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Aspergillus flavus: A potential Bioremediator for oil contaminated soils

By

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Abstract:

Biodegradation is cost-effective, environmentally friendly treatment for oily contaminated sites by the use of microorganisms. In this study, laboratory experiments were conducted to establish the performance of fungal isolates in degradation of organic compounds contained in soils contaminated with petrol and diesel. As a result of the laboratory screening, two natural fungal strains capable of degrading total organic carbons (TOC) were prepared from isolates enriched from the oil contaminated sites. Experiments were conducted in Erlenmeyer flasks under aerobic conditions, with TOC removal percentage varied from 0.7 to 32% depending on strains type and concentration. Strains *Phanerocheate chrysosporium* and *Aspergillus niger* exhibited the highest TOC removal percentage of 32 and 21%, respectively, before nutrient addition. TOC removal rate was enhanced after addition of nutrients to incubated flasks. The highest TOC reduction (45%) was estimated after addition of combination of nitrogen, phosphorus and sulphur to *Phanerocheate chrysosporium* strains. Results of experimental work carried out elucidate that the fungi like *Phanerocheate chrysosporium* and *Aspergillus niger* were capabled of producing enzymes at a faster rate to decompose the substrate hydrocarbon and released more CO_2 and hence these potential fungi can be utilized effectively as agents of biodegradation in waste recycling process and Bioremediation of oil contaminated sites.

Key words: Biodegradation, Total organic carbon *(TOC), Soil Fungi, Phanerocheate chrysosporium, Aspergillus niger.*

1. Introduction

Large quantities of organic and inorganic compounds are released into the environment every year as a result of human activities. Soil contamination is a typical side-effect of industrial activity. Among the technologies available to deal with contaminated soils, bioremediation based on the metabolic activity of microorganisms has certain advantages (Rajiv et al 2009 & Kaviyarasan and Karthikeyan 2003). Environmental pollution with petroleum and petrochemical products (complex mixtures of Hydrocarbons) has been recognized as one of most serious current problems. Bioremediation has become an alternative way of

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remediation of oil contaminated sites, where the addition of specific microorganisms (Bacteria, Cyanobacteria, Algae, Fungi, Protozoa) or enhancement of microorganisms already present can improve biodegradation efficiency in both *in-situ* and or *ex-situ* (In reactors) procedures.

Physical, chemical and biological factors have complex effects on hydrocarbon biodegradation in soil (Bossert and Compeau 1995). For this reason, experts frequently recommend that soil bioremediation projects begin with treatability studies to empirically test the biodegradability of the hydrocarbon contaminants and to optimize treatment conditions. On the other hand, it is possible that the expense of such treatability studies could be avoided or minimized, if certain soil characteristics could be measured and used to predict the potential for bioremediation of a site, the kinetics of hydrocarbon removal or the optimal values for certain controllable treatment conditions. For example, certain co-contaminants such as heavy metals might preclude hydrocarbon bioremediation. Or, soil particle size distribution might partly dictate the potential rate and extent of hydrocarbon removal.

We examined hydrocarbon biodegradation in microcosms with three selected strains in soil samples from petrol and diesel contaminated sites. We determined the effects on biodegradation, including (i) Intrinsic soil properties (particle size, C content, water holding capacity), (ii) Soil contaminants (heavy metals), (iii) Controllable conditions (temperature, N and P content) and (iv) Inoculation with hydrocarbon-degrading microorganisms. We addressed the question of whether measuring soil characteristics could allow prediction of the outcome of soil bioremediation. Also, we identified treatment options which generally appear to benefit bioremediation of oil contaminated soils. This is the first such comprehensive study characterizing hydrocarbon biodegradation in oil contaminated soils of Petroleum servicing stations and automobile servicing stations.

2. Materials and Methods

Ten hydrocarbon-contaminated soil samples were taken during August and November 2011 from five Petroleum servicing stations and automobile servicing stations of each present within the city limits of Visakhapatnam. Samples were taken from the top 10 cm of soil with clean trowels or scoops and placed in glass bottles. Samples were brought to laboratory with necessary precautions. Soil samples were sieved (4.7 mm mesh) and well mixed prior to use. Ten characteristics of each soil sample were measured. Soil pH was determined in slurry with distilled water. Water holding capacity (WHC) was determined gravimetrically (6). The following measurements were performed by methods

described by Michael (10). Soil Analysis: total C and organic C, available P as well as % gravel, % sand, % silt and % clay.

We carried out three treatments to evaluate the efficiency of petrol and diesel oil degradation in the contaminated soils, using sterilized soils as controls. The treatments were: (a) Natural attenuation (soil's natural ability to degrade the contaminant); (b) Biostimulation (adding nutrients to improve the natural biodegradation rate); and (c) Bioaugmentation (addition of a microbial consortium from selected species isolated from a contaminated soil plus nutrients).

These experiments were conducted to examine the ability of consortium 7, 13, and 16 to degrade oil collected from Oil contaminated soils. The laboratory tests were carried out in duplicate under aerobic conditions in Erlenmeyer flasks (250 ml) as incubation reactors. Flasks were shaken on a rotary shaker at 200 rpm at 35 and 28 °C (field temperature range of the studied area). The oil degrading efficiency of different consortia was screened on minimal salt medium. In three sets of Erlenmeyer flasks, oil (100 ml sample) was inoculated with two fugal strains ,Phanerocheate chrysosporium *and* Aspergillus niger *prepared at different concentration of minimal salt medium (0.1, 1 and 0.05%) designated as A, B, and C, respectively. The total final volume of oil, nutrient broth and consortia was 100 ml. The final consortia concentration was maintained as indicated. In addition control samples on fungal mycelia -free basis were run in parallel. Flasks were incubated for 10 and 18 days. TOC% degradation was determined as mentioned in the above, which was considered as our base line control sample.*

Respirometry experiments were conducted with soil, soil with nutrients nitrogen (N) and phosphorus (P) and soil with nutrients supplemented with glucose (0.2 g), an easily assimilable substrate, in order to assess the real and potential metabolic activity of indigenous microorganisms. A 20 g sample of sieved soil (<2 mm) was placed in a plastic vial. Field holding capacity was adjusted with water to 60% in all treatments. N and P were added as NH_4Cl *(150 mg) and* K_2HPO_4 *(20 mg). Vials containing soil were placed in closed 1-l glass jars. A glass vial containing 10 ml 0.2 N NaOH was placed in each jar to trap* CO_2*. 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.*

3. Results and Discussion

The Physico – Chemical characters of the soil samples were represented in Table 1. The evolution of cumulative CO_2 in soil, inoculated with *Phanerocheate chrysosporium* indicated a progressive increase in respiratory activity, especially when nutrients or glucose were added. In contrast, soil, inoculated with *Aspergillus niger* showed a null response in the first 5 days, followed by a slight increase when glucose was added. This difference could be attributed to the absence of assimilable sources of carbon and energy or to a presence of toxic compounds in soil (Table 2). The measurement of carbon dioxide released during the biodegradation process may be used as an index of hydrocarbon degradation

(Michael 1986).Table 2, summarizes the CO_2 released during biodegradation in a comparative account for *Phanerochaete chrysosporium* and *Aspergillus niger*. On the fifth day of degradation the CO_2 release by *Phanerocheate chrysosporium* was observed to be 12.6mg where as it was 7.02mg in *Aspergillus niger*. On the tenth day of degradation CO_2 evolved by *Phanerocheate chrysosporium* was maximum of 19.50mg and that of *Aspergillus niger* was 16.81mg. On the fifteenth day of degradation the CO_2 release by *Phanerocheate chrysosporium* and *Aspergillus niger* was 11.73mg and 11.2 mg respectively. After the twentieth day of degradation the CO_2 release was observed to be 7.64mg by *Phanerocheate chrysosporium* and 7.4mg by *Aspergillus niger* (Fig 1).

Similar biodegradation experiments were carried both in sterilized and unsterlized soils to study the soil's natural ability to degrade the contaminant.(Table 3).The results observed are the CO_2 release during 5^{th}, 10th, 15^{th} and 20^{th} days was in the order of sterilized *Phanerocheate chrysosporium* showing maximum followed by sterilized *Aspergillus niger* the next being unsterlized , *Phanerocheate chrysosporium* followed by unsterilized *Aspergillus niger* and the least being the unsterilized soil with the native species.

The different level of biodegradation in sterilized and unsterilized soils was due to the competition with indigenous species and the nutrition requirements available. The unsterilized soils have microflora indigenous to them, and the types of microbes present depend on upon many factors like the type of feed, storage conditions and weather conditions etc. On wetting the substrate, the microbes become active and start growing by getting nutrients from the substrate. When the pure cultures of the fungi were inoculated, the inoculums has to compete with the indigenous microflora. The competition between the two decides the fate of treatment process (Gardner 1965).

The competition among the species was studied by the antagonism studies where in the growth of *Phanerocheate chrysosporium* in competition with *Aspergillus niger* was observed to be equally scattered in the Petriplates which was same with *Aspergillus niger* in competition with *Phanerocheate chrysosporium*. This was also proved from the CO_2 release and biodegradation of hydrocarbon in soil samples where in *Phanerocheate chrysosporium* was able to degrade effectively in competition with the indigenous species, which does not contains any antagonistic characters for *Phanerocheate chrysosporium*(Fig 2).

Physico – Chemical Characteristics of soil of pre and post degradation studies (Table 4) and these results elucidates that there was decrease in the pH of the soils which was due to the degradation by the fungal species; similar results were observed in the experiments conducted by Kaviyarasan et.al 2003 and Kamra 1998. The degradation in terms of decrease in pH was also observed in the studies conducted by Artuchelvan et.al (2). The highest TOC% reduction was achieved (81.2-57.3) after 10 days incubation period by *Phanerocheate chrysosporium*,

followed by *Aspergillus niger* (78.3.–50.1).These results suggested that fungal strains prepared from isolates grown at 37 °C were more active in TOC degradation than fungal strains prepared from isolates grown at 28 °C. The highest TOC% reduction rate obtained in this study was under aerobic condition, which was in agreement with other reported studies where aerobic conditions were generally considered necessary for extensive degradation of hydrocarbons in the environment (Alexander 1980, Baker and Herson 1990 & Rehm and Reiff 1981). The decrease in the carbon content and nitrogen content in the soil characteristics was due to the degradation process that has been taken place, which further gives the fertility to the soil by altering the C/N ratio of the soil Artuchelvanet al. 2003). All these observations revealed that indigenous soil fungus *Aspergillus niger* was a potential hydrocarbon degrader in comparison with *Phanerocheate chrysosporium* (Klien 2000 and Moore 1972).

All observations revealed that the discharged service station effluent or waste in long run will be a potential hazard due to accumulation of mutagenic and carcinogenic aromatic hydrocarbons in soil. Therefore, periodic monitoring of Service Station effluent and recipient soil is strongly suggested for quantitative assessment of potential hazardous contaminants entering into the soil system. This was important not only in tracing the overall impact of land spreading but also in determining how the effluent reapplication should be made and how successful certain management practices will be in achieving the goal of rapid, environmentally safe hydrocarbon degradation and contaminant demobilization.

This study on soil Physico-Chemical properties, microbial ecology and biodegradative potential of micro flora in oil contaminated soils concluded that the fungi like *Phanerocheate chrysosporium* and *Aspergillus niger* were capable of producing enzymes at a faster rate to decompose the substrate hydrocarbon and released more CO_2 and hence these potential fungi can be utilized effectively as agents of biodegradation in Waste recycling process. The soil fertility can be easily amended from fertility viewpoint with the help of local available fungi.

The present investigations elucidated that the aerobic digestion of the hydrocarbon contaminated soils could be carried out by inoculating them with white rot fungus *Phanerocheate chrysosporium* under optimal cultural and process conditions. Bioremediation can effectively remove the petroleum hydrocarbons, and shorten the remediation period.

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References

Alexander M (1980) Biodegradation of chemicals of environmental concern, *Science*. pp. 132-211.

Artuchelvan V, Venkatesh KR, Kanakasaabai V, Nagarajan S **(**2003) Degradation of Lignin From Pulp And Paper Industry Effluents By *Sporotrichum Pulverulentum*. Ind. *Jr. of Microbiol*. 24,234-237.

Baker HA, Herson DS (1990) In situ bioremediation of contaminated aquifers and subsurface soils, *Geomicrobiol. J*. 8: 133–146.

Bossert I D, Compeau G C (1995) Cleanup of petroleum hydrocarbon contamination in soil. In: Young, L.Y; Cerniglia, C.E. Editors, 1995. *Microbial Transformation and Degradation of Toxic Organic Chemicals*, Wiley-Liss, New York, pp. 77–125.

Gardner W H (1965) Water content. In: Black, C.A., Editor. *Methods of Soils Analysis. Part 1*, American Society of Agronomy, Madison, WI, pp. 82–127.

Kamra D N (1998) Biodelignification of Lignocellulose Feeds With White Rot Fungi For The Feeding Of Ruminants. pp 177.

Kaviyarasan Mohan V, Ebenezer Karthikeyan (2003) Biodegradation of Azo Dye by *Pleurotus ostreatus* and *Tricholoma lobaynese***.** J. Poll Res. 22 (1), 77 – 80.

Klien J (2000) Possibilities, limits, and future developments of soil bioremediation. In: Rehm, H.J., Reed, G. (Eds.), Environmental processes II. Soil Decontamination, Biotechnology, Vol. 11b, 2nd Edition. Wiley-VCH, Weinheim, FRG, pp 465–476.

Michael P (1986) Ecological methods for Field and laboratory investigation. Tata McGraw Hill Publishing Co. Ltd., New Delhi, India, pp 95-130.

Moore E Landecker **(**1972**)** Fundamentals of the Fungi. Prentice – Hall India Pvt.Ltd, New Delhi, pp 295-296.

Rajiv K Sinha, Dalsukh Valani, Shanu Sinha, Shweta Singh, Sunil Herat (2009) Bioremediation of contaminated sites: a low-cost nature's biotechnology for environmental clean up by versatile microbes, plants & earthworms. In: Solid Waste Management and Environmental Remediation: 978-1-60741-761-3

Rehm H J, Reiff T (1981). Mechanism and occurrence of microbial oxidation of long chain alkenes, *Adv. Biochem. Eng.* (19**)**, 175–215.

Table 1. Physical and chemical characteristics of soil samples

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Soil 7	Soil 8	Soil 9	Soil 10
Composition (%)										
Sand	95.2	93.7	85.2	88.8	89.6	83.8	89.1	88.2	78.2	68.6
Gravel	19.5	58.8	50.0	26.0	11.0	17.8	19.9	31.8	13.8	45.3
Silt &	4.2	4.7	11.2	6.9	5.3	4.2	9.5	10.0	12.7	24.4
Clay	0.65	1.6	3.6	4.3	5.1	0.6	1.4	1.8	9.1	7.0
WHC(gg^{-1})	0.33	0.30	0.18	0.19	0.19	0.17	0.19	0.18	0.36	0.44
pH	7.3	7.4	7.7	7.8	7.9	8.2	7.8	7.5	7.8	7.8
C%(Total)	5.2	3.8	1.2	1.5	1.1	0.2	1.2	0.6	0.2	0.2
C%(Organic)	2.25	1.25	1.2	1.1	0.89	0.19	1.5	0.81	0.30	0.15
Total Nitrogen($\mu g\ g^{-1}$)	900	800	300	200	100	200	100	300	100	100
Avail P ($\mu g\ g^{-1}$)	9.5	6.1	6.9	5.6	4.8	1.0	0.8	3.8	2.6	2.3
Copper($\mu g\ g^{-1}$)	27.0	20.2	18.2	24.8	18.2	11.6	16.2	5.1	7.1	14.2
Zinc($\mu g\ g^{-1}$)	28.5	27.2	15.3	24.8	21.3	11.3	14.6	28.6	20.1	12.6
Chromium ($\mu g\ g^{-1}$)	20.0	22.4	16.4	18.2	20.4	14.8	17.6	21.6	18.8	16.6

Table 2. ***Microbial activity estimated by of CO_2 (mg) released in degradation assays (relative to the control) of oil contaminated soils***

Day	*Phanerocheate chrysosporium*			*Aspergillus niger*		
	Attenuation	Biostumulation	Bioaugmentation	Attenuation	Biostumulation	Bioaugmentation
05	5.49	7.34	12.6	1.42	2.32	7.2
10	6.82	10.64	19.5	3.48	6.84	16.81
15	4.62	8.24	14.31	1.9	4.01	13.08
20	2.32	4.23	11.73	0.96	2.26	11.0 2

All values are expressed in mg.

Table 3 CO_2 Released (mg) during Biodegradation in sterilized and unsterilized soils

Day	Unsterilized soil			Sterilized soil	
	Unsterilized soil	*Phanerocheate chrysosporium*	*Aspergillus niger*	*Phanerocheate chrysosporium*	*Aspergillus niger*
5	4.9	3.86	2.6	1.62	1.5
10	6.5	5.8	5.0	5.6	3.4
15	15.1	26	24	45.2	37.8
20	7.2	8.4	7.3	18.2	15.7

All values are expressed in mg.

Table 4 Characterization of soil samples of during pre and post exposure to Biodegradation.

Type of soil	Name of the species	pH		Final TOC% estimation		Organic carbon (mg/g)		Nitrogen (mg/g)		C/N ratio (%)	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Unsterilized soil	*Phanerocheate chrysosporium*	7.6	6.21	30.30	68.3	0.78	0.63	0.09	0.14	8.66	4.5
	Aspergillus niger		6.46		50.2		0.57		0.12		4.75
	Unsterilized(without Inoculants)		6.93		20.1		0.72		0.09		8
Sterilized soil	*Phanerocheate chrysosporium*		5.42		81.2		0.55		0.16		3.43
	Aspergillus niger		5.61		78.3		0.40		0.10		4

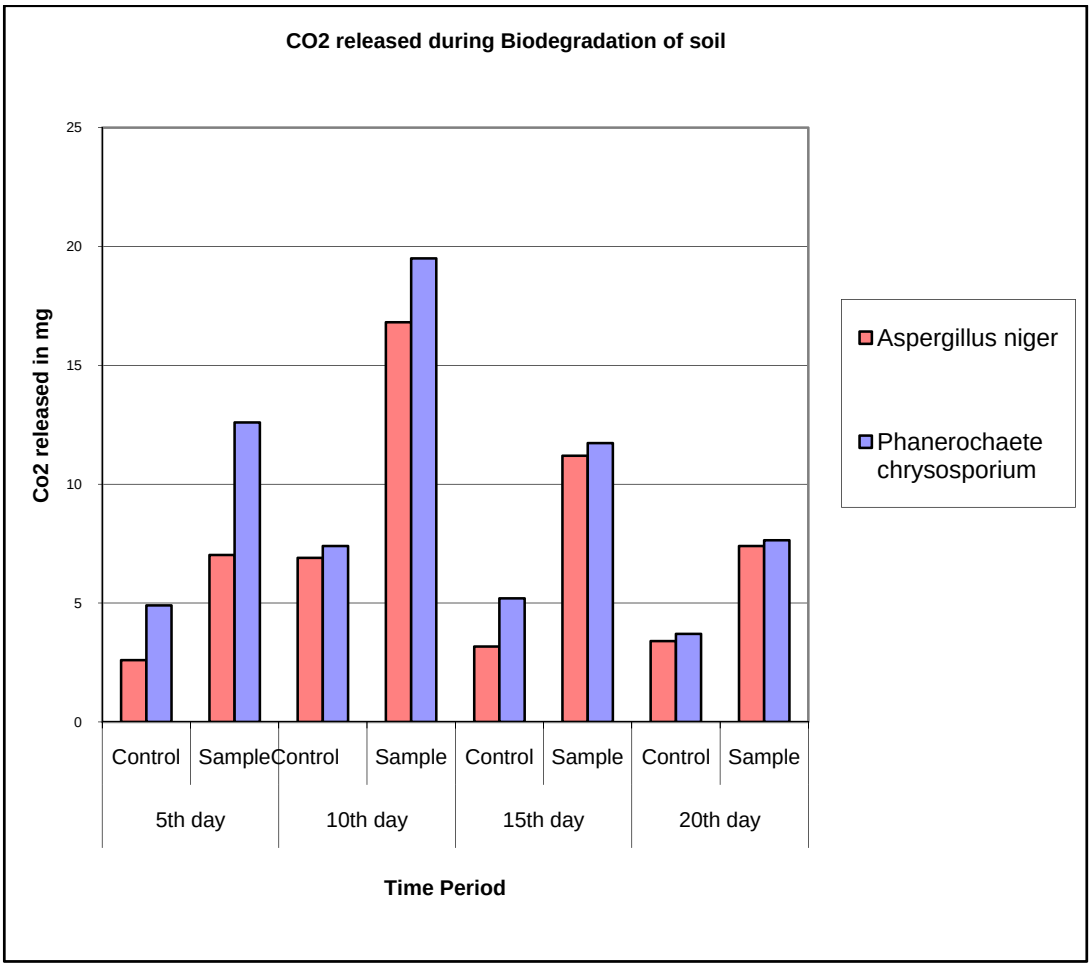

Fig 1 CO_2 released during the Biodegradation in sterilized and unsterilized soils

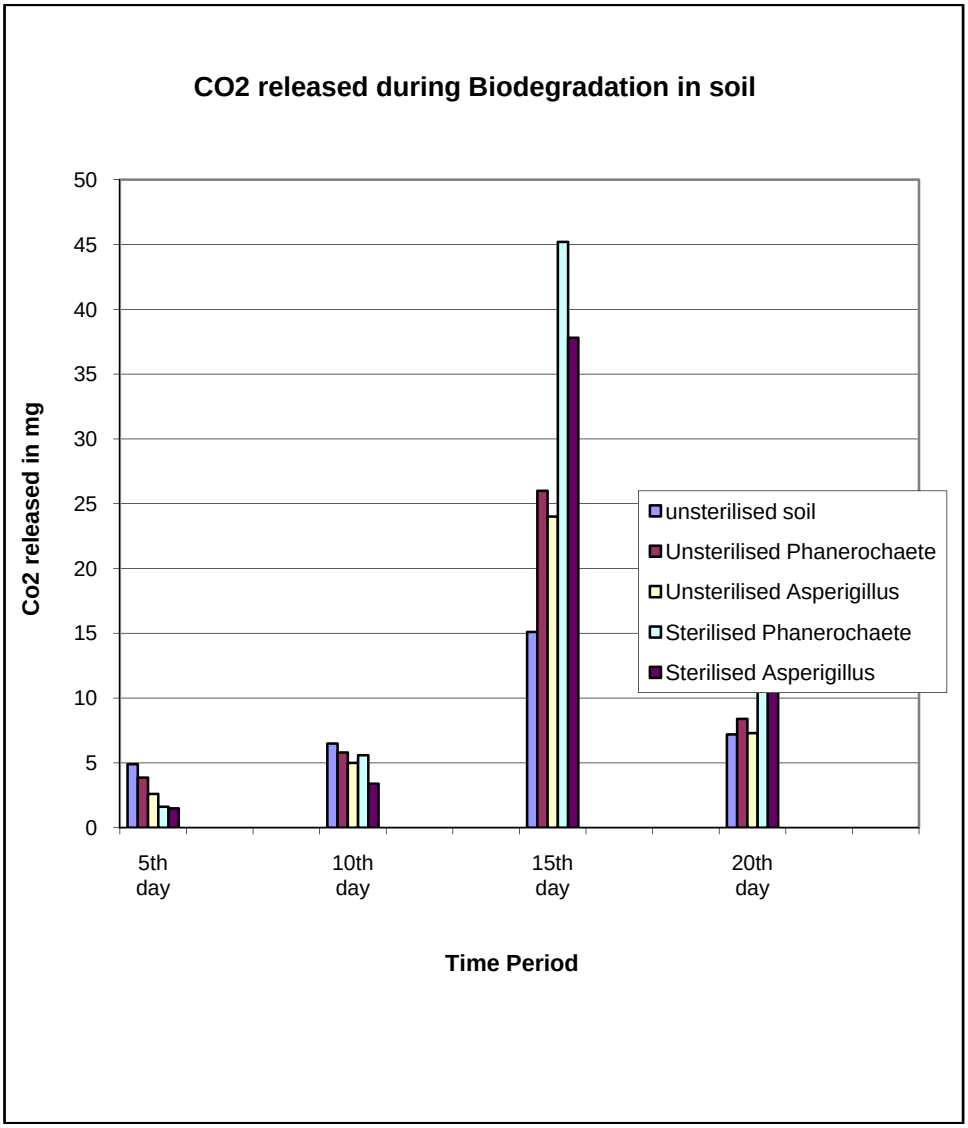

Fig 2 CO_2 released during the Biodegradation in sterilized and unsterilized soils