Polycyclic Aromatic Hydrocarbons are formed by two or more fused benzene rings in linear, angular or cluster arrangements and are predominantly available in the aquatic and terrestrial environments due to several polluting anthropogenic activities and incomplete combustion of organic material. Among all pollutants, the toxic, mutagenic, and carcinogenic properties of polycyclic aromatic hydrocarbons (PAHs) have motivated scientists in putting efforts to remediate PAHs from the environment (Samanta et al., 2002). They have been recognized as a potential health risk due to their intrinsic chemical stability, high recalcitrance to different types of degradation and high toxicity to living organisms. The United State Environmental Protection Agency (USEPA) has identified 16 PAHs compounds as priority pollutants based on their abundance and toxicity.

PAHs are degraded by a range of naturally occurring microorganisms. As a group, PAHs show varying ability to induce cancer and is difficult to identify the structural features associated with their carcinogenic activity. Furthermore, some environmental transformation products of PAHs may also react directly with DNA, causing mutations and possibly cancer (Pitts et al., 1982). The lipophilic character of aromatic hydrocarbon can alter the membrane fluidity, permeability, causes lipid bilayer disruption, diminish the energy transduction, affects activity of membrane associated carbohydrate and proteins. PAHs induced responses in cyanobacteria, interference with membrane-mediated physiological and biochemical processes like membrane permeability, disruption in activity of photosynthetic pigments, PS-II and PS-I, enzymatic dysfunction, and interaction with biomolecules like carbohydrates, proteins, amino acids and phenol.

Cyanobacteria are known to live in wide range of environmental conditions and play a vital role in primary production, decomposition, nutrient cycling, and energy flow. The success of cyanobacteria in many past and modern environments can be attributed to large extent to their metabolic versatility, flexibility and reactivity. Cyanobacteria have a choice of different metabolic pathways and physiologies. They are very reactive and respond instantaneously to the changing condition. Besides, cyanobacterial mats have also been shown to degrade many organic compounds including trinitrotoluene, chrysene, naphthalene, anthracene, benzopyrene, hexadecane, phenanthrene, polychlorinated biphenyl, trichloroethylene and certain pesticides (Kuritz and Wolk, 1995).
In recent years, studies have focused exclusively on the role of bacteria and fungi in the degradative processes. Algae remain poor investigated organisms by the environmental microbiologist, in spite of their ubiquitous distribution, their central role in the fixation, turnover of carbon, other nutrient elements and recognition of their heterotrophic abilities. In addition, some studies suggested that the cyanobacteria not only capable of bio accumulating heavy metals, but they also capable of biotransforming some of environmental pollutants including high molecular weight PAHs.

In present investigation, an attempt has been made to evaluate the toxicological effects of model PAHs- Anthracene and Pyrene on three different cyanobacteria- *Nostoc muscorum*, *Anabaena fertilissima* and *Synechocystis* sp. in laboratory conditions. The effect of model PAHs on morphological variations, heterocyst frequency, some biochemical parameters such as Photosynthetic pigment contents (chlorophyll-
\*a*, carotenoids and phycobiliproteins), cellular metabolites (total carbohydrates, total proteins, total amino acids, total phenol) and enzyme activity (Nitrate reductase, Glutamine synthetase, Succinate dehydrogenase) were determined after every 4-day interval up to 16 days. For further verification of the results molecular level approaches like Functional group variation using FTIR, and protein profiling (SDS-PAGE) was carried out. In addition, biodegradation of model PAHs and identification of biotransformants was determined using GC-MS. Study showed significant differences in morphological, biochemical and molecular pattern of these test cyanobacteria. In regard to above aspects following significant observations were recorded.

**Toxicological effects and Bioremediation of model PAHs on selected Cyanobacteria in vitro studies:**

- Among all three cyanobacterial species, maximum chlorophyll *a* reduction was encountered in *Nostoc muscorum* by 91.62%, followed by *Anabaena fertilissima* by 81.65% and *Synechocystis* sp. by 76.8% of Anthracene doses after 16 days. While Pyrene treated cultures exhibited maximum reduction of chlorophyll-a content of *Nostoc muscorum* (4.0 ppm) by 95.91%, *Anabaena fertilissima* by 84.8% and *Synechocystis* sp. by 92% after 16 days of exposure period.
The great fall of carbohydrates (92.4%), amino acids (94%) and proteins (81%) was encountered for *Nostoc muscorum* when treated with 4.0 ppm of Anthracene after 16 days. Moreover, phenols expressed a consistent raise in its content by 45% by the end of the experiment. The low inhibitory effect of Pyrene was observed in *Synechocystis* sp. and reflected low drop of carbohydrates (80%) followed by amino acids (78.4%) and proteins (68%) as well as increment in phenols (37%) after 16 days. Enzyme activities of all the three enzymes-nitrate reductase, glutamine synthetase and succinate dehydrogenase were highly inhibitory in response to the model PAHs doses.

GC-MS based biodegradation studies revealed that *Synechocystis* sp. could degrade 41.9% of 3.5 ppm of Anthracene and 36.7% of 2.5 ppm of Pyrene after 16 days. Moreover, bioremediation of Anthracene and Pyrene showed some important intermediate bio-transformants after 4 day and 16 days. Anthracene exposure to *Anabaena fertilissima* produced a common metabolite 2, 4-Dimethyl-1-heptene (C\textsubscript{9}H\textsubscript{18}) and 9-Octadecene (C\textsubscript{18}H\textsubscript{36}) in all the treated concentrations after 4-days. Besides, Hexane (C\textsubscript{6}H\textsubscript{12}), 2, 3, 4-Trimethylhexane and Erucic acid (C\textsubscript{22}H\textsubscript{36}O\textsubscript{2}) were recorded in all the three treatments when compared with Pyrene standard after 16 days.

FT-IR spectroscopy showed that new peaks of nitro compounds; N-O symmetric stretch, 1°, 2° amines; N-H wag, aromatic amines; C-N stretch, aromatics; C-C stretch (in-ring) and 1° amines; N-H bend were observed in Pyrene treated *Synechocystis* sp. whereas Anthracene treatment induced alcohols, carboxylic acids, esters, ethers; C-O stretch, 1° amines (N-H bend) and aliphatic amines; C-N stretch.

Protein expression displayed that synthesis of several proteins declined with increasing duration of exposure to the PAHs. Some unique bands of 29 kDa, 33 kDa, 48 kDa and 61kDa were also generated in cells of *Synechocystis* sp. after 16 days of Anthracene treatment. Majority of polypeptide bands were absent in PAHs treated *Nostoc muscorum* as compared to biotic control.

The trend of resistance capacity of the test species is as follows; *Synechocystis* sp. > *Anabaena fertilissima* > *Nostoc muscorum*.

In nature, most microalgae including cyanobacteria are found in association with other aerobic or anaerobic microorganisms. It is evident that the molecular oxygen from algal photosynthesis is used as an electron acceptor by bacteria to degrade organic matter. Carbon dioxide (CO\textsubscript{2}) from the bacterial mineralization
completes the photosynthetic cycle. The principle of self-oxygenation by natural systems can be effectively employed for remediation of many pollutants (Muñoz and Guieysse, 2006). In addition, Park et al (2008) suggested that long-term laboratory algal cultures have maintained symbiotic relationship with bacteria. Therefore, in another approach to improve/ enhance the bioremediation capacity of chief degrading PAHs cyanobacterial species, an artificial cyanobacterial- bacterial consortium was constructed.

Enrichment isolation, and identification of model PAHs degrading bacterial strains was carried out from common industrial effluent canal (Amalakhadi) and crude oil polluted site (Telva), near Ankleshwar Industrial Area, Gujarat, India. Furthermore, determination of toxicological effects of model PAHs on pigment contents, metabolites and activity of enzymes of artificially constructed Synechocystis sp. and bacteria consortia- JPNKA7, JPNKB2, and JPNKA7B2 was carried out at every four days intervals up to sixteen days. Biodegradation efficacy of consortia JPNKA7, JPNKB2 and JPNKA7B2 was determined using GC-MS at every four days interval up to sixteen days of treatments.

Toxicological effects and Bioremediation of model PAHs on defined artificial cyanobacterial and bacterial consortia in vitro studies:

- The results of artificial consortia constructed with indigenous PAHs degrading bacteria, indicated the involvement of bacteria could enhance the growth of Synechocystis sp. in terms of Chl-a by three to four fold then the monoculture of Synechocystis sp. Moreover, the declining trend of chlorophyll a, carotenoids and phycobiliproteins content was sharply lowered at the end of the experiment compared to monoculture of Synechocystis sp.

- Two PAHs degrading bacterial strains Bacillus benzoevorens AX7 (Gene bank Accession number AX7-1346516) and Pseudomonas indoxyladons BX2 (Gene bank Accession number BX2-236735) were isolated using enrichment culture technique from crude oil polluted site-Telva, and Industrial effluent canal (Amalakhadi) near Ankleshwar, Gujarat, India.

- In order to improve the tolerance and biodegradation efficacy of chief degrading cyanobacterial species Synechocystis sp. the predefined artificial cyanobacterial -
bacterial consortia were constructed and labeled as JPNKA7, JPNKB2 and JPNKA7B2. The LC_{50} doses for all three consortia were selected as 70, 100 and 150 ppm for Anthracene and 40, 70 and 100 ppm for Pyrene after 16 days.

- Amongst three different combinations of consortia, JPNKA7B2 could degrade 62 ppm of Anthracene and 50 ppm of Pyrene by 97% and 94% respectively after 16 days where monoculture of *Synechocystis* sp. could degrade up to 41% and 36% at 3.5 ppm of Anthracene and 2.5 ppm Pyrene respectively. Consortium JPNKB2 produced anthracene-1, 2-diol which is key metabolite and formed during Meta cleavage pathway of Anthracene degradation. The degradation of Pyrene showed the major key metabolites phenanthrene-4 carboxylate, 1, 2 naphthenic acid and acenaphthalene after four days of treatment which ensures ring cleavage of four rings containing compound Pyrene.

- Thus, the degree of degradation capacity of model PAHs by different consortia are in the following order: consortium JPNKA7B2 > consortium JPNKB2 > consortium JPNKA7 > *Synechocystis* sp. monoculture.

*In vitro* study suggested the great potential of defined artificial cyanobacterial-bacterial consortia JPNK to remediate model PAHs. Therefore, another attempt has been made to evaluate the effect of environmental ingredients during bioremediation of PAHs by cyanobacterial and bacterial consortia in *ex situ* studies:

- The *ex situ* bioremediation of model PAHs was performed in the month of January to March 2014 by applying different consortia in microcosm. Results suggested that among all three, consortium JPNKA7B2+NPK displayed highest degradation by 97.1% at 200 ppm Anthracene and 94.16% at 150 ppm of Pyrene with combined bioaugmentation and biostimulation approach and effective in stimulating the indigenous microbial population, confirms the indigenous microorganisms were physiologically adapted and capable of degrading PAHs in the contaminated environment. However, *Synechocystis* sp. monoculture did not resulted in active remediation of the Anthracene and Pyrene due to the inefficiency of the microbial population to strive against the PAHs contamination.

- Present study suggested that degradation of model PAHs was more pronounced at alkaline pH ranged from 8.2 ± 0.7 and 23±0.2 °C temperature. Physicochemical
properties of water and sediment samples in microcosm - pH, temperature and conductivity, salinity, nutrient levels are key factors in degradation rate of model PAHs.

These findings would be first report of photosynthesis-enhanced biodegradation of Anthracene and Pyrene by microalgal–bacterial consortium in a one-stage treatment. Thus, artificial cyanobacterial-bacterial consortium further may recommend for remediation of Anthracene, Pyrene and other toxic environment pollutant contaminated sites in situ.