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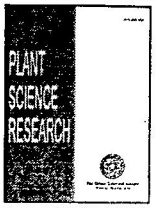

Influence of selected heavy metals on the aerobic degradation of γ-hexachlorocylohexane (γ-HCH) by HCH-degrading bacterial isolates

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ABSTRACT

Zinc, lead, cromium and cadmium are well known for their potential toxicity towards microbial and other life forms. Reports regarding the occurrence of these toxic metals as pollutants in rice fields suggest that there is a shift in the structural diversities and catabolic capacities of soil bacteria when subjected to heavy metals in any metal contaminated soil. Experiments were conducted to study the influence of some heavy metals like Zn, Pb, Cd and Cr on the aerobic degradation of γ-hexachlorocyclohexane. The HCH-degrading bacteria used for this study, viz; *Bacillus pumilus, Coryneform* sp. and *Ochrobacterium anthropi* were collected from different rice soils varying widely in their physico-chemical characteristics. These were isolated and identified in the Soil Microbiology Laboratory at the Cental Rice Research Institute, Cuttack basing on their morphological, physiological and biochemical characteristics. The toxic levels of these metals on these bacteria were studied. Results demonstrated that at 50 μg g^{-1} amendment of these heavy metals in the media, γ-HCH degradation by these bacteria was only marginally affected. Thus they can be used in the bioremediation programme of xenobiotics as the results indicated that *B. pumilus, Coryneform* sp. and *O. anthropi* could effectively degrade the HCH isomers in environment contaminated with Zn, Pb, Cr and Cd each up to 50 μg g^{-1} level.

1. Introduction

Heavy metals are inherent components of soils, but in recent years there is a concern over their accumulation in soil due to man-made applications. There are reports of occurrence of heavy metals as pollutants in rice fields (Takijima *et al.*, 1973; Foy *et al.*, 1978; Nayar, 1987). One of the sources of heavy metal pollution in rice fields is the widely used phosphate fertilizers, super phosphate and rock phosphates in particular, which contain heavy metals like Cd, Cr, Pb, and Ni as contaminants (Machelette *et al.*, 1984; Dash, 1982). Likewise effluents of tanneries can serve as a potential source of Cr and tannin contamination in rice-fields and water resources (groundwater, river). Tannery effluents inhibit micro-organisms and consequently adversely affect the productivity of the soil (Muthukumar, 1980; Muthukumar and Mahadevan, 1982).

There are a number of reports regarding the toxicity of heavy metals. The toxic level of Cd to *Pseudomonas aeruginosa* and *Aeromonas* sp. in a synthetic medium was reported to be 6.45 μM and 2.00 μM respectively (Walker and Houston, 1981). Abbas and Edward (1989) reported that the growth of *Streptomyces coelicolour* was inhibited by 50% after 16 hr of culture in presence of 0.14 mM Cd^{2+}. Although Cd has been reported as a very toxic metal for microorganisms, some selected microbes like *S. lividans* TK 24 was found to be resistant to all Cd^{2+} concentrations (Amoros *et al.*, 1998). Wenderoth and Reber (1999) reported that, in a metal-contaminated soil, catabolically less versatile strains of gram-negative phototropic bacteria are more tolerant to heavy metals

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like Zn^{2+}, than that of more versatile ones. They also suggested that, there was a shift in the structural diversities, gram-reactions and catabolic capabilities of soil-bacteria when subjected to toxic metals in a metal-contaminated soil. Also there is a shift in the spectrum of substrates utilized, due to the Zn contamination of soil.

In the present experiment attempt has been made to study the influence of some toxic metals on the degrading metabolism of pesticides, particularly γ-hexachlorocylohexane (γ-HCH), by bacteria albeit the fact that in a contaminated environment, an interaction of several factors including heavy metals are imminent influencing factors for degrading pesticides by microbes.

2. Materials and methods

2.1 Soil properties

Five soils of varying physico-chemical characteristics from different rice growing tracts of the country were used in this study. A deltaic alluvial soil from the experimental farm of Central Rice Research Institute, Cuttack, Odisha, two acid sulphate soils - Pokkali and Kari from Kerala, one coastal saline soil, Canning soil from West Bengal and a laterite soil from Bhubaneswar, Odisha were used in various experiments. The soils were air-dried in shade and after breaking the clods, sieved through a < 2mm mesh and stored in polyethylene bags at room temperature.

The physico-chemical properties of the soils (Table 1) were determined by the following methods (Jackson, 1973):

- Soil pH was measured by 1:1.25 soil to water ratio using a digital pH meter with Calomel glass electrode assembly.
- Organic carbon content of the soils was determined by Walkley and Black method and the organic matter was calculated by multiplying the organic carbon values with 1.72.
- Total nitrogen content of the soils was estimated by Kjeldahl method.
- The cation exchange capacity (CEC) of soils was determined by 1 N ammonium acetate (pH 7.0) by summation of exchangeable Na, K, Ca, Mg and H.
- Physical analysis for clay, silt and sand fractions was measured by employing the Bouyoucos hydrometer method (Black, 1965).

2.2 Source of pesticide isomers

Technical formulation of α-, β-, γ- and δ-isomers of hexachlorocyclohexane (HCH) (99.1% purity) used in studies on their degradation by different soil suspensions and in pure culture of different bacteria, were obtained from M/s Lachat chemicals, Mequon, Wisconsin, U.S.A. Commercial formulation of HCH containing 50% active ingredient (γ-HCH) was obtained from M/s Das Enterprise, Calcutta, India.

2.3 Isolation of HCH-degrading bacteria from suspension of HCH-treated soils

For isolation of HCH-degrading bacteria, 10 ml portions of MS medium containing γ-HCH (8-9 $\mu g.ml^{-1}$)

Table 1
Physico-chemical characteristics of the soils used in the study

Location	Soil type	Taxonomic group	pH[a]	Organic matter[b] ($g.kg^{-1}$)	Total Nitrogen[c] ($g.kg^{-1}$)	SO_4 ($\mu g.g^{-1}$)	EC ($dS.m^{-1}$)	CEC[d] (cMoles. g^{-100} soil)	Soil Separates ($g.kg^{-1}$)[e]		
									clay	silt	sand
Cuttack, Odisha	Alluvial	Aeric endoaquept	6.16	8.20	0.90	10.20	0.51	95.50	25.90	21.60	52.50
Bhubaneswar, Odisha	Laterite	Haplustulf	5.89	7.10	0.90	N.D	0.84	145.00	9.00	11.20	79.80
Aleppy, Kerala	Acid sulphate	Sulfaquept	3.41	246.10	3.70	321.64	6.93	289.10	29.40	6.80	63.80
Cochin, Kerala	Acid sulphate	Sulfaquept	5.40	55.10	2.10	543.20	3.20	192.00	45.60	7.80	46.60
Canning, West Bengal	Coastal saline	Haplaquept	6.69	8.52	1.30	162.40	17.23	191.00	40.60	49.60	9.80

[a] Measured by taking 1:1.25 soil water ratio by Elico-digital pH meter
[b] Determined by Walkey-Black method
[c] Estimated by Kjeldal method
[d] Estimated by Ammonium-acetate pH (7.0) method
[e] Estimated by Bouyoucos hydrometer method

was inoculated with 1 ml suspension of soil from HCH-retreated flooded pots and incubated under aerobic conditions at room temperature (28 ± 2°C). After complete disappearance of γ-HCH from the inoculated medium in about 5 to 10 days, 1 ml of this medium was added to a fresh set of Erlenmeyer flasks containing 10 ml MS medium supplemented with γ-HCH and incubated for a further period of 5 days. This was repeated 5 times at 5 d intervals for selective enrichment of γ-HCH degrading bacteria. After 5th transfer, 1 ml of the inoculated medium was serially diluted in sterile distilled water up to 10^{-8} dilution. Sterile MS medium-containing γ-HCH was inoculated separately with 1 ml of each dilution. Uninoculated medium served as control. Inoculated flasks were incubated at room temperature (28 ± 2°C). After 5 days, 1 to 2 ml samples were withdrawn aseptically from each flask for each dilution and analyzed for γ-HCH by GLC after extraction with hexane.

The maximum dilution, from which γ-HCH disappeared completely in 5 to 10 days, was plated on to MS medium supplemented with glucose (1%), peptone (0.1%), agar (1.8%) and γ-HCH. Distinct types of bacterial colonies appeared on the agar plate within 3 days of incubation at room temperature (28 ± 2°C). MS medium (10 ml) containing γ-HCH (as the sole carbon source) was inoculated with individual bacterial colonies that appeared on the agar plates to test for their ability to degrade γ-HCH. Five distinct bacterial colony types showing exceptional degradation of γ-HCH were selected. These bacteria were further purified by several transfers on plates containing MS medium with glucose and peptone (MSGP) and γ-HCH.

These bacteria were subsequently identified as *Nocardia* sp. and *Bacillus pumilus* collected from alluvial soil; *Burkholderia gladioli* and *Coryneform* sp. from acid sulphate soil and *Ochrobactrum anthropi* from saline canning soil based on their morphological, physiological and biochemical characteristics. The identity of the cultures was later confirmed by the identification services of the International Mycological Institute, Kew, U.K.

2.4 Characterization of bacterial isolates

2.4.1 Bacillus pumilus

This bacterium, also isolated from HCH-retreated alluvial soil, was gram positive, cylindrical, motile aerobes, usually grow in chain, endospore forming, highly resistant to heat, other destructive agents and desiccation, both catalase and oxidase positive, usually white in colour and especially produces carbohydrate capsule.

Members of this species are found widely in nature, primarily in soil where their spores are reported to occur more frequently than those of *Bacillus subtilis,* and they are known to be resistant to adverse conditions such as heat and desiccation.

2.4.2 Coryneform sp.

This bacterium was isolated from HCH-retreated *Kari* soil and is a gram positive, slightly curved, rod-shaped, non-motile aerobes, and usually greenish white in colour. It was oxidase-positive, phytopathogen, sensitive to triphenyl tetrazolium chloride (TTC) and required organic growth factor for luxurious growth. This bacterium was identified as a closely related to *Clavibacter.*

It is widespread in nature and found in soils and on plant materials. In general, members of this genus are phytopathogens and restricted to their host plants. However, there are cases where farm machinery and storage facilities can become contaminated.

2.4.3 Ochrobactrum anthropi

This bacterium was isolated from the saline *Canning* soil retreated with HCH and was a gram negative, circular, pinpoint type, strictly aerobe, motile especially characterized by the gummy consistency of the colony, and having very small colony size. It produced acids from glucose, xylulose and a gray-white pigment.

2.5 Laboratory methods

2.5.1 Mineral salts medium

A mineral salts medium [0.5 g $(NH_4)_2HPO_4$; 2 g $MgSO_4,7H_2O$; 0.001 g $FeSO_4.7H_2O$; 0.1 g K_2HPO_4; 0.01 g $Ca(NO_3)_2$ and 1 l distilled water, pH 7.0] was shaken with analytical grade of 0.01 γ-HCH for 48 hr on a mechanical stirrer and subsequently sterilized by filtration through a Millipore® filter (0.3 μm). 10 ml portions of this medium were aseptically dispensed in sterile 100 ml Erlenmeyer flasks and later inoculated with 1ml of soil suspension (flooded) from untreated or HCH-treated pots, collected 15 days after first and successive application of HCH. The uninoculated medium served as control. The samples were incubated under intermittent shaking (for 4 hr after every 4 hr interval) to provide aerobic conditions. At periodic intervals, 1 ml portion of the inoculated and uninoculated medium were withdrawn aseptically from duplicate flasks and shaken with 2-4 ml of hexane for 2-3 min for extraction of HCH residues. HCH residues in hexane fraction were analyzed by GLC.

2.5.2 Extraction of heavy metals

The effect of heavy metals on the aerobic degradation of γ-HCH by selected HCH-degrading bacteria, viz. *B. pumilus* from alluvial soil, *Coryneform* sp. from acid-sulphate soil and *O. anthropi* from saline soil were examined. Different concentrations of aqueous solutions of salts of heavy metals (Zn as $ZnSO_4$; Pb as $Pb(NO_3)_2$; Cd as $CdCl_2$ and Cr as $K_2Cr_2O_7$ were prepared and added to the γ-HCH supplemented MS medium to provide final concentrations ranging from 5 $\mu g.ml^{-1}$ to 50 $\mu g.ml^{-1}$. Analysis of the medium by atomic absorption spectrophotometer showed that heavy metals, Cd and Zn were in solution at the concentrations used. MS medium, thus supplemented with respective heavy metals and γ-HCH was sterilized by filtration through a Millipore® filter (0.3 μm). 10 ml portions of this sterile medium dispensed in 100 ml Erlenmeyer flasks were separately inoculated with 0.1 ml of the bacterial suspension (48 hr old culture grown on MS medium supplemented with γ-HCH) from their respective dilutions containing equal no. of cells. Uninoculated medium served as control. At periodic intervals, 1 to 2 ml samples from each variety of inoculated and uninoculated media were withdrawn aseptically from duplicate flasks and analyzed for γ-HCH by GLC after its extraction with hexane.

2.5.3 Extraction and analysis of HCH isomer

Residues of HCH isomers remaining in both uninoculated and inoculated medium were extracted with hexane. Portions (1 to 2 ml) of liquid MS medium were withdrawn aseptically from each flask and shaken with 1 to 6 ml of hexane and 50 mg of sodium sulphate for 5 minutes. When HCH isomers were applied to the medium at concentrations above their solubility range, HCH residues remaining in the medium were extracted by shaking the medium in the whole flask with hexane (medium: hexane of 1:1 or 1:2) for 20 min in a rotary shaker.

2.5.4 Gas liquid chromatography (GLC)

HCH isomers, extracted from liquid medium or soil in hexane, were analyzed in a Varian gas chromatograph model 3400, equipped with a ^{63}Ni detector and a metal column (2m length, 1/8" OD) packed with 3% OV-17 on chrom WHP 80/100 mesh. Column, injector and detector were maintained at 220, 240 and 240°C, respectively with a flow rate of the carrier gas (95% argon in 5% methane) at 20 $ml.min^{-1}$. Under these conditions, the retention time for γ-HCH was 2.42 min, for α-HCH 2.0 min, for β-HCH 2.8 min and 3.42 min, for δ-HCH. Retention time of the bacterial metabolite of β-HCH was 4.0 min. The residues of γ-HCH were quantified using a standard curve linear over 0.5 to 3.0 ng.

The recovery of the parent isomers of HCH, by the extraction and analytical procedures used in this study immediately after application to the MS medium ranged from 95 to 100%.

3. Results

Industrial and agricultural practices often lead to an increase in the release of toxic metals into the environment (Brookes, 1984; Giovanni *et al.*, 1995). Since only very small amounts of heavy metals leach from the soils, they readily accumulate in the soil environment causing concern over their effect on the functioning of the soil biological systems (Baath, 1989; Doleman, 1985; Doelman and Jensen, 1994; Salomons and Stigliani, 1995; Witton, 1992). Heavy metals affect the growth, morphology and metabolism of microorganisms of soils through functional disturbance, protein denaturation or the destruction of the integrity of the cell membrane (Baath, 1989; Babich and Stozky, 1980; Leita *et al.*, 1995).

Investigations on the effect of heavy metals on the diversity and catabolic versatility of soil bacteria using aromatic acids suggested that, with the reduction in available energy in metal-contaminated soils, micro-organisms could eventually loose a part of the catabolic capabilities that are related to energetically poor substrates (Reber, 1992; Burkhardt *et al.*, 1993). It was, therefore considered prudent to study the impact of select heavy metals on the degradation of γ-HCH by HCH-degrading bacterial isolates.

Effect of selected heavy metals viz. Zn, Pb, Cr and Cd on the aerobic degradation of γ-HCH by three selected HCH-degrading bacteria including *B. pumilus, Coryneform* sp. and *O. anthropi* was studied in the present experiment. Degradation of γ-HCH by *B. pumilus* in a mineral salts medium without Zn amendment was 95% after 72 hr of incubation that decreased gradually with the increase in concentration of Zn in the media. At 50 $\mu g.g^{-1}$ amendment of Zn in the medium γ-HCH degradation was reduced by 88%. Similarly, γ-HCH degradation by *Coryneform* sp. in control MS medium was 88% after 72 hr of degradation and reached 86% at 50 $\mu g.g^{-1}$ amendment of Zn during the same period. The degradation of γ-HCH was observed to be highest at 10 $\mu g.g^{-1}$ amendment of Zn in the media. Thus, Zn did not affect γ-HCH degradation by *Coryneform* sp. γ-HCH degradation by *O. anthropi* in control medium was 95% that was reduced to 84% at 50 $\mu g.g^{-1}$ of Zn

amendment during incubation for 72 hr. γ-HCH degradation by all the bacteria was not affected 5 and 10 μg.g^{-1} of Zn-amendment (Table 2).

γ-HCH degradation by *B. pumilus, Coryneform* sp. and *O. anthropi* in mineral salts medium without Pb amendment was 94%, 97% and 99% respectively after 72 hr of incubation. There was a gradual but little decline in the concentration of γ-HCH in the media inoculated with *B. pumilus* and *Coryneform* sp. at 5, 10 and 50 μg.g^{-1} amendment of Pb. The only exception was in case of *O. anthropi.* In this case, the rate of γ-HCH degradation remained at the same level as in media amended with 5 and 10 μg.g^{-1} Pb. At 50 μg.g^{-1} Pb amendment in the media, γ-HCH degradation by *B. pumilus* was 84%, by *Coryneform* sp. 82% and by *O. anthropi* 89% after 72 hr of incubation (Table 3).

In MS medium without Cr^{2+} amendment, γ-HCH degradation by *B. pumilus* was 94%, by *Coryneform* sp 99% and by *O. anthropi* was 99% after 72 hr of incubation. In all the cases, like that of Zn and Pb, there was also a gradual decrease in γ-HCH degradation when the media was amended with 5 and 10 μg.g^{-1} of Cr and reached 82%, 80% and 80% by *B. pumilus, Coryneform* sp. and *O. anthropi* respectively in the media amended with 50 μg.g^{-1} of Cr^{2+} during the same period (Table 4).

Almost the entire amount of γ-HCH in mineral salts medium without Cd^{+} amendment was degraded by *B. pumilus, Coryneform* sp. and *O. anthropi.* There was a marginal inhibition in γ-HCH degradation in media amended with 5 and 10 μg.g^{-1} Cd^{2+} in case of *B. pumilus,* but remained at same level as that of unamended control in case of *Coryneform* sp. after 72 hr of incubation. At 50 μg.g^{-1} amendment of Cd^{2+}, γ-HCH recovery was 16% by *B. pumilus*, 18% by *Coryneform* sp. and 15% by *O. anthropi* (Table 5).

4. Discussions

Heavy metals are a group of soil pollutants. The contamination by heavy metals causes a serious problem because they cannot be naturally degraded like organic pollutants and they accumulate in different parts of food-chain. Under stress conditions caused by advanced anthropogenic effects such as dissemination of chemical pollutants, the development and biochemical activities of soil microorganism undergo several alternations.

Lead (Pb) is a common environmental contaminant found in soils. Unlike other metals, Pb has no biological role, and is potentially toxic to microorganisms. Pb has detectable effects upon the community directly even at the concentration level of 1ppm (Sober *et al.,* 2008). The

Table 2
Effect of Zn on the degradation of γ-hexachlorocyclohexane (γ-HCH) in mineral salts medium by different bacteria (x10^8 cells)

Different bacteria	Incubation period (hr)	γ-HCH[a] recovered (μg.ml^{-1})[b]							
		Zn amendment (μg.ml^{-1})							
		0		5		10		50	
		U.I.	I.	U.I.	I.	U.I.	I.	U.I.	I.
B. pumilus	0	9.7±0.1	7.4±0.02	9.5±0.01	8.4±0.0	9.9±0.1	8.2±0.2	9.7±0.1	8.5±0.1
	6	8.6±0.02	4.3±0.01	7.7±0.3	4.6±0.02	8.8±0.0	4.2±0.01	8.9±0.01	5.4±0.0
	24	7.3±0.1	1.3±0.0	6.0±0.0	1.8±0.2	8.0±0.0	1.7±0.01	8.5±0.0	2.1±0.0
	48	6.6±0.0	0.9±0.02	5.9±0.1	1.2±0.0	6.7±0.1	1.0±0.0	7.0±0.0	1.7±0.02
	72	6.1±0.0	0.3±0.0	5.8±0.2	0.5±0.0	6.2±0.02	0.4±0.0	6.4±0.02	1.4±0.01
Coryneform sp.	0	9.7±0.1	7.9±0.0	9.5±0.01	8.0±0.0	9.9±0.1	8.2±0.02	9.7±0.1	8.9±0.03
	6	8.6±0.02	5.6±0.01	7.7±0.3	4.3±0.03	8.8±0.0	4.6±0.01	8.9±0.01	5.2±0.01
	24	7.3±0.1	1.7±0.0	6.0±0.0	1.3±0.0	8.0±0.0	1.5±0.05	8.5±0.0	2.8±0.0
	48	6.6±0.0	1.0±0.02	5.9±0.1	0.87±0.01	6.7±0.1	0.9±0.01	7.0±0.0	1.0±0.01
	72	6.1±0.0	0.76±0.0	5.8±0.2	0.61±0.1	6.2±0.02	0.5±0.0	6.4±0.02	0.92±0.0
O. anthropi	0	9.7±0.1	9.0±0.0	9.5±0.01	8.0±0.0	9.9±0.1	8.2±0.02	9.7±0.1	8.5±0.05
	6	8.6±0.02	6.5±0.05	7.7±0.3	4.6±0.02	8.8±0.0	4.6±0.01	8.9±0.01	5.2±0.0
	24	7.3±0.1	2.6±0.2	6.0±0.0	0.9±0.02	8.0±0.0	2.1±0.01	8.5±0.0	3.8±0.1
	48	6.6±0.0	1.1±0.0	5.9±0.1	0.8±0.0	6.7±0.1	1.3±0.0	7.0±0.0	1.3±0.2
	72	6.1±0.0	0.34±0.0	5.8±0.2	0.37±0.01	6.2±0.02	0.67±0.1	6.4±0.02	1.0±0.1

[a] γ-HCH was added to the mineral salts medium in aqueous solution at 10 mg.ml^{-1}
[b] Average of two replicate observation Mean ± S.D.; U.I.-Uninoculated; I.-Inoculated.

Table 3

Effect of Pb on the degradation of γ-hexachlorocyclohexane (γ-HCH) in mineral salts medium by different bacteria (x10^8 cells)

Different bacteria	Incubation period (hr)	γ-HCH[a] recovered (μg.ml^{-1})[b]							
		Pb amendment (μg.ml^{-1})							
		0		5		10		50	
		U.I.	I.	U.I.	I.	U.I.	I.	U.I.	I.
B. pumilus	0	10.0±0.0	9.4±0.05	9.5±0.05	9.1±0.01	9.3±0.0	8.4±0.2	9.4±0.02	9.3±0.01
	6	9.6±0.03	4.6±0.0	9.2±0.1	4.6±0.02	9.2±0.0	5.1±0.1	9.2±0.03	6.0±0.0
	24	8.2±0.05	0.9±0.0	8.0±0.0	1.4±0.02	8.5±0.1	3.1±0.1	8.6±0.1	3.5±0.01
	48	7.6±0.01	0.4±0.3	6.8±0.02	1.2±0.1	6.7±0.5	1.6±0.01	7.3±0.1	2.7±0.1
	72	6.3±0.1	0.23±0.05	5.9±0.0	0.5±0.1	5.9±0.2	0.7±0.0	6.1±0.02	1.0±0.01
Coryneform sp.	0	10.0±0.0	9.5±0.1	9.5±0.5	9.3±0.2	9.3±0.0	9.2±0.05	9.4±0.02	9.3±0.05
	6	9.6±0.0	4.3±0.2	9.2±0.1	3.9±0.2	9.2±0.0	4.7±0.0	9.2±0.0	5.1±0.1
	24	8.2±0.1	1.9±0.0	8.0±0.0	1.3±0.0	8.5±0.1	1.9±0.0	8.6±0.1	2.0±0.0
	48	7.6±0.0	0.7±0.2	6.8±0.0	1.2±0.0	6.7±0.0	1.5±0.3	7.3±0.2	1.8±0.0
	72	6.3±0.1	0.2±0.0	5.9±0.0	0.6±0.0	5.9±0.0	0.9±0.1	6.1±0.0	1.1±0.0
O. anthropi	0	10.0±0.0	8.6±0.0	9.5±0.1	8.9±0.2	9.3±0.0	8.8±0.2	9.4±0.0	9.1±0.0
	6	9.6±0.3	6.7±0.0	9.2±0.1	4.7±0.1	9.2±0.0	4.2±0.1	9.2±0.0	4.5±0.0
	24	8.2±0.1	1.2±0.0	8.0±0.0	1.5±0.1	8.5±0.1	2.0±0.0	8.6±0.1	2.3±0.0
	48	7.6±0.0	0.8±0.	6.8±0.0	1.5±0.0	6.7±0.1	1.3±0.1	7.3±0.2	1.4±0.1
	72	6.3±0.1	0.1±0.0	5.9±0.0	0.9±0.1	5.9±0.0	0.9±0.1	6.1±0.0	1.3±0.1

[a] γ-HCH was added to the mineral salts medium in aqueous solution at 10 mg.ml^{-1}

[b] Average of two replicate observation Mean ± S,D,; U.I.-Uninoculated; I.-Inoculated.

Table 4

Effect of Cr on the degradation of γ-hexachlorocyclohexane (γ-HCH) in mineral salts medium by different bacteria (10^8 cells)

Different bacteria	Incubation period (hr)	γ-HCH[a] recovered (μg.ml^{-1})[b]							
		Cr amendment (μg.ml^{-1})							
		0		5		10		50	
		U.I.	I.	U.I.	I.	U.I.	I.	U.I.	I.
B. pumilus	0	9.8±0.0	8.8±0.02	9.7±0.1	9.0±0.03	9.9±0.03	9.2±0.2	9.9±0.1	9.5±0.1
	6	9.4±0.1	7.6±0.1	8.3±0.3	5.6±0.0	8.7±0.1	6.1±0.0	9.4±0.3	6.5±0.0
	24	8.4±0.1	2.4±0.2	6.5±0.0	2.6±0.5	6.8±0.2	2.0±0.1	6.7±0.05	2.6±0.3
	48	7.6±0.02	0.9±0.0	5.3±0.2	1.0±0.0	6.2±0.0	1.1±0.0	6.2±0.2	1.9±0.0
	72	7.0±0.5	0.3±0.0	5.1±0.5	0.5±0.0	5.8±0.02	0.6±0.2	6.1±0.1	1.1±0.0
Coryneform sp.	0	9.8±0.0	9.2±0.1	9.7±0.1	9.0±0.2	9.9±0.3	9.4±0.2	9.9±0.1	8.8±0.5
	6	9.4±0.1	7.1±0.5	8.3±0.3	4.6±0.0	8.7±0.1	6.0±0.5	9.4±0.3	6.5±0.5
	24	8.4±0.1	2.3±0.0	6.5±0.0	2.4±0.2	6.8±0.2	1.7±0.0	6.7±0.5	2.9±0.0
	48	7.6±0.2	0.7±0.1	5.3±0.2	0.7±0.0	6.2±0.0	0.9±0.01	6.2±0.2	2.0±0.1
	72	7.0±0.5	0.1±0.0	5.1±0.5	0.4±0.1	5.8±0.2	0.6±0.2	6.1±0.01	1.2±0.1
O. anthropi	0	9.8±0.0	9.4±0.5	9.7±0.1	9.6±0.0	9.9±0.3	9.4±0.4	10.0±0.1	8.8±0.5
	6	9.4±0.1	5.8±0.2	8.3±0.3	4.8±0.2	8.7±0.1	6.0±0.1	9.4±0.3	6.2±0.5
	24	8.4±0.1	1.9±0.2	6.5±0.0	1.9±0.1	6.8±0.2	2.1±0.5	6.7±0.5	2.9±0.2
	48	7.6±0.2	0.8±0.2	5.3±0.2	1.2±0.02	6.2±0.0	1.3±0.0	6.2±0.2	2.0±0.0
	72	7.0±0.5	0.1±0.0	5.1±0.5	0.7±0.1	5.8±0.2	0.66±0.1	6.1±0.01	1.2±0.1

[a] γ-HCH was added to the mineral salts medium in aqueous solution at 10 mg.ml^{-1}

[b] Average of two replicate observation Mean ± S,D,; U.I.-Uninoculated; I.-Inoculated.

soil microbial activity is also affected by concentrations of heavy metals viz Cd, Zn,and Pb. The decrease in CFU is more significant in case of oligotrophic and spore forming bacteria. Significant inhibition of C-biomass occurs in soils highly contaminated by heavy metals (Smejkalova *et al.*, 2003).

Many reports indicate that heavy metals interfere with the biochemistry of diverse group of microorganisms isolated from their natural environments (Alloway 1995; Sani *et al*, 2003; Utgikar *et al.*, 2004; Pennanen *et al.*, 1996). Reports indicate that the rate of respiration of soil microbial population is inhibited by the metals like Cu, Zn and Ni (Nwuche and Vgoji, 2008). However, information relating to the sensitivity of whole soil bacterial communities to heavy metals is not common (Diaz Ravina *et al.*, 1994; Chander and Brooks, 1993). Microorganisms do not live in isolation but in complex biological communities within which exists complex interactions arising from biotic and abiotic influences (Peterson and Klug, 1994; Chefetz *et al.*, 1996)

Human activities such as agriculture and industry are the source of soil pollution with the heavy metals Zn and Cd. Elevated levels of Zn and Cd have toxic effect on microorganisms which play an important role in soils (Patricia *et al.*, 2008). The environmental risk of heavy metal pollution is pronounced in soils adjacent to large industrial complexes. The microbes collected from the polluted soils near a copper smelter in China were studied for the potential effects of heavy metals on microbial biomass, activity and community composition and the result showed that the microbial biomass was negatively affected by the elevated metal levels and was closely correlated with heavy metal stress. Enzyme activity was also greatly depressed by the conditions in the heavy metal contaminated sites (Wang *et. al.*, 2007).

Thus, the present study is an attempt to evaluate one of the biological parameters in the indication of soil quality to prevent the negative ecological consequences as described by Filip (2002).

There are a number of reports regarding the toxicity of heavy metals to microorganisms (Walker and Houston, 1981; Abbas and Edward, 1989; Amoroso *et al.*, 1998). Results from the present study demonstrate that even at 50 $\mu g.g^{-1}$ amendment of Cd^{2+}, γ-HCH degradation by *B. pumilus, Coryneform* sp. and *O. anthropi* was only marginally affected (Table 5). Similar was the case with 50 $\mu g.g^{-1}$ amendment of other heavy metals like Zn, Pb and Cr in the media, where approximately 80% or more γ-HCH was degraded by the three bacteria used in the experiment (Table 2-4). Wenderoth *et al.* (1999) reported

Table 5
Effect of Cd on the degradation of γ-hexachlorocyclohexane (γ-HCH) in mineral salts medium by different bacteria ($x10^8$ cells)

Different bacteria	Incubation period (hr)	γ-HCH[a] recovered ($\mu g.ml^{-1}$)[b]							
		Cd amendment ($\mu g.ml^{-1}$)							
		0		5		10		50	
		U.I.	I.	U.I.	I.	U.I.	I.	U.I.	I.
B. pumilus	0	10.0±0.0	8.8±0.0	9.8±0.2	9.0±0.0	9.9±0.1	8.8±0.0	9.9±0.2	9.6±0.4
	6	9.4±0.2	7.1±0.3	8.2±0.0	4.4±0.0	8.4±0.5	5.6±0.0	9.0±0.0	6.2±0.0
	24	8.2±0.0	1.4±0.0	6.3±0.1	1.7±0.1	6.7±0.0	2.0±0.0	7.1±0.0	3.4±0.3
	48	7.9±0.1	0.9±0.0	5.8±0.1	1.1±0.0	5.9±0.1	1.2±0.1	6.8±0.2	1.9±0.3
	72	7.1±0.1	0.3±0.1	5.2±0.0	0.4±0.1	5.5±0.2	0.6±0.0	6.2±0.1	1.0±0.1
Coryneform sp.	0	10.0±0.0	9.0±0.0	9.8±0.2	9.4±0.2	9.9±0.1	9.6±0.0	9.9±0.2	9.4±0.2
	6	9.4±0.2	6.9±0.2	8.2±0.0	5.4±0.1	8.4±0.5	5.4±0.05	9.0±0.0	5.9±0.1
	24	8.2±0.01	1.2±0.03	6.3±0.5	2.4±0.02	6.7±0.2	1.8±0.1	7.1±0.01	2.6±0.1
	48	7.9±0.1	0.7±0.1	5.8±0.1	1.4±0.5	5.9±0.1	1.5±0.05	6.8±0.2	1.6±0.2
	72	7.1±0.5	0.1±0.0	5.2±0.2	0.7±0.2	5.5±0.1	0.7±0.3	6.2±0.1	1.1±0.1
O. anthropi	0	10.0±0.0	9.3±0.05	9.8±0.2	8.7±0.1	9.9±0.1	8.7±0.05	10.0±0.2	9.2±0.1
	6	9.4±0.2	6.5±0.5	8.2±0.0	3.9±0.03	8.4±0.5	5.6±0.02	9.0±0.0	5.6±0.05
	24	8.2±0.1	2.0±0.0	6.3±0.5	1.9±0.05	6.7±0.2	2.4±0.2	7.1±0.1	2.5±0.05
	48	7.9±0.1	0.8±0.02	5.8±0.1	1.3±0.0	5.9±0.1	1.6±0.07	6.8±0.2	1.8±0.02
	72	7.2±0.05	0.1±0.0	5.2±0.01	0.35±0.1	5.5±0.2	0.5±0.4	6.2±0.1	0.9±0.2

[a] γ-HCH was added to the mineral salts medium in aqueous solution at 10 $mg.ml^{-1}$
[b] Average of two replicate observation Mean ± S,D,; U.I.-Uninoculated; I.-Inoculated.

that in metal-contaminated soil, catabolically less versatile strains of Gram-negative prototropic bacteria are more tolerant to heavy metals like Zn^{++} than that of more versatile ones. They also suggested that, there was a shift in the structural diversities, gram-reactions and catabolic potentials of soil-bacteria when subjected to heavy metals in a metal-contaminated soil. Also there is a shift in the spectrum of substrates utilized due to the heavy metal (Zn) contamination of soil (Wenderoth *et al.*, 1999).

Micro-organisms represent the richest repertoire of molecular and biological diversities in nature as they comprise the most diverse forms of life. They are nature's original recyclers, converting toxic organic compounds to usable end products, often CO_2 & H_2O. Ever since, it was discovered that microbes have the ability to transform and/or degrade xenobiotics, scientists have been exploring the microbial diversity, particularly of contaminated areas in search for organism that can degrade a wide range of pollutants. Microbial diversity offers an immense scope of environment friendly options for mineralization of contaminants or their transformation into less harmful hazardous compounds.

Extrapolation of these findings from solution phase to field situations warrants caution, because these toxic metals, entering into the complex soil systems will be subjected to fixation and sorption processes and may further modulate the effect of these metals in solution phase on the bacterial degradation of γ-HCH. Use of a particular bacterium in the bioremediation of a xenobiotic would expose that bacterium to hostile environment with several environmental obstacles including the toxic metals. Results reported in the present study indicate that *B. pumilus, Coryneform* sp. and *O. anthropi* can effectively degrade the HCH isomers in environment contaminated with Zn, Pb, Cr and Cd up to 50 $\mu g.g^{-1}$ level.

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