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Evaluation of bioremediation effectiveness on sediments Contaminated with industrial wastes

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ABSTRACT

A treatability study was conducted to determine the most effective bioremediation strategies for the decontamination of sediments dredged from industrially polluted Gandigudem Lake. Microcosm experiments were performed with the addition of a microbial consortium and nutrients. The performance of each treatment was examined by monitoring biological parameters such as basal respiration, microbial biomass carbon (C_{mic}), metabolic quotient (qCO_2), C_{mic}/TOC ratio, dehydrogenase activity, and phytotoxicity. Results of the study suggest that the addition of nutrients to the contaminated sediments accelerated bioremediation and the application of an enriched native microbial consortium in concentration greater than the indigenous microbial population further increased the bioremediation efficiency. The study also demonstrated the effectiveness of combining bioassays with chemical monitoring for evaluation of bioremediation effectiveness and assessment of the de-contaminated/ stabilized sediments.

Keywords: Sediments, bioremediation, microcosm experiments, microbial consortium, bioassays.

1. Introduction

Polluted sediments are a serious environmental risk for human beings and ecosystems. The city of Hyderabad, India is located in a semi-arid portion of the country and has about 80 lakes in and around the city. Recent industrial growth in the city has resulted in the development of eight large industrial estates in the precatchment areas that unscrupulously discharge their effluents into the lakes thereby severely impacting the natural ecological balance of these surface water bodies .These concerns continue to drive the need for development and application of the remedial technologies.

Biostimulation is a bioremediation approach that relies on stimulating the activity of indigenous microorganisms by introducing electron acceptors, electron donors and/or nutrients into the contaminated matrices. The indigenous microorganisms may or may not primarily target the organics as a food source. However, the organics are assumed to degrade more quickly in comparison to natural attenuation due to the increased numbers of microorganisms caused by increased levels of nutrients. The process of bioaugmentation, on the other hand, involves the introduction of microorganisms that have been cultured to degrade specific organic compounds in a contaminated system. The cultures may be derived from the contaminated soil or they may be obtained from a stock of microbes that have been previously proven to degrade organic compounds. Once introduced into the system, the

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cultured microorganisms selectively consume the contaminants of concern. The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have been widely demonstrated (Margesin and Schinner, 2001).

A variety of chemical and biological parameters are used to describe the stage of organic matter stabilization during different biological treatments. Chemical data alone are not sufficient to evaluate the biological effects, since it is impossible to analyze all the compounds and synergistic effects contributing to toxicity. Biological and biochemical properties including the rate of CO_2 release or oxygen consumption, microbial biomass and activities of enzymes are considered to be sensitive indicators of remediation progress due of their relevance in cycling of organic matter. These indicators also provide information about the presence of viable microorganisms and impact of pollutants on the metabolic activity in the contaminated media (Huang et al., 2006). Parameters such as the microbial biomass carbon (C_{mic}), microbial metabolic quotient (qCO_2) and C_{mic}/total organic carbon (TOC) ratio can provide valuable information about the biochemical processes occurring during bioremediation. There is growing evidence that such biological parameters have a great potential as early and sensitive indicators of sediment ecological stress and restoration progress.

The study reported in this manuscript evaluated the potential of different bioremediation approaches to decontaminate dredged sediments. The study also investigated the usefulness of selected biological parameters for monitoring and assessing the results of the bioremediation process.

2. Materials and Methods

2.1 Site and Sediment Characterization

The sediments used in this study were collected from Lake Gandigudem which is situated in the highly industrialized Medak district of Andhra Pradesh, India. This lake is a 0.21 km^2 reservoir that was developed for regional flood control. It has a hydraulic capacity of approximately 0.9 million m^3. Several medium and large-scale industries including pharmaceutical, bulk drug and pesticide manufacturing units discharge untreated or partially-treated effluents into an open channel, which ultimately flows into the reservoir.

In this study, sediment samples obtained from the lake bed were air-dried ground, sieved (< 2mm), and characterized for physico-chemical properties (pH, TOC, total nitrogen, total phosphorous, and cation exchange capacity) according to standard methods (APHA.,1998). Heavy metals were extracted from the sediment samples following ISO method 11466 (1995). The extracts were analysed for heavy metals by inductively coupled plasma spectrometry according to ISO method 11885 (1996).

2.2 Microbial Consortium for Bioremediation:

Composite samples of contaminated soil were collected from various points of potential contamination in the industrial zone of Medak district. A mixed culture was revived in a culture medium consisting of 2 g NH_4Cl, 0.2 g NaCl, 0.2 g $MgSO_4\,7H_2O$, 0.5 g KH_2PO_4, in 1

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liter H_2O. Subcultures were prepared every 5 to 7 days by transferring 2 ml of a full-grown culture into 125 ml of fresh medium in a 250 ml Erlenmeyer flask. The carbon source was derived from the contaminants added during the acclimation and/or treatment procedures. The cultures were placed on an orbital shaker operated at 35°C and 250 rpm. The culture solution provided the microorganisms with the mineral nutrients necessary for survival and cell growth.

2.3 Acclimation Procedure

The acclimation procedure involved an initial addition of 0.01 g of the composite sample of contaminated sediments to the 24 h culture maintained at 35°C and 250 rpm. After 5 to 7 days, approximately 1 ml of the culture was transferred to 125 ml of fresh revival solution. After 1 day, which allowed an adequate cell population to grow, 0.1 g of sediment was added. This process was repeated with incremental additions of 0.1 g of sediment until a total of 0.5 g was added. After several enrichment steps the culture was harvested in its mid log phase (10^9 CFU/ml) and this consortium was used in bioremediation studies. The consortium was tested for its ability to utilize the complex organics in the sediments under aerobic conditions (data not shown).

2.4 Bioremediation Experiments

Sediment samples were collected from the bottom of Gandigudem Lake by dredging about 0.25 m of the top layer of the lake bed. Laboratory-scale bioremediation tests were conducted using microcosms. The experimental protocol consisted of four treatments and sterilized controls.

2.5 Microcosm Description

2.5.1 Basic treatment

Five pans with a surface area of 450 cm^2 and a volume of 1794 cm^3, each containing 500 g of contaminated sediments were prepared. The sediments were mixed weekly with a sterile spatula to provide sufficient air and oxygen. The microcosms were covered with aluminum foil and stored at room temperature (35°C). Deionized water was then added every week to the pans to achieve sediment moisture content of approximately 60% of the water holding capacity. These conditions were applied to all treatments.

- The sediment in Pan 1 (control) was sterilized three times by autoclaving at 121°C for 30 min.
- Biostimulation with simple aeration was evaluated in Pan 2, which did not receive any nutrient or culture supplementation.
- Biostimulation with aeration and nutrient addition was evaluated in Pan 3 which received urea, $(NH_4)_2SO_4$ and K_2HPO_4 to provide a C: N: P molar ratio of 100:10:1.
- Bioaugmentation with aeration and nutrient addition was evaluated in Pan 4, which received nutrients and a 50 ml inoculum of $3.2x10^9$ CFU/ml of the microbial consortium previously enriched from the contaminated soil.

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2.6 Sediment Analyses

Sediments in the treatment units were sampled at 0, 2, 4, 6, 8, and 12 weeks for chemical and microbiological analyses. Composite samples were collected from five different areas of the microcosm. A portion of the composite sediment mixture was placed in sterile bottles for microbiological analyses, and the remainder was analyzed for residual chemical contaminants.

The modified Walkley-Black dichromate oxidation method was used for the determination of total organic matter . TOC was determined using the formula given by Navarro et al., 1990):
TOC = (TOM -9.33/1.745)
Where, TOM represents the total organic matter of the sample.

Respiratory measurements were conducted in triplicate without (basal respiration) and after addition of a growth substrate to the samples (substrate induced respiration, SIR). Microbial biomass carbon (C_{mic}) was estimated by the SIR method. Growth substrate for the SIR test was prepared according to Palmberg and Nordgren., 1993, Which consisted of 80 g glucose, 13 g $(NH_4)_2SO_4$ and 2 g KH_2PO_4. The substrate ingredients were mixed and thoroughly ground in mortar. Approximately 100 mg of the substrate was mixed with 10 g (dry weight) of sediment removed from the treatment unit.CO_2 monitoring was performed by transferring 2 g sediment samples from different treatment units into a plastic vial. The vials were placed in closed 1-liter glass jars. A glass vial containing 10 ml 0.2 N NaOH was placed in each jar to trap CO_2 resulting from substrate mineralization The NaOH trap was periodically replaced. $BaCl_2$ (10 ml) was added to the NaOH trap and the amount of CO_2 produced by each microcosm determined by titration with 0:1 N HCl. C_{mic} was estimated as mg microbial biomass-C/kg dry sediment using a conversion factor of 30 (Frish and Hoper 2003). The metabolic quotient (qCO_2) was calculated as the ratio of basal respiration to C_{mic}.

Dehydrogenase activity was determined by monitoring the rate of reduction of 2, 3, 5-triphenyltetrazolium chloride to triphenyl formazan as described by Alef 1995 . Dehydrogenase activity was calculated as µg of formazan per gram of soil after 24 h, and expressed as relative activity (%) in relation to the control activity (100%).

2.7. Phytotoxicity

Germination index (GI) was used to evaluate the phytotoxicity of sediments to garden cress, *Lepidium sativum.* Water was added to the sediment sample to obtain moisture content of 85%. After 2 h, the sediment extract was separated by centrifugation (6000 rpm) and filtered with a 0.8 µm pore size filter paper. The filtrate was diluted with distilled water to 3:1 and 1:1 ratios of extract to water (v/v). Five milliliter of each dilution was distributed in five Petri dishes with 10 seeds each of *Lepidium satvium*. The seeds were incubated at 27◦C for 24 h in the dark. The number of germinated seeds and the root elongation of the sample compared to the control (distilled water) were used to calculate the GI according to the following equation:

$$GI\ (\%) = GsLs\ /GcLc \times 100$$

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Where, *G*s and *G*c are the average number of germinated seeds in the sample and in the control replicates, respectively and *L*s and *L*c represent the average root elongation in the sample and in control replicates, respectively.

Sediment samples were collected and analyzed for total PAHs by USEPA Method 3550 and total phenols by APHA 5530. The nitro- and chloro-aromatic compounds were extracted from the sediments and determined using Method 8330 (US Environmental Protection Agency, 1997).1,2 Dichlorobenezene and 1,4 Dichlorobenezene were determined using the method 8121 (EPA 1994) and method 8021(EPA 1996). All analyses were conducted by a commercial laboratory and results were reported on a dry weight basis.

3. Results and Discussion:

This section presents a discussion of results obtained from the experiments described above.

3.1 Sediment Analyses

The general physico-chemical characteristics of the sediments collected from the contaminated lake bed of Gandigudem Lake are summarized in **Table 1**.

Table 1: Physical and chemical characteristics of Sediments

Parameter	Sediment
Composition (Sand:Silt:Clay)	60:4:32
TOC (%) w/w	19.6
Total Nitrogen % w/w	0.31
pH	7.8
Nitrate and Nitrite (mg kg^{-1})	0.93
Total Phosphorus (mg kg^{-1})	1.5
[a]CEC(CMol/kg)	8.2

* Presented data are arithmetic means of two sub samples per homogenized sediment sample.[a] Cat ion exchange capacity

3.2 Respiration Measurements

The microorganism metabolic activity (respiration) increased significantly with time in all the treatments. The metabolic activity of indigenous microorganisms was determined as production of CO_2 evolution (basal respiration). The basal respiration reflects the activity of sediment micro flora, which may be related to the biodegradation of organic compounds in sediments (Franco et al., 2004). Figure 1 illustrates the CO_2 evolution data for the different treatments performed on the sediments. Respiration measurements give an idea of the microbial activity in sediments and the quantity and quality of substrates susceptible to mineralization (Frische & Hoper., 2003). The cumulative CO_2 data indicated a progressive

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increase in respiratory activity especially when nutrients or culture was added. The highest CO_2 evolution was observed in Pan 4 (22.4 gm/d/dw) till the 8th week. This treatment unit received bioaugmentation with nutrient addition and aeration. This was followed by Pan 3 (21.3 gm/d/dw), which received biostimulation with nutrient amendment and aeration. Similarly, Pan 2, which was subjected to biostimulation with aeration only showed some increase in CO_2 production (8.7 gm/d/dw) as compared with Pan 1 (control).

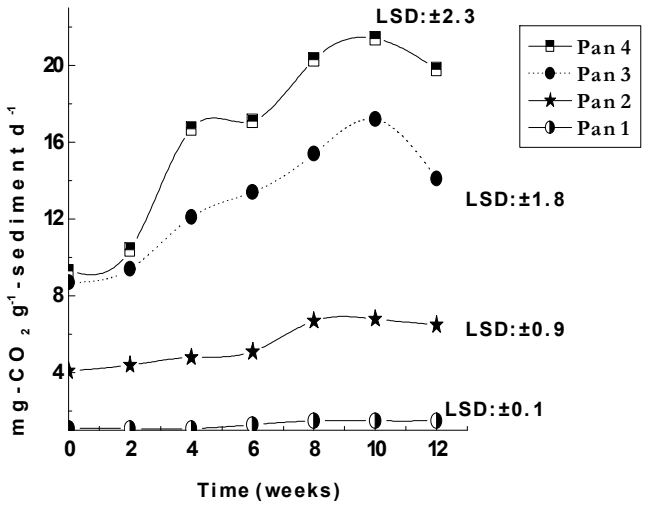

Figure 1: Changes in basal respiration (CO_2 – C g day $^{-1}$ per g^{-1} sediment (dry weight) during bioremediation of contaminated lake sediments

Microbial biomass in sediments is an agent of biodegradation, the primary mechanism by which dissolved organic matter is decomposed and organic pollutants are removed from the soil (Marschner and Kabitz., 2003). The microbial biomass carbon, C_{mic}, as determined by substrate induced respiration, demonstrated maximum values after 5 weeks of treatment. However, contrary to basal respiration, C_{mic} was observed to fluctuate, most likely due to changes in microbial biomass caused by microbial succession (Figure 2). Both respiration and biomass were significantly higher in Pan 4 (bioaugmentation) and Pan 3 (biostimulation with nutrient addition and aeration). The impact of bioaugmentation was clearly visible beyond week 6 where the microbial biomass carbon in Pan 4 was observed to decrease more gradually than that in Pan 3.

The metabolic quotient (qCO_2) reflects the efficiency with which carbon is used by soil microbes (Marin et al., 2005). It can also serve as an index of microbial stress in the face of contamination. (Wardle and Ghani, 1995). Changes in the metabolic quotients calculated during the 12 weeks of treatment were compared with the initial status.

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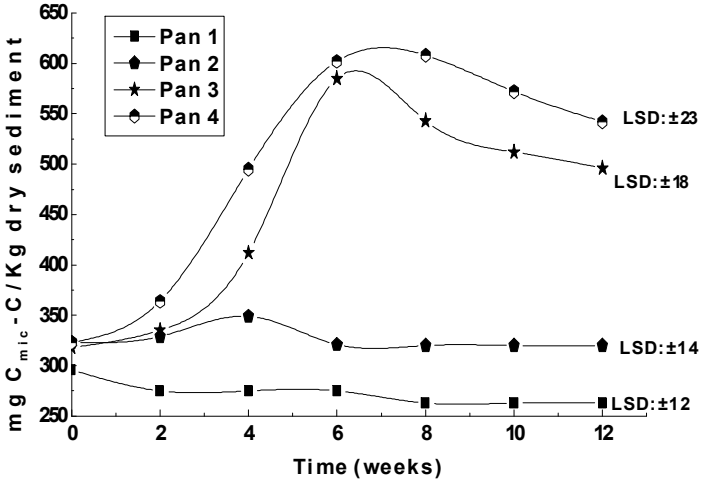

Figure 2: Changes in microbial biomass carbon (C_{mic})

Samples taken from the Pans 3 and 4 showed significant increases in qCO_2 values when compared to Pan 2 during the first 8 weeks of treatment. However, these values decreased beyond week 8 with the decrease in the microbial activity (Figure 3). Elevated qCO_2 values indicate a rather small, but active microbial biomass. At the end of the experiment the values of qCO_2 tend to decrease because the system reached a new equilibrium after the disappearance of biodegradable organics. Pan 1 (control) showed lower metabolic quotients and remained unchanged after treatment.

The C_{mic}/TOC ratio was found to increase with the increase in the metabolic activity during treatment. It showed a highest response in Pan 4 when compared to the Pan 3 (Figure 4). It is clear that the microbial biomass increases in quantity to help in the biodegradation of organic substrates during bioremediation. At the end of the bioremediation process the ratio decreased because most of the biodegradable organics present in the sediments were degraded by the indigenous or added microorganisms. All four parameters described above indicated that microbial activity diminished towards the end of 12 weeks.

3.3 Dehydrogenase Activity

Enzymatic activity was investigated to evaluate the efficiency of the microbial community in utilizing organic matter. Dehydrogenase activity in sediments is used to monitor microbial activity and as an index of the total oxidative activity in a sample. Biological oxidation of organic compounds can result in dehydrogenation processes that are catalyzed by dehydrogenase enzymes. These enzymes play an essential role in the oxidation of organic matter by transferring hydrogen from microbial populations capable of degrading organic substrates to the electron acceptor. The assay of dehydrogenase in contaminated sediments can be used as a simple method to examine the possible inhibitory effects of contaminants on microbial populations (Bento et al., 2005).

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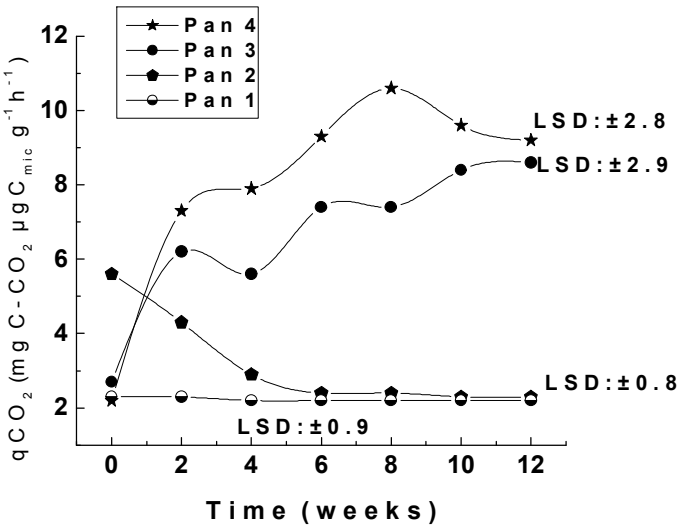

Figure 3: Changes in metabolic quotient

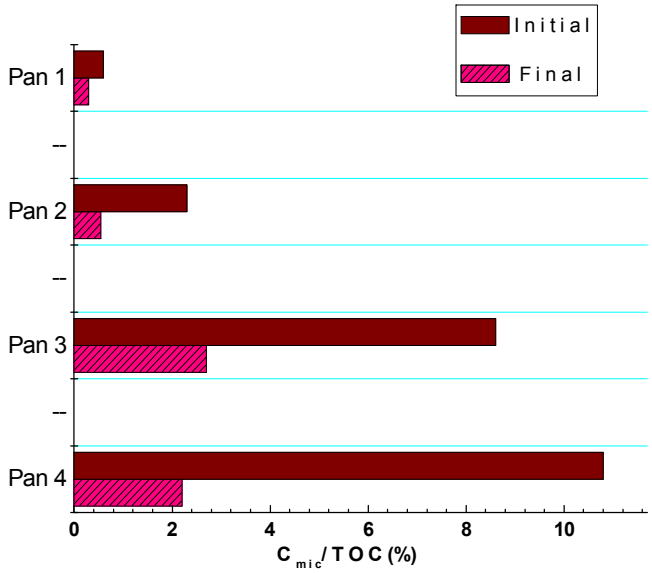

Figure 4: C_{mic}/TOC ratio

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Figure 5 illustrates the relative activity of dehydrogenase during treatment. The highest microbial activity was observed to occur in Pan 4 as indicated by the high dehydrogenase activity**.** The addition of an enriched microbial consortium increased the dehydrogenase activity from 5.8 to 125.8 µg INTF/gm dw by week 8. Biostimulation with nutrient addition increased dehydrogenase activity from 5.8 to 95.6 µg INTF/gm dw by week 9. Biostimulation with aeration only (Pan 2) also had a positive impact on enzymatic activity where a slight increase in dehydrogenase activity, from 5.9 to 27.3 µg INTF/gm dw was observed by week 8. After 9 weeks, dehydrogenase activity diminished in all treatment units since all labile organics were degraded.

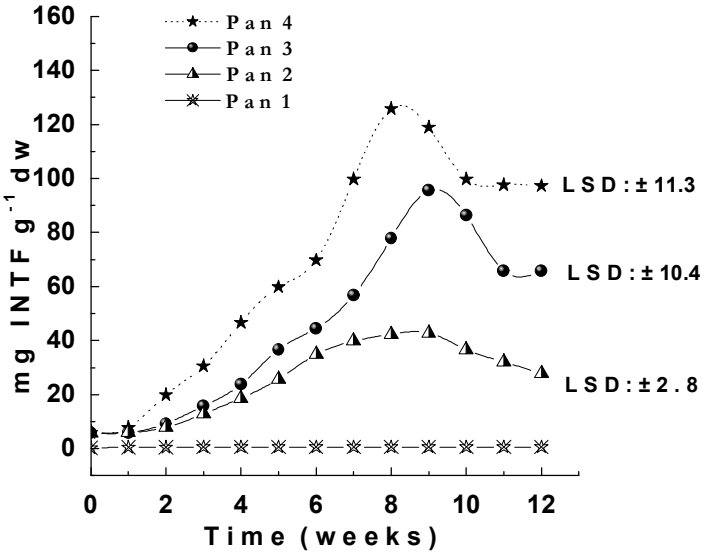

Figure 5: Changes in Dehydrogenase activity (Iodo tetrazolium formazan (INTF) g $^{-1}$ of sediment (dry weight)

3.4. Phytotoxicity Assay

A phytotoxicity assay was performed using *L. sativum* to evaluate the rate and extent of detoxification during bioremediation. This assay can measure low toxicities affecting root growth and high toxicities affecting germination. The toxicity assay was expected to provide information complementary to the microbial activity measurements described above. Data illustrated in Figure 6 show that all treatments resulted in decrease of toxicity in the sediments as indicated by the increase in the values of germination index (GI). Phytotoxicity level decreased dramatically in sediments in which an enriched culture of microorganisms was added (Pan 4). A comparatively lower decline in phytotoxicity was observed in Pan 3 in which indigenous microbial activity was stimulated by the addition of nutrients and aeration**.** A small increase in GI values was also observed for Pan 2 which received biostimulation

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with aeration only. Controls showed low values of germination index which did not change during treatment.

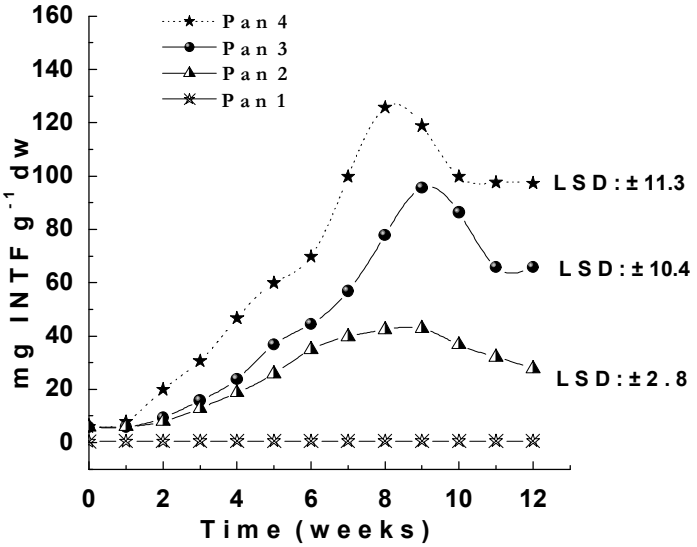

Figure 6: Changes in Germination Index (GI)

3.5 Chemical Monitoring

Chemical analysis of the sediment samples revealed contamination with a number of toxicants. Besides phenolic compounds as the major contaminants, the sediments were also contaminated with benzene, xylene, toluene, chlorobenezenes, PAHs and heavy metals as co contaminants. Figure 7 illustrates the change in chemical concentrations in different treatment units during the 12 week treatment period.

Chemical monitoring data revealed a significant decrease in the concentration of all organics in the treatment units (Pans 2 to 4). Pan 4 showed the highest percentage of contaminant removal during 12 weeks of incubation, even when part of the loss was attributed to volatilization as observed in control samples (Table 2).

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Table 2: Contamination of sediment samples taken from the experimental Pans installation (0 months) and after 12 weeks of remediation

PARAMETER (mg/kg dry wt)	PAN 4	PAN 3	PAN 2
0 weeks			
Benezene	59.5	59.5	59.6
Toulene	38.6	38.8	38.6
Xylene	16.8	16.8	16.8
Total PAH's	9.8	9.82	9.8
1,2 DCB	32.14	32.16	32.14
1,4 DCB	26.7	26.4	26.6
Total Phenols	346	346	346
Arsenic(As)	24.5	24.8	23.6
Copper(Cu)	38.9	33.5	33.4
Chromium(Cr)	30.3	32.2	34.5
Nickel(Ni)	43.3	46.5	42.3
Lead (Pb)	213.4	218.7	213.4
Zinc(Zn)	260.6	256.8	254.3
12 weeks			
Benezene	9.9	17.0	46.8
Toulene	3.9	13.4	33.8
Xylene	3.9	5.3	14.7
Total PAH's	4.1	4.9	8.0
1,2 DCB	7.1	12.4	25.3
1,4 DCB	5.4	9.7	22.9
Total Phenols	19.6	46.9	249.6
Arsenic(As)	20.5	22.6	23.4
Copper(Cu)	27.5	29.8	30.6
Chromium(Cr)	20.3	24.5	30.9
Nickel(Ni)	19.8	32.4	41.5
Lead (Pb)	196.7	204.8	210.3
Zinc(Zn)	240.9	245.3	250.9

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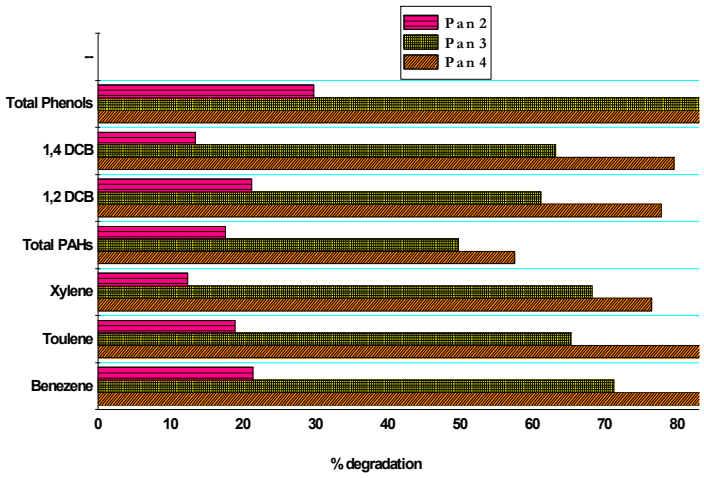

Figure 7: % Degradation of various organics during different bioremediation treatments

4. Conclusion

This study reports changes in various microbial and enzymatic parameters during microbial decontamination of sediments contaminated with industrial wastes. The combination of biological parameters used in this study proved to be appropriate for monitoring remediation efficiency during bioremediation. The parameters studied reliably and consistently indicated bioremediation progress during treatment. The study also demonstrated that bioaugmentation was more effective in removing a variety of organic contaminants from the lake sediments as compared to biostimulation with aeration and nutrient addition. The latter was significantly more effective than biostimulation with aeration only. The microorganisms utilized the sediment organic matter and reduced phytotoxicity as demonstrated by increasing dehydrogenase activity, respiration and germination index during first 8 weeks of treatment. Increasing C_{mic}, metabolic quotient and C_{mic}/TOC ratio also indicated that the metabolic efficiency in the conversion of carbon substrates and biomass increased during the first 8 weeks. This study shows that bioaugmentation or biostimulation with nutrient addition was required to initiate and sustain degradation of the contaminants in the lake sediments. Our observations support the prevailing view that in many contaminated sediments, microorganisms with the appropriate metabolic capabilities occur naturally, and these microorganisms can be stimulated by nutrient amendment. Results from this study provide valuable information for developing remediation approaches to manage lake bed sediments contaminated with organic pollutants.

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