Biodegradation, Biotransformants.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are aromatic hydrocarbons with two or more fused benzene rings with natural as well as anthropogenic sources. Natural sources are forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees while anthropogenic sources of PAH include burning of fossil fuel, coal tar, wood, garbage, refuse, used lubricating oil and oil filters, municipal solid waste incineration and petroleum spills and discharge. They are widely distributed environmental contaminants that have detrimental biological effects, toxicity, teretogenicity, mutagenecity and carcinogenicity. They do not degrade easily under natural conditions, besides their persistence increases with increase with the molecular weight. They are biodegraded/ biotransformed into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) and rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium.

Both bacteria and fungi have been studied upto a certain extant for their ability to degrade xenobitics including PAHs. In recent years, new technology employing microorganisms to remove PAHs from the contaminated environment via bioadsorption and biotransformation has been proposed (Juhasz and Naidu, 2000; Chávez-Gómez *et al.*, 2003). Moreover, previous studies have been focused mainly by bacteria and fungi, but relatively little attention has been paid on the role of microalgae despite, algae applied in various wastewater treatment processes and shown to be able to remove toxic organic pollutants by autotrophic growth (Semple *et al.,* 1999; Lei *et al.,* 2002;Tam *et al*., 2002).

Canet *et al.*, (2001); Tang *et al.*, (2005) undertaken on the uptake and/or metabolism of a single PAH

contaminant, but in nature, these are common to have a mixture of PAH contamination, and interact with each other. However, the possible stimulatory, antagonistic, competitive uptake and metabolism of the combined PAHs received little exploration (Dean-Ross *et al.*, 2002). Representative cyanobacterial genera which degrade hydrocarbons under aerobic conditions are *Aphanocapsa, Anabaena, Microcoleus, Nostoc, Oscillatoria* and *Phormidium* was given by Prince (1998). Smaller PAHs, such as naphthalene, phenanthrene and anthracene, have been studied with regards to their degradation by bacteria, due to early oxidation products, and aliphatic intermediates by Tian *et al.* (2002).The present experiment therefore focused to evaluate the some intermediate bio-transformants during biodegradation of high molecular weight phenanthrene and fluoranthene and a mixture of two by cyanobactrial species – *Aulosira fertilissima* Ghose. Fluoranthene was selected because it is the main representative among PAHs, predominant in air, sediment and water, and was often occured in contaminated environments as stated by Tang *et al.* (2005). Nirmalkumar *et al*. (2013) also carried out the biodegradation and molecular characterization by 16S rDNA amplification were carried out to evaluate differential effects of 2,4-D ethyl ester and pencycuron on *Anabaena fertilissima, Aulosira fertilissima* and *Westiellopsis prolifica*. Various compounds like 2,4-D methyl ester, 2,4-D isobutyl ester, Isobutyric acid allyl ester, 3-Bromobutyric acid, 2,4-D butyl ester, Hydroxyurea, Trifluroacetic acid, 2-Methyl propyl ester, Acetic acid 2-propenyl ester and Acetic acid (2,3-dichlorophenoxy) were transformed from 2,4-D ethyl ester while Benzoxazole Benzoxazole was the only compound generated from pencycuron treated *Westiellopsis prolifica.*

Experimental Section

Cyanobacterial species and culture conditions

The selected cyanobacterial species *Aulosira fertilissima* Ghose was grown and maintained in BG-11 medium (Rippka *et al.*, 1979), free of combined nitrogen source (C-N). Culture was grown under controlled illumination of 30µE.m-² s-¹ light for 14:10 h light/dark periods at 25±2 temperature under aerobic and static conditions. Exponentially grown cyanobacterial cells were used throughout the experiment. The selected species of cyanobacteria was made auxenic by treating with streptomycin and benzyle penicillin in the following concentration for 24 hrs (Table. 1) (Ferris and Hirsch, 1991).

Table 1. Different concentration of streptomycin and benzyl penicillin treatment

		1	2	3	4	5
Benzyl penicillin	PPM	500	250	125	62.5	31.75
Streptomycin	PPM	250	125	62.5	31.75	15.80

Structure of *Aulosira fertilissima* Ghose: Expanded stratum, dark blue green, membranous; trichomes straight or am little flexuous, parallel or densely intricate with very short pseudo branches; 6-11µm broad and 7-10µm long, cylindrical when young, later barrel shaped; contents granular, sheath thick; at first gelatinous an hyaline, later firm and brown; heterocyst, intercalary, oblong or elliptical, 8-9µm broad and 10-14µm long, spores in series usually alternating with dead cells, generally oblong-elliptical sometimes angular due to compression, 10-13µm broad and 18-24µm long (Desikachary, 1959).

Experimental set-up

For each compound, 27 sugar tubes and 3conical flask (250ml) each containing 2ml culture+18ml media. Nutrient media were prepared by 162ml distilled water+162µl media and autoclaved. After sterilization, 0.1% stock solution of fluoranthene or phenanthrene, or a mixture of both, prepared in acetone with 0.05gm/ 50 ml each PAH and for mixture of both 0.025gm/50ml. Different doses of 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 30.0 ppm of fluoranthene or phenanthrene or mixture were prepared in the8 sugar tubes and control is maintained separately without the addition of any chemical. A total of 81 tubes were prepared for the one algal species (*Aulosira fertilissima*) and triplicate set were maintained. The tubes were shaken on a rotary shaker at 160 rpm at a light intensity of 50 mol s^{-1} m^{-2} under the same room temperature in the environmental chamber. Appropriate amounts of cyanobaterial culture of *Aulosira fertilissima* were then inoculated into each tube. Triplicate tubes of the control and the PAH treatments were retrieved after 1-,4- and 7-days of incubation. Simultaneously, 27 sugar tubes, each containing 18ml autoclaved BG11 culture medium, were prepared. These were divided into three groups, nine with fluoranthene, nine with phenanthrene and the remaining nine with a mixture of fluoranthene and phenanthrene, to monitor degradation and biotic removal of PAHs. Triplicate tubes from each group were collected after 1, 4, and 7-days incubation.

Fig:1.Phenanthrene

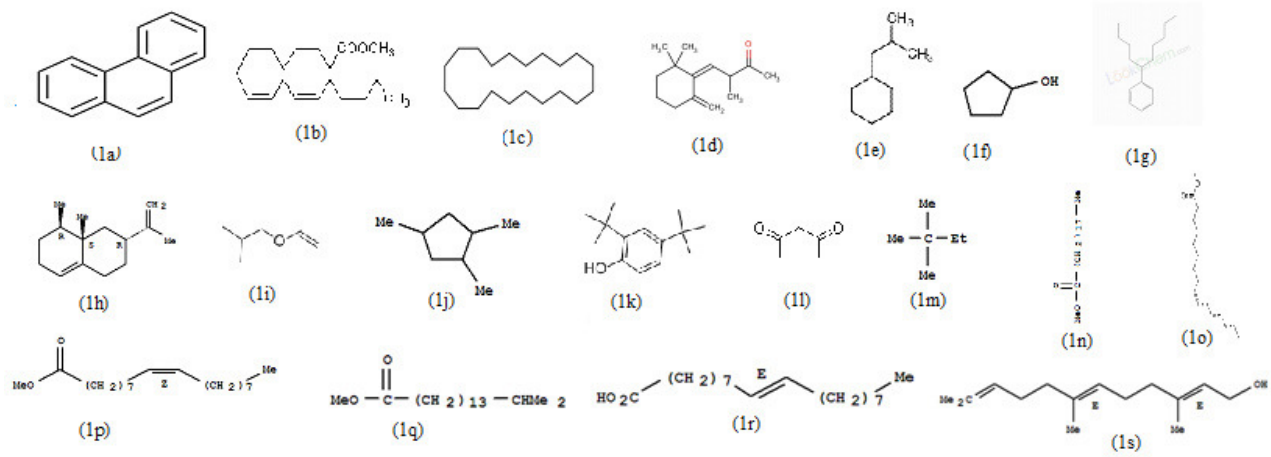

Fig. 1. Intermediates of phenanthrene degradation by *Aulosira fertilissima.*

(1a)Phenanthrene mol wt 178.23, (1b) methyl linoleate mol wt of 294.47, (1c)cycloeicosane mol wt 296.57,(1d)2-butane,4(2,2-dimethyl-6-methylenecyclohexylidene)-3-methyl-,(z) mol wt 332.35, (1e)Isobutylcyclohexane mol wt 168.23, (1f)cyclopentanol mol wt 86.13, (1g) 1- phenyldecane mol wt of 218.38, (1h)Naphthalene,1,2,3,4,5,6,7,8,8a-octahydro-1,8a -dimethyl-7-(1-methylethenyl)-,(1R,7R,8aS) mol wt of 128.17, (1i)Isobutyl vinyl ether mol wt 100.16, (1j)Cyclopentane,1,2,4-trimethyl mol wt 112.21, (1k)2,4-di-tert-butylphenol mol wt of 206.32, (1l)2,4-pentanedione mol wt 100.13 (1m)2,2-dimethylbutane mol wt 86.18, (1n)Nonadecanoic acid,methyl ester mol wt 312.53, (1o)Palmitoleic acid methyl ester mol wt 268.53, (1p)9-octadecenoic acid(9z)-,methylester mol wt 296.55, (1q)Hexadecanoic acid,15-methyl ester mol wt 284.48, (1r)9-octadecenoic acid mol wt 296.48 ,(9E)1 octadecene 252.48, (1s)(E,E)-Farnesol mol wt 222.47

Fig:2. Fluoranthene

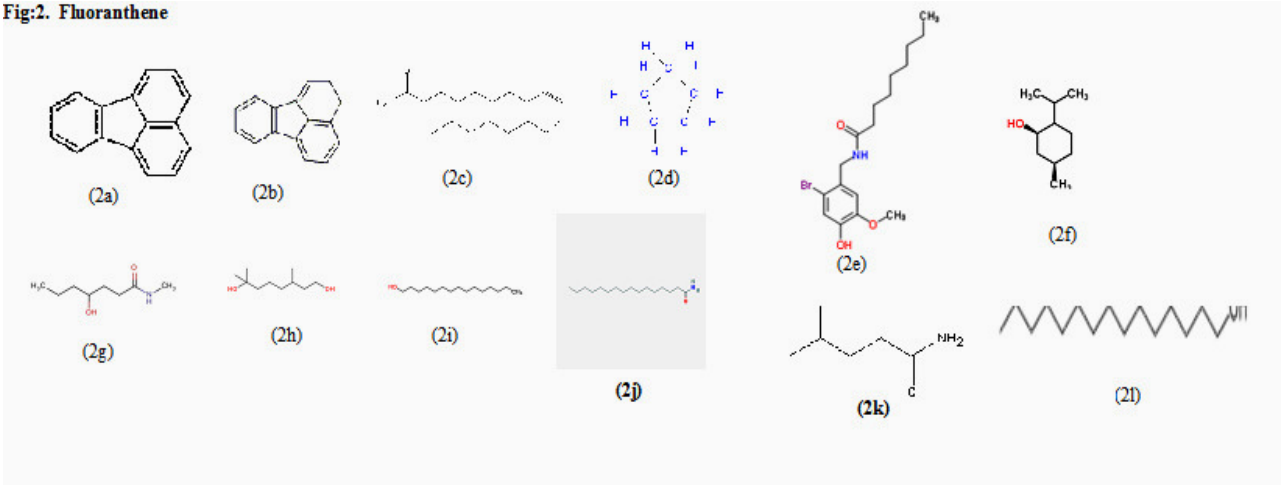

Fig. 2. Intermediates of fluoranthene degradation by *Aulosira fertilissima.*

(2a)Fluoranthene mol wt 202.26, (2b)2,3-dihydrofluoranthene mol wt 204.27, (2c)9-octadecenamide mol wt 281.50, (2d)cyclopentane mol wt 70.13, (2e)N-(2-bromo-4-hydroxy-5-methoxybenzyl)nonanamide mol wt 293.40, (2f)1R,1R-2-isopropyl-5-methylcyclohexanol mol wt 156.26, (2g)Heptanamide,4-hydroxy-n-methyl mol wt 387.52, (2h)Hydroxycitronellol mol wt 174, (2i)1-pentadecanol mol wt 228.41, (2j)Hexadecanamide mol wt 255.43, (2k)Pentanamide,4-methyl mol wt 115.17, (2l)1-pentadecanol mol wt 228.41.

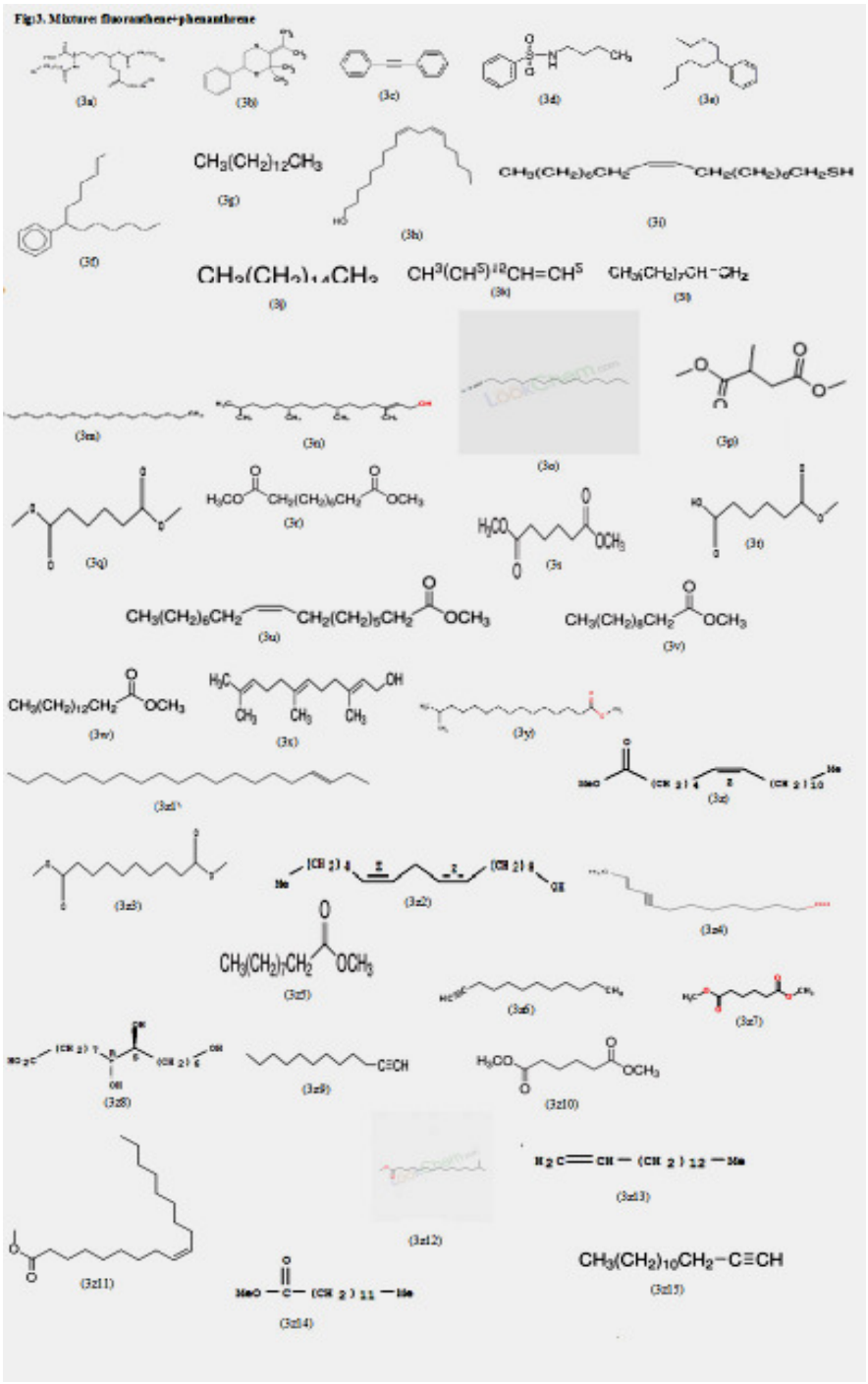

Fig. 3. Intermediates of mixture of phenanthrene and fiuoranthene degradation by *Aulosira fertilissima.*

(3a)3-eicosene,(E)- mol wt 280.53, (3b)3-isopropylidene-2,2-dimethyl-6-phenyl-1,4-oxathiane mol wt 248.38, (3c)diphenylacetylene mol wt 178.23, (3d) *N*-Butylbenzenesulfonamide mol wt 213.30, (3e)decan-5-ylbenzene mol wt 218.37, (3f)7-phenyltridecane mol wt 260.45, (3g) cis,cis-9,12-octadecadienol mol wt 266.46, (3h)tetradecane mol wt 198.39, (3i)cis-9-octadecene-1-thiol mol wt 284.54, (3j)hexadecane mol wt 266.44, (3k)1-octadecane 254.49, (3l)tetradecene mol wt 196.37, (3m)1-nonadecene mol wt 266.50, (3n)phytol mol wt 296.53, (3o) Hex1-ene mol wt 84.16, (3p)dimethyl methylsuccinate mol wt 160.17, (3q) N-butylbenzenesulfonamide mol wt 213.30, (3r)Methyl undecanoate mol wt 200.32, (3s)dimethyl adipate mol wt 174.20, (3t)dimethyl glutarate mol wt 160.16, (3u)methyl cis-9-octadecenoate,oleic acid methyl ester mol wt 296.49, (3v)1 pentadecene mol wt 210.40, (3w)methyl pentadecanoate mol wt 256.42, (3x)Trans,trans-farnesol mol wt 222.37, (3y)14 methyl pentadecanoic acid methyl ester mol wt 270.45, (3z)6-octadecanoic acid, methyl ester ,(6Z) mol wt 296.49, (3z1)dimethyl sebacate mol wt 230.30, (3z2)9,12-octadecadien-1-ol.(9Z,12Z) mol wt 266.46, (3z3)N- butylbenzenesulfonamide mol wt 213.29, (3z4) (E) -8-dodecen-1-ol, mol wt 226.36, (3z5) farnesol mol wt 222.37, (3z6) 1 dodecyne mol wt 166.30, (3z7) dimethyl adipate mol wt 174.20, (3z8) Hexadecanioc acid,9,10,16-trihydroxy-,(9R,10S)-rel- mol wt 304.48, (3z9) 1 dodecyne mol wt 166.30, (3z10) methyl decanoate mol wt 186.29, (3z11) (Z)-9-octadecanoic acid methyl ester mol wt 296.49, (3z12) 1-nonadecene mol wt 266.50, (3z13) 1- pntadecene mol wt 210.40, (3z14) tridecanoic acid, methyl ester 228.37, (3z15) 1-tetradecene mol wt 196.37

Table 2. The maximum uptake concentration

Compound	Maximum % Uptake 4th day	Maximum % Uptake 7th day
Fluoranthene	45% at 1ppm	66.4% at 2.5 ppm
Phenenthrene	77% at 1ppm	80% at 1 ppm
Fluoranthene in mixture	29% at 5 ppm	12.38% at1 ppm
Phenanthrene in mixture	35.15% at 2.5 ppm	39.28% at 1ppm

Sampling and Analysis.

After the incubation, the tubes were collected, the cyanobacterial pellet was separated from the medium by centrifugation at 5000 rpm for 10min at 4°C. The pellet was crushed in 5 ml of absolute methanol, and the crud methanolic extract of cyanobacterial species treatment were subjected to GC-MS (AutoSystem XL GC, PerkinElmer, USA) at Sophisticated Instrumentation Center for APPLIED Research and Testing (SICART), Vallabh Vidhya Nagar, Gujarat. GC-MS analysis was performed using GC apparatus attached to a PE-5MS fused silica capillary 5% phenyl/95% methylpolysiloxane column (30 mm x 50 mm, 0.25 µm film thickness, Perkin elmer, USA). The column temperature was initially 80°C, held for 5 min, then ramped at 10˚C/min from 80˚C to 290˚C and detector temperature was set at 250˚C. Helium 1 ml/min was used as the carrier gas.

Samples (1 µl) were injected in the split mode (1:40). MS condition were run in EI+ through a Perkin Elmer Turbo Mass mass spectrometer as follow: ionization energy -70 ev; scan rate 1.6 scans/sec; interscan delay 0.01 sec; source temperature 250˚C; mass unit range 30 to 650 m/z; solvent delay 3.00 min. Mass spectra of the chromatographic peaks were compared by spectra in the Wiley NIST/EPA/NIH Mass spectral Library 2005 (Aseer *et al.*,2010). Quantification was based on the external standard, fluoranthene and phenanthrene calibration curves, and the uptake of the PAHS calculated by the peak area difference from the 1st day to 7th day. (Lei *et al*. 2002).

Statistical analysis

The finding of present investigation was analyze using significant statistical test such as PPA and one way ANOVA test with the help of Ky Plot (2006). Pearson Product Analysis (PPA) performed for uptake of fluoranthene and phenanthrene at different days interval by *Aulosira fertilissima* Ghose**.** Whereas the effect of incubation times for *Alosira fertilissima* Ghose and each individual PAH treatments/concentrations would be explored by One-Way ANOVA test.

Results and Discussion

GC-MS analysis of two different high molecular weight compounds phenanthrene, fluoranthene and their mixture showed intermediate bio-transformants during biodegradation by cyanobacterial species – *Aulosira fertilissima* Ghose.

The intermediates found to be rare at lower concentration treatment:

Retention time of 20.37 methyl cis 9 octa decanoate mol wt 296.49, Rt of 18.69 methyl nonadecane mol wt 345, Rt of 20.70 9 –octadecanamide mol wt 281.47, Rt of 21.22 hydroxycitronellol mol wt 174.28, Rt of 22.90 nonanamide mol wt 371.10 for fluoranthene, while Rt of 28.33 napthelene octahydro dimethy mol wt l204.35, Rt of 18.41 methyl palmitoleate mol wt 268.43, 1-pentadecenebutane 2-2 dimethyl, Rt of 22.90 cycloeicosane mol wt 280.53 for phenanthrene, whereas Rt of 15.68 dimethyl succinate mol wt 160.17, Rt of 10.16 dimethyl adipate mol wt 174.19, Rt of 8.05 dimethyl gluterate mol wt 160.17,Rt of 14.21 methyl decanoate mol wt 186.29, Rt of 20.65 methyl undecanoate mol wt 200.32, Rt of 24.30 9-hexadecanoic acid mol wt 254.40,Rt of 20.35 methyl oleate mol wt 296.49, Rt of 18.66 14 penta decanoic methyl ester mol wt 270.45 for mixture were recorded as rare at lower concentration.

The intermediates found to be rare at higher concentration treatment

Rt of 20.39 2,3 dihydrofluoranthene mol wt of 204.26,Rt of 20.70 9 octadecanamide mol wt of 281.47, Rt of 21.20 1- pentadecanol molecular weight of 228.41, for fluoranthene while Rt of 26.24 EE-farnesol mol wt of 222.37, Rt of 17.23 cyclopentanetrimethyle mol wt of 112.21, Rt of 21.14 methyl linoleate mol wt of 294.47, Rt of 20.74 9, octadecanoic acid 9(E) mol wt of 282.52, for phenanthrene whereas, Rt of 20.65 methyl undecanoate mol wt of 200.32, Rt of 18.66 14-methyl pentadecanic acid methyl estermethyl pentadecanol mol wt of 270.45, Rt of 20.28 8, dodecen 1ol(E) mol wt of 226.35, Rt of 12.67 N butyl benzene sulfonamide mol wt of 213.30, Rt of 17.66 benzene 1 butyl hexyl mol wt of 218.37,Rt of 16.60 7phenyl tridecane mol wt of 260.35 for mixture were registred as rare intermediates at higher concentration.

The intermediates found to be very rare at lower concentration treatment

Rt of 14.05 phenol, 2-4 bis(1,1-dimethylethyl) mol wt of 206.32, Rt of 15.57 hexadecane mol wt of 226.44 hexadecane, Rt of 18.43 cis-6-octadecenoic methyl ester mol wt of 296.48, Rt of 18.69 methyl nonadecane mol wt 345, Rt of 21.12 pentanamide 4 methyle mol wt of 115.17, Rt of 20.90 nonadecanamide mol wt of 297.51 for fluoranthene while, Rt of 21.10 3octadecene mol wt of 252.47 , Rt of 14.03 2,4 Di tert butyl phenol mol wt of 206.32, Rt of 18.41 methyl palmitoleate mol wt of 268.43, Rt of 19.35 1octadecane mol wt of 254.49, Rt of 22.90 cycloeicosane mol wt of 280.53, for phenanthrene where as, Rt of 18.00 1tetradecyne mol wt of 194.36, Rt of 8.05 dimethyl glutarate mol wt of 160.17, dodecyne mol wt 166.30, for mixture were encountered as very rare at lower concentration.

The uptake of phenanthrene and fluoranthene was registered higher at 1 ppm however increasing concentration decline the uptake by the treated cyanobacteria (Fig. 4 and 5) after 4th and 7th days of different doses of PAHs. Moreover, the uptake of phenanthrene is higher than the fluoranthene, as well as the synergetic effect of fluoranthene on phenanthrene were encountered.

The maximum percentage uptake of fluoranthene, phenanthrene, fluoranthene in mixture and phenanthrene in mixture by *Aulosira fertilissima* at the end of 4-days incubation were recorded by 45%, 77%, 29.13%, and 35.15% respectively. The maximum percentages uptake of fluoranthene, phenanthrene, flupranthene in mixture and phenanthrene in mixture by *Aulosira fertilissima* at the end of 7-days incubation were 66.4%, 80%, 12.38%, 39.28%. The presence of fluoranthene had an enhancement effect on degradation and transformation of phenanthrene as the percentage loss of phenanthrene was higher than that in a mixture of fluoranthene and phenanthrene. Under both single and mixed conditions, phenanthrene had higher percentage of loss than fluoranthene, suggesting that phenanthrene was less recalcitrant and was easier to biotransform(Table. 2).

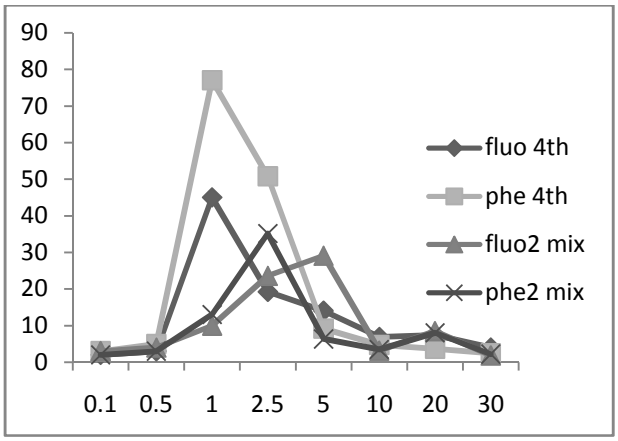

Fig. 4. Uptake of phenanthrene, fluoranthene and both by *Aulosira fertilissima* after 4 day.

Statistical analysis:

Pearson Product Analysis (PPA) shown compound pairs (PAHS) for uptake of fluoranthene and phenanthrene by *Aulosira fertilissima* registered highest correlation in between single fluoranthene and single phenanthrene(F*P) compound (0.944) at the end of 4th day and lowest correlation in single phenanthrene and fluoranthene in mixture while remaining are moderately correlated (Table. 3). One-Way ANOVA for uptake of fluoranthene and phenanthrene by *Aulosira fertilissima* reveal the uptake of fluoranthene ($P \leq 0.05$) and phenanthrene is highly significant ($P \leq 0.001$),while uptake of fluoranthene in mixture and uptake of phenanthrene in mixture are less significant ($P > 0.05$) (Table. 4). In the present investigationintermediates were observed like Methyl linoleate, 4-(2,2- dimethyl-6-methylenecyclohexylidene)-3-methyl-,(Z)- etc. for phenanthrene biodegradation process while 2,3-dihydrofluoranthene, (1R,5R)-2-isopropyl-5-methylcyclohexanol, for fluoranthene degradation. Moreover, 3-isopropylidene-2,2-dimethyl-6-phenyl-1,4-oxathiane, 7- phenyltridecane, diphenylacetylene, for mixture of two PAHs applied. Similar observations were encountered from the cultures of *Microcoleus chthonoplastes* and *Phormidium corium* were able to degrade n-alkanes has been supported by the work of Al-Hasan et al. (1998). *Oscillatoria sp.* and

Agmenellum quadruplicatum oxidize naphthalene to 1-naphthol (Cerniglia and Gibson 1979; Cerniglia *et al.* 1980a). Moreover, *Oscillatoria sp.* strain JCM oxidizes biphenyl to 4-hydroxybiphenyl (Cerniglia *et al.* 1980b) and *A. quadruplicatum* metabolizes phenanthrene into trans-9,10-dihydroxy-9,10dihydro- and 1-methoxy- (Narro *et al.*, 1992). Several other strains can degrade crude oil and other complex organic compounds such as surfactants and herbicides (Yan *et al.*, 1998; Radwan and Al-Hasan 2000; Raghukumar *et al.*, 2001; Mansy and El-Bestway, 2002).

Table 3. Pearson Product Analysis (PPA) of Compound Pairs (PAHS) for Uptake of Fluoranthene and Phenanthrene by *Aulosirafertilissima*

Compound Pairs	Day 4	Day 7
F x P	**0.944**[a*]	0.732[b]
F x FM	0.352[c]	0.776[b]
F x PM	0.470[c]	0.586[b]
P x FM	**0.338**[c*]	0.774[b]
P x PM	0.674[b]	0.899[a]
FM x PM	0.599[b]	0.473[c]

[a*]: Highest correlation (F x P: 0.944), [a]: High correlation [b]: Moderate correlation, [c]: Low correlation [c*]: Lowest correlation (P x FM: 0.338)

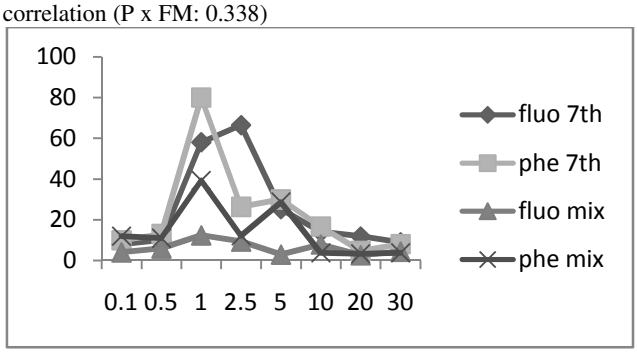

Fig. 5. Uptake of phenanthrene, fluoranthene and both by *Aulosira fertilissima* after 7 days.

Table 4: One-Way ANOVA for Uptake of Fluoranthene and Phenanthrene by *Aulosira fertilissima*

ANOVA	F (cal)	P
F	3.873[a]	* (P≤ 0.05)
P	15.829[b]	*** (P<=0.001)
FM	1.004	N.S. (P>0.05)
PM	1.457	N.S. (P>0.05)

F: fluoranthene, P: phenanthrene, FM: fluoranthene mixture, PM: phenanthrene in mixture

The removal of organic compounds by cyanobacterial uptake and metabolism is species specific, and is also toxicant dependent. The molecular weight, water solubility and lipophilicity of the compound would affect the bioaccumulation and degradation by microorganisms. The 4-ring PAHs, Xuoranthene and pyrene, were easier to remove and degrade by *Selenastrum capricornutum* than phenanthrene, a 3-ring PAH (Chan *et al.* 2013). Similar to microalgae, Potinet *et al.* (2004) reported that two filamentous fungi isolated from PAH-contaminated soil, namely *Coniothyrium sp.* and *Fusarium sp.* preferentially degrade high molecular weight PAHs (5–6 ring) than low molecular weight PAHs. On the contrary, PAHs with low molecular weight such as 2-ring naphthalene and 3-ring phenanthrene were more susceptible to bacterial degradation than PAHs with more than 3-rings (Juhasz and Naidu, 2000; Yu *et al.*, 2005). In the present study also *Aulosira fertilissima* showed higher efficiency in the removal of phenanthrene 3 ring structure than fluoranthene 4 ring structure. These results indicate that fluoranthene was comparatively more stable, recalcitrant, and difficult to remove by cyanobacteria. The mechanism of naphthalene oxidation by this cyanobacterium is discussed by Tiehm. Fritzsche (1995) suggested that the degradation of soluble pyrene by a bacterial strain, *Mycobacterium*, enhanced in presence of another PAH, phenanthrene. Similarly, naphthalene could stimulate degradation of phenanthrene and pyrene by a bacterial isolate *Pseudomonas putida* KBM-1 (McNally *et al.*, 1999). However, inhibitory interaction and competition between mixed PAHs was also reported (Stringfellow and Aitken, 1995; Bouchez *et al.*, 1995).In the current investigation too, the production of biotrnasformants are more and degradation of fluoranthene stimulate phenanthrene where the results are corroborated with the findings of Dean-Ross *et al.*(2002) who found that utilization of pyrene by a bacterial strain, *Mycobacterium xavescens*, was slower in the presence of Xuoranthene than its absence, although it could utilize the two PAHs simultaneously.

Conclusions

From the experiment, we could conclude that *Aulosira fertilissima* has higher efficiency of forming biotransformants during biodegradation of PAHs, especially phenanthrene a three ring structure and having low molecular weight as compared with fluoranthene. The degradation and uptake of phenanthrene by *Aulosira fertilissima* is high. The biodegradation of phenanthrene was also stimulated by presence of fluoranthene in mixture of both PAHs compounds utilized.

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