SOIL METHANOTROPHS COMPOSITION AND MICROBIAL BIOMASS LEVELS FROM LINDANE CONTAMINATED SITES

THESIS

Submitted to the

Babasaheb Bhimrao Ambedkar (A Central) University Lucknow

For the award of the degree of

Doctor of Philosophy

In

Environmental Microbiology

Submitted by

Siddharth Boudh

(Enrolment No. 964/14)

Under the guidance of

Dr. Jay Shankar Singh

Assistant Professor

Department of Environmental Microbiology

School for Environmental Sciences

Babasaheb Bhimrao Ambedkar University

(A Central University, NAAC Accreditation 'A' Grade)

Vidya Vihar, Raebareli Road Lucknow-226025

(U.P.), INDIA

2019

Dedicated to
My Beloved Family

CERTIFICATE

This is to certify that the Thesis titled **"SOIL METHANOTROPHS COMPOSITION AND MICROBIAL BIOMASS LEVELS FROM LINDANE CONTAMINATED SITES"** submitted by **Mr. Siddharth Boudh** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The Thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 16/08/2019

Supervisor

Dr. Jay Shankar Singh
Assistant Professor
Deptt. of Environ. Microbiology
B.B. Ambedkar (Central) University
Lucknow-226025, Uttar Pradesh, India

16/08/2019

Head of Department

Head
Department of Microbiology
B.B.A.U., Lucknow-226 025

STUDENT DECLARATION

This is to certify that the material embodies in the present Ph.D. work entitled **"SOIL METHANOTROPHS COMPOSITION AND MICROBIAL BIOMASS LEVELS FROM LINDANE CONTAMINATED SITES"** is original research work done by me. It has not been submitted in part or full for any other diploma or degree in any other University. In this Thesis, matter written, data presented and plagiarism, if any, is the sole responsibility of the student Mr. Siddharth Boudh. If any allegations/query/question arises regarding the Thesis, I will be solely responsible and answerable.

I also declare that the Thesis is essentially free from all kinds of plagiarism.

Mr. Siddharth Boudh
S/o D.D. Indramani
House No. 27/5, Kanhaiyapuram,
Rajghat Colony, Jhansi-284003. U.P., India
Mob. No. 9935679092
E-mail : boudh.siddharth@gmail.com

ACKNOWLEDGEMENTS

It has been a long time here in Babasaheb Bhimrao Ambedkar (Central) University, Lucknow and I had the good fortune of coming in contact with many marvellous people.

My Heartfelt Gratitude to the Fatherly figure **Dr Jay Shankar Singh**. He has been absolutely wonderful and has guided me not only professionally but also in my personal life and has been a pillar of support during my research work. His constant trust in me and push for excellence is the reason I am able to complete the present work. I could be difficult at times but he never lost patience with me. Sir I thank you from the bottom of my heart.

I would like to thank **Professor Rajesh Kumar**, Head Department of Environmental Microbiology, BBAU, for providing me necessary facilities for my research work and he has been really kind to me and has supported me at any number of times. I am highly thankful to all the faculties of the department **Professor Ram Chandra, Dr RN Bharagava, Dr VS Baghel, Dr Digvijay Verma, Dr Pankaj Arora, Dr Ravi Gupta and Dr Harish Chandra** for their valuable suggestion and support. I would also like to thank Dr Monali Rahalkar and Dr Pranitha Saurabh Pandit Bioenergy group, Agharkar Research Institute, Pune, Maharastra for healthy discussion on methanotrophs community analysis.

I would like to thank Dr Mannickam, Dr Preeti Chaturvedi and Dr DK Patel for providing me research facilities and GC-MS/MS analyses at Indian Institute for Toxicology Research (IITR), CSIR, Lucknow. I would also like to thank late Professor Surendra Singh Department of Botany, BHU for his help in analyses of soil microbial biomass -C, -N and -P. This endeavour would not have been possible without the support of my family they have been patient with me since childhood and continue to love and nurture me with their unconditional support there are no words which could express my gratitude towards them. My father **Shri. DD Indramani** and My mother **Smt. Vidya Devi** my greatest source of achievement, pride and inspiration and taught me about unconditional love, reinforced the importance of giving back and taught me how to be a better person in life have always supported me. Big shout out for my brother **Rahul** and sisters **Jyoti didi** and **Preeti** who were always loving and caring.

Family reminds me of my friends who have been so loving and caring towards me that I never felt I was not at home. My stay in BBAU has led to some lifelong friendships. I was blessed with some wonderful seniors and friends like Dr Seema Paliwal, Dr Virendra Singh, Dr Pankaj chowdhary, Dr Vishwas Hare, Dr Abhay Prakash Rawat, Dr Vineet Rudra, Dr Rachna, Dr Sandhya Mishra, Dr Prem Chandra, Dr Mukesh, Dr Atul Upadhya, Dr Vimal Chandra Pandey and my Batch mate Beenu Shastri, My juniors Adarsh, Sheel, Ajay, Shitij, Roop Kishor, Ranjan, Vinay, Raman, Manjhi, and all my Labmates Dr Shobhit, Dr Chhatrapal, Dr Shashank, and Dr Arun who has been the source of joy in sometimes difficult phases of PhD. I would like to sincerely acknowledge nonteaching staff Mr. Ranjeet Sharma, Chand bhai, Dhiraj, Nancy and Rahul who has been very supportive and loving towards me. I am also thankful to all the Persons and Institutions inovolved and helped me in overcoming the difficulties faced by me during my research work.

Siddharth Boudh

CONTENTS

LIST OF FIGURES

List of Tables

LIST OF ABBREVIATIONS AND SYMBOLS

α	:	Alpha
ANOVA	:	Analysis of Variance
~	:	Approximately
CO_2	:	Carbon dioxide
cm	:	Centimeter
CTAB	:	Cetyl Trimethylammonium Bromide
COD	:	Chemical Oxygen Demand
°C	:	Degree Celsius
EC	:	Electrical Conductivity
FTIR	:	Fourier Transform Infrared Spectroscopy
GC-MS	:	Gas Chromatography Mass Spectrophotometry
GHGs	:	Green house gases
g	:	Gram
g/L	:	Gram per litre
HPLC	:	High Pressure Liquid Chromatography
HCH	:	Hexachlorocyclohexane
IUPAC	:	International Union of Pure and Applied Chemistry
Fe	:	Iron
K	:	Kelvin
GC-MS/MS	:	Gas Chromatography-Mass Spectrometry
µg	:	Microgram
µL	:	Microlitre
µm	:	Micrometer
mL	:	Milliliter
MgL^{-1}	:	Milligram per litre
µ	:	Miu
mg	:	Milligram
NMSM	:	Nitrarate Mineral Salt Medium
min	:	Minute
M	:	Molarity

nm	:	Nanometer
NIST	:	National Institute of Standards and Technology
-	:	Negative
OD	:	Optical Density
%	:	Percentage
+	:	Positive
POPs	:	Persistent organic pollutant
rpm	:	Revolution Per Minute
RNA	:	Ribonucleic acid
SEM	:	Scanning Electron Microscope
SMB	:	Soil Microbial Biomass
UV	:	Ultra Violet
U/mL	:	Unit per milliliter
H_2O	:	Water
w/v	:	Weight over volume

CHAPTER-1
Introduction

CHAPTER 1

INTRODUCTION

1.1 Lindane

Lindane (isomer of hexachlorocyclohexane γ-HCH), an organochlorine compound, has been listed as a persistent organic pollutant (POPs) under Stockholm Convention on June 29, 2005 (Abhilash, 2009). More than 52 countries not only globally banned its formulation but also use of it in any form. This toxic insecticide is ranked among the top chemicals of concern by the US agency for toxic substances and disease registry (Abhilash et al., 2008). Out of the known eight isomers of HCH (α, β, γ, and δ), 'γ' is the only isomer having insecticidal property to kill the variety of insects. Its agricultural use has been banned in most of the developed countries; however, some developing countries are continuing its use due to its low cost (Johri et al., 1998). Due to its continuous use throughout the world, lindane-contaminated sites are prominent worldwide. Once HCH enters the environment, it can distribute globally (Simonich and Hiteis, 1995) and can also persist in various environments (Abhilash et al., 2008; Abhilash, 2009). The various sites of lindane contamination have been reported in different countries, viz., Europe (Concha-Grana, et al., 2006), America (Osterreicher-Cunha et al., 2003; Phillips et al., 2006), and Asia (Prakash et al., 2004; Zhu et al., 2005).

The half-life period reported for lindane in soil and water is 708 and 2292 days, respectively (Beyer and Matthies, 2001). Lindane causes various environmental impacts and persists in the soil for long periods. Therefore, toxicity and threats of environmental contamination are of great concern, and this problem can be solved through biodegradation-based approaches. The need of the hour is to develop procedures that could remove these toxic compounds by converting them to non-toxic form intermediate simple compounds. The various approaches of decontamination of HCH like chemical treatment, incineration, and land filling available, but they lack widespread application due to their cost factor and toxicity concerns to the living system. The bioremediation technology has been proposed as a promising tool for in situ detoxification of pesticide-contaminated sites. The various soil microorganisms capable of degrading and utilizing the organochlorine γ-hexachlorocyclohexane as a source of carbon have been reported over the last two decades at various places (Sahu et al., 1990; Adhya et al., 1996; Okeke et al., 2002; Nawab et al., 2003). Although the use of HCH has been banned in most of the countries, γ-HCH and its non-insecticidal isomers α, β, and δ still continue to pose environmental and health hazard (Pavilikova et al., 2012).

Currently, India is the largest consumer and manufacturer of lindane in the world (Sagar and Singh, 2011). India had a total lindane production capacity of 1,300 tons per year, with two companies producing: Aditya Birla Chemicals Pvt Ltd with a capacity of 1,000 tons per annum (tpa), and India Pesticides Limited (IPL), UPSIDC Industrial Area, Deva Road, Chinhat, Lucknow (U.P.) with 300 tpa capacity. Aditya Birla Chemicals Pvt Ltd has now stopped Lindane production (Abhilash and Singh 2009). Now India Pesticide Limited (IPL, Lucknow) is the only operating plant worldwide. For each ton of lindane produced, 8–12 tons of wastes are generated (generally enriched in the and β isomers). Thus these production residues were deposited in an uncontrolled manner around the various production sites around the world (Vijgen 2006a, b) and because of their toxicity, lipophilic nature, long range transport capability through wind, persistence behaviour and easily bio-accumulate in food chain of various organisms, make these stockpiles one of the globe's largest hazardous organic chemical waste sites. Lindane residues are readily found in milk (Dhanya et al., 2012), meat (Aulakh et al., 2006), water (Kaushik et al., 2007), fish (Amaraneni et al., 2011), human blood (Dhananjayan et al., 2012), butter and ghee (Singh et al., 2007), Honey sample (Choudhary et al., 2008), breast milk (Kalra et al., 2003) and Maternal and cord blood (Pathak et al., 2008) etc. Hence, an increasing concentration of this pesticide in the biosphere is a matter of great concern (Hewitt 1998). Therefore, developing suitable remediation packages are utmost important for the clean-up of lindane contaminated soil sites.

Among the various clean-up technologies, bioremediation is widely recognized as an innovative technique for the decontamination of pesticide contaminated soil sites. Research on isolating and monitoring microorganism with catabolic potential has improved the prospectus of using microbial bioremediation as an alternative to chemical or engineering based remediation strategies. Literature provides ample evidence on the catabolic potential of microbes against pesticides (Rigas et al., 2005; Barragan-Huerta et al., 2007) and their role in natural remediation processes. Microbial diversity and their biomass offer a variety of eco-friendly options for mineralization of pesticides or their transformation into less toxic metabolites. Previous studies reported the microbial degradation of various pesticides under aerobic and anaerobic conditions (Nagata et al., 2005; Elcey and Kunhi, 2010).

In nature most of the soil microorganisms have adopted to mineralize and biodegrade the pesticides to satisfy their energy and nutrient requirements (Bhuyan et al., 1992). Mineralization and degradation of wide variety of toxicants such as lethal heavy metals, toxic dyes and poisonous pesticides have been reported by bacteria and fungi (Tekere et al., 2001). Microbial mediated bioremediation are eco-friendly and less harmful because during bioremediation process less by products are released in the environment. The lindane mineralization has been also reported in species of white rot fungi such as *Pleurotus* sp, *Cyathus bulleri*, *Phanerochaete chrysosporium*, *Trametes hirsutus*, etc. under aerobic conditions (Singh and Kuhad, 2000; Tekere et al., 2002). Further, it has been demonstrated that *Fusarium ventricosum* can be a potential bio-agent in degradation of lethal endosulphan pesticide. It seems that there is large number of microbes which are still to be identified with respect to lindane and other pesticide degradation in the soil and environment. Therefore, in the present research work is an attempt has been made to isolate and identify the methanotrophs from lindane contaminated sites and to assess their capability in degradation of lindane. For the first time, the degradation of lindane by methanotrophs is being reported via this investigation.

1.2 Methanotrophs

Methanotrophs plays an important role in global methane (CH_4) budget by consuming a significant amount of CH_4 in soils (Singh, 2011; Singh and Singh, 2012; Singh, 2013). This CH_4 consumption mediated by soil methanotrophs can contribute up to 15% to the total global CH_4 destruction (Singh and Pandey, 2013). In addition, to their ability to oxidize CH_4, the CH_4-monooxygenase (MMO) enzymes in methanotrophic bacteria can also co-metabolize diverse types of organic pollutants, and so application of methanotrophs in bioremediation of such compounds has been widely investigated (Jiang et al., 2010). It is interesting, and perhaps fortunate, that the conditions that exist in some of the specific ecosystems appear to favour the growth of the methanotrophs and the synthesis of MMO, which is essential for the rapid degradation of organic toxicants. Methanotrophic bacteria also have considerable potential for use in biotechnology and in bioremediation due to the amenability of these bacteria to large-scale cultivation (Semrau et al., 2010). Hasin et al. (2010) have provided an example of a methanotrophs that converts a toxic heavy metal species into a less toxic and insoluble form, thus extending the bioremediation potential of methanotrophic bacteria. Although, several recent reports indicated that methanotrophs may be useful tool to extend the utility of these bacteria for several organic pollutant degradation including insecticides and pesticides (Semrau et al., 2010; Jiang et al., 2010; Overland et al., 2010; Semrau et al., 2010). In 1996 Kumaraswamy and his colleagues study the effect of γ- HCH on methane oxidation. Mertens et al. (2005) analyse stereospecific effect of HCH on activity and structure of soil methanotrophic communities using methane oxidation assay and PCR DGGE analysis respectively.

Methanotrophic bacteria also have considerable potential for use in biotechnology and in bioremediation due to the amenability of these bacteria to large-scale cultivation (Semrau et al., 2010; Jiang et al., 2010; Overland et al., 2010; Semrau et al., 2010). It has been suggested that methanotrophs influence the speciation and bioavailability of heavy metals in the environment (Choi et al., 2006). Hasin et al. (2010) as in the reductive transformation of soluble and more toxic Cr (VI) into a less toxic Cr (III) species, which is insoluble and tends to get precipitated at high pH. There is possibility of reverse methanogenesis, where anaerobic methane oxidation can be coupled to iron or manganese reduction due to concerted activity of archaea and methanotrophic bacteria. It is still unclear how methane oxidation is coupled to metal reduction process. Perhaps bacteria solely responsible for anaerobic oxidation of methane may prefer coupling of manganese reduction (Bala et al., 2009). The flexibility in survival of methanotrophs confers them added advantage and makes them ideal for remediation of hazardous environmental wastes under a diverse range of habitats (i.e., terrestrial, marine, Arctic and Antarctic Polar Regions).

During, *in-situ* bioremediation, the growth of indigenous populations of methanotrophs is augmented after the supply of CH_4 and oxygen (Hazen et al., 2009) as the degradation of pollutants by methanotrophs is typically a co-metabolic process and it can be sustained only in the presence of growth substrate. Further, there are reports about limitations offered by methanotrophs in biodegradation of pollutants (Semrau et al., 2010), which may also arise due to toxicity of pollutants to methanotrophs. Pfiffner et al. (1997) have reported significant increases in the population densities of methanotrophs in the soil contaminated by lindane (γ-HCH) - the most widely used insecticide. A possible way to improve this bioaugmentation efficiency can be enhanced by the application of native methanotrophic bacteria into the contaminated site, but it is infrequently made. Further, Mertens et al. (2005) demonstrated promising results for a slow-release bio-augmentation approach using encapsulated *Sphingomonas* sp. cells for the biodegradation of lindane in laboratory condition. Therefore, bioremediation based on methanotrophic bacteria might be an emerging tool and has been receiving more attention as an eco-friendly and efficient means of lindane remediation (Mertens et al., 2005).

Though the application of pesticides in agriculture has offered considerable economic benefits by significant enhancement in the production and yield of crops but on the other hand these pollutants may cause severe environmental hazards and threat to living beings. It is not known, how the HCH isomers quantities impact the methanotrophic microbial community and their biomass in the soils. Therefore, identification and quantification of HCH residues in soils may provide important information regarding their correlation with methanotrophic microbial community and their role in degradation of HCH residues.

1.3 Soil microbial biomass

The soil microbial biomass (SMB), considered as an important source of available plant nutrients in several nutrient deprived ecosystems. Land use changes or land use covers and cutting of forests not only affect the availability of required nutrients but also influence the soil microbial community and their biomass levels. It is proposed that several toxic soil pollutants such as heavy metals, insecticides and pesticides, etc. severely influencing the beneficial soil microbial activities and biomass (Singh and Gupta, 2018). Hence, any such toxic pollutions and disturbances are due to environmental drivers (soil pH, temperature, salinity, etc.) in soil could be one of the major factors which may affect the SMB pools and nutrient availability to plants. The previous investigations showed that several environmental drivers (soil organic matter, gravimetric moisture, soil pH, etc.) govern the dynamics of SMB (Singh et al., 2018). However, no investigations have been conducted in the present selected regions to find out the levels of SMB in soils contaminated with HCH isomers. Further, it is also not known how the HCH residues concentrations impacts the SMB–C, –N and –P levels. The earlier studies of in present region were conducted to find out the impact of forest fires, seasonality, vegetation cover or topographical variations on SMB-C, -N and -P dynamics (Singh and Kashyap, 2007; Singh et al., 2009; Singh et al., 2010). Therefore, study of SMB–C, –N and –P contaminated with HCH and OCPs residues may provide new information about the region of Vindhyan plateau. It is hypothesised that the higher HCH isomers concentrations in soil may lead to several unfavorable conditions to the soil microbial diversity, abundance and their biomass. Therefore, the consequences of changes in HCH isomers concentrations and soil physico-chemical properties should be investigated on SMB levels and correlation between HCH isomers concentrations, SMB values and soil physico-chemical properties.

In India, limited research work has been carried to evaluate the role of microbes in remediation of pesticides from lindane contaminated sites. Majority of the Indian studies used different bacterial strains cultured and maintained in laboratory rather than the contaminated fields and evaluated the biodegradation potential of microorganism in pesticide containing nutrient media. To the best of our knowledge there is no information about the methanotrophs diversity and soil microbial biomass from lindane contaminated sites. Hence, there is urgent need to study the soil methanotrophs composition and microbial biomass levels from lindane contaminated sites. It is also not known whether different contaminated sites have similar community composition of methanotrophs. Further, we don't know about their diversity in lindane contaminated soils. It is expected that present proposed investigation would provide answer to some of the questions raised here. Further, understanding soil microbial biomass and the types of methanotrophic community structure and its potential capability to degrade the lindane is important for verifying the significant contribution of these microbes in cleaning the soil system contaminated by the lindane. The objectives of the present study are:

Objectives

1. To analyse the lindane residues in collected soil samples from lindane contaminated sites/area.
2. To study the soil methanotrophs compositions and microbial biomass-C, -N and -P from lindane contaminated sites.
3. To monitor the methanotrophic strains for lindane degradation tolerance level.
4. To assess the lindane degradation potential of identified methanotrophic strains at different environmental conditions.
5. To examine the end products during lindane degradation by methanotrophs in laboratory conditions.

CHAPTER-2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

2.1 Lindane

The organochlorine pesticides (OCPs) like lindane have long residual action and have persistent nature without losing their toxicity for a long time (Agbeve et al., 2014). The OCPs are so named because they include carbon, hydrogen, and chlorine in their constituents. There are mainly three major subclasses of OCPs as diphenylaliphatics, cyclodienes and hexachlorocyclohexane (HCH). It has been estimated that about 10 million tons of the technical HCH have been used globally from 1948 to 1997 (Walker et al., 1999; Li, 1999). The OCPs have been commonly used across the world to control agricultural pests and vector born diseases (Abhilash and Singh, 2009; Zhang et al., 2011). The OCPs contaminated soils, turning into gradual toxic sources, has been damaging environment with more potential (Niu et al., 2016). The use of Xenobiotic compounds in most of the countries has banned, however, some are still using the lindane (γ-HCH) due to its low cost value and therefore, spontaneously contaminating the soils (Nagata et al., 2005). In 2009, the lindane (α- and β-HCH) has been added to the list of POPs under the Stockholm Convention, which has been a matter of global concern (Vijgen et al., 2011). The use of HCHs (a mixture of α, β, γ, δ, and ε-HCH isomers) such as purified lindane (γ-HCH, the only isomer with insecticidal properties) in the agricultural sector is a great cause for public health concern (Wang et al., 2009; Chang et al., 2011). The use of γ-HCH has resulted into serious contamination problems globally due to their persistence nature in aquatic, soil and air (Dominguez et al., 2016; Vega et al., 2016; Khan et al., 2017; Madaj et al., 2017). It has been proved that there are huge accumulations of HCHs in different environmental samples worldwide (Peng et al., 2015). For many years, humans have enjoyed the benefits of using pesticides to control weeds, insects, pathogenic fungi, parasites and rodent pests in crops. Pesticides are the only effective means of controlling pathogens, weeds or insect pests in many circumstances (USEPA, 2012).

In the environment, lindane has potential to transform into a variety of chemicals; most of them are volatile in nature. The HCH is the collection of the five isomers i.e. alpha (α), beta (β), gamma (γ), delta (δ), and epsilon (ε). The HCH isomer manufactured as lindane is not produced in the United States since 1977; hence it is imported in multiple forms for pharmacologic and industrial purposes. The use of lindane has been restricted by the EPA; however, it can be applied only by certified pesticide sellers. The three HCH isomers (α, ß, and γ-HCH), have been included in the list of POPs in the Stockholm Convention (Vijgen et al., 2011). The waste of HCH isomers, generated during lindane production, with no insecticidal properties are commonly called as 'muck' (65–70% of α-HCH, 7–10% of ß-HCH, 75% of δ-HCH, 1–2% of ε-HCH, <2% of η-and θ-HCH) (Willet et al., 1998). The illegal dumping of waste HCH isomers (called as "muck") has created large number of HCH dump sites all over the world from where these harmful HCH isomers are leaking into the different types of ecosystems (Willett et al., 1998; ATSDR, 1999). It has been estimated that more than 52 countries has globally banned the HCH in its any kind of use in different formulations. The countries involved in lindane formulation are also creating lindane dumping sites which are causing environmental problems to living beings.

Apart from this, the scientists get involved in working on its degradation and trying to find out the easiest, cheapest and safest way to decontaminate the lindane from polluted sites. Bacteria present in lindane contaminated sites that have evolved the ability to degrade HCH. The HCH degradation potential of various HCH isomers by bacterial strains is mediated primarily by two genes: The *LinA* and *LinB* which encode for enzyme dehydro-chlorinase and halo-alkane de-halogenase, respectively. Long distance transport of lindane is evidenced by its presence in the Arctic Ocean, where it has never been used. Most of the lindane is present in the water environment, although a significant amount is also found in the soil/sediment and some in air. Lindane has also been shown to bio-accumulate in the fatty tissue of living organisms.

For many years, we are continuously using pesticides to control the weeds, insects, pathogenic fungi, parasites and rodent pests. The pesticides are the only effective means of controlling pathogens, weeds or insect pests in many circumstances (USEPA, 2012). Apart from the beneficial role, the use of pesticides also has some drawbacks or side effects, such as potential toxicity to humans, birds and other aquatic animals. The HCH isomers are one of the most abundant organochlorine contaminants in the Arctic Ocean (USEPA, 2006). Production of one ton of lindane generates a waste of around 6-10 tons of other isomers (Vijgen, 2006). A massive 10 million tons of the technical HCH have been used world over from 1948 to 1997 (Walker et al., 1999; Li, 1999). India started the production of technical HCH in the year 1952 (Gupta, 1986) and perhaps was the largest user of technical HCH and DDT in the world. Technical HCH and DDT amounted to 70% of total insecticide production in the 1980's.

Lindane has been recently listed as a persistent organic pollutant (POP) under the United Nations' Economic Commission for Europe Convention on Long-range Transboundary Air Pollution (LRTAP POPs Protocol) and the Great Lakes Binational Toxics Strategy between United States and Canada. It is also the subject of a joint re-evaluation in the US and Canada under NAFTA's (North American Free Trade Agreement) Technical Working Group on Pesticides. In the environment, lindane is potentially transformed into a variety of chemicals, most of which are volatile. These include γ-pentachlorocyclohexene, γ-3,4,5,6-tetrachlorocyclohexene, α-HCH (Bintein and Devillers, 1996; Cornacoff et al., 1988). The ratio of α-HCH to γ–HCH concentration in air has been used as an indicator to estimate the possible origin of the air mass in the long-range transportation of contaminants (Iwata et al., 1993). Earlier, bio-isomerization of γ -HCH to α-HCH was thought to be the principal route for long range contamination; however, current field studies have found that only a small percentage of γ -HCH is converted to α-HCH due to involvement of microbial activities (Waliszewski, 1993; Singh et al., 1991).

2.2 Physico-chemical properties of lindane

Lindane is the common name for γ-hexachlorocyclohexane. The chemical formula for lindane is $C_6H_6Cl_6$ and its chemical structure has been shown in Figure. 2.1. It has a molecular weight of 290.83 g/mol. Lindane (γ-HCH) is an insecticide and fumigant which have been utilized for the control of an extensive variety of soil-dwelling and plant eating (phytophagous) insects.

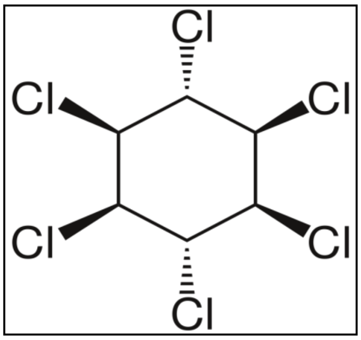


Figure 2.1 Molecular structure of lindane

It is commonly utilized on a wide variety of crops, in stockrooms, in public health to control insect-borne diseases and with fungicides for the seed treatment.Lindane is also presently used in lotions, creams, and shampoos for the control of lice and mites (scabies) in humans (USFDA, 2003). Trade or other names for lindane include Aphitiria, Agrocide, Aparasin, Ambrocide, Benesan, Benexane, benhexachlor, benzene hexachloride, BHC, gamma-BHC, Borer-Tox, BoreKil,Exagama, Gallogama, Gamaphex, Gamasan, Gammex, Gammexane, Gexane, Isotox, Jacutin, Kwell, Lorexane, Lindafor, Lintox, Lindagronox, Lindaterra, Lindatox, ,Noviagam, Quellada, Steward, Streunex and Tri-6 (USEPA, 1983) (Table 2.1). Lindane is a white powder, that evaporates in to the air, with a musty (odourless when pure), odour at concentration of 12 ppm and more (NTP, 1998). Lindane is known to be steady in air, light, heat, carbon dioxide, and strong acids and half-life for lindane in soil and water is accounted for as 708 days and 2292 days individually (Beyer and Matthies, 2001).

Table 2.1 Physical and chemical properties of lindane.

Common name	**Lindane**
Chemical class	Organochlorine
Chemical name	1,2,3,4,5,6-hexachlorocyclohexane, γ-isomer, γ-HCH
CAS Registry number	58-89-9
Chemical formula	$C_6H_6Cl_6$
Molecular weight	290.83
Melting point	112.5°C
Boiling point	323.4°C
Solubility in water at	25°C 7.52 mg/L
Partition coefficients Log KOW	3.3, 3.61
Log KOC:	3.0, 3.57
Bioaccumulation factor:	
1. In human fat	19 ± 9
2. In aquatic animals:	2.5 ± 0.4
Vapor Pressure at	20 °C or 25°C 5.3 ± 1.4 x 10-3 Pa
Henry's law constant at	25°C 3.2x10-4 KPa m^3/mol
Conversion Factors	

Air 1 ppm	1.18 mg/m^3
Water 1ppm	1 mg/L
Carcinogenicity classification	
ACGIH	A3
EPA	NA
IARC	2B

NA= not available

2.3 Commercial production of lindane and its applications

After the second world-war, numerous organizations started to produce HCH. They use various methods to produce technical HCH. These methods include catalyser**,** Butylacetate-method, Benzine-Dioxane Method, Modified Benzine-Dioxane Method, Benzine Method, Methanol-circulation-method etc. Table 2.2 shows countries mainly associated with lindane production.

Table 2.2. Brief introduction of lindane producing countries.

Country	Period of production	References
Albania	1982 till 1990	UNEP (2002)
Argentina	1947-1949	UNEP (2002)
Austria	1965-1990	Dept of the Environment and Heritage (2004), Li (1999).
Azerbaijan	1960 – 1985	Okan (2004)
Belgium	--	Stoffbericht (1993)
Brazil	1962-1985	UNEP (2002); Lopez (1999)
Bulgaria	Till 1966	Hauzenberger (2004)

Canada	Till 1974	CEC (2000); NRTEE (2000)
China	1995-2000	Li (2001, 2004)
Czech Republic	1954-April 1977	Hauzenberger (2004)
Croatia	--	UNECE (2000)
Denmark	Till 1977	De Bruin (1997)
Egypt	---	Li (1999)
France	1977-1979	De Bruin (1979)
Eastern Germany	1967-1982:	Heinisch (1994)
West Germany	1955-1971	De Bruin (1979)
	Discontinued 1972	
Ghana	--	UNEP (2002)
Hungary	1953-1964	Barczi (1994)
India	Till 2003	Jensen (2004)
Japan	1948-1987	UNEP (2002);
		Questionnaire Ministry of Agriculture (2004)
Poland	1956-1982	Questionnaire (2004)
Romania	---	Hauzenberger (2004)
(Former) Soviet Union	Till 1990	Li et al. (2005)
Switzerland	From 1935 to 1965	Bentz (2004)
Turkey	Till 1982	Türkman et al. (1993)
USA	1950s-1970s	Weston (1993)

Technical-grade HCH is produced as a mixture of isomers (primarily the, β, γ, δ, and ε isomers) by photo-chlorination of benzene, a response that can be begun by free-radical initiators such as visible or ultraviolet light, x-rays, or γ-rays (ATSDR, 2005).

The active γ-hexachlorocyclohexane (lindane) can be concentrated by treatment with methanol or acetic acid, followed by fractional crystallization, which produces technical grade lindane containing 99.9% γ-HCH isomer. Commercial production of lindane in the United States began in 1945 and peaked in the 1950s, when 17.6 million pounds was manufactured (IARC, 1979). Lindane is no longer produced commercially in the United States, but it is produced by 13 manufacturers worldwide, including 7 in India and 4 in China (SRI, 2009), and is available from 42 suppliers, including 19 U.S. suppliers (Chem Sources, 2009). U.S. imports of HCH increased from 310,000 lb to 1.4 million pounds between 1989 and 1999 imports, declining to zero in 2005 and remaining zero through 2008 except in 2006, when 73,000 lb was imported. U.S. exports of HCH increased from zero in 1990 to 1.5 million pounds in 2005, declining to 154,000 lb in 2008 (USITC, 2009).

2.4 Application of lindane

Before getting banned lindane was used in various field like agriculture, forestry, household uses, seed treatment etc. as shown in Figure. 2.2.

1. Lindane is utilized for louse control and as a wood preservative in few nations (UNEP 2002).
2. Lindane is registered for the control of carpet beetles and clothes moths in home.
3. It is reregistered by the EPA for control of aphids, cucumber beetles, cutworms, melon worm, pickleworm, squash bug, squash vine borer, white grub and other insects on cucurbits.
4. For control of the following insects found in the home: ants, centipedes, clothes moths, house fly, odorous house ant, cockroaches, bed bugs, chats, little black ants, sow bugs, mosquitoes, scorpions, spiders, water bugs and silverfish.

5. It is also use for controlling wood boring beetles generally called powder post beetles and also for dry wood termites.
6. All the major arthropods pests that attack pets, including ticks, flies, lice, mites and pet premises. Ohio is the only reported state that recommended lindane for control of fleas on cats.
7. It has also been used as pharmaceutical treatment for lice and scabies, formulated as a shampoo or lotion.
8. For control of phylloxera and shoot curculio on pecans.
9. Lindane is the only pesticide registered for the control of all the insect pests attacking Christmas trees.
10. For all the major insects and leaf miners on woody ornamentals, floral and foliage plants.
11. Lindane is special EPA registration for the control of symphylans on Hawaiian pineapple production. It is used in conjugation of 4 soil fumigants.
12. For the use on a variety of livestock arthropod pests and on many classes of livestock- beef, cattle, hogs, pigs, sheep, goats and horses and livestock premises-barns, pens, sleeping quarters and shelters.
13. In forest lands (commercial, seed orchards and naval stores), lindane is the only registered chemical for use on living trees for insect control.
14. Lindane is insecticide for use as a seed treatment on the minor crops such as peas, beans, sunflower, lentils and vegetables and major crops including sorghum and sugar beets.

2.5 Sources of lindane contamination in environment

The primary routes of potential human exposure to lindane and other hexa-chlorocyclohexane isomers are ingestion, inhalation, and dermal contact (HSDB 2009).

The general population potentially is exposed through consumption of foodstuffs contaminated with pesticide residues. According to U.S. Food and Drug Administration's Total Diet Survey, lindane was detected in 279 of 2,168 samples and in at least one sample of all 54 different food items analyzed (FDA, 2006). Most of the food items in which lindane were detected had significant fat content.

Figure 2.2 Application of lindane for different purposes

However, the highest lindane concentrations were in pickles and raw mushrooms, which have low fat content. Daily dietary intake of HCH isomers by the adult U.S. population was estimated at 0.010 µg/kg of body weight for all isomers and 0.002 µg/kg for lindane. For 1982 to 1984, the estimated dietary intake of lindane was 1.9 ng/kg of body weight for infants aged 6 to 11 months and 7.9 ng/kg for toddlers aged two years, who had the highest average daily intake. By 1986 to 1991, daily intake had fallen to 0.8 ng/kg for infants and 3.2 ng/kg for toddlers (ATSDR, 2005).

Dermal introduction happens when shampoos and creams containing lindane are utilized for the treatment of lice and scabies (FDA, 2009). The highest average blood concentration of lindane measured in children after scabies treatment with one of these products was 0.028 μg/mL (ATSDR, 2005). According to the U.S. Environmental Protection Agency toxics release inventory, environmental releases of lindane ranged from 314 and 2,118 lb between 1988 and 1997. In 1998, over 25,000 lb was sent to a hazardous-waste landfill. By 2006, releases had declined to 10 lb. In 2007, five facilities released a total of 1,555 lb of lindane, mostly off site for unspecified management (TRI, 2009). Lindane was found in at least 189 hazardous-waste sites currently or formerly on the National Priorities List; it occurred in air at 9 sites, surface water at 33 sites, sediment at 36 sites, and soil at 90 sites. The non-occupational pesticide exposure study, published in 1990, collected personal air samples at one U.S. location with high pesticide usage and one with low to medium usage. The range of mean γ-HCH concentration was 7 to 22 ng/m^3 at the high-usage site and 0.7 to 5 ng/m^3 at the low- to medium-usage site (ATSDR, 2005).

The HCH isomers have been detected in human fatty tissue, blood, and breast milk. The National Human Adipose Tissue Survey (NHATS), conducted in 1982, found β-HCH residues in 87% of composite post-mortem samples of fatty tissue. According to NHATS data, the mean concentration of β-HCH in fat decreased from 0.45 ppm in 1970 to 0.16 ppm in 1981. The levels were highest in the southern United States. In the 1970s, the National Health and Nutrition Examination Survey (NHANES) found β-HCH in blood at a median concentration of 1.7 ppb. When the NHANES was repeated in 1999 to 2000, the geometric mean concentration of β-HCH and γ-HCH in serum lipid was 9.68 ng/g for individuals over 12 years of age (ATSDR, 2005). The β-HCH was measured in breast milk at a concentration of 0.6 ng/g in Canadian populations living near the Great Lakes. In the Netherlands, concentrations of γ-HCH in breast-milk fat in 1988 ranged from 0.01 to 0.24 mg/kg (HSDB 2009). Many other studies in populations throughout the world, especially Arctic populations, have found HCH isomers in blood, fat, and breast-milk samples. The HCH isomers have been measured at higher concentrations in all types of samples in areas of the world where lindane is still extensively used for pest control, such as India and Africa.

2.6 Toxicological effects of lindane contamination in environments

Lindane produces central nervous system interruption through a variety of mechanisms, the most important being its ability to act as a non-competitive GABA antagonist interacting with the picrotoxin site, both in membranes and in intact cultured neurons, thereby inhibiting the GABA-induced C1-flux following activation of either the GABA (A) or GABA (C) receptor (Pomes et al., 1994; Aspinwall et al., 1997). Rosa et al. (1997) demonstrated that lindane induces significant changes in the intracellular Ca^{2+} homeostasis of central neurons and has a significant impact in the cerebellum. Lindane principally influences the dantrolene-sensitive intracellular Ca^{2+} stores, causing a release of calcium from these stores and altering the sensitivity of membranes.

The neurotoxic effects of lindane are predominantly mediated through its non-competitive antagonism of the GABA (A) receptor. It was noted that following poisoning and seizure, the concentrations of dopamine and its primary metabolite (DOPAC) were increased throughout the mesencephalon and the striatum. In cases of chronic intoxication, dopamine levels fluctuated. These fluctuations, following repetitive exposure to lindane, may account for some of the Parkinsonian-like symptoms our patient experienced. Attia et al. (1991) have shown that chronic lindane poisoning causes an enhanced night time rise in pineal N-acetyltransferase (NAT) activity and increased central melatonin secretion, as well as increased serum melatonin levels. Chronic lindane poisoning also caused a significant reduction in central serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA). This change in central serotonin may well be the factor which mediates the anxiety and depressive changes seen in our patient, as well as the disruption of her sleep cycle. Nedkova-Bratanova et al. (1979) have shown that chronic lindane poisoning, even at low levels, diminishes di-peptidase activity and disrupts the activity of the intestinal di-saccharidases for up to 90 days following low-level poisoning. Sucrase is the most effected di-saccharidase. This disruption of the di-saccharidases may be responsible for the persistent diarrhoea experienced by our patient.

The acute (short-term) health effects may occur immediately or shortly after exposure to Lindane such as contact can irritate the skin and eyes; breathing lindane can irritate the nose; throat and lungs. Lindane can cause headache, nausea, vomiting, dizziness and seizures, irritability, restlessness, muscle weakness and twitching, convulsions and coma (Nantel et al., (1977). The chronic (long-term) health effects can happen sooner or later after exposure to Lindane and can keep going for a considerable length of time or years. Lindane may be a carcinogen in humans since it has been shown to cause liver, lung, endocrine gland and other types of cancer in animals. Four cases of leukaemia were reported in men exposed to γ-HCH with or without other chemicals (IARC 1979; Sidi et al. 1983). Technical-grade, α and β-HCH and the γ-HCH produced liver tumours in mice when administered orally (Kashyap et al. 1979; Munir et al., 1983). The Lindane may damage the developing foetus and decrease fertility in females. High exposure to Lindane may damage the blood cells causing anaemia. Exposure to lindane has been linked with blood disorders known as blood dyscrasias, and in particular the disorder aplastic anaemia where the formation of platelets and white cells is disrupted (Morgan et al., 1980). Lindane can cause abnormal heart rhythm (arrhythmia). Lindane may damage the liver and kidneys. Lindane is fat soluble and this contributes to its tendency to bio-accumulate through food chains. Lindane residues have been detected in the kidneys, livers and adipose tissue of a wide variety of wild animals and birds. It is highly toxic to aquatic invertebrates and fish.

2.7 Lindane bioremediation approaches

Currently there are several dumping sites present in the world from where lindane, POPs and other hazardous chemicals are easily come in contact with biotic and abiotic component of environment. Lindane-residues from "muck" due to rain water, wind and because of illegal discharge in water bodies, dry deposition, snow can enter the environment and cause toxicity. Lindane residues are readily found in milk, meat, water, soil, packed water bottles, fish, human blood, butter and ghee, honey sample, vegetables, breast milk, maternal and cord blood (Pathak et al., 2008).

In order to remove lindane from contaminated environments, several laboratories have isolated and characterize the microorganisms that can cause the degradation of lindane and other HCH-isomers under anaerobic and aerobic conditions. Figure 2.3 shows the role of microorganisms in bioremediation of lindane.

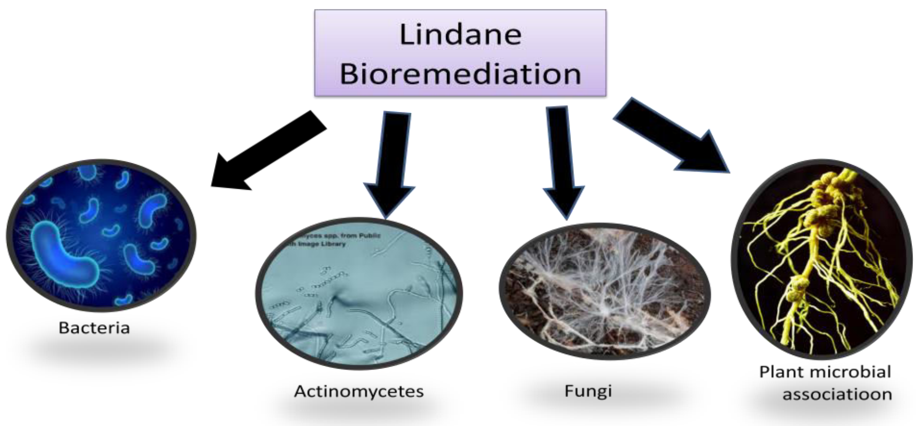


Figure 2.3 Role of microorganisms in bioremediation of lindane

2.8 Role of bacteria in lindane remediation

In the beginning it was believed that lindane biodegradation is largely an anaerobic process, and various levels of anaerobic degradation of α, β, γ, and δ-HCH has been observed. Mac Rae et al. (1969) reported degradation of lindane by anaerobic *Clostridium* sp. Initial study suggested that the anaerobic microorganisms use lindane as a sole carbon and energy source. Later studies demonstrated that lindane is used as an electron acceptor under anaerobic conditions rather than as a carbon source. Other isolates capable of degrading one or more of the other four HCH isomers under anaerobic conditions includes *C. sphenoides* (Heritage and MacRae, 1977), *C. butyricum* and *C. Pasteurianum* (Jagnow et al., 1977). Ohisa and Yamaguchi (1978) reported that under anaerobic pure culture condition, strain of *C. rectum* degrade lindane optimally at pH 7-8. Lindane degradation can also be done by *Escherichia coli* which was isolated from rat faeces. About 10% of the added lindane was metabolized by the bacterium in trypticase soy broth containing the pesticide lindane. A single metabolite, 2, 3, 4, 5, 6-pentachloro-1-cyclohexene (PCCH) was detected and identified by gas chromatography and mass spectrometry (Francis et al., 1975). Members of the family *Sphingomonadaceae* appear to have an important role in aerobic lindane degradation.

The pathway for the degradation of lindane has been comprehensively worked out in the bacterium *S. paucimobilis* UT26, and the genes for its different enzymes have been characterized (Nagata et al., 1999). The catabolic genes associated with the degradation of lindane were initially discovered in *S. japonicum* UT26, and termed *Lin* genes (Nagata et al., 1999). Six structural *Lin* genes (*LinA–LinF*) (Nagata et al., 1994; Miyauchi et al., 1999; Endo et al., 2005) and one regulatory gene (*LinR*) (Miyauchi et al., 2002) are involved in the complete mineralization of lindane in *S. japonicum* UT26.

In addition, a *LinX* gene, encoding a protein that has activity similar to that of *LinC*, was also characterized (Nagata et al., 1994). The *LinA*-encoded HCH dehydrochlorinase (*LinA*) mediates the first two steps of dehydrochlorination of lindane. In addition to mediating the second step in the degradation of lindane in *S. Japonicum*UT26, *LinB* has been reported, recently, to transform β-HCH to 2,3,4,5,6-pentachlorocyclohexanol (PCHL) (Nagata et al., 2005). The PCHL has lower hydrophobicity and lower chemical stability than β-HCH, and the bacteria that degrade and use it might exist in the polluted environment, enabling the complete degradation of β-HCH by a combination of biological pathways. The location and stability of *Lin* genes encode the lindane catabolic pathway, have been explored in the lindane-degrading *S. francense* strain sp1+, and in two non lindane-degrading mutants (Sp1- and Sp2-) (Ceremonie et al., 2006).

A strain of *Pseudomonas paucimobilis* isolated from paddy field rhizosphere soil was demonstrated to degrade lindane (Sahu et al., 1990). About 98% of lindane was aerobically degraded by *S. paucimobilis* after 12 days of incubation (Johri et al., 1998). The *Pseudomonas aeruginosa* ITRC-5 can degrades α-, β-, γ- and δ- HCH, in both liquid-culture and contaminated soils, and the degradation of β- and δ-isomers is enhanced in the presence of α- or γ-HCH (Kumar et al., 2005). Incubation of 'muck' with the isolated bacterium *P. aeruginosa* ITRC-5 under optimized conditions i.e. 1.7 mM input concentration, pH 9.0, and temperature 20–30 ±1°C, causes substantial degradation of HCH-isomers, which is accompanied with reduction of their toxicity (Chaudhary et al., 2006). Bioconversion and biological growth kinetics of *P. aeruginosa* degrading technical HCH was investigated in batch process under aerobic condition by Lodha et al. (2007). At lower technical HCH concentrations (1-10 mg/L), degradation (above 99%) was observed whereas at higher concentration (20-50 mg/L), the degradation efficiency was reduced. A gram-positive *Microbacterium* sp. strain, ITRC1, has the capacity to degrade all four major isomers of HCH present in both liquid cultures and aged contaminated soil was isolated and characterized by Manickam et al. (2006). For the first time a *Xanthomonas* sp. was isolated from a contaminated soil which utilized lindane as sole carbon and energy source by successive de-chlorination (Manickam et al., 2007).

2.9 Role of actinomycetes and fungi in lindane remediation

Actinomycetes for bioremediation are an attractive approach, since these microorganisms have already adapted to the various habitat (Shelton et al., 1996). Pesticide-degrading actinomycetes belonging to *Arthrobacter, Brevibacterium, Corynebacterium, Clavibacter micromonospora, Mycobacterium, Nocardia, Nocardioides, Rhodococcus* and *Streptomyces* (De Schrijver and De Mot, 1999). Actinomycetes have a great potential for bioremediation of toxic compounds (Benimeli et al., 2007). Most of the lindane degrading fungi known to date are the members of the family of white rot fungi, and a very few non-white rot fungi has been noted to degrade the lindane. The lindane biodegradation is accomplished with the action of extracellular oxidative enzymes, produced by the fungus to decompose woody substrates, such as laccase, manganese peroxidise, lignin peroxidase (Rigas et al., 2005). Bumpus and Aust (1987) published the first report on the biodegradation of lindane by white rot fungi *Phanerochaete chrysosporium*. Mougin et al. (1997) reported the enhanced mineralization in soils supplemented with lindane by *Phanerochaete* sp. and the fungus seemed to modify lindane degradation pathway by increasing the conversion of volatile intermediates to CO_2. Biodegradation of lindane up to 85 to 95% by white rot fungi such as *Pleurotus ostreatus, P. sajorcaju* and *Trametes hirsuta* has been reported by earlier workers. Singh and Kuhad (1999) investigated lindane degradation ability of the white-rot fungus *Trametes hirsuta* in liquid culture, which was compared *P. chrysosporium*. It was shown that *T. hirsuta* degraded lindane faster than *P. chrysosporium*. Singh and Kuhad (2000) studied lindane degradation capability of two white rot fungi, *Cyathusbulleri* and *P. sordid* and reported that *C. bulleri* degraded lindane more efficiently than *P. sordida*.

The degradation of lindane at various concentrations by a sub-tropical white rot fungus was studied in batch and packed bed bioreactor systems. About 82 ± 6% degradation of lindane was achieved in batch cultures and 81% degradation was noted in packed bed reactor (Tekere et al., 2001). Bioremediation process was evaluated in polypore fungus, *Ganoderma australe* in mixtures of a sandy soil and wheat straw doped with lindane (Rigas et al., 2007). Maximal degradations of 94.5% were attained after 30 days for lindane by the white-rot fungus *Bjerkanderab adusta* in a slurry batch bioreactor (Quintero et al., 2007). Biodegradation of lindane by *Phycomyceteous* and *Conidiobolus*, a non-white rot fungus was reported by Nagpal et al. (2008). The bracket-like polypore fungus, *Ganoderma australe* was selected for its potential to degrade lindane in liquid agitated sterile cultures. The maximum lindane biodegradation (3.11 mg g^{-1} biomass) was obtained with addition of nitrogen supplements during 5 days of cultivation time (Dritsa et al., 2009). Two *Fusarium* species (*F. poae* and *F. solani*) isolated from the pesticide contaminated soil showed better degradability of lindane while used as a sole carbon source (Sagar and Singh, 2011).

2.10 Government and private initiatives on lindane concern

Many registered companies used lindane for various purposes which include direct treatment of livestock, pet products, ornamentals, home lawns, fallow areas, commercial food processing facilities and storage areas, greenhouses, wood treatment, forestry, Christmas tree plantations, military use on human skin and clothing (EPA, 2006). Lindane is classifies by the U.S. EPA as one of twenty-two “Bio-accumulative Chemicals of Concern” in the Great Lakes. Now release of these chemicals is restricted into "mixing zones" because of "proceeding with confirmation that the exceptionally bio-accumulative nature of these lethal chemicals displays a huge potential hazard to human wellbeing, aquatic life and wild life." (Great Lakes Initiative, Fact Sheet). The U.S. EPA likewise arranged lindane as an "Extremely Hazardous Substance" in Section 302 of the Emergency Planning and Community Right-to-Know Act.11.12. Under the Clean Water Act. The U.S. EPA characterizes lindane as a "Priority Pollutant" for the security of sea-going life and human wellbeing in surface water" (USEPA Water Quality Criteria). Lindane is incorporated into the U.S. EPA's Toxic Release Inventory (TRI) Program, which "requires facilities in specific enterprises, which produce, process, or utilize critical measures of harmful chemicals, to report every year on their release of these chemicals.

Lindane and the other HCH isomers are mobile in environment, and through long-extend environmental transport, are deposited in the Arctic, where they have been recognized in air, surface water, groundwater, residue, soil, ice, snowpack, fish, wild life, and human beings (EPA Risk Assessment Fact Sheet 2006). All utilizations with the exception of pharmaceutical uses are currently limited. "On August 2, 2006, EPA reported that registrants Chemtura USA Corporation, trailed by AGSCO Inc, Drexel Chemical Co. furthermore, JLM Industries, Inc. asked for to intentionally wipe out all residual pesticide enlistments of the organochlorine pesticide lindane. EPA also made an assurance that the rest of the employments of lindane are not qualified for re-enlistment (Lindane RED Addendum 2006).

The U.S. Agency for Toxic Substances and Disease Registry(ATSDR) (2005) ranks lindane 32nd of the 275 substances on its list of CERCLA (Superfund) "Priority Pollutants." "This list reflects a "prioritization of substances in view of a mix of their recurrence, poisonous quality, and potential for human introduction at NPL (National Priorities List) sites."Beginning January 1, 2002, any item utilized for the treatment of lice or scabies in individuals that contains the pesticide Lindane should not be utilized or sold as a part in state of California." (California Assembly Bill 2000).Lindane is banned for use in 52 nations, and limited or extremely confined in 33 nations."(EPA Risk Assessment Fact Sheet 2006).The International Agency for Research on Cancer (IARC), the chief office on cancer-causing agent classification, at present considers HCH, the class of chemicals to which lindane has a place, as possibly carcinogenic to humans (IARC 1998). On 13 July 2000 the European Union Standing Committee on Plant Health voted in favour of a restriction on lindane with the support from UK and other member states. The European commission confirmed the choice in December 2000, which ought to happen before the end of 2001. The boycott will cover all agricultural and amateur gardening uses of lindane. The 13th international HCH pesticides forum was organized in Zaragoza 3-6 November 2015 by international HCH and pesticides association (IHPA), the Aragon Government and SARGA to attract consideration regarding the huge ecological and money related issues in the locale of Aragon (Spain) created by the former production of Lindane by Inquinosa manufacturing plant and the legacies made by the wild dumping of gigantic amounts of HCH-waste in the region encompassing Sabinanigo and the Gallego River.

2.11 Soil microbial biomass

Soil microbial biomass (SMB) plays a crucial role in nutrient cycling and has been considered as an important parameter of soil health and environmental sustainability (Amador et al., 2012; Yang et al., 2016). It has been reported that the SMB is a very sensitive indicator of the soil dynamics and involved in C and N nutrient cycles (Liu et al., 2012). In soil composition, approximately 2 -3 % of total organic carbon is contributed by the soil microbial biomass and referred as labile fraction of soil organic matter (Yang et al., 2016). The SMB pool can be revived by afforestation on deforested land with the help of mixed plantation in association with suitable bio-inoculants enhancing the ecosystem productivity.

It is now well accepted that soil microbial biomass play a vital role in nutrient cycling in agro-ecosystem and affected by several limiting factors such as soil water availability and etc. (Rangel-Vasconcelos et al., 2015). The SMB is an important parameter of ecosystems as it is the fraction of soil organic matter (SOM) which is most rapidly decomposed by the action of microorganisms. The dynamics of SMB are determined by both biotic and abiotic components of the ecosystem and vary in different location along with time in both natural and man-made ecosystem (Wardle and Hungria, 1994; Rangel-Vasconcelos et al., 2015). The variation in soil microbial assembly over certain time period is closely associated with changes in water availability in soil (Patel et al., 2010), and associated with the studies related to the understanding of release and mineralization pattern of nutrient available for plant (Wardel, 1998; Rangel-Vasconcelos et al., 2015; Luizao et al., 1992). The pattern in temporal variation of SMBin temperate climate are already well understood as it may be closely associated with seasonal variations in temperature and hydrological regime (Wardel, 1998).

The SMB is referred as an important driver of ecosystem functioning and it may be consider as sensitive key biological indicator of perturbations owing to soil disturbances (Zornoza et al. 2009). The microbial diversity and biomass have been described and reviewed at local and regional scales. A unifying driver, or set of environment drivers affecting SMB pattern scales need to be investigated as suggested by earlier workers (Singh and Gupta, 2018). The SMB has been considered widely as the index of soil fertility and ecosystem productivity. The escalating soil stresses due to land degradation and climatic variability are directly correlated with loss of microbial diversity and abundance or biomass dynamics (Singh and Gupta, 2018). The main ecological factors which stabilize the SMB and minimize its turnover, are supposed to play a vital roles in the ecosystem sustainability by maintaining essential function of soil health, through carbon and nutrient turnover process (Singh and Gupta, 2018). Even after disturbances, an ecosystem with a higher microbial diversity and biomass may have a higher capacity to sustain the ecological stability. Above ground (plant litter quantity and quality) and below ground (Soil microbial flora and fauna diversity) play significant roles in controlling the functioning of ecosystem (Wardle et al., 2004). The N deprived soils in Arctic, Alpine Tundra and Temperate forest ecosystem release nutrients after death and decay of soil micro-flora and it has been pointed out as an important source of plant nutrient (van der Heijden et al., 2008). Moreover, many microbial communities have restricted bio-geographic distribution (e.g. N_2- fixing rhizobacteria in tropical forests and mycorrhiza in boreal temperate forests). It is suggested that the disparity in the size of SMB can impact variability of functioning of various ecosystem types. The disturbances in climate conditions and anthropogenic intervention are the significant drivers to regulate the existence and survival of indigenous microbial diversity, and consequently, the essential soil functioning of the ecosystem (Singh ang Gupta, 2018). The macroclimate topography and soil characteristics are main factors influencing SMB dynamics across different ecosystems locations (Wardle, 1992). The temporal dynamics of SMB may be involved in shaping the soil contents and release of immobilized labile nutrient due to microbial cell death, decay and decomposition. The availability of released nutrients are generally involved in plant growth and functioning of the ecosystem (Wardle, 1998). The factors which provide viability to the soil microbial community therefore, are assumed to enhance the conservation of soil nutrients in the form of higher SMB size (Singh and Gupta, 2018). Climate variability, land use types and the vegetation cover are some of the key factors for assessing SMB variability across different ecosystem types along the large geographical area (Singh and Gupta, 2018). The variation in the quantity and quality of substrate (Organic C and N) inputs caused by varying plant residues types (litter and fine roots) is associated with nutrient specificity and can be crucial drivers influencing the SMB across the ecosystem types. Accordingly, the higher SMB in soil of some particular ecosystem having vegetation cover with giant plant species is due to the greater availability of organic substances. The persistence and fewer distributed ecosystem had higher SMB values than the most disturbed one as reported by Wardle (1998), which indicates that temporal SMB variability is exclusively governed by the dominant vegetation. Therefore, it has been suggested that the forest ecosystem having insignificant disturbances may have higher SMB pool as compares to other disturbed areas soil. In addition, Zhang et al. (2016) demonstrated that shifts in plant species composition during the influence the microbial community composition dynamics and SMB by changing soil organic nutrient content. Though nutrient status, seasonality, temperature, soil and other factors are being play an important role in controlling of dry tropical forest ecosystem. The SMB pool may be one of the vital factors affecting output in the tropical dry deciduous forests, as experiences in the Vindhyan plateau (Singh et al., 2010). For Indian point of view, the previous studies concerning SMB across the tropical dry deciduous forest ecosystems have been conducted in one or the other sites on a temporal scale, and without deciphering differences in SMB status and its role in distribution and variations of the dominant vegetation composition (Singh and Gupta, 2018).

In nutrient poor ecosystems, the SMB acts as the major reservoir of essential nutrients, and plays very important role in the plant growth (Vimal et al., 2017; Singh et al., 2018). In the dry tropical deciduous forest ecosystem, nutrient withdraw from the decaying leaves and undergo immobilization in the SMB pool, has been considered as the nutrient assimilating adjustments in response to nutrient scarcity, and thus SMB constitutes available nutrient source for the survival and growth of various plant communities (Singh et al., 2010; Singh, 2015; Singh and Gupta, 2018). Thakur et al. (2015), explained that plant diversity is a key component in controlling of SMB pool, therefore, any variation in the soil physico-chemical properties due to forest clearing may have significant impact on stability of SMB and its turnover rates (Singh and Gupta, 2018). Since, the deforestation is a common practice among human beings for the generation of managed agricultural land use systems; however, afforestation has resulted into establishment of SMB pool in soil due to the regain status of soil nutrients via decomposition of litter residues (Singh et al., 2010). The elevated level of agricultural land expansion enhances deforestation and consequently causing disturbances in SMB trends in soil (Singh and Gupta, 2018).

The SMB pool is more helpful in estimation of most satisfactory primary productivity of any restored degraded land system (Do Couto et al., 2016). Consequently, the ratio of SMB to the total organic nutrients present in soil may be considered as a reliable parameter for the success of degraded land rehabilitation. The re-established SMB status in soil and its dynamics under prevailing environmental drivers may provide available information about the restoration progress and productivity potential for agricultural purposes (Singh and Gupta, 2018).

Rainfall may causes osmotic stress in microbial cells and promote cell lysis, resulting in the release of essential soil nutrients (Wardle, 1998; Yang et al., 2008) that become available to the soil micro-biota and various plants (Singh et al. 1989). The ecosystems at higher elevation favour the growth of microorganisms as the higher amounts of precipitation result into high level of plant biomass. In India, the MBC content in undistributed tropical forest soil is found higher as compared with savannah and grassland soils (Singh et al., 2010). It is reported that the disturbed region, which may have the result of nutrient release from dead SMB and the decomposition of leaf litter and litter, plays a crucial role in the tropical and sub-tropical ecosystems as they are mainly involved in providing both a short term supply of nutrients and substrate for synthesis of SOM in the long-term supply of nutrients (Fernandes et al., 1997). The ecosystem having less disturbances may have higher level of SMB content in the soil if the soil is not intervened by natural or anthropogenic activities.

2.12 Methanotrophs

Methanotrophs are cosmopolitan in their occurrence and are well known for oxidation of potent greenhouse gas methane (CH_4) in various upland soil ecosystems (Singh and Strong, 2016). Diverse methanotrophs genera reported from various types of ecosystems are shown in Fig. 4. In order to metabolize their growth substrate, the methanotrophs synthesize both particulate and soluble forms of methane monooxygenases (MMOs), which exhibit ability to co-metabolize diverse types of hydrocarbons and halogenated toxic compounds. The significant pollutants like heavy metals, petroleum hydrocarbons and trichloroethylene (TCE) are known to be easily degraded by application of methanotrophs (Shukla et al., 2009; Jiang et al., 2010).

Methanotrophs are an efficient and unique bioremediation tool to decontaminate the polluted sites.

The major challenge before the researchers has been to enhance the activity of microorganisms and develop means to bring the contaminant into direct contact with these microorganisms to achieve an optimal efficiency of bioremediation (Singh, 2015). The ever expanding horizons of biotechnology offers an effective tool to overcome many metabolic limitations in the microorganisms which can be exploited to achieve the desired changes in the microorganisms and stimulate the specific activity of indigenous or introduced microorganisms (Singh et al., 2014).

The various types of methanotrophs with potential to contribute in bioremediation process are given in Figure 2.4

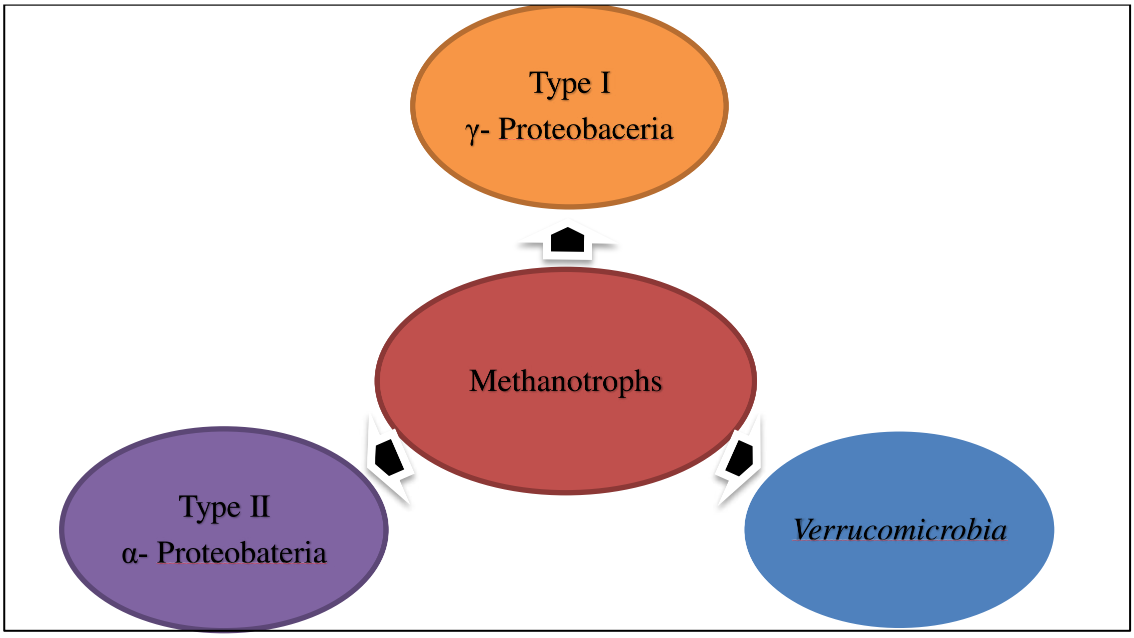


Figure 2.4 Types of methanotrophs reported from various types of ecosystems

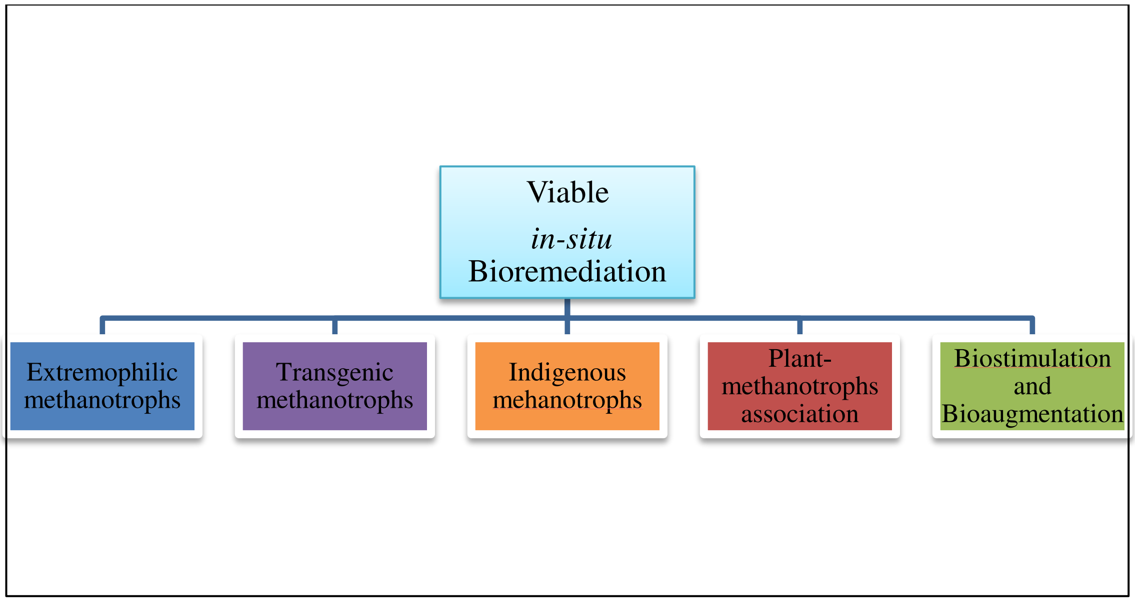


Figure 2.5 A proposed diagram with different factors that can enhance the *in-situ* bioremediation by methanotrophs

The MMO is known to exist in at least two forms. One form, the pMMO is found in most known aerobic methanotrophs as well as *M. oxyfera* and is located in the cytoplasmic membrane (Semrau et al., 2010). Another form, the soluble methane monooxygenase (sMMO) is found in some aerobic methanotrophs and is located in the cytoplasm (Semrau et al., 2010). A great majority of the methanotrophs are known to produce particulate methane-monooxygenase (pMMO) except few strains. The *Methylcella palustris* -a known producer of soluble methane-monooxygenase (sMMO), capable of oxidizing a wider range of organic compounds including aliphatic, aromatic hydrocarbons and their halogenated derivatives (Trotsenko and Murrell, 2008). Thus, sMMO-containing methanotrophs exhibit ability to utilize a relatively broad range of substrates for their growth (Shigematsu et al., 1999) and show faster pollutant turnover kinetics i.e., a fast decline in the pollutants than that observed in pMMO-producing methanotrophs. On the contrary, pMMO works on a very narrow spectrum of carbon substrate (alkanes and alkenes). Further, it has been observed that the MMO is not constitutively present in all the methanotrophic bacteria. The type-II methanotrophs of the genus *Methylobacter* dominate the methane-oxidizing flora of Mono Lake, but molecular signals (*pmoA* amplicons) that were found in type-II methanotrophs of the *Methylocystis* genus (Lin et al., 2005) is considered to have come from conjugative transfer of DNA between *Gammaproteobacteria* and *Methylobacter*. However, type-I methanotrophs have the Calvin–Benson–Bassham pathway of C assimilation, while the genome of *Methylobactor* has annotation for the serine pathway, a feature of type-II methanotrophs of the *Alphaproteobacteria*. In the absence of natural substrate, the conditions existing in some of the specific ecosystems appear to favour the growth of type-II methanotrophs (Lee et al., 2006), which synthesize methane monooxygenase (MMO) enzyme, which can easily mediate the rapid degradation of low-molecular-weight halogenated hydrocarbons like TCE and some other (Shukla et al., 2009). Very recently it has been demonstrated that the facultative methanotrophy and utility of methanotrophs is very useful in biodegradation of several organic pollutants (Im and Semrau, 2011). A summary of the current genera of methanotrophs known to synthesize MMOs, responsible for bioremediation of diverse inorganic and organic pollutants is presented in Fig. 2.5.

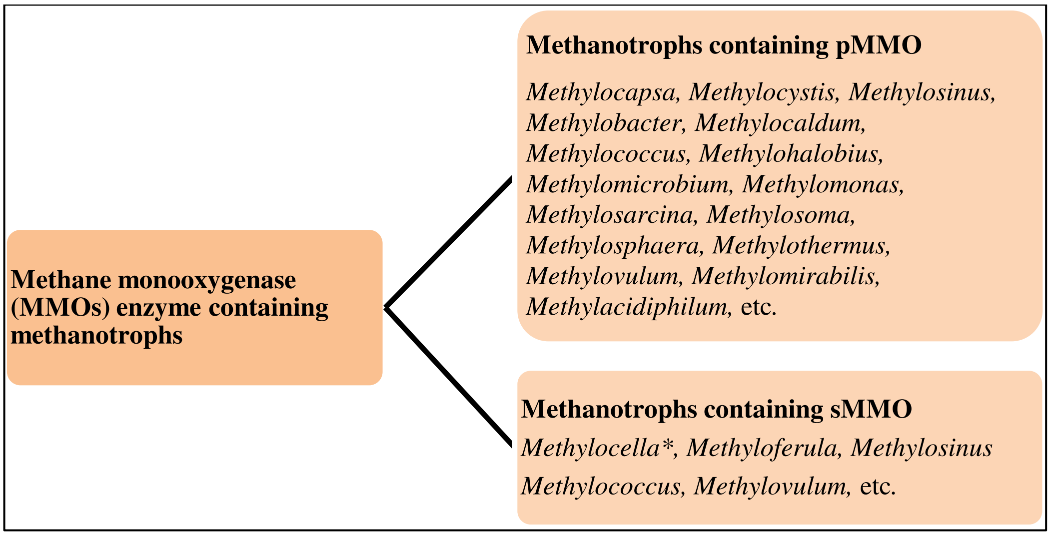


Figure 2.6 Distribution of pMMO and sMMO among different know methanotrophic genera. *Some *Methylocella* spp. expresses sMMO exclusively.

It is now well established fact that both the sMMO and pMMO are involved in the degradation of halogenated hydrocarbons and have potential application in environment and human health (Bolt, 2005; Scott and Chiu, 2006). In contrast to other microbes that are recognized to degrade halogenated hydrocarbons via reductive pathways (Maymo-Gatell et al., 1999), the biodegradation of chlorinated hydrocarbons by methanotrophs occurs under aerobic condition mediated by an oxidative process (Lontoh et al., 2000). The oxidative biodegradation carried out by MMOs is apparently more significant than the reductive dehalogenation of chlorinated ethenes, such as TCE and tetrachloroethylene, which often results into accumulation of several toxic intermediates, e.g., vinyl chloride, a known potent carcinogen (Maymo-Gatell et al., 1999). The MMOs mediated oxidative mechanisms of degradation of halogenated compounds by the methanotrophs does not accumulate hazardous intermediates (McCue et al., 2002). Thus, the applicability of methanotrophic degradation of halogenated hydrocarbons for *in-situ* bioremediation of contaminated ecosystems can be a major focus of the future studies. The role of different methanotrophic MMOs in bioremediation of inorganic and organic pollutants is shown in Fig. 2.6.

Methanotrophic bacteria (MB) also have considerable potential for their application in bioremediation due to the amenability of these bacteria for large-scale cultivation (Semrau et al., 2010; Overland et al., 2010; Pandey et al., 2014). It has been suggested that methanotrophs influence the speciation and bioavailability of metals in the environment (Choi et al., 2006). Hasin et al. (2010) as observed in case of transformation of soluble and more toxic Cr (VI) into a less toxic Cr(III) species, which is insoluble and therefore, tends to get precipitated at high pH. There is a possibility of reverse methanogenesis by methanotrophs, where anaerobic methane oxidation can be coupled to iron or manganese reduction due to co-metabolic activity of *Archaea* and methanotrophic bacteria. It is still not clear, how methane oxidation is coupled to metal reduction process. The flexibility in survival of methanotrophs confers them added advantage and makes them an ideal tool for remediation of hazardous environmental wastes under a diverse range of habitats (i.e., terrestrial, marine, Arctic and Antarctic Polar Regions).

2.13 Methanotrophs in heavy metal remediation

The relevance of reducing the heavy metal toxicity by methanotrophs is associated with Cu-containing protein molecule present in methanotrophs which can work even in the typically distinct microaerophilic zones. In such locations, intense redox cycling leads to active precipitation of Mn and Fe oxides (Ferris et al., 1999). The CH_4 oxidation requires presence of Cu (due to its high reactivity), which, in turn, demands a strong intracellular Cu defense system. The molecular carrier for Cu, termed as methanobactin (*mb*) - a 1216-Da fluorescent metal binding chromo-peptide (Kim et al. 2004), confers protection to the cells both from external and internal Cu toxicity. The study of Knapp et al. (2007) provided a strong evidence about the *mb* mediated Cu release from the mineral stage, which changes the availability of Cu and allows pMMO gene expression in methanotrophs. Therefore, *mb* might be particularly critical for ecological succession of methanotrophs in such metal polluted environments where *mb* like proteins allow the selective acquisition of Cu, while protecting the methanotrophs against other similar potentially toxic metals.

By using microorganism based bioremediation of heavy metals, highly toxic and soluble form of Cr(VI), produced from metal plating, tanning, paper making industries (Zayed and Terry, 2003; Hasin et al., 2010) is detoxified by transforming the metal to less toxic and less soluble form of Cr (III). Hasin et al. (2010) reported a well characterized model of methanotrophs *Methylococcus capsulatus* (Bath), capable of bioremediation of chromium (VI) pollution over a wide range of concentrations (1.4-1000 mg L^{-1} of Cr^{6+}). The genome sequence of *M. capsulatus* (Bath) suggested at least five genes for the chromium (VI) reductase activity in this bacterium. Thus, the use of methanotrophic bacteria in remediation of such toxic heavy metals from the contaminated sites could be an emerging innovative tool, offering a more eco-friendly, low cost sustainable technology for bioremediation.

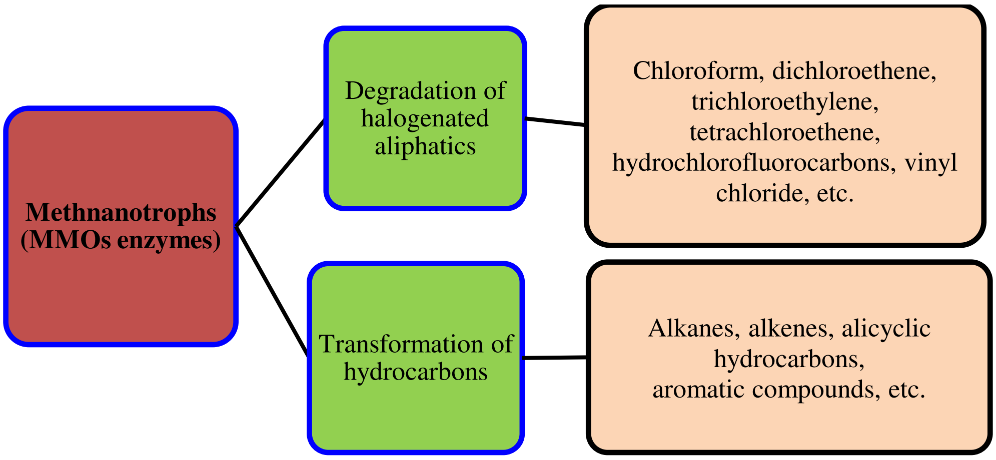


Figure 2.7 Role of different methanotrophic MMOs enzymes in bioremediation of inorganic and organic pollutants

2.14 Methanotrophs in halogenated hydrocarbon remediation

The pollution of natural environment like groundwater and soil by halogenated hydrocarbons has become a serious ecological problem (Kikuchi et al., 2002; Takeuch et al., 2005). Low-molecular-weight halogenated hydrocarbons are susceptible to degradation by anaerobic and aerobic bacteria as described by Hanson et al. (1990). Methanotrophic bacterium *Methylosinus trichosporium* 0B3b degrades TCE more rapidly than other bacteria and a correlation between the synthesis of sMMO and TCE biodegradation was confirmed. The chlorinated ethenes are synthetic compounds with no recognized natural sources and are commonly applied in diverse business practices including degreasing operations, dry cleaning, dying, textile production, etc. (Bakke et al., 2007). The reductive anaerobic bioremediation of chlorinated hydrocarbons, for example tetrachloroethylene to ethene through TCE, dichloroethylene (DCE), and vinyl chloride (VC) as intermediates, has been known for some time (Maymo-Gatell et al., 1999). However, *in-situ* application of anaerobic bio-dechlorination has been imperfect as this process does not result in complete dechlorination in the presence of sulfate due to metabolic competition with the sulfate reducing bacteria for hydrogen. Thus, incomplete dechlorination leads to accumulation of TCE, cis-dichloroethylene (c-DCE), trans-dichloroethylene (t-DCE) and VC (Maymo-Gatell et al., 1999).

There have been dearth of information on aerobic bacterial strains that can consume halogenated hydrocarbons such as chlorinated ethenes as growth substrates (Verce et al., 2000; Coleman et al., 2002) or co-metabolize these toxic compounds (Futamata et al., 2001). In rhizosphere soils of vascular plants contaminated with a variety of chemicals, including TCE showed significantly higher number of methanotrophic bacteria (Brigmon et al., 1999).

Methanotrophic bacteria are one of those groups of microbes which are capable to degrade the hazardous compounds via co-oxidation. Due to their omnipresence in diverse environment, these bacteria have been widely applied for cleaning the sites contaminated with chlorinated ethenes (Hanson and Hanson, 1996; Semrau et al., 2010). Due to capability of methanotrophs to degrade a wide variety of potential pollutants including halogenated hydrocarbons has encouraged the workers to study their potential applications in bioremediation (Nikiema et al., 2005; Lee et al., 2006). Application of high levels of bio-stimulating substances could cause other problems with environmental pollutants due to their interaction with organic compounds. In contrast, methanotrophs induce the MMO involved in TCE degradation only in the presence of CH_4 (Takeuchi et al., 2005; Shukla et al., 2009). Since the CH_4 is one of the natural end products of anaerobic microbial processes it should not cause environmental problems at the levels of bioremediation of organic contaminants. Due to all the required considerations and precautions, *in-situ* stimulated bioremediation by augmenting the methanotrophic populations is under way in many laboratories (Pfiffner et al., 1997; Iwamoto et al., 2000).

An increase in the population of indigenous methanotrophs due to addition of nutrients and methane gas to a sand column demonstrated that that the TCE was degraded to carbon dioxide (Wilson and Wilson, 1985). The abundance of methanotrophs in the TCE contaminated aquifers in a natural gas field implied that the coarse sand stratum plays an important role for *in-situ* bioremediation (Takeuchi et al., 2001). Expanding this idea of *in-situ* bioremediation, additional considerations for selection of microbes as well as suitable habitat are the primary requirement for successful bioremediation of toxic compounds. The diversity of the methanotrophic community involved in degradation of TCE from non-contaminated environment provides an indication of the *in-situ* bioremediation potential of natural soil environments (Newby et al., 2004; Erwin et al., 2005).

Lindane contaminated soils cause potentially serious problems to surface and ground water quality, especially when its concentration is high due to unwarranted spills or discharges. It is still considered to be a serious threat to the environment due to its persistent nature in environment and its potential to bio-accumulate in the food chain. Though the γ-HCH is bio-degradable, but higher concentrations are inhibitory to the degradation potential of applied microbes (Abhilash et al., 2008). A significant increase in the population densities of methanotrophs in the soil contaminated by γ-HCH indicated the survival capacity of these microbes against the insecticide lindane.

A possible way to improve the bioremediation efficiency is possible through application of native methanotrophic bacteria adapted to the contaminated site. Slow-release bio-augmentation approach, using encapsulated *Sphingomonas* sp. cells, for the biodegradation of lindane in laboratory condition has also been used. Therefore, bioremediation based on methanotrophic bacteria might be an emerging tool and has been receiving more attention as an eco-friendly and efficient means of lindane remediation (Mertens et al., 2005). There is need for further improvement in the technology in order to achieve a reliable bio-augmentation technology for bioremediation of lindane contaminated sites. Emphasis should be laid on the enhanced effort to screen for more indigenous methanotrophic population and appropriate inoculation practice to optimize the technology.

CHAPTER-3
Study sites and soil samples

CHAPTER 3

STUDY SITES AND SOIL SAMPLING

3.1 Geographical location and weather conditions of study sites

In present study, two study regions namely Lucknow and Renukoot (Sonebhadra district) located in Uttar Pradesh state of India, were considered. The six sampling sites distributed in Lucknow and Renukoot districts of Uttar Pradesh, India are mentioned in Table 3.1

Table 3.1 Different study sites of Lucknow and Renukoot region.

Study sites		Coordinates
Lucknow region		
1.	S1 (IPL Chinhat)	Latitude: 26°54'50.54"N; Longitude: 81° 4'7.58"E
2.	S2 (Chakhar village)	Latitude: 26°54'50.23"N; Longitude: 81° 4'4.48"E
3.	S3 (Maati village)	Latitude: 26°54'51.11"N; Longitude: 81° 4'3.05"E
Renukoot region		
4.	S4 (Adjacent to ABCIL)	Latitude: 24°12'8.93"N; Longitude: 83° 2'53.68"E
5.	S5 (Sheo Park Bazar)	Latitude: 24°12'16.99"N; Longitude: 83° 2'48.95"E
6.	S6 (Donki Nala)	Latitude: 24°12'18.11"N; Longitude: 83° 3'18.08"E

3.1.1 Lucknow region

The Lucknow, a large city in northern India, is the capital of the state of Uttar Pradesh. The city stands at an elevation of approximately 123 metres above sea level. Lucknow is situated on 26.30 & 27.10 North latitude and 80.30 & 81.13 East longitude. Lucknow covers an area of 3,244 km^2. Situated in the middle of the Indus-

Gangetic Plain, the Lucknow city is surrounded by rural towns and villages. The Lucknow district is surrounded on the eastern side by district Barabanki, on the western side by district Unnao, on the southern side by Raebareli and on the northern side by Sitapur and Hardoi districts. It has been listed as the 17th fastest growing city in India and 74th in the world. The Gomti River, Lucknow's chief geographical feature, meanders through the city and divides it into the Trans-Gomti and Cis-Gomti regions.

Lucknow has been considered as a land locked city. The distance from the ocean gives Lucknow an extreme type of continental climate with the prevalence of continental air in major portion of the year. The 4 months (June to September) does the air of sea origin travels to this region and causes humidity, cloudiness and precipitation. About 75 % of the total rainfall in the area is realized during June to September. The whole year can be broadly divided in to four seasons. The winter (December – February), summer (first fortnight of June – till September) and rainy (two post monsoon months of October and November). The summers in Lucknow are extremely hot and winters incredibly cold. The temperature may go up to about 45 ^{o}C or higher in summers, however the average temperature is experienced around 39-40 ^{o}C. Although the winters are not resentfully cold on most of the days, the temperature may drop to 2-3 ^{o}C for a few days in winters when the cold waves from the Himalayan region moves towards plains. The winter experiences dense fog in the mornings and sometimes visibility are too reduced to driving. Fog is quite common from mid-December to late January. Lucknow has a humid subtropical climate with cool, dry winters from mid-November to February and dry hot summers with thunderstorms from late March to June. The summer months (May to June) are extremely hot and winters (December and January) are extremely cold. The rainy season (July to September) experiences and gets an average rainfall of 896.2 millimetres from the south-west monsoon winds, and occasionally little rainfall in January.

In Lucknow, the India Pesticide Pvt. Ltd (IPL) was established in 1992, with an installed capacity of lindane (technical) production of 25 metric ton (MT) per month (300 MT per annum). Apart from lindane production, the industry has also in-house formulation facility for producing various lindane formulations. The IPL utilizes its full production capacity and produces 300 MT/annum of lindane. Well known products of the IPL are captan, folpet, thiram, ziram, monocrotophos, gamma benzene hexachloride (γ-HCH), tolnaftate, dye intermediates, etc.

The IPL claims about having all necessary clearances and pollution abatement measures as per the environmental protection law. However, still today it can be seen that muck yard situated adjacent to the industry and illegal “muck” dumps in nearby villages, shows the gross violations of environmental laws by this industry. According to the current people residing the nearby area and villages, IPL dumped all its “muck” in a deep pit at a village called Chakhar situated right behind the main IPL factory. But after few years due to complaints regarding intolerable smell and mysterious deaths of cattles which used to grazed in the lindane contaminated area, IPL had to change the location of its dumping sites. During the last few years, a low-lying area in a village called Ummaria, about 20 km away from the IPL, has been selected on leased from a local people to dispose of and dumping the “muck”. Even though the peoples of the area have protested against such illegal dumping of wastes, the combined power of the IPL and the landholders has been forced to accept and permit these toxic waste deposits in their village.

3.1.2 Renukoot region

The Renukoot region, situated in Sonebhadra district (area 6,788 km^2) of Uttar Pradesh was also selected for present study. The Sonebhadra is the second largest district of Utter Pradesh (India), previously a part of southern Mirzapur. It is bounded by Mirzapur, district in the northwest, Chandauli district in the north, Bihar state to the northeast, Jharkhand state to the southeast, Chhatisgarh state to the south and Madhya Pradesh state to west. The Renukoot area is commonly called as “Sonaghati” (golden valley) due to the richness of minerals, rocks and its natural resources. Red coloured and fine textured sandstone (Dhandraul orthoquartzite) is the most important rock of the region. The Sonbhadra district is called the power capital of India, as the region has many electrical and thermal power plants. Thermal power project is located in Anpara, Obra, Renusager and Pipri. The potential natural vegetation of the Renukoot region is tropical dry deciduous forest and exhibits various xerophytic characters. The soil, which is completely exposed during summer season, bears a beautiful canopy of green plants in rainy season, belong to diverse taxa.

The Renukoot region experiences a semi arid type of climate, with three seasons in a year, i.e. summer (March to June), rainy (July to October) and winter (November to February). The maximum monthly temperature varies from 21 - 45 ºC during the year from January to June. One of the characteristic features of the climate of Renukoot, Sonebhadra is the extreme months of winters and summers. The average temperature starts increasing from March and notice the peak during May and June while coldest month was January with yearly average minimum temperature as 8.9 ^{o}C while May-June was the hottest months with average maximum temperature of 44.7 ^{o}C.

The Aditya Birla Chemicals India Ltd. (ABCIL) [formerly known as Kannoria Chemicals & Industries Ltd. (KCIL)], located on the northern bank of Rihand river, is mainly a Chlor Alkali plant producing caustic soda using mercury cell technology, which was set up in 1964 in technical collaboration with Kerbs & Co A.G. of Switzerland to produce 16,500 tonnes annually of caustic soda. In 1993, a lindane (technical) plant was commissioned to utilise chlorine produced as a by-product of the caustic soda process. Initial capacity of the lindane plant was 600 tonnes annually, which was expanded to its present capacity of 1000 tonnes annually in 1995. Subsequently, various formulations of lindane were added to the production capacity of the plant. The ABCIL discharges its "treated" effluents to nearby area and mainly into the Donki Nala (rain water drain), which further meet the Gobind Sagar Dam or the Rihand Dam, about 1.5 km from the factory. There are villages and forests within 500 meters of the factory where wastes are dumped illegally by the factory.

The six sampling sites distributed in Lucknow and Renukoot Region districts of Uttar Pradesh, India have been shown in Figure 3.1 In Lucknow region three sites namely i) IPL Chinhat (S1), ii) Chakhar (S2) situated right behind the IPL nearby village and iii) Maati village (S3) adjacent to IPL. We also selected three sites in Renukoot region namely djacent to ABCIL (S4), ii) Sheo Park Bazar (S5) and iii) Donki Nala (S6).

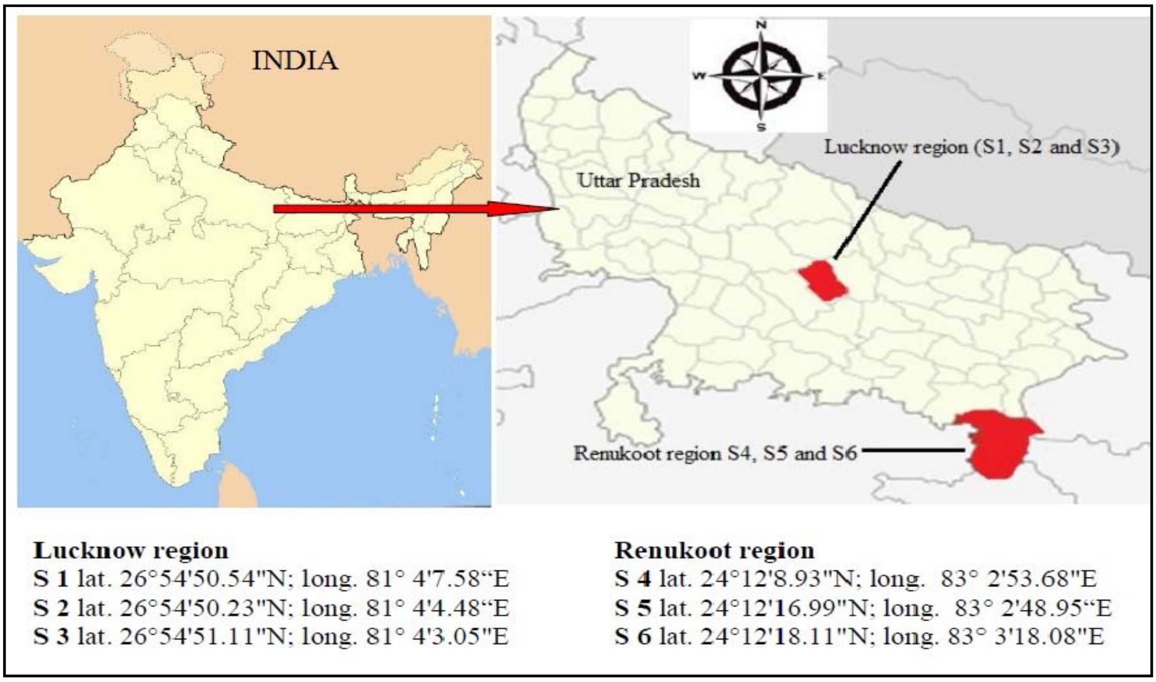


Figure 3.1 Location of selected study sites (S1, S2, S3, S4, S5 and S6) distributed in Lucknow and Renukoot region of Uttar Pradesh, Northern India.

3.2 Soil sampling

For the present study the soil samples were collected randomly in triplicate at depth of 0-20 cm from selected sites situated in Lucknow (S1, S2 and S3) and Renukoot region (S4, S5 and S6) Figure 3.2 The collected soil samples were wrapped in aluminium foil and put in sterilized plastic zipped air tight polythene bags and transported to the laboratory in an ice box and immediately stored at 4°C for further processing of physico-chemical, estimation of lindane and other residues of hexachlorocyclohexane and further explore the soil microbial biomass methanotrophs community composition from the selected study sites.

Figure 3.2 Collection of soil samples from six selected sites from Lucknow and Renukoot region, Uttar Pradesh, India.

CHAPTER-4
Soil physico-chemical properties and lindane residues

CHAPTER 4

SOIL PHYSICO-CHEMICAL PROPERTIES AND LINDANE RESIDUES

4.1 Introduction

The physico-chemical properties of soil are directly correlated with the interaction of various soil formation biotic and abiotic factors. The various soil physico-chemical characteristics of soils vary in space and time due to variation in topography, climate, physical weathering processes, vegetation cover, microbial activities and several other biotic and abiotic drivers (Paudel and Sah, 2003). The properties of soils along with its abiotic and biotic factors play an important role in agriculture productivity (Ahire et al., 2013). The deterioration of soil physico-chemical properties occurred due land uses, addition of pollutants, deforestation and addition of chemicals (Sumithra et al., 2013). The changes in soil physico-chemical properties may negatively impact the growth of plant and crop yields (Jha and Singh, 1991). The industrialization and development in agriculture are necessary for development, but is should be sustainable in nature. During the last few years, effects of urbanization along a rural-urban development on soil microbial diversity and its impact on physico-chemical properties have been investigated in India (Rai et al., 2018). The biological properties of soil too directly affect the crop productivity and yields. In order to enhance the crop yield, farmers are continuously using pesticides and chemical fertilizers in excess amount, which causes serious soil contamination and other environmental problems. Therefore, it is important to find out the viable option for the restoration and re-establishment of soil productivity continuously facing the problems of soil degradation.

The organochlorine pesticides (OCPs) mainly include lindane, DDT, DDD, endosulfan, kelthane, chlorobenzilate, chloropropylate, methoxychlor, aldrin, dieldrin, heptachlor, isodrin, isobenzan, endrin, chlordane, toxaphene, mirex and kepone. These are synthetic chemicals used extensively to control the diseases of humans and domestic animals caused by insects and mites. These chemicals cause great damage to agricultural crops (Lal and Saxena, 1982). Lindane (gamma-1, 2, 3, 4, 5, 6,-hexachlorocyclohexane; γ-HCH) is an insecticide and fumigant which have been used for the control of a wide range of soil-dwelling and plant eating (phytophagous) insects. It is commonly used on a wide variety of crops, in 8 warehouses, in public health to control insect-borne diseases and with fungicides for the seed treatment. Although OCPs have a long history (over 30 years) of usage in India, very limited information is available on the presence of OCP residues in the air, soil and water systems (Ali et al., 2014; Sharma et al., 2014). Also, study of the status of OCPs in the soil of northern India such as Lucknow and Renukoot region is lacking. The present study aimed to monitor and determine the soil physico-chemical properties, concentration of HCH isomers and OCPs in lindane contaminated sites of Lucknow and Renukoot region.

4.2 Materials and methods

4.2.1 Physico-chemical analyses of soil samples

The detailed description about study sites and collection of soil samples has been described earlier in Chapter 3. In order to determine the physico-chemical properties of soil such as temperature, pH, electrical conductivity (EC), total-C, -N and -P were nalysed as per standard procedures.

4.2.1.1 Temperature

The temperature of soil is directly linked to the temperature of the atmosphere because soil is an insulator for heat flowing between solid earth and the atmosphere.

Procedure

1. Cleaned the surface of the probe rod of the Thermometer.

2. Inserted the probe rod of digital Thermometer into the soil up to 5-6 inches. Note the temperature immediately
3. Washed the probe rod with distilled water.
4. Again inserted the probe rod into the soil and note the temperature.
5. At least 2-3 reading were taken per location

4.2.1.2 Soil pH

The pH meter with a range of 0-14 pH, pipette/dispenser, Beaker, Glass rod.

Reagents

Buffer solution (pH 4, 7 and 9) and deionized water

Procedure

1. Air dried soil for 1-4 days depending on the relative humidity and soil properties. Then grind the air-dried soil to pass 2mm sieve and mix well.
2. Calibrated the pH meter, using buffers of pH 7.0, 4.0 and 9.2.
3. Weighed 10 g of air dried soil sample into 50 or 100ml beaker, add 20ml deionised water.
4. Allowed the soil to deionised water without stirring and then thoroughly stir for 10 second using a glass rod.
5. Stirred the suspension for 30 minutes and record the pH on the calibrated pH meter.

4.2.1.3 Electrical conductivity

Electrical conductivity (EC) is expressed in units of milli-Siemens per meter (mS/m). Soil EC measurements may also be reported in units of deci-Siemens per meter (dS/m), which is equal to the reading in mS/m divided by 100.

Apparatus

Electrical conductivity meter, Beakers (25 ml), Erlenmeyer flask (250 mL) and Pipettes, Filter paper

Reagents

0.01M Potassium chloride solution: Dried potassium chloride at 60 ºC for two hours. weighed 0.7456 g of it and dissolve in distilled water and make the volume to one litre. This solution gives an electrical conductivity of 1.412 mS/cm at 25 °C.

Procedure

1. Take 20 g of soil into 50 ml Erlenmeyer flask, add 40 ml of distilled water, stirred well the flask and filtered through Whatman No. 1 filter paper. The filtrate kept for the measurement of conductivity.
2. Washed the conductivity electrode with distilled water and rinsed with standard KCl solution.
3. Calibrated the EC meter with KCl solution into a 25 ml beaker and dip the electrode into the solution and adjust the conductivity meter to read 1.412 mS/cm
4. Washed the electrode and dip it in the soil extract.
5. The reading in mS/cm of electrical conductivity is the measure of the soluble salt content in the extract, and an indication of salinity status of the soil. The conductivity can also be expressed as m mhos/cm

4.2.1.4 Estimation of total organic carbon (µg g^{-1} soil)

Organic carbon in the soil sample was estimated by rapid dichromate oxidation method as described by Walkey and Black (1964). The detailed steps are as follows:

i. About 0.50 g of soil samples (passed through 0.2 mm sieve) was taken in 500 mL conical flask was kept on asbestos sheet.

ii. Added 10 mL of 1N $K_2Cr_2O_7$ in flask and gently swirled. The flask was kept in asbestos sheet.

iii. Added 250 mL of conc. H_2SO_4 (containing 1.25% $AgSO_4$) very carefully from a measuring cylinder. Gently swirled few times. kept this flask for 30 minutes and protects from droughts

iv. Added 20 mL of diphenylamine indicator and titrated with ferrous ammonium sulphate till the colour flashes from blue violet to green. Similarly control was run without soil

v. The data was computed as formula mentioned below

Organic C (%) = 10(B-T)/B×0.003×100/S

Where

B = Volume of ferrous ammonium sulphate required for blank titration in mL

T = Volume of ferrous ammonium sulphate required for soil sample in mL

S = Weight of soil in g

4.2.1.5 Estimation of soil total nitrogen (μg g^{-1} soil)

Total-N was estimated by potassium permanganate method by Subbaih and Asija,1956. The detailed steps are given below.

i. Taken 20 g of soil sample in 800 mL Kjeldal flask, added 20 mL of water 100 mL of 0.32% $KMNO_4$, and 100 mL of 2.5% NaOH solution

ii. The excess frothing during heating was prevented by adding few glass beads.

iii. The digested product was collected in a flask containing 20 mL boric acid and indicator solution.

iv. With the absorption of NH_3 gas the pinkish colour boric acid solution turns to green.

v. Nearly 100 mL of distillate was collected and titrated with 0.02 N H_2SO_4

vi. The blank correction (without soil) was made for final calculation.

Total N (ppm) = (A-B) × N× 14×10^3/W

Total N (%) = N in ppm/10,000

Available N (Kg/ha) = N (%) × 22500

Where, A = Volume of H_2SO_4 Consumed for blank in mL

B = Volume of H_2SO_4 Consumed for soil sample in mL

N= Normality of H_2SO_4

W = Weight of soil sample

4.2.1.6 Estimation of soil total phosphorous (µg g^{-1} soil)

Total-P in soil was estimated by Olsen method (Olsen et al., 1954). The steps are mentioned below:

i. Added 2.5 g of soil sample in 50 mL of extracting solution ($NaHCO_3$) in 250 mL conical flask.

ii. Flask shaken with magnetic shaker and filter suspension with Whatman filter paper No. 4.

iii. Added activated Carbon (free of P) to obtain clear filtrate.

iv. Shaken the flask immediately before pouring the suspension into the funnel.

v. Taken the 5 mL of extract into 25 mL volumetric flask and added 5 mL of Dickman and Brays reagent drop by drop with constant shaking till the effervescence stopped

vi. The neck of the flask was washed and contents were diluted to about 22 mL (if pH is less than 5.0 then acidify with $5NH_2SO_4$ to pH 5)

vii. The colour was stable for 24 h and the maximum intensity was obtained in about 10 minutes.

viii. The data was calculated with the formula below.

Oslen P (Kg/ha) = $R \times V/v \times 1/S \times 2.24 \times 10^6/10^6$

Where, V= Total volume of extract

V= volume of aliquots taken for analysis

S= Weight of soil

R= Weight of P in the aliquots in µg (from standard curve).

4.2.2 Extraction and cleanup of collected soil samples for lindane residues analyses

4.2.2.1 Chemicals and reagents

All the chemicals and reagents used in present study were of analytical reagent (AR) grade. Individual standard of α-HCH, β-HCH, γ-HCH, δ-HCH, Aldrin α-endosulfan, PPP-DDD, OP-DDT, PP-DDT were purchased from Sigma Aldrich (India). All the used solvents; acetone, dichloromethane, n-hexane, ethyl acetate, petroleum ether, sodium sulphate anhydrous were of HPLC grade and purchased from Merck Pvt. Ltd. India. Pre activated alumina was procured from Hi-media, India**.**

The extraction and analysis of soil samples was carried out by the method described by Concha-Grana et al. (2006). An amount of 1.0 g each of soil samples (in triplicate), collected from selected sites was extracted in 20 mL of solvent consisting of hexane: acetone (1:1, v/v) sonicate (Digital ultra Sonicator model: LMUC 4 Labman scientific instrument) for 5 minutes with 30 second of interval at a power of 6 Watt. The extract was centrifuged at 2000 × g for 2 minutes to obtain the clear solvent phase. Further, it was dried using rotatory evaporator (IKA RV 10.) and the resulting HCH residues were dissolved in appropriate volumes of hexane. This was passed through pre activated alumina and anhydrous sodium sulphate column (10 cm length 2 cm internal diameter) and eluted it with 20 mL hexane. Dried the filtrate in rotatory evaporator and subsequently dissolved in hexane and analysed using GC (Agilent 7890A, USA) and GC-MS/MS (Trace GC Ultra TSQ Quantum XLS, Thermo Fisher Scientific). Efficiency of extraction procedure was examined by recovery experiments conducted with spiked soil samples in triplicate. Uncontaminated garden soil sample (1 g) was spiked with HCH isomers at a concentration level of 200 μg g^{-1} soil and extracted by the same method. The same spiked soil served as the blank and processed along the samples.

4.2.2.2 GC analyses for separation of organochlorine pesticides

Gas chromatograph (Agilent, USA) was employed for all sample analyses. The system was equipped with electron capture detector (μECD) and an auto sampler. Analytes were separated on a DB-5, 30 m 0.25 mm 0.25 μm column (Agilent, USA). The carrier gas was nitrogen at a flow rate of 1.0 mL $minute^{-1}$. The column was connected to the electron capture detector (μECD). The ECD was maintained at 300° C, respectively. Nitrogen was the makeup gas for the ECD (30 mL $minute^{-1}$). The oven temperature were programmed as initially at 170 °C held 1 minute, increased at the rate of 5°C to 220 °C which was held for 25 minutes, increase to 280 °C held for 5 minutes. Injector port temperature was held at 250°C. Sample extract volumes of 2 μL were injected in the split mode i.e. 20:1. HP Chemstation, gas chromatography software was used for the instrument control, data acquisition and processing.

4.2.2.3 GC-MS/MS analyses of extracted compounds

The 100 μL of sample extract was taken into 1.5 mL Eppendorf tube, to this 80 μL of methoxyamine hydrochloride and 100 μL of BSTFA were added and then these samples were kept in shaker (BRTH-100 and BR Biochem) at 65 °C and 1400 rpm speed for 1 h. Samples were filtered through 0.22 μ syringe filter after incubation. Then the derivatized phase was transferred into GC-MS/MS vial. The GC-MS/MS was used for the identification of different unknown components present in the sample fraction. The sample was injected on DB-5MS (30 m × 0.25 mm ID, 0.25 μm film thickness) column. Helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1.2 mL minute^{-1} with split less mode, and 1μL of sample was injected in the GC-MS/MS system. The injector port temperature was maintained at 150 °C and the mass transfer line temperature was maintained at 290 °C. The oven temperature was programmed at 65 °C (hold for 2 minute), with an increase of 6 °C minute^{-1} to 230 °C, then 10 °C minute^{-1} to 290°C, ending with a 20 minute^{-1} hold. The solvent delay was 6.5 minute and the total GC-MS/MS running time was 55 minute with full scan mode. Various components were identified by their retention time and based on MS library research (NIST) has derived mass fragmentation pattern of all the fractions. GC-MS/MS method was used to detect the chemical constituents present in all the unknown samples

4.3 Statistical analyses

The data generated from the present investigation are expressed as the mean of three replicates ± SE. All the observations from each selected sites were carried out in triplicate to improve the analytical precision of the experimental data. To confirm the variability of data obtained and validity of results, the data were subjected for the statistical analysis using one way Analysis of Variance (ANOVA) by SPSS software (IBM SPSS Statistics version 20). The correlation analysis was also performed to examine the relationship between soil physico-chemical properties, pesticide concentrations, soil microbial biomass levels and methanotrophs.

4.4 Results and Discussion

4.4.1 Soil physico-chemical properties

The analysed data on soil physico-chemical characteristics of soil samples collected from study sites are presented in Table 4.1. The results revealed that soil samples collected from Lucknow and Renukoot are slightly acidic in nature with pH values ranged from 6.1 to 6.9. The acidic nature of soils of present study regions could be due the presence of high concentration of acidic organic residues and other organic hydrocarbons in the soil (Manickam et al., 2010). The moisture content (%) for both the study region varied from 2.77 to 6.90 % (Table 4.1). The electrical conductivity (EC) was found between 305.2 to 676.6 µS cm^{-1}across the study sites. Across the study sites, the total-C was found to be maximum (11, 250.5 µg g^{-1} soil) in soil samples of Renukoot region and minimum (6,105.2 µg g^{-1} soil) in soils of Lucknow region. Similarly, the total-N was measured maximum (705.6 µg g^{-1} soil) at S5 (Renukoot site) and minimum (500.5 µg g^{-1} soil) at S1 (Lucknow site), respectively. Across study sites the total-P was found minimum (567.5 µg g^{-1}) at S1 and maximum (766.2 µg g^{-1}) at S5 respectively, Lucknow and Renukoot sites. The soil NH_4-N content was found maximum in S6 (17.2 ± 1.9 µg g^{-1}) site of Renukoot region and minimum at S1 (6.8 ± 0.83 µg g^{-1}) site of Lucknow region. The higher total–C, –N and –P in the soils of Renukoot region compared to Lucknow region could be because soils of Renukoot region receives more plant litters via forest vegetation compared to Lucknow urban area. Higher organic carbon and organic matter content due to the high influx of litter and microbial activities is also suggested by earlier workers Mohn et al., 2006; Hitch and Day (1992). The favourable conditions of soil factors at Renukoot sites may favours the greater microbial mediated decay and decomposition process and consequently, a greater amount of organic and inorganic nutrients in soils of Renukoot region, covered by dense forest vegetation might be expected. The results of FTIR spectra of soil samples showed different surface characteristics and functional groups from two different location of Uttar Pradesh, India as shown in Figure 4.1. The peak observed at 3414.9 and 3448.7 cm^{-1} are may be due to the O-H stretching and deformation respectively assigned to the water adsorption at both the sites (Paknikar et al., 2005; Massoud et al., 2017). The two prominent peaks were observed at 2933.2 and 2520.4 cm^{-1} in Lucknow region, corresponding to C-H stretching whereas as no peaks were observed at this region in Renukoot soil samples.

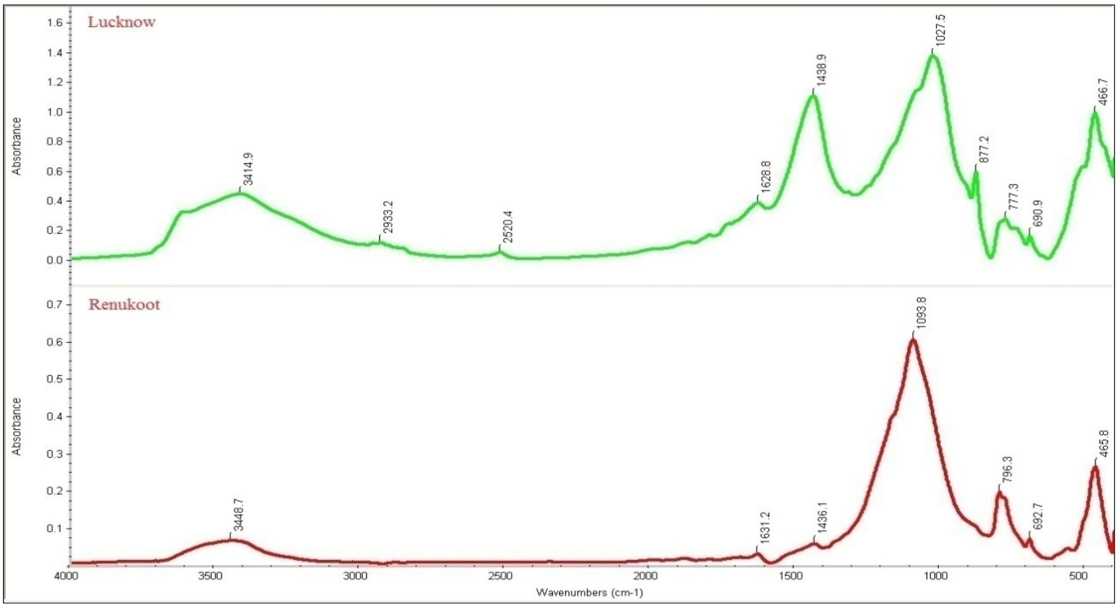


Figure 4.1 IR- spectra (FTIR) of soil samples obtained from Lucknow and Renukoot region.

Table 4.1 Physico-chemical properties of HCH contaminated soil sample of Lucknow and Renukoot Region, Uttar Pradesh, India. The values given are means of three replicates ±SE.

Soil parameters	**Lucknow Region**			**Renukoot Region**			**ANOVA (N=18)**
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	
Soil pH	6.3 ± 0.26^{a}	6.9 ± 0.31^{a}	6.5 ± 0.4^{b}	6.1 ± 0.098^{c}	6.3 ± 0.40^{b}	6.4 ± 0.17^{b}	F=2.05, P<0.01
Temperature (°C)	42.3 ± 1.45^{a}	39.5 ± 1.52^{b}	44.3 ± 0.66^{c}	46.2 ± 0.57^{c}	39.6 ± 1.85^{b}	43.6 ± 1.45^{c}	F=4.15, P<0.02
Moisture content (%)	2.77 ± 0.39^{a}	4.33 ± 0.35^{b}	4.10 ± 0.60^{c}	6.86 ± 0.076^{c}	6.90 ± 0.36^{b}	6.06 ± 0.56^{d}	F=4.47, P<0.01
EC (µS cm^{-1})	316.5 ± 9.46^{b}	313.6 ± 10.72^{b}	305.2 ± 13.22^{b}	674.8 ± 17.50^{a}	676.6 ± 15.89^{a}	658.3 ± 8.33^{a}	F=229.40, P<0.001
Total -C (µg g^{-1} soil)	6105.2 ± 112.8^{b}	6160.5 ± 115.8^{b}	6170.2 ± 118.8^{b}	$11{,}060.2\pm877.5^{c}$	11250.5 ± 875.3^{c}	11025 ± 856.8^{c}	F=828.75, P<0.001
Total-N (µg g^{-1} soil)	500.5 ± 12.5^{b}	500.8 ± 10.3^{d}	502.5 ± 11.8^{d}	700.8 ± 12.8^{c}	705.6 ± 13.5^{c}	630.4 ± 12.7^{e}	F=154.23, P<0.001
Total-P (µg g^{-1} soil)	567.5 ± 4.33^{a}	569.2 ± 3.78^{a}	579.6 ± 9.59^{b}	651.5 ± 16.74^{c}	766.2 ± 17.02^{d}	756.6 ± 14.40^{d}	F=125.45, P<0.001

Different letters indicate significant difference between treatments for each pesticide ($P < 0.05$).

4.4.2 Lindane pesticide residues

The GC and GC-MS/MS analysis of collected soil samples showed that the pesticides residues of HCH were found in collected soils of both the Lucknow and Renukoot region. The results confirm the presence of HCH isomers and OCPs such as α-HCH, β-HCH, γ-HCH, Aldrin, PP-DDE, β- Endo, PP-DDD, OP- DDT and PP- DDT in soil both the regions (Figure 4.2).

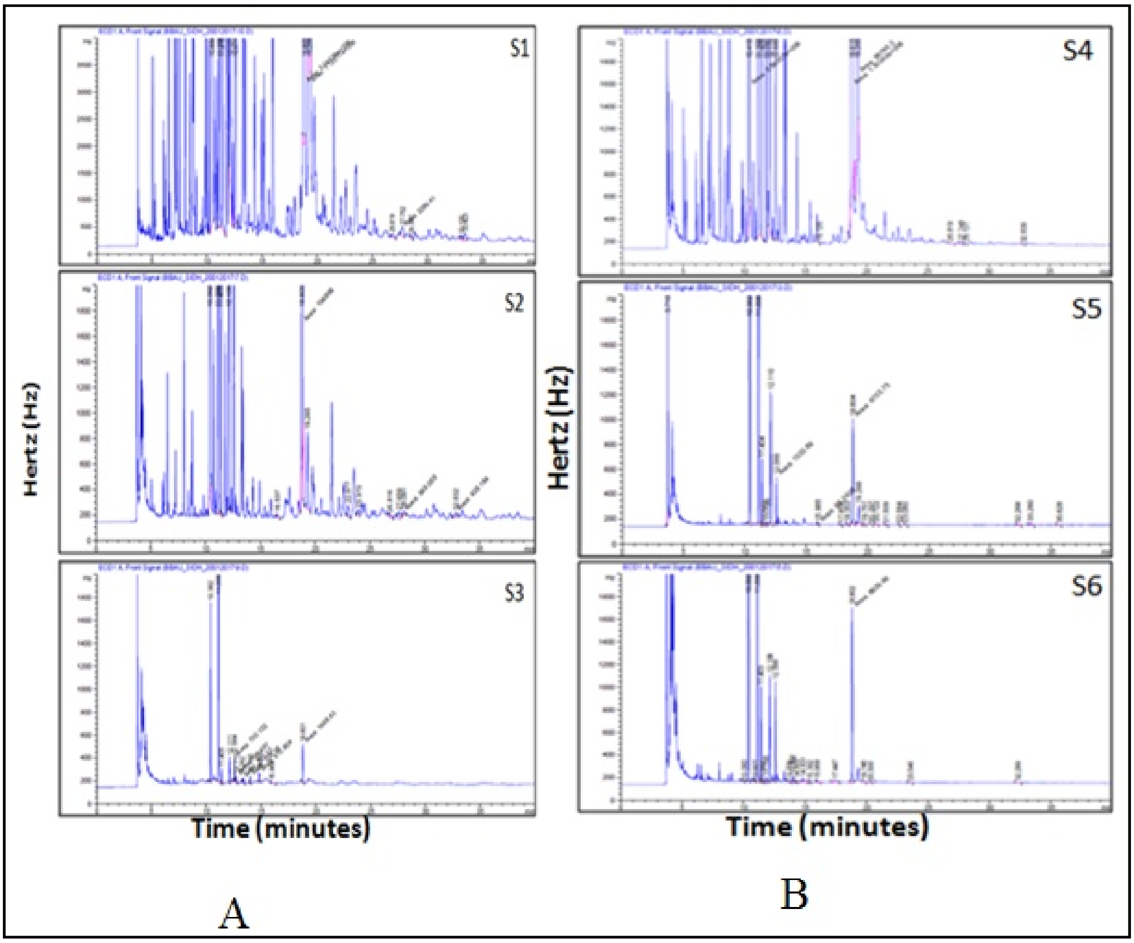


Figure 4.2 GC chromatogram of soil samples collected from six different sites (S1 to S6) of Lucknow and Renukoot region.

The HCH residues in soil samples in Lucknow region were found comparatively higher concentration as compared to Renukoot region and varied significantly due to sites (Table 4.2). Among the HCH isomers, the concentration of α-HCH and γ-HCH were found higher in soil samples of Lucknow region and varied from 309.97 and 182.12 µg g^{-1} soil, respectively. The β-HCH was highest (169.15 µg g^{-1} soil) at S4 while lowest (5.93 ± 1.05 µg g^{-1} soil) at S5 site. The Aldrin was highest (0.02 µg g^{-1} soil) at S6 and lowest (0.006 µg g^{-1} soil) in soil of S4 site. The low concentration of pesticide residues in soils of Renukoot region compared to Lucknow region could be low anthropogenic interference because of lesser dense human population and agriculture activities. Further, the denser forest vegetation and greater soil microbial diversities may be involved in bioremediation of these pesticides. The intensity of pesticides biodegradation depends upon environmental factors, such as temperature, soil type, pH, moisture and organic carbon content (Cousins et al., 1999; Afful et al., 2010). It has been estimated that the degradation of DDT in soil ranges from 4 to 30 years, while other chlorinated OCPs may remain stable for many years after their use (Doleman et al., 1990). The higher values of γ-HCH in this study indicated that this isomer is more persistent in environment due to low decay properties under aerobic conditions (Loibner et al., 1998) and are more resistant to microbial degradation than other HCH isomers. Further, in the present investigation, the overall results confirmed the presence of β-endosulphan in soil samples of selected sites. In natural environment β-isomers disappears faster as it has less half-life and perhaps that might be the reason it was not detected in earlier studies (Sutherland et al., 2002). Apart from targeted OCPs, some of the non-targeted OCPs of unknown impurities were also detected.

Table 4.2. Residues of HCH and organochlorine pesticides detected in soil samples of study sites. The values given are means of three replicates ±SE.

Pesticides ($\mu g\ g^{-1}$ soil)	RT (minutes)	Lucknow region			Renukoot region			ANOVA (N=18)
		S1	S2	S3	S4	S5	S6	
α –HCH	10.38	111.25±5.69^c	11.87±1.04^d	309.97±6.93^a	194.44±4.04^b	1.10±0.22^d	1.24±0.20^d	F=982.81, P<0.001
β –HCH	11.10	31.69±1.85^d	51.95±2.02^c	98.10±6.68^b	169.15±9.02^a	5.93±1.05^e	9.11±0.67^e	F=175.15, P<0.001
γ –HCH	11.40	3.25±0.19b^d	0.39±0.08^c	182.12±9.69^a	14.66±1.82^b	0.12±0.02^c	0.22±0.04^c	F=328.94, P<0.001
ALDRIN	16.16	ND	ND	0.01±0.005^a	0.006±0.003^b	0.01±0.006^a	0.02±0.005^a	F=3.418, P<0.005
PP-DDE	23.09	0.04±0.01^a	0.01±0.005^b	ND	ND	ND	ND	F=10.510, P<0.002
B-ENDO	26.81	0.05±0.005^b	0.03±0.01^b	0.08±0.02^a	ND	ND	ND	F=20.00, P<0.001
PP-DDD	27.65	0.09±0.008^a	ND	0.32±0.03^b	ND	ND	ND	F=194.01, P<0.001
OP-DDT	28.18	0.07±0.01^a	ND	ND	0.01±0.006^b	ND	ND	F=13.286, P<0.001
PP-DDT	32.95	0.08±0.005^b	0.08±0.01^b	0.13±0.01^a	0.01±0.006^c	ND	ND	F=51.950, P<0.001

RT=Retention time; S=site; ND=not detected; Different letters indicate significant difference between three replicates for each pesticide (P < 0.05).

The results also showed that Folpet (fungicide) was also detected in soil of Lucknow region (Figure 4.3). The Captan compound was also detected in soils of Renukoot region. In Lucknow region on RT- 21.81, α-lindane and on RT 28.18 Captan, on RT 28.35 Folpet was confirmed by GC-MS/MS and at Renukoot region on RT-28.18 Captan was detected. All these results shows that the soil samples of sites S1 and S2, S3 were also contaminated with the persistent organic pollutants.

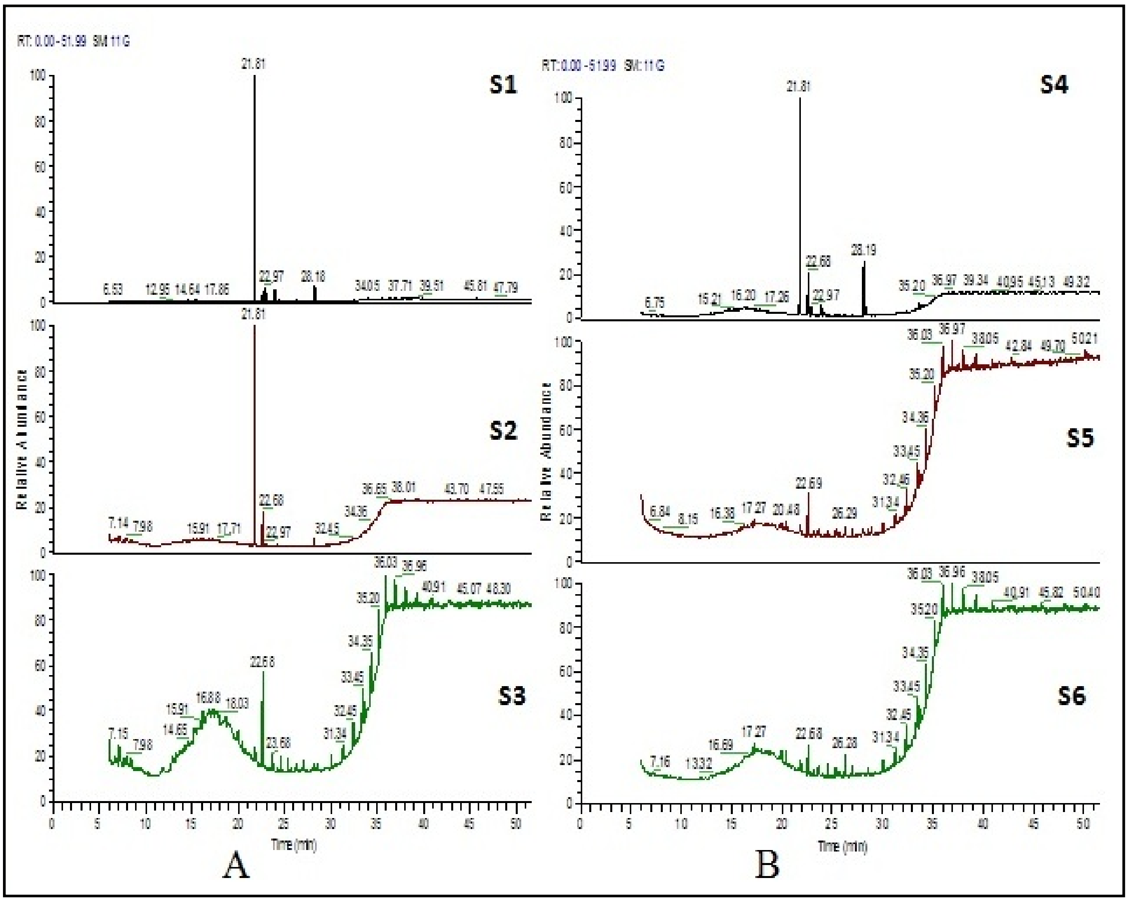


Figure 4.3 The GC-MS/MS chromatogram of soil samples collected from six different study sites (S1 to S6) of Lucknow and Renukoot region.

4.5 Conclusions

The selected sites (S1 to S6) distributed in Lucknow and Renukoot region varied significantly in terms of soil physico-chemical properties (total-C, -N, -P, moisture contents, pH, etc.), pesticides (HCH and OCPs) residues and SMB-C -N and -P values. The soils of Lucknow region sites, having higher amount of pesticides residues, may reduces the SMB-C -N and -P quantity compared to sites of Renukoot region. A higher litters and plant residues available at sites of Renukoot region due to dense natural forest soil as compared to Lucknow urban region sites, may considerably enhances the favourable soil conditions and therefore, a higher SMB-C, -N and -P values at sites of Renukoot region might be expected. A negative correlation between pesticides and SMB-C, -N and –P suggest that higher HCH and OCPs present in soil may adversely influence the growth and multiplication of microbial diversity. The results of this study strongly confirm that variations in soil physico-chemical properties and HCH and OCPs residues may significantly affect the SMB-C, -N and –P compositions. Therefore, removal of lethal HCH and OCPs residues from soils via bioremediation/phytoremediation can be a viable and eco-friendly soil management practice to improve the beneficial species of soil microbial communities and biomass which may consequently support the soil, agriculture and environmental sustainability. This study suggests that continuous routine assessment and monitoring of HCH, OCPs and other lethal pesticides in the Lucknow, Renukoot and other regions is essential for the control, prevention and to minimize contamination and health risk of concerned people.

CHAPTER-5
Methanotrophs composition and soil microbial biomass –C, -N and –P from lindane contaminated sites

CHAPTER 5

METHANOTROPHS COMPOSITION AND SOIL MICROBIAL BIOMASS -C, -N & -P FROM LINDANE CONTAMINATED SITES

5.1 Introduction

The methanotrophs, aerobic bacteria, are considered as important regulators of atmospheric CH_4 fluxes (Tiwari et al., 2018). Methanotrophs are subgroup of methylotrophs, and generally characterized by their ability to use methane as their source of carbon and energy (Hanson and Hanson, 1996). The methane monooxygenase (MMO) is the main methanotrophic enzyme which occurs as both particulate (pMMO) and soluble (sMMO) forms. Generally, the *pmoA* gene encodes the α-subunit of pMMO enzyme and is listed in the genome majority of known methanotrophs except *Methylocella* and *Methyloferula* (Dedysh et al. 2000**).** Based on morphologies, physiologies, and phylogenies, the proteobacterial methanotrophs can be further divided into type I (γ-*Proteobacteria*, families *Methylococcaeae* and *Methylothermaceae*) and type II (α-*Proteobacteria*, families *Methylocystaceae* and *Beijerinkiaceae*) (Trotsenko and Murrell 2008).

Methanotrophs were traditionally classified as type I (*Gamma Proteobacteria*) or type II (*Alpha Proteobacteria*), primarily according to their use of the ribulose monophosphate pathway (type I) or serine pathways (type II) for formaldehyde assimilation and arrangement of internal structures (Hanson and Hanson 1996). They were further subdivided into a type X group, consisting of *Gamma Proteobacteria* that had biochemical capabilities associated with type II methanotrophs. The traditional classification scheme had its shortcomings, as the methanotrophic bacteria are more diverse and have greater biochemical capability than previously imagined. A recently discovered phylum that consists of thermophiles: *Verrucomicrobia* (*Methylacidiphilum* and *Methylacidimicrobium* spp.) has also been added (Sharp et al., 2014; Kalyuzhnaya et al., 2015; Strong et al., 2015). The particulate methane monooxygenase (pMMO), enzyme of methanotrophic bacteria is found in all methanotrophs except *Methylocella* and *Methyloferula* (Kizilova et al., 2014).

The isolation and iidentification of soil methanotrophic bacteria always seems to be a challenging task among microbiologists as well as other group of researchers because many of the methanotrophs are not easy to culture in laboratory. However, presently, the application of culture-independent molecular tools has been recognized as a reliable method to identify soil methanotrophs community composition in natural and agro-ecosystems. During the last few years, various molecular approaches has been successfully applied to study the ecology of soil methanotrophs in various natural and agriculture environmental conditions (McDonald et al., 1995; Horz et al., 2001; Lin et al., 2004; Lau et al., 2007; Chen et al., 2008; Rastogi et al., 2009). In order to differentiate the type I and type II methanotrophic bacteria, group-specific PCR primers are designed to specifically amplify the 16S rRNA gene of methanotrophs to find out their close relationship with other known methanotrophs at the family or genus level (Henckel et al., 1999; Wise et al., 1999; Gulledge et al., 2001; Chen et al., 2007). As suggested by earlier researchers, besides the 16S rRNA target gene, methanotrophic functional genes such as *pmoA* were used to find out the abundance of methanotrophs in soil (Fjellbirkeland et al., 2001; Horz et al., 2001). In view of the above earlier investigations the present study used metagenomics approach to find out the methanotrophic community composition and diversity in lindane contaminated soil samples collected from Lucknow and Renukoot region.

The soil microbial biomass (SMB), considered as an important source of available plant nutrients in several nutrient deprived ecosystems. Land use changes or land use covers and cutting of forests not only affect the availability of required nutrients but also influence the soil microbial community and their biomass levels. It is proposed that several toxic soil pollutants such as heavy metals, insecticides and pesticides, etc. severely influencing the beneficial soil microbial activities and biomass (Singh and Gupta, 2018). Hence, any such toxic pollutions and disturbances due to environmental drivers (soil pH, temperature, salinity, etc.) in soil could be one of the major factors, which may affect the SMB pools and nutrient availability to plants. The previous investigations showed that several environmental drivers (soil organic matter, gravimetric moisture, soil pH, etc.) govern the dynamics of SMB (Singh et al., 2018). However, no investigations have been conducted in the present selected regions to find out the levels of SMB in soils contaminated with HCH isomers. Further, it is also not known how the HCH residues concentrations impacts the SMB–C, –N and –P levels. The earlier studies of in present region were conducted to find out the impact of forest fires, seasonality, vegetation cover or topographical variations on SMB-C, -N and -P dynamics (Singh and Kashyap, 2007; Singh et al., 2009; Singh et al., 2010). Therefore, study of SMB–C, –N and –P contaminated with HCH and OCPs residues may provide new information about the region of Vindhyan plateau. It is hypothesised that the higher HCH isomers concentrations in soil may lead to several unfavorable conditions to the soil microbial diversity, abundance and their biomass. Therefore, the consequences of changes in HCH isomers concentrations and soil physico-chemical properties should be investigated on SMB levels and correlation between HCH isomers concentrations, SMB values and soil physico-chemical properties.

In India, limited research work has been carried to evaluate the role of microbes in remediation of pesticides from lindane contaminated sites. Majority of the Indian studies used different bacterial strains cultured and maintained in laboratory rather than the contaminated fields and evaluated the biodegradation potential of microorganism in pesticide containing nutrient media. To the best of our knowledge there is no information about the methanotrophs diversity and soil microbial biomass from lindane contaminated sites. Hence, there is urgent need to study the soil methanotrophs composition and microbial biomass levels from lindane contaminated sites. It is also not known whether different contaminated sites have similar community composition of methanotrophs. Further, we don’t know about their diversity in lindane contaminated soils. It is expected that present proposed investigation would provide answer to some of the questions raised here. Further, understanding soil microbial biomass and the types of methanotrophic community structure and its potential capability to degrade the lindane is important for verifying the significant contribution of these microbes in cleaning the soil system contaminated by the lindane. The outcome of this study would provide some base line data about the microbial biomass levels and methanotrophic community compositions existing in lindane contaminated soils.

5.2 Material and methods

5.2.1 Soil sampling

The detail soil sampling methods have been described earlier in Chapter 3. The collected soil samples stored at 4 $^{\circ}$C were used for the analysis of soil methanotrophs community composition and soil microbial biomass -C, -N and -P content.

5.2.2 Methanotrophs abundance and community composition

The soil samples collected from Lucknow and Renukoot region were used to identify the methanotrophs abundance and community composition. The detail outline of culture dependent and culture independent techniques used for methanotrophs identification are given in Figure 5.1

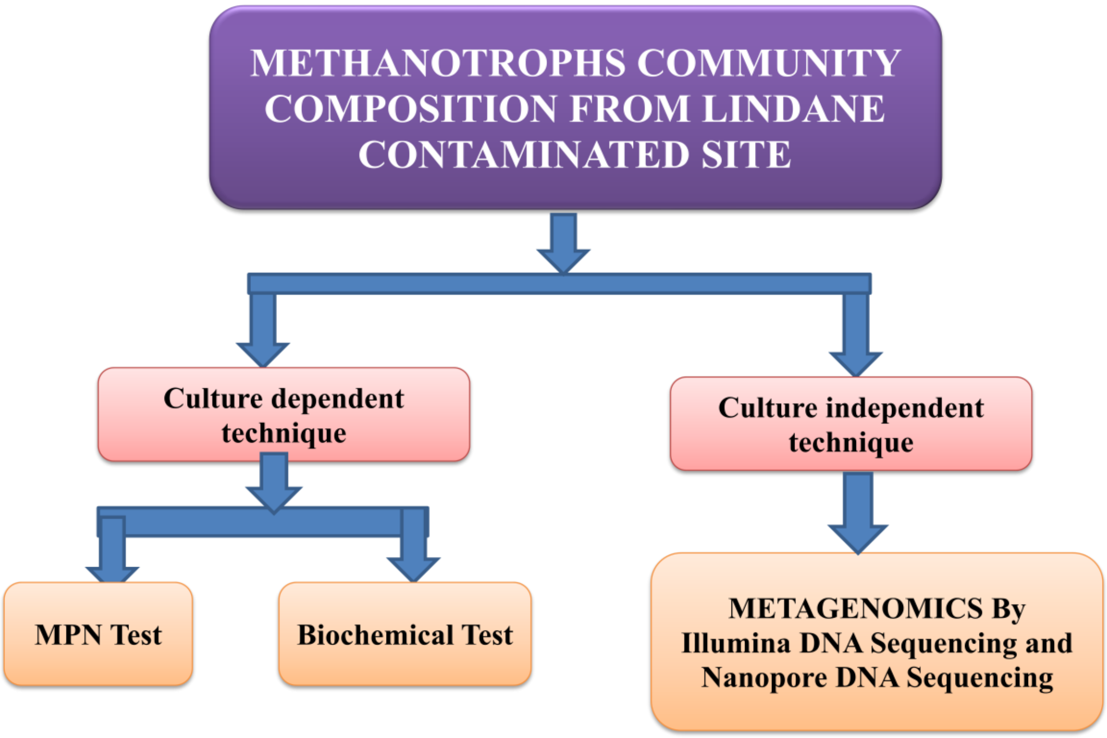


Figure 5.1 Methods used in identification of soil methanotrophs abundance and community composition

5.2.3 Culture dependent techniques

5.2.3.1 MPN methods

The culture enrichment techniques was used for culturable methanotrophic community, its structure was identified using modified most probable number (MPN) (Saitoh et al., 2002) method. A modified nitrate mineral salt (NMS) medium (Whittenbury et al., 1970) was used to isolate the methanotrophs. The NMS media contains $MgSO_4.7H_2O$, 1 g ; KNO_3, 1 g; $Na_2HPO_4.12H_2O$, 0.71 g; ferric ammonium EDTA, 5 g, chelated iron solution 2.0 mL which contains ferric (III) ammonium citrate 0.01 g or ferric chloride 0.05 g, EDTA sodium salt 0.2 g, HCl 0.3 mL, distilled deionized water 100 mL and Trace element solution 1 mL. The composition of trace element solution is DiSodium EDTA, 50 mg; $FeSO_4$.7H2O; 20 mg; H_3BO_3, 3 mg; $CoCl_26H_2O$, 2 mg; $CuSO_4$- $5H_2O$; 3 mg; $ZnSO_47H_2O$; $MnCl_24H_2O$, 3 mg; $Na_2MoO_42H_2O$, 3 mg; $NiCl_26H_2O$, 2 mg. The pH of the medium was maintained at 6.8. The media was autoclaved at 121 ºC for 15 min for sterilization. The serially diluted soil samples of different sites (S1, S2, S3, S4, S5 and S6) were spread on Petri plates containing NMS media for the enumeration of methanotrophs abundance. The CH_4 gas was injected with 1:1 ratio with air in the Petri plates as carbon source for the growth of methanotrophs which was incubated at 30 °C in a rotatory shaking incubator at 120 rpm for 2-3 weeks in anaerobic chamber (Figure 5.2). After the growth on NMS medium, the methanotrophic bacterial isolates from different soil samples were considered for biochemical characterization according to Chandra and Singh (2014).

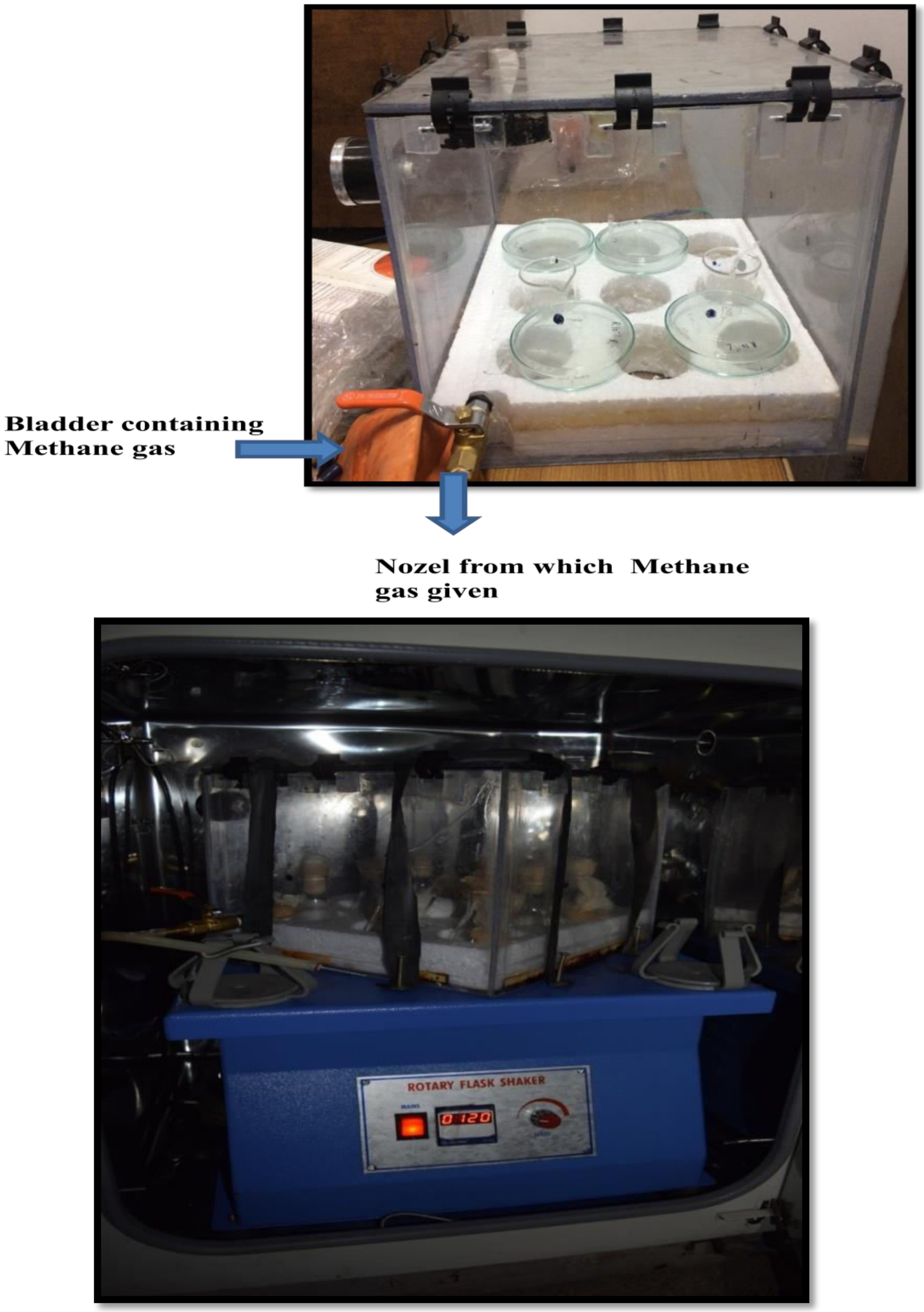
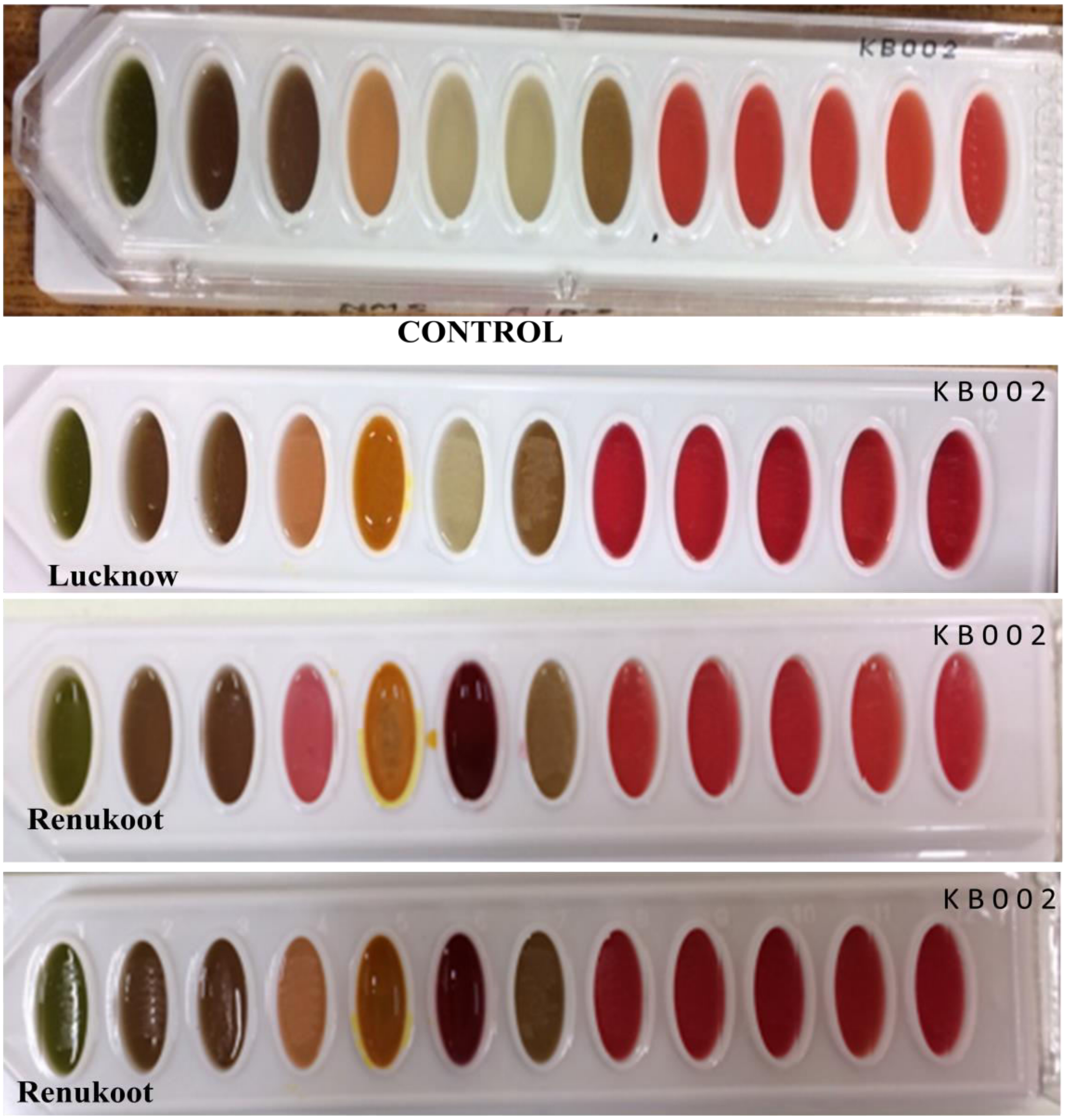


Figure 5.2 Anaerobic chamber for incubation of methanotrophs

5.2.3.2 Biochemical characterization

After the growth on NMS medium, the methanotrophic bacterial isolates was considered for biochemical characterization according to Chandra and Singh (2014). These isolates were tested for Gram staining, catalase test, oxidase test, urease test, citrate utilization test, H_2S production test etc. The biochemical test of soil methanotrophs of different sampling sites is shown in Figure 5.3.



Results

Figure 5.3 Biochemical test of soil methanotrophs

5.2.4 Culture independent techniques

5.2.4.1 Illumina sequencing

Methanotrophs community composition of India Pesticide limited (IPL), Lucknow soil sample was studied by Illumina sequencing. Metagenomics is a molecular tool used to analyze DNA acquired from environmental samples in order to study the community of microorganisms present without the necessity of obtaining pure culture. The methanotrophic bacterial diversity such as *Methanobacterium*, *Methanobrevibacter*, *Methanocella*, *Methanosaeta*, *Methanosarcina, Verrucomicrobia* (*Methylacidiphilum* and *Methylacidimicrobium* spp.) were particularly observed in soil samples of Lucknow region. Generally, the methanotrophs are not easy to culture and it becomes a very tedious work for researchers to isolate methanotrophs by culture dependent techniques therefore, metagenomics is introduced to study the diversity of microorganisms is important to soil and human health. Metagenomics can also be used in find the organisms that can grow in toxic wastes and can then extract useful genes from these organisms. Metagenomics-based approaches were found to be more helpful in the identification and isolation of new genes from the uncultivable sources and provide insights for future research. The 16S V3-V4 metagenome sequencing and analysis (methanotrophs) in soil sample of IPL Lucknow has been described in Figure 5.4.

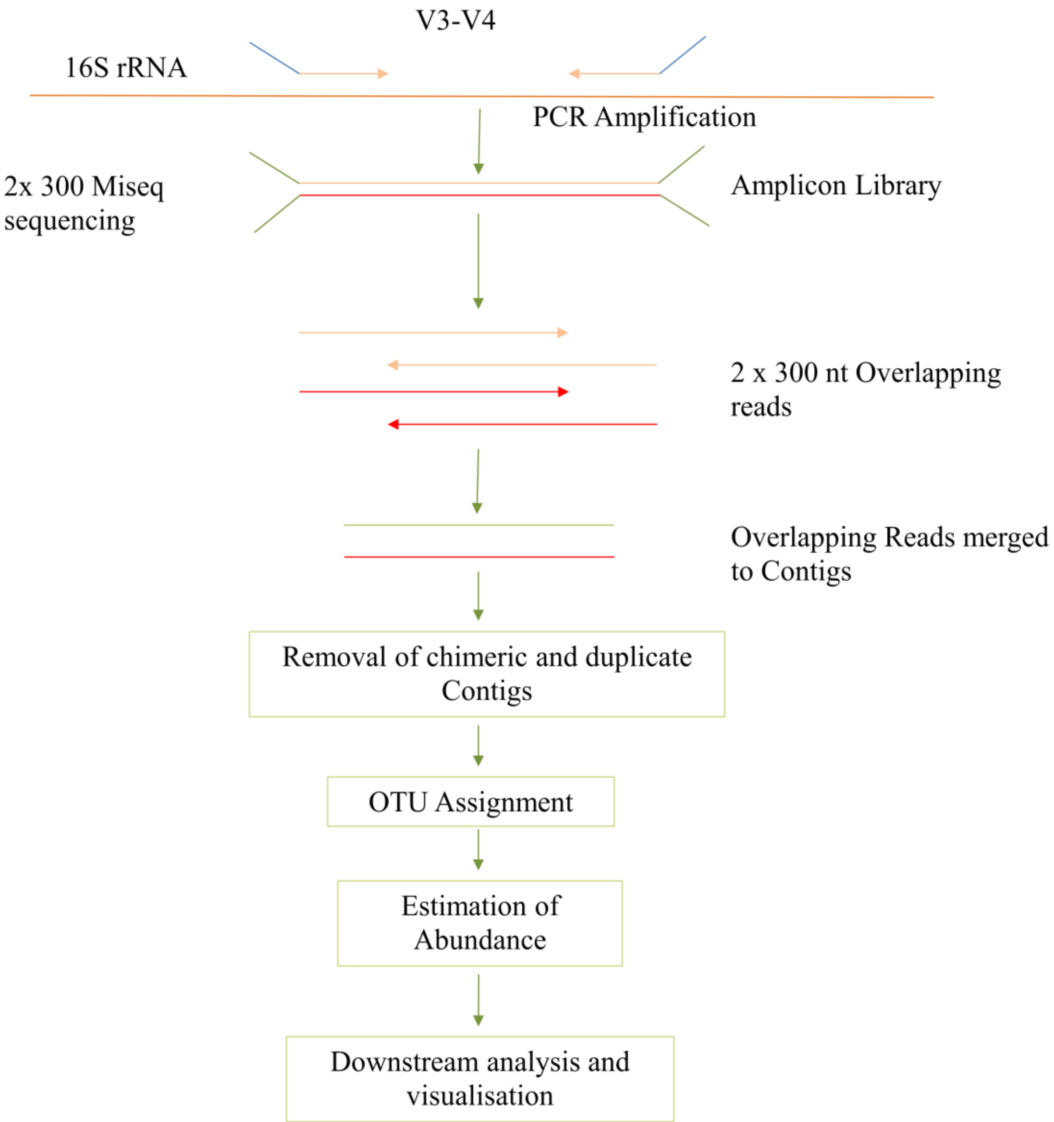


Figure 5.4 16S rRNA Metagenome Workflow

An amount of 25 ng of DNA was used to amplify 16S rRNA hyper variable region V3-V4. The reaction includes KAPA HiFi HotStart Ready Mix and 100 nm final concentrations of modified 341F and 785R primers (Klindworth et al., 2013). The PCR involved an initial denaturation of 95°C for 5 min followed by 25 cycles of 95°C for 30s, 55°C for 45s and 72°C for 30s and a final extension at 72°C for 7 min. The amplicons were purified using Ampure beads to remove unused primers. Additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. The sequence data quality was checked using Fast QC (Andrews et al., 2017) Multi QC software. The data was checked for base call quality distribution, % bases above Q20, Q30, %GC, and sequencing adapter contamination. The sample has passed QC threshold (Q20 > 95%). The primer sequence and Summary of raw sequence data are given in Table 5.1 and 5.2, respectively. The histogram of reads with average sequence quality scores have been shown in Figure 5.5 and 5.6

Table 5.1. The primers used in PCR.

SNo	Primer name	Primer sequence 5'→ 3 '
1	V3V4F	CCTACGGGNGGCWGCAG
2	V3V4R	GACTACHVGGGTATCTAATCC

Table 5.2 Summary of raw sequence data and quality.

Sample ID	Number of reads	Read length	GC%	% Bases > Q20
IPL	113276	301	56.5	99.01

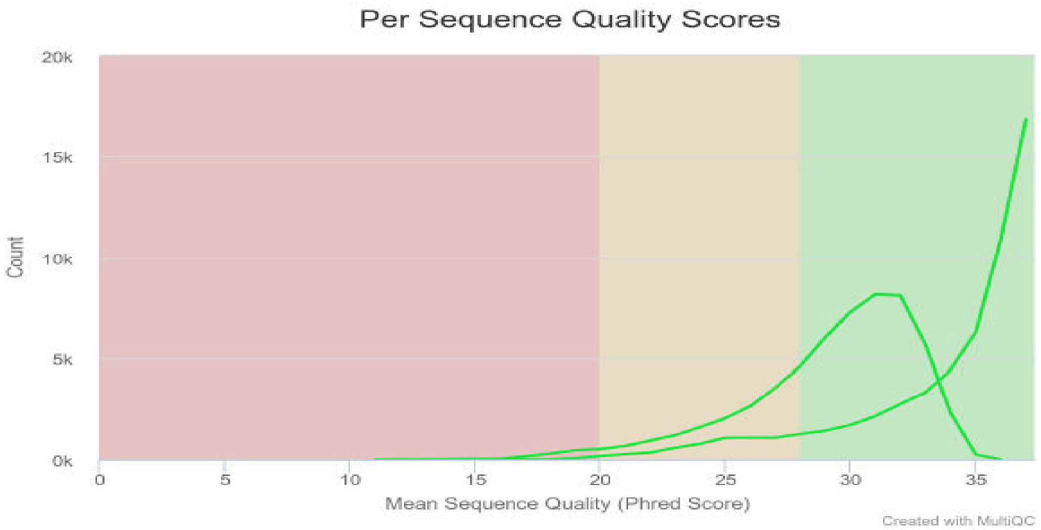


Figure 5.5 Histogram of reads with average sequence quality scores.

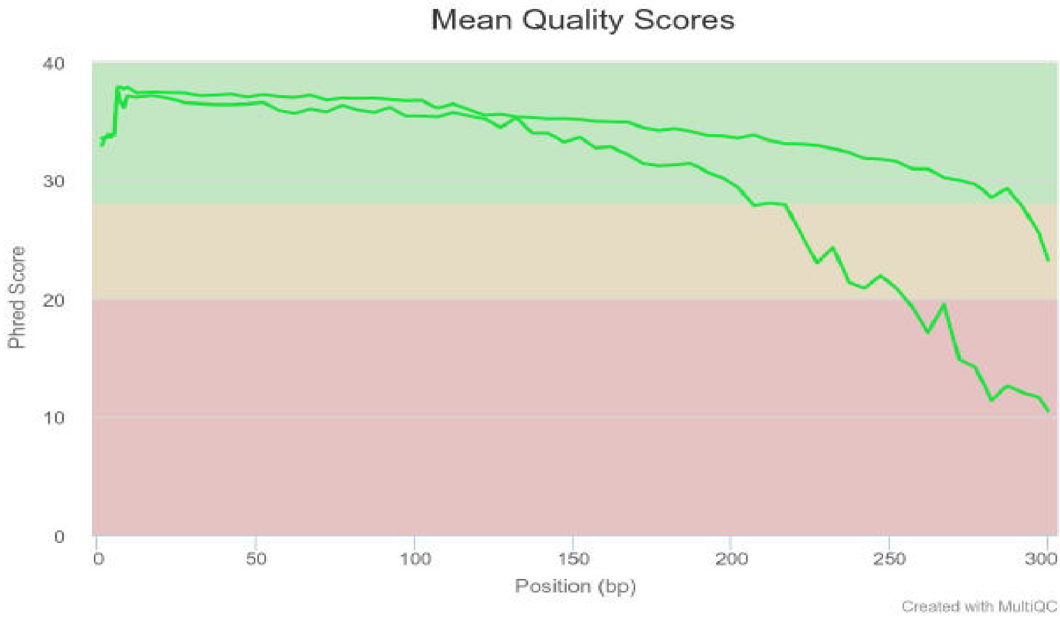


Figure 5.6 Histogram of reads with average base sequence quality scores.

The reads were trimmed (20 bp) from 5'end to remove the degenerate primers. The trimmed reads were processed to remove adapter sequences and low quality bases using Trimgalore (Babraham bioinformatics 2017). The QC passed reads were imported into mothur (Schloss et al., 2009) and the pairs were aligned with each other to form contigs. The contigs were screened for errors and only those between 300bp and 532bp were retained. Any contig with ambiguous base calls were rejected. The high quality contigs were checked for identical sequences and duplicates were merged.

Although the primers for the experiment were designed for 16s bacterial rRNA, there are good chances for non specific amplification of other regions. To correct for this we align the contigs to a known database for 16s rRNA. Depending on the variable region being amplified, most of the contigs will align to its respective region on the database. Any ambiguous contigs aligning to other region on the database were discarded.

After this process the gaps and the overhang at the ends from the contigs were removed and processed for chimera removal which may have formed due to PCR errors. UCHIME algorithm (Edgar et al., 2011) was used to flag contigs with chimeric regions. A known reference of all the chimeric sequences was used to identify and remove possible chimeric sequences.

The filtered contigs were processed and classified into taxonomical outlines based on the GREENGENES v.13.8-99 database (Desantis et al., 2006). The contigs were then clustered into OTUs (Operational Taxonomic Unit). After the classification, OTU abundance was estimated. 788 OTUs were identified out of which only 9 Methanotrophs were filtered out for further analysis. The alpha diversity calculations were performed for these Methanotrophs using Phyloseqence (McMurdie and Holmes, 2013). An R package for metagenome analysis Beta diversity analysis cannot be performed for this sample as it requires at least 2 samples or groups to check the differences in microbial abundances between two samples or groups. PICRUSt (Langille et al., 2013) was used to predict gene family abundance. PICRUSt program was designed to estimate the gene families contributing to a metagenome by bacteria or *Archaea* identified using 16S rRNA sequencing. The 16s RNA copy numbers were normalized by PICRUSt's precalculated files. The metagenomes were predicted using predict metagenomes.py script. The predicted pathways were collapsed into higher categories. OTU contributions for the particular functions were estimated by metagenome contributions.py script.

5.2.4.2 Nano-pore sequencing

In nano-pore DNA sequencing we use electric current or voltage in which nucleotide sequence pass through very small pore approx 1nm. We detect the alteration of current when a nucleotide is passing through that pore by doing that we get the idea of nucleotide we are dealing with. 1nm is sufficient to pass the nucleotide because nucleotide is much thinner than 1nm diameter. The DNA extraction was performed by using the suitable method for the sample type from commercially available kits such as QIAGEN, ZYMO RESEARCH, Thermo-Fisher DNA extraction as per the manufacturer's recommendation. The PCR conditions and primer details has been given in Table 5.3 and 5.4, respectively. The sequencing protocol involved Nanopore sequencing which was performed by using 1µg of DNA template. Then after, end repair/dA tailing Ligation of Barcode Adapter, Barcoding PCR, End repair/dA tailing, Blunt end Adapter Ligation, Purification using AM Pure XP bead binding, priming and loading the SpotON flow cell were followed in the present study.

Table 5.3 The PCR conditions for extraction of DNA.

Reaction mixture (50 µL)		**Cycling conditions**		
Template DNA	100 ng	Initia	2 minutes at 95 ºC	
Forward Primer	0.3 µM	Denaturatio	15 seconds at 95	25 Cycles
Reverse Primer	0.3 µM	Annealing	15 seconds at 60	
Master Mix	25 µL	Extension	2 minutes at 72 ºC	
Nuclease Free water	Volume makeup 50 µL	Final Extension	10 minutes at 72ºC	

Table 5.4 Primer details for the extraction of DNA

SNo	Primers name	Sequence (5` → 3`)	Temp (°C)	GC-Content
1	27F	AGAGTTTGATCM TGGCTCAG	56.3	47.5%
2	1492R	TACGGYTACCTTGT TACGACTT	55.3	45%

5.2.4.3 Bioinformatics protocol

EPI2ME 16S analysis work flow all used to perform genus-level identification from single reads; with access to base called files for detailed investigations at the species and sub-species level. The phylogeny analysis of query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment. The workflow is designed to BLAST base called sequence against the NCBI 16S bacterial database, which contains 16S sequences from different organisms. Each read is classified based on % coverage and identity. The 16S workflow will be useful in identifying the composition of a methanotrophic community. The 16S workflow for identification of methanotrophic community has been shown in Figure 5.7

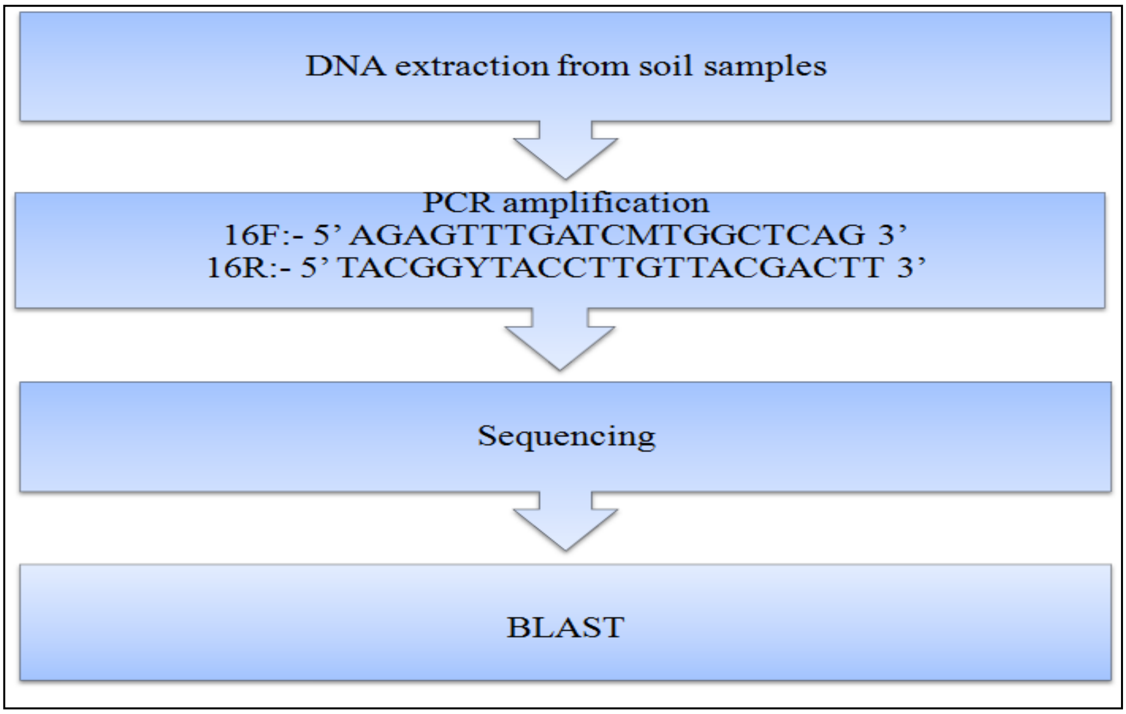


Figure 5.7 16S workflow for identification of methanotrophic community.

Extracted DNA from the sample was subjected to Nano Drop and GEL Check before being taken for PCR amplification: The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality. The amplified 16S PCR Product is purified and subjected to GEL Check and Nanodrop QC. The NanoDrop reading of 260/280 at a value of 1.8 to 2 issued to determine the DNA's quality. After the 16S PCR Amplification, the products are purified using QIAGEN GEL Purification Kit. 10 µL of Purified product is taken in a separate tube and volume is made up to 45µl with Nuclease free water. End-repair and DA Tailing is performed as follows 45µl of volume adjusted purified sample , 7 µL of Ultra II End-prep buffer (from NEB) and 3 µL of Ultra II End-prep Enzyme is added along with 5µl Nuclease free water – incubate at 20 ºC for 5 minutes and at 65 ºC for 5 minutes. 60 µL of AMpure XP Beads are added and purification is performed along with two washes of 180 µL with 70% Ethanol, purified product is eluted using 31µl of nuclease free water. 20 µL of Barcode adapters from Oxford Nanopore kit (EXP-PBC096 & SQK-LSK109) are added along with 30 µL of END prep DNA and 50 µL of Blunt TA/Ligase Master Mix (From NEB) - incubate at RT for 10 minutes. 40 µL of AMpure XP Beads are added and purification is performed along with two washes of 180 µL with 70% Ethanol, purified product is eluted using 25µl of nuclease free water. Barcoding PCR is performed using the EXP-PBC096 Kit while any one barcode is used per sample (volume – 1 µL) with 2 µL of adapter ligated template and 25 µL of Long AMPTaq 2x Master Mix and 22 µL Nuclease free water. Barcoding PCR conditions are as follows: Initial Denaturation at 95 ºC for 3 minutes, Denaturation at 95 ºC for 15 sec Annealing at 62 ºC for 15 second extension at 65 ºC for 180 second total of 18 cycles, final extension at 65 ºC for 7 minutes. The PCR purification using QIAGEN GEL purification Kit is carried out.

Total of 1µg of pooled DNA (different barcoded samples are added) in 47 µL of volume – End PREP is performed as follows – 47 µL of template, 1 µL of DNA CS, 3.5 µl of NEBNext FFPE DNA repair buffer, 2 µL of NEBNext FFPE DNA repair mix, 3.5µl of Ultra II End-prep buffer, 3 µL of Ultra II End-prep enzyme and incubated at 20 ºC and 65 ºC for 5 mins respectively. 60 µl of AMpure XP Beads are added and purification is performed along with two washes of 200 µl with 70% Ethanol, purified product is eluted using 61 µL of nuclease free water. This is taken forward for sequencing adapter ligation and clean up – 60 µL of template from previous step is added to 25 µL of ligation Buffer, 10 µL of NEBNext Quick T4 DNA Ligase, 5 µL of Adapter Mix and incubated at RT for 10 minutes. 40 µL of AMpure XP Beads are added and purification is performed along with two washes of 250 µL S fragment buffer, purified product is eluted using 15 µL of elution buffer. The prepared library is taken forward for sequencing in the Oxford Nanopore Platform.

5.3 Soil microbial biomass (SMB) analyses

5.3.1 SMB-C

An amount of 50 g of conditioned soil samples were saturated with purified liquid chloroform (Analytical reagent grade, Qualigens, India) for 10-20 hrs and subsequently removed with the help of evacuation (Srivastava and Singh, 1998). Then after, the soil was extracted using 0.5M K_2SO_4 (1:4, soil: extract) and shaken for 30 minutes. The same protocol was followed for the extraction of unfumigated soil sample. The MB-C in 0.5 M K_2SO_4 soil extract was measured by using dichromate digestion (Vance et al., 1987) and estimated by the following equation:

$$MB\text{-}C = 2.64 \times Ec \qquad (1)$$

Where, Ec is the difference between C extracted from fumigated and un-fumigated soils, both expressed as $\mu g\ g^{-1}$ oven dry soil basis (Jenkinson and Ladd, 1981).

5.3.2 SMB-N

The conditioned soil samples (25 g) were saturated with purified liquid chloroform (Analytical reagent grade, Qualigens, India) for 18-20 hrs. The extraction procedure was similar as described for SMB-C. The 0.5 M K_2SO_4 extract of fumigated and un-fumigated soil samples were analysed for SMB-N using the micro Kjeldahl digestion procedure (Brookes et al., 195). The MB-N was calculated by the equation given as follows:

$$MBN = X - Y/Kn \qquad (2)$$

Where X represent total N in K_2SO_4 extract of fumigated soil and Y denotes total N in K_2SO_4 extract of un-fumigated soil, Kn stands for fraction of biomass-N extracted after $CHCl_3$ treatment. A K_N of 0.54 (Brookes et al., 1985) was taken by assuming that 54% of MB-N was extracted by $CHCl_3$ treatments.

5.3.3 SMB-P

An amount of 5 g of conditioned soil samples were saturated with purified liquid chloroform (Analytical reagent grade, Qualigens, India) for 18-20 hrs. Two portion of soils (fumigated and un-fumigated) were extracted with 0.5 M $NaHCO_3$ solution. MB-P was determined as inorganic-P (Pi) in 0.5 M $NaHCO_3$ extract of fumigated and un-fumigated soil samples by ammonium molybdate-stannous chloride method (Sparling et al., 1985). The MB-P was calculated from $CHCl_3$ released Pi dividing by a Kp value of 0.40 (Brookes et al., 1982) by assuming that 40% of P in biomass is related to Pi release by $CHCl_3$ treatment (Brookes et al., 1982; Srivastava and Singh, 1988).

Soil samples were collected from different selected sites and analysed for the SMB-C, -N and -P. the SMB-C, -N and -P (µg g^{-1} dry soil) by using the chloroform, fumigation- extraction procedure as described by earlier workers (Brookes et al., 1985; Vance et al., 1987). Liquid chloroform after purification was used for the soil fumigation (Shrivastava and Singh, 1988). The 0.5 mol L^{-1} K_2SO_4 (1:4 soil: extract) was used for extraction for about 30 minutes. Similar extraction procedures were also followed for non-fumigated soil samples. The SMB-C and -N in 0.5 mol L^{-1} K_2SO_4 soil extract was determined by dichromate digestion (Vance et al., 1987). The SMB-P was analysed as inorganic P (Pi) in 0.5 mol L^{-1} $NaHCO_3$ extract of both fumigated and non-fumigated soil samples by ammonium molybdate-stannous chloride method as described earlier (Sparling et al., 1985).

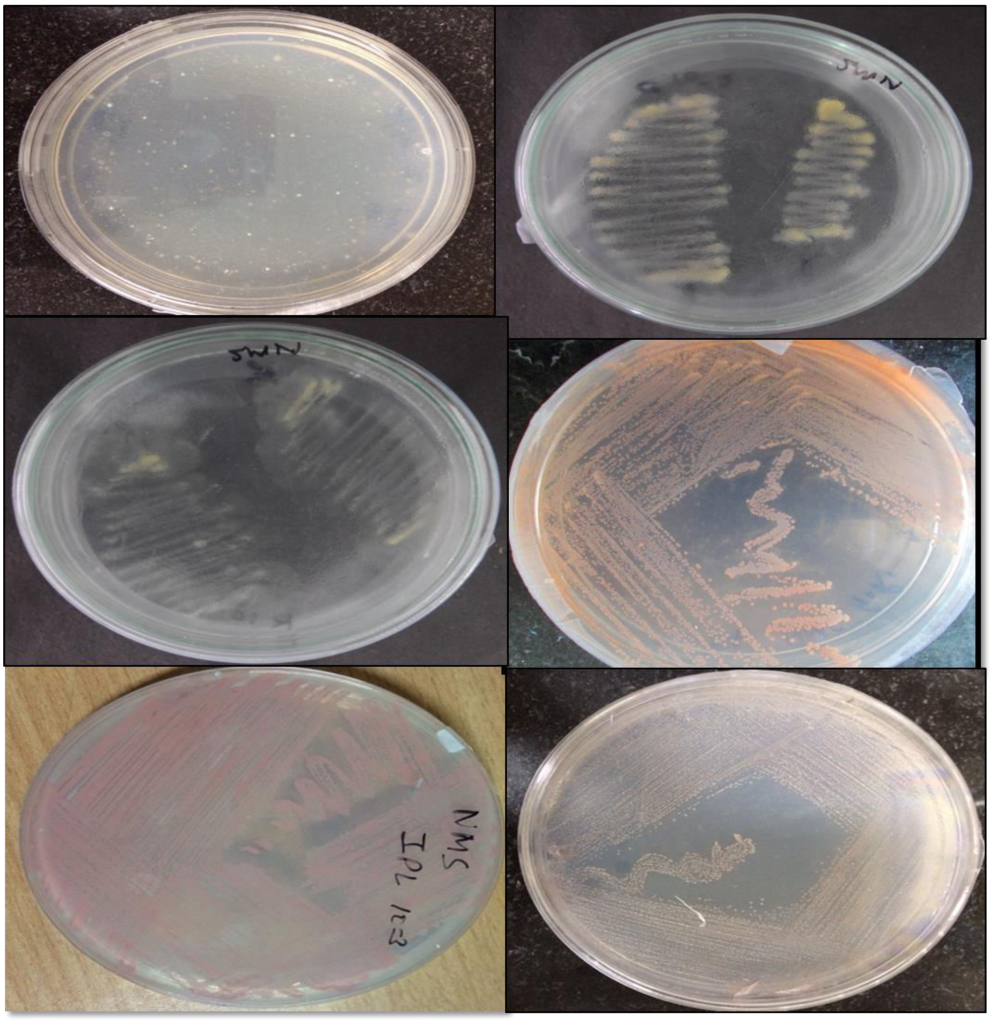
5.4 Statistical analysis

The data generated from the present investigation are expressed as the mean of three replicates ± SE. All the observations from each selected sites were carried out in triplicate to improve the analytical precision of the experimental data. To confirm the variability of data obtained and validity of results, the data were subjected for the statistical analysis using one way analysis of variance (ANOVA) using SPSS software (IBM SPSS Statistics version 20).

5.5 Results and Discussion

5.5.1 Methanotrophs abundance/community composition

The cream and pink colour colonies (Figure 5.8) on NMS (nitrate mineral salt media) specific media for the isolation of methanotrophs confirmed the growth of aerobic methanotrophs. After several rounds of streaking from plate to plate, pure colonies were obtained on the plates. The colonies were observed to be cream or pink in colour, round and measuring about 1 mm in diameter. The cells were found to be motile and Gram-stain negative.Different morphology of methanotrophs was observed during cultivation period. After incubation period (2-3 weeks), the methanotrophic bacterial growth on Petri plates was observed which indicates the presence of methanotrophs in lindane contaminated site of Lucknow and Renukoot region.



A **B**
Lucknow soil samples **Renukoot soil samples**

Figure 5.8 The colonies of aerobic soil methanotrophs growing on NMS media after 2-3 weeks of incubation at 30 ºC.

The result demonstrated that methanotrophic population in Renukoot region was significantly higher than Lucknow region due to large forest area and soil having higher amount of organic contents which contributes growth of methanotrophs in well aerated soil (Megonial and Guenther, 2008; Shoemaker et al., 2014). The competitive inhibition of NH_4^+ to CH_4 was noticed as a main inhibitory effect for methanotrophs multiplication (Schimel et al., 1993). Zheng et al. (2008) demonstrated inhibitory effect of soil N amendments on the methanotrophic population density and community composition in Chinese paddy soil. Morphological and biochemical test of bacterial isolates in soil samples collected from lindane contaminated sites are given in Table 5.5. The SEM images (Figure 5.9) of soil methanotrophic bacterial isolates from study sites showed rod shaped bacteria present in the soil samples.

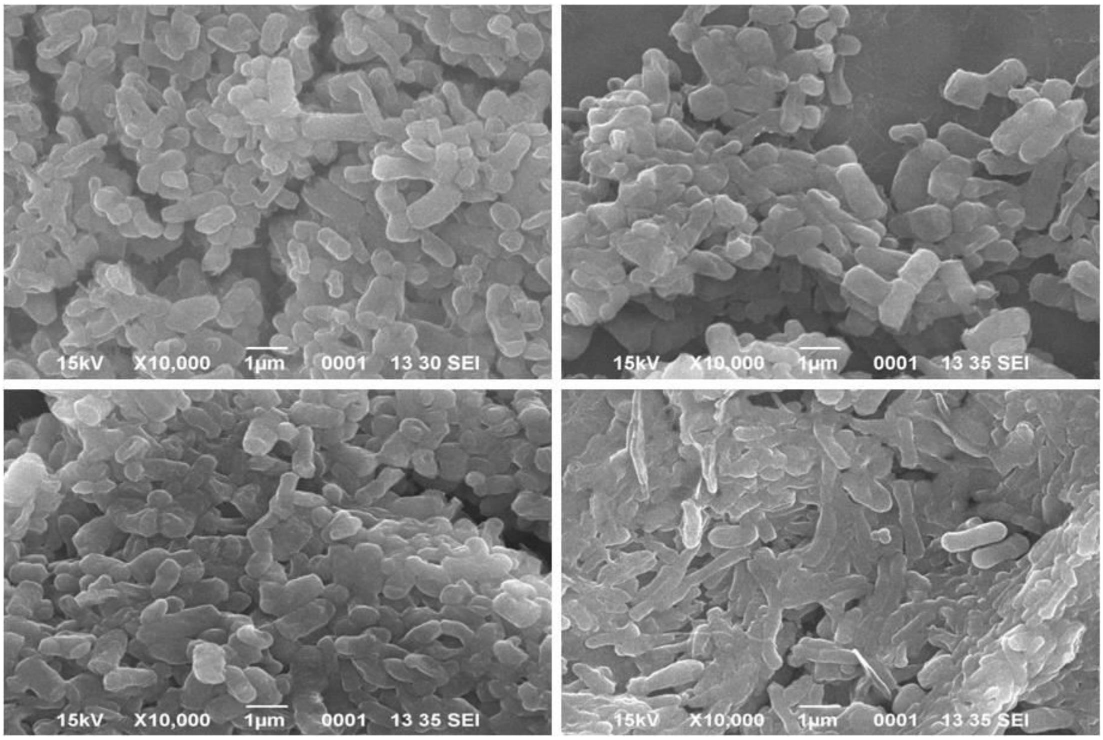


Figure 5.9 SEM of soil methanotrophic bacterial isolates from study sites

Table 5.5 Morphological and biochemical test of some bacterial isolates from soil sample collected from lindane contaminated sites of Lucknow and Renukoot region.

Isolated strains	Gram staining	Colour	Motility test	Catalase test	Oxidase test	*Amylase production*	Urease test	Citrate utilization
SBRJS01	–	Pink	+	+	+	–	–	–
SBIJS02	–	Pink	+	+	+	–	–	–
SBJS03	–	Cream	+	+	+	–	–	–
SBJS04	+	Pink	–	–	–	–	–	–
SBJS05	+	Cream	–	+	+	–	–	
SBJS06	–	Cream	–	–	–	+	+	
SBJS07	–	Pink	+	+	–	–	–	–
SBJS08	–	Cream	+	–	–	+	–	–
SBJS09	–	Cream	–	–	–	+	–	+
SBJS10	+	Cream	–	–	–	–	–	+
SBJS11	+	Pink	+	+	–	–	–	–
SBJS12	–	Pink	+	–	+	–	+	–
SBJS13	–	Cream	+	–	+	–	–	–
SBJS14	–	Cream	–	–	–	–	–	–
SBJS15	+	Cream	+	–	–	+	+	–

5.3.2 Methanotrophs community compositions of Lucknow region (Illumina sequencing)

Metagenomics is used for the detection, identification and relative quantification of environmental microorganisms. The growing accessibility of next generation DNA sequencing (NGS) methods has greatly advanced our understanding of microbial diversity in medical and environmental science. In this study, high-throughput metagenomic sequencing provided a powerful strategy to investigate the methanotrophs community structure and functional potential associated in lindane contaminated soil. Alpha diversity is a measurement of richness and relative abundance of bacteria within the sample. Methanotrophs alpha diversity statistics and methanotrophs genus abundance are presented in Table 5.6 and 5.7, respectively.

In community ecology, α-diversity is mainly used to reflect the diversity of each sample, which estimates the number of species in the microbial community as well as the abundance and diversity of species in environmental communities through a series of statistical indices Figure 5.10 Represent different alpha diversity.

The Phyla abundance distribution, genus abundance distribution and KEGG orthology predicted using PICRUSt is shown in Figure 5.11 (a), (b) and (c). Krona plot a new visualization tool that allows intuitive exoloration of relative abundances within the complex hierarchies of metagenomic classification as shown in Figure 5.12.

Table 5.6 Methanotrophs alpha diversity statistics.

Sample	Observed	Chao1	Se.chao1	ACE	Se.ACE	Shannon	Simpson	Inv Simpson	Fisher
IPL	9	9	0.236	9.43	1.321	1.888	0.813	5.356	2.873

The indices for community diversity calculation include:

ACE : An index to estimate the number OTU in communities. It was proposed by the Chao and is commonly used to estimate the total number of species in ecology. (http://www.mothur.org/wiki/Ace)

Chao : It is an index that uses Chao 1 algorithm to estimate the OTU number of samples. Chao commonly used in ecology to assess the total number of species. (http://www.mothur.org/wiki/Chao)

Shannon: Commonly used to reflect the diversity index α for the estimation of microbial diversity.

(http://www.mothur.org/wiki/Shannon)

Simpson : Simpson diversity index, proposed by Edward Hugh Simpson in 1949 and is commonly used in ecology to quantify biological diversity in a region.

(http://www.mothur.org/wiki/Simpson).

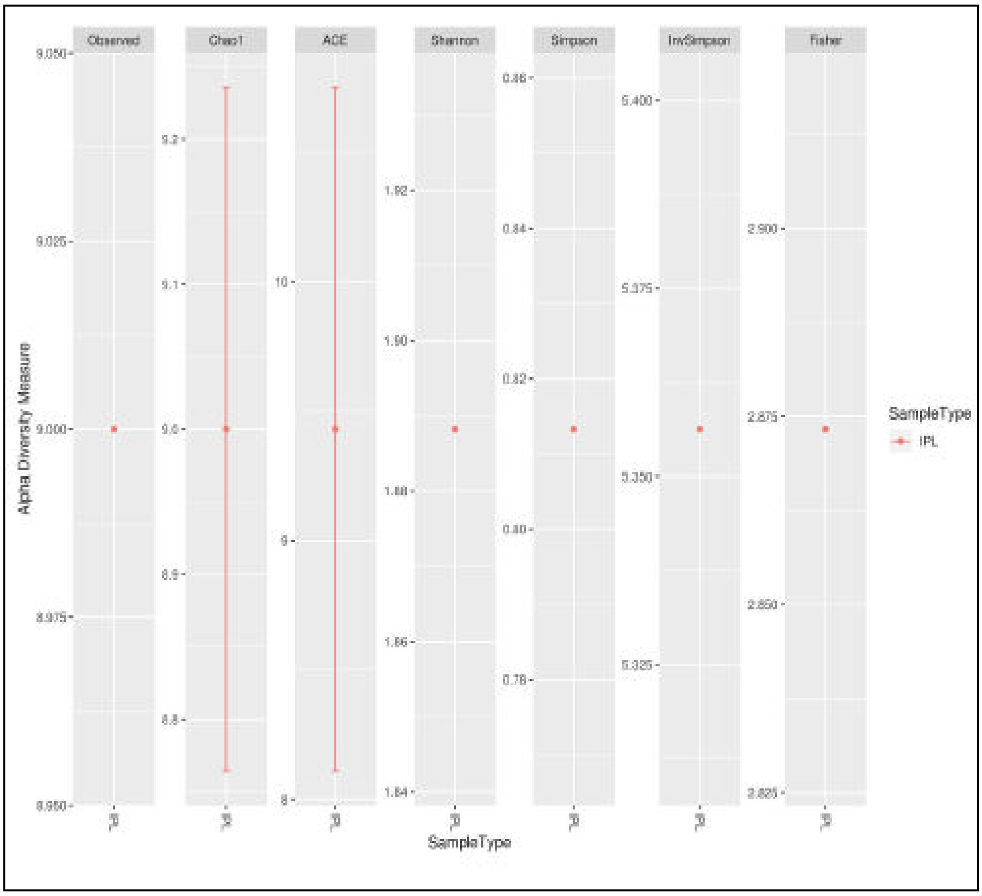


Figure 5.10 Represent different alpha diversity indices of which Chao1 and ACE represent the richness of the sample and Shannon, Simpson, InvSimpson and Fisher represent both richness and relative abundance.

Table 5.7 Methanotrophs community composition in soil of Lucknow region.

Genus	Methanotrophs abundance
Methanocella	20
Methanosarcina	9
Methanomicrobia (unclassified)	6
Methanobrevibacter	5
Methanobacterium	7
Methanosaeta	2

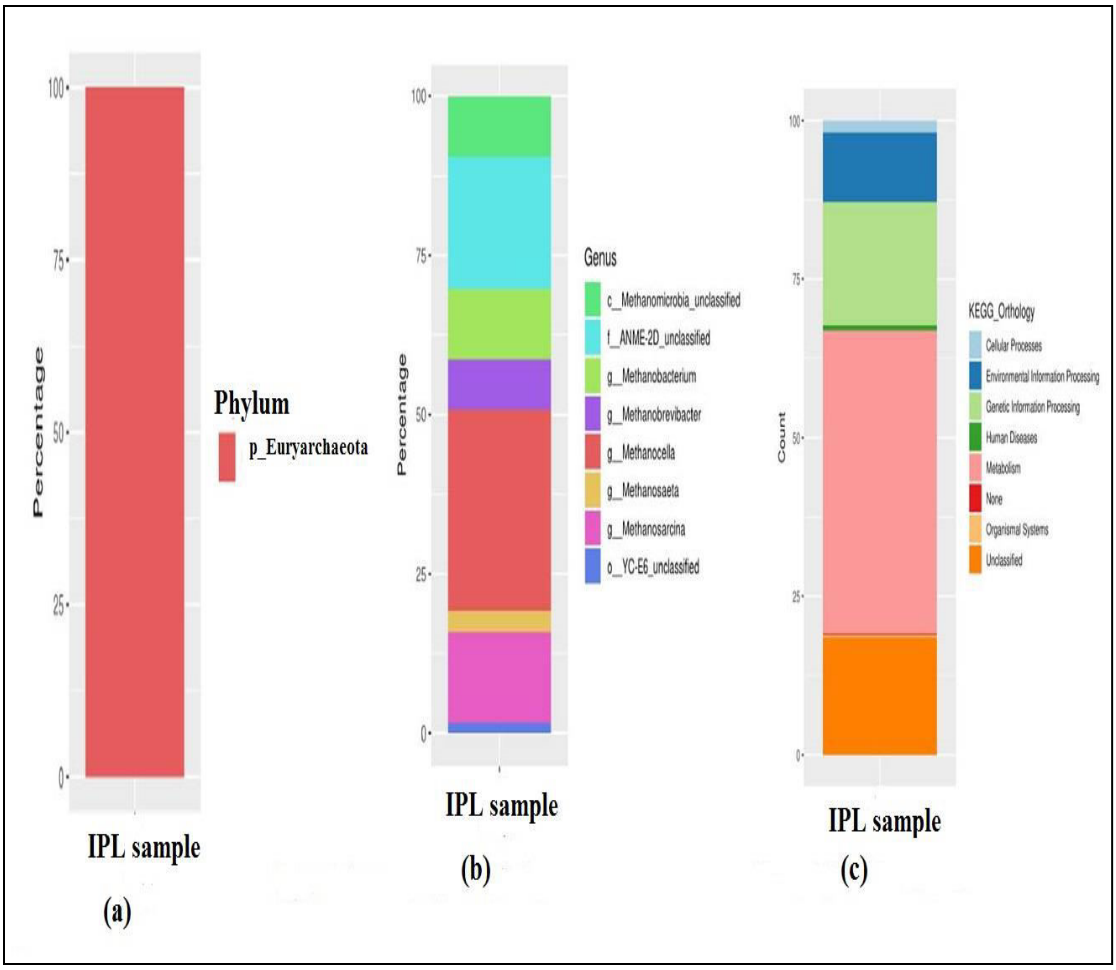


Figure 5.11 (a) Phyla abundance distribution (b) Methanotrophs genus abundance (c) KEGG orthology predicted using PICRUSt.

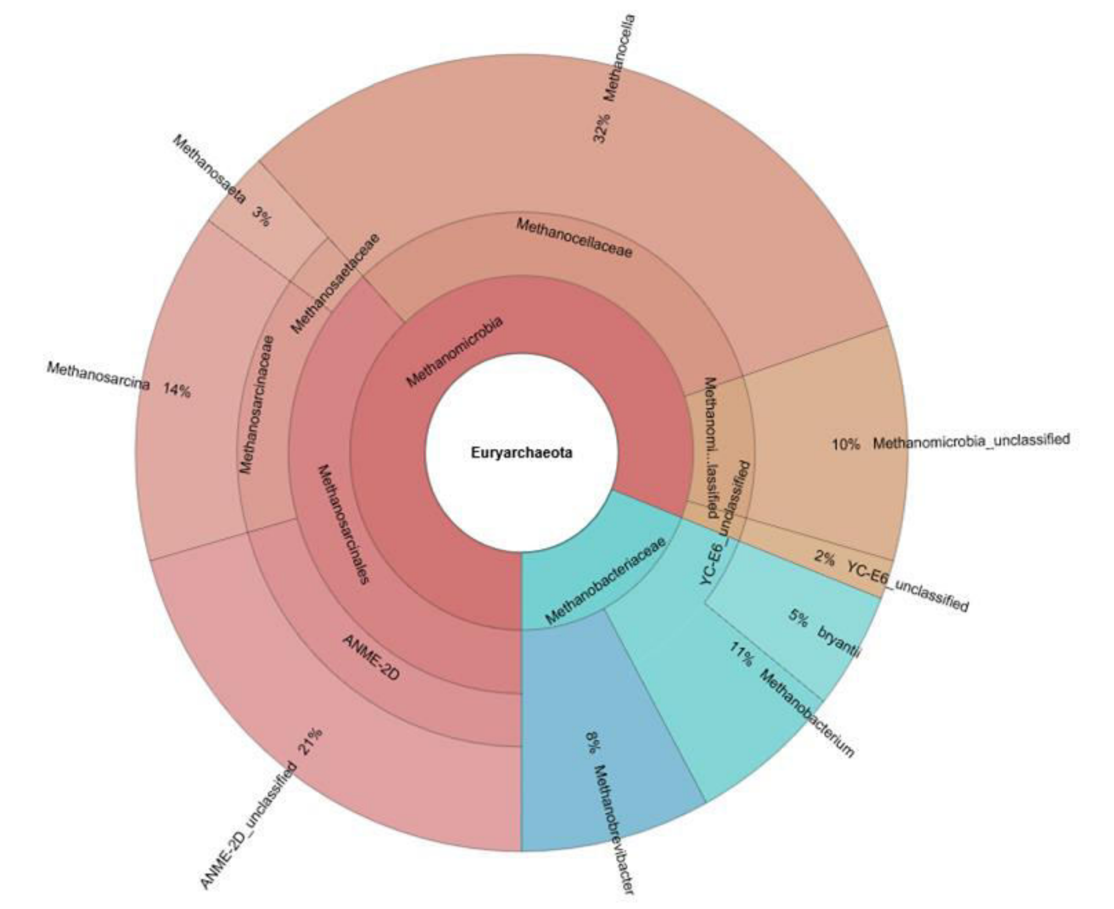


Figure 5.12 Krona plot showing methanotrophic community composition in lindane contaminated soil of Lucknow region.

5.3.3 Methanotrophs community in Renukoot region (Nano-pore sequencing)

The contaminated soil contained 44,946 reads having 42,981 classified and 1,965 non-classified reads of bacterial population as shown in Figure 5.8. The metagenomics of soil sample showed that the total number of methanotrophs present in selected site was 676. Further, the methanotrophic community composition in lindane contaminated Renukoot soil are given in Table 5.8

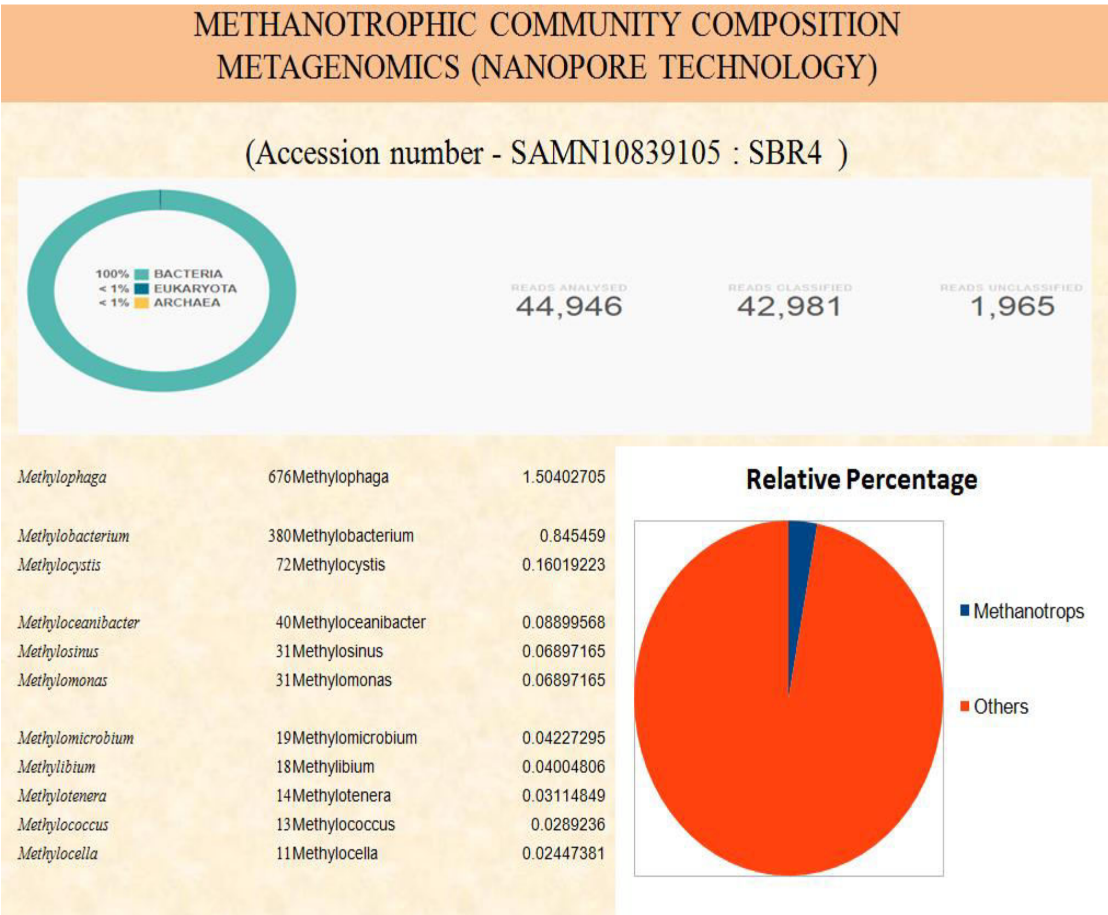


Figure 5.13 Methanotrophic community composition in soil of Renukoot region.

Table 5.8 Methanotrophic community composition in lindane contaminated soil of Renukoot region.

Family	**Methanotrophs**
Methylobacteriaceae	*Methylobacterium nodulans* ORS 2060; *Methylobacterium aquaticum Methylobacterium phyllosphaerae; Methylobacterium radiotolerans* JCM2831; *Methylobacterium extorquens; Methylobacterium populi*;
Methylophilaceae	*Methylotenera mobilis* JLW8; *Methylophilus* sp. TWE2; *Methylobacillus flagellates* KT
Methylocystaceae	*Methylocystis bryophila; Methylocystis* sp. SC2; *Methylosinus trichosporium* OB3b
Methylacidiphilaceae	*Methylacidiphilum* sp.
Methylophilaceae	*Candidatus*sp.; *Methylopumilis turicensis; Candidatus Methylopumilis Planktonicus; Methylovorus*sp.
Methylococcaceae	*Methylococcus capsulatus* str. Bath; *Methylomicrobium alcaliphilum* 20Z
Methylophilaceae	*Methylomonas denitificans*; *Methylomonas methanica* MC09;
Sterolibacteiaceae	*Methyloversatilis* sp. RAC08;
Beijerinkiaceae	*Methylocella silvestris* BL2
Piscirickettsiaceae	*Methylophaga frappieri; Methylophaga nitratireducenticrescens*

Significant differences in methanotrophs community composition were found among soils exposed to distinct land utilization patterns and lindane contaminated sites of Lucknow and Renukoot region. The lindane concentration and soil organic matter concentrations seem to have major effects on methanotrophs community composition and abundance. The lower number of methanotrophs community in soil of Lucknow region (Table 5.7 and Figure 5.11) compared to Renukoot region soil (Table 5.8 and Figure 5.12) could be due to higher concentration of lindane residues at the soil of former site. Given the fact that the dense vegetation covers at Renukoot site could be a critical factor for the greater abundance and community composition of soil methanotrophs compared to Lucknow site. Similar to this study the investigation of Chen et al. (2008) also demonstrated that the abundance and community composition size of methanotroph was decreased because of removal of the standing vegetation cover. The high proportion of physiologically distinct methanotrophs abundance in forest uplands (Renukoot) versus disturbed urban (Lucknow) soils clearly indicated that land use changes and anthropogenic disturbances could particularly select for a certain community of methanotrophs, possibly by altering ecological niches such as nutrient concentration and chemical (lindane) status of targeted bacteria.

5.3.4 Soil microbial biomass-C, -N and –P

Plant litters and soil microbial flora are the main drivers which play fundamental roles in controlling the ecosystem processes (Singh and Gupta, 2018). The variations in the size of SMB can impact the functioning of various ecosystem types (Singh et al., 2009). Differences in the quantity and quality of substrates (organic C and N) inputs might be due to varying in plant residues types/quantity as suggested by Singh and Gupta (2018). The data on SMB-C, -N, -P ($\mu g\ g^{-1}$ soil) across different study sites of

Lucknow and Renukoot region are presented in Table 5.9. ANOVA revealed significant variation ($P < 0.001$) in SMB-C, -N and –P values due to sites and being highest in soils of Renukoot region. The SMB-C quantity across the sites ranged from 102.7 (S1) to 389.3 (S5) µg g^{-1} soil. Similarly the SMB-N ranged from 15.8 (S3) to 69.1 (S5) µg g^{-1} soil. The values of SMB-P ranged between 12.2 (S3) to 32.9 (S5) µg g^{-1} soil (Table 5.9). A significant variation in values of SMB-C, -N, -P in both the study region may be because the organic contents and quality due to the variation in vegetation compositions and anthropogenic activities (Singh et al., 2010). Since the six selected sites and regions of present study varied in terms of soil physico-chemical properties, HCH and OCPs residues concentrations and vegetation composition therefore, significant variations in SMB–C, –N and –P contents among sites may be also expected. It possible that across Lucknow and Renukoot region that high SMB-C, -N and -P at S4, S5 and S6 sites of Renukoot region may be because of more suitable soil conditions, sufficient soil moisture contents, organic matters via availability of sufficient quantity of plant litters. The unfavourable soil moisture condition and low organic matter contents at S1, S2 and S3 sites of Lucknow region could suppress the SMB quantity too.

Table 5.9. Measured values of soil microbial biomass across different study sites. The values given are means of three replicates ±SE.

Soil microbial biomass (SMB) (μg g^{-1} soil)	Lucknow region			Renukoot region			ANOVA (N=18)
	S1	S2	S3	S4	S5	S6	
SMB-C	102.7±25.8^{c}	214.8±11.6^{d}	99.7±6.5^{a}	189.4±11.4^{b}	389.3±41.2^{d}	365.2±36.2^{d}	F=353.25, P<0.001
SMB-N	23.3±7.4^{d}	45.2±8.6^{c}	15.8±6.2^{b}	56.6±9.5^{a}	69.1±11.5^{e}	65.5±6.7^{e}	F=123.55, P<0.001
SMB-P	21.2±5.7b^{d}	23.8±9.8^{c}	12.2±9.69^{a}	22.3±7.8^{b}	32.9±8.3^{c}	26.8±5.4^{c}	F=98.56, P<0.001

S=site; Different letters indicate significant difference between treatments for each parameter (P < 0.05).

The selected sites (S1 to S6) distributed in Lucknow and Renukoot region varied significantly in terms of soil physico-chemical properties (total-C, -N, -P, moisture contents, pH, etc.), pesticides (HCH and OCPs) residues and SMB-C -N and -P values. The soils of Lucknow region sites, having higher amount of pesticides residues, may reduces the SMB-C -N and -P quantity compared to sites of Renukoot region. A higher litters and plant residues available at sites of Renukoot region due to dense natural forest soil as compared to Lucknow urban region sites, may considerably enhances the favourable soil conditions and therefore, a higher SMB-C, -N and -P values at sites of Renukoot region might be expected. A negative correlation between pesticides (lindane residues) concentrations and SMB-C, -N and -P suggests that higher HCH and OCPs present in soil may adversely influence the growth and multiplication of microbial diversity/biomass (Table 5.10). The results of this study strongly confirms that variations in soil physico-chemical properties and HCH and OCPs residues may significantly affects the SMB-C, -N and -P compositions. Therefore, removal of lethal HCH and OCPs residues from soils via bioremediation/ phytoremediation can be a viable and eco-friendly soil management practice to improve the beneficial species of soil microbial communities and biomass which may consequently support the soil, agriculture and environmental sustainability.

Table 5.10. Pearson's correlation (2-tailed) between pesticides and soil microbial biomass. Total soil samples analysed was N=18 (6 sites × 3 replicates).

Parameters ($\mu g\ g^{-1}$ soil)	α –HCH	β –HCH	γ –HCH	ALDRIN	PP-DDE	B-ENDO	PP-DDD	OP-DDT	PP-DDT	SMB-C	SMB-N	SMB-P
α –HCH	1.000											
β –HCH	0.724**	1.000										
γ –HCH	0.832**	0.359	1.000									
ALDRIN	-0.157	-0.255	0.570	1.000								
PP-DDE	-0.043	-0.221	-0.232	-0.452	1.000							
B-ENDO	0.625**	0.111	0.743**	-0.260	0.299	1.000						
PP-DDD	0.807**	0.217	0.937**	-0.077	0.450	0.841**	1.000					
OP-DDT	0.089	-0.050	-0.214	-0.384	0.629**	0.230	0.018	1.000				
PP-DDT	0.626	0.194	0.711**	-0.427	0.271	0.935**	0.818**	0.192	1.000			
SMB-C	-0.789**	-0.539**	-0.529*	0.550*	-0.449	-0.746**	-0.649**	-0.485*	-0.796**	1.000		
SMB-N	-0.664**	-0.158	-0.684*	0.364	-0.437	-0.913**	-0.830**	-0.374	-0.921**	0.862**	1.000	
SMB-P	-0.855**	-0.558*	-818**	0.141	0.006*	-0.805**	-0.800**	-0.079	-0.796**	0.802**	0.782**	1.000

*Significant at P<0.05; **significant at P<0.01

5.4 Conclusions

Present investigation indicates a wide variation in methanotrophs abundance/community composition and soil microbial biomass-C, -N and –P in soil samples of Lucknow and Renukoot region. The study showed that soil lindane contamination/concentration has the potential to decrease the methanotrophic species and SMB levels in soil having greater concentration of lindane residues. The soil microbial biomass-C, -N and –P and methanotrophic community and abundance were found to be higher in soils of ABCIL (Renukoot) as compared with IPL (Lucknow) region due to less anthropogenic disturbances, lower lindane residue contamination and greater forest cover in that particular area. The presence of high soil moisture and organic matter ultimately increased the count of soil methanotrophs numbers/diversity and SMB-C, -N and -P in soils of upland Renukoot forest region. Since soil parameters are indices of soil microbial community stability and ultimately control the ecosystem services. This study suggested that methanotrophs community abundance/diversity and SMB levels serve as potential ecological indicators of soil quality. More study based on advanced molecular approaches is required in these regions to find out the number/diversity of methanotrophs to minimize the lindane residue contamination and enhancement of SMB levels.

CHAPTER-6

Lindane tolerance level and degradation potential of methanotrophs at different environmental conditions

CHAPTER 6

LINDANE TOLERANCE LEVEL AND DEGRADATION POTENTIAL OF METHANOTROPHS AT DIFFERENT ENVIRONMENTAL CONDITIONS

6.1 Introduction

Lindane (γ-hexachlorocyclohexane or γ-HCH) is a cyclic, saturated and organochlorine pesticide (OCP) which is resistant to microbial degradation (Phillips et al., 2005; Manickam et al., 2008). Its presence in the environment may cause heath problems in living organisms via entering to the food web and chain (Szabo and Loccisano, 2012; Sheng et al., 2013). In spite of these it is still being produced by local and small scale industries for various purposes (Lal et al., 2010; Bajaj and Singh, 2015). Lindane and other HCH isomers contaminants are taken up by crop plants or leached into ground water (Wauchope et al., 1992). Therefore, removal of lindane residues from the contaminated soil and other ecosystems are need of the hour.

Microbial bioremediation is one of the cost effective option for biodegradation of pesticides present in soil and other environments. Many microbial species in the form of pure or mixed cultures have been reported to degrade lindane (Salam and Das, 2013). The investigation of lindane tolerant microorganisms are seems to be a potential bioresource for bioremediation in the field conditions. A wide range of microorganisms includes fungi (Rigas et al., 2007; McErlean et al. 2006) and bacteria (Gupta et al., 2000; Boltner et al., 2005) have potential in lindane isomers degradation. Methanotrophs, a group of unique bacteria are reported from diverse habitats, including soils, peat bogs, wetlands, sediments, lakes, fresh waters, and marine waters (Ge et al., 2014; Strong et al., 2015). The methanotrophic enzyme MMO catalyses conversion of

CH_4 to methanol and also co-metabolizes the TCE (Whittenbury et al., 1970; Oldenhuis et al., 1989; Dedysh et al., 2005). It has been reported that the MMO in methanotrophs exists as soluble methane monooxygenase (sMMO) and as particulate methane monooxygenase (pMMO) (Hanson and Hanson, 1996). Both forms of the MMO co-metabolize the TCE but sMMO is more capable in degrading the TCE (Lontoh and Semrau, 1998). In contrast to the above, no reports are available on methanotrophs and their use in lindane tolerance and degradation.

The environmental parameters such as temperature, pH, moisture, etc. can vary temporally and spatially which affect their proliferation and degradation potential with respect to various toxic compounds present in the environment (Siddique et al., 2002; Bastos and Magan, 2009; Abrusci et al., 2011). The previous studies related to lindane degradation mediated by different microbial species are limited only at mesophilic temperature (25 to 30 ºC) (Siddique et al., 2002; Mertens et al., 2006). The degradation capability of lindane degrading strains depends on various environmental factors (pH and temperature) (Quintero et al., 2005b). Many researchers have reported that pH and temperature can greatly influence the biodegradation ability of bacteria (Okeke et al., 2002; Quintero et al., 2005a). Generally, lindane degraded at wide range of pH and it was observed that most of the bacteria are able to reduce lindane either at pH 7.0 or near to neutral pH (6.5-7) (Rijnaarts et al., 1990). The *Methylosinus trichosporium* OB3b has been is reported to involve in co-metabolism of contaminants (Oldenhuis et al., 1991; Fitch et al., 1996), oxidation of propene (Hou et al., 1979) and synthesis of polyhydroxybutyrate (PHB) (Williams, 1988; Doronina et al., 2008). The *M. trichosporium* OB3b possesses two systems for methane oxidation, a pMMO, expressed under high biomass/copper ratios and a sMMO, expressed at low copper conditions (Hakemian and Rosenzweig, 2007; Semrau et al., 2010). Some methanotrophic strains capable in nitrogen fixation are reported (Oakley and Murrell, 1988; Auman et al., 2001). The study related to degradation potential of methanotrophs at different environmental conditions is almost lacking therefore, this study also explores the lindane degradation tolerant and potential at different environmental conditions (pH and temperature). This study also assesses the identification of end product during lindane degradation by methanotrophs isolated from soil of Lucknow and Renukoot region.

6.2 Material and methods

6.2.1 Chemicals and reagent

Lindane acetone, n-hexane, acetone, ethyl acetate, methanol, isopropanol, mercuric thiocynate, ferric alum solution and all chemicals were of analytical grade and were purchased from Sigma-Aldrich or other commercial sources.

6.2.2 Methanotrophs isolates

Out of 15 methanotrophs isolates (Chapter 5) from soil of Lucknow and Renukoot region, identified on the basis of biochemical tests, only three isolates SBRJS01, SBIJS02 and SBJS03 were selected for lindane tolerance and degradation potential at different environmental conditions. The *Methylosinus trichosporium* URRH3 was used as control methanotrophs.

6.2.3 Lindane tolerance potential of SBRJS01, SBIJS02 and SBJS03 isolates

The nitrate mineral salts (NMS) medium (composition of NMS medium is given in previous Chapter 5 and Table 5.5 was used to study the lindane tolerance level of SBRJS01, SBIJS02 and SBJS03 isolated and a control at varying lindane concentrations. The different lindane concentration solution was prepared by dissolving 0.05 g of lindane powder in 10 mL acetone as described by Pannu and Kumar (2014). The control URRH3 and SBRJS01, SBIJS02 and SBJS03 isolates were inoculated in a flask containing 100 mL NMS medium with the various concentration of lindane (10 mg L^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1}, 50 mg L^{-1} and 60mg L^{-1}) and incubated in at 30 $^{\circ}$C at 120 rpm and observes the turbidity. The OD_{600} was taken periodically up to one month using UV-visible spectrophotometer (SPECORD 210 Plus, Analytik Jena). The SBRJS01, SBIJS02 and SBJS03 isolates were finally selected for molecular identification. Subsequently chloride release during incubation from the same flasks was also estimated to monitor the lindane mineralization by methanotrophic isolates.

6.2.4 16S rRNA identification of lindane tolerant SBRJS01, SBIJS02 and SBJS03 isolates

The methanotrophic SBRJS01, SBIJS02 and SBJS03 isolates were identified by 16S rRNA sequencing. The genomic DNA of bacterial isolates, grown on NMS agar plates, was amplified using high-fidelity PCR polymerase. The 16S Forward primer and 16S Reverse primer were used for PCR amplification of the 16S rRNA. The genomic DNA template was amplified with a 35-cycle PCR initial denaturation at 95°C for 5 min, subsequent denaturation at 94°C for 30 sec, annealing temperature at 50°C for 30 second, extension temperature 72 °C for 1.30 minutes and final extensions at 72°C for 7 minutes. The PCR product was analyzed on 1% agarose gel and purified. The purified PCR products were sequenced by (Chromous Biotech, Pvt Ltd. Bangalore, India) on ABI 3500 Genetic Analyzer, using the Big Dye Terminator version 3.1”. The partial 16S rRNA gene sequence was compared with known sequence in the NCBI database by using BLASTn to identify the most similar sequence alignment. Similar sequence were downloaded and aligned with Muscle; a phylogenetic tree was constructed using UGPMA clustering method using MEGA software by Neighbor-joining Method (Kumar et al., 2016).

6.2.5 Dechlorination assay

Mineralization of lindane by methanotrophic isolates was examined by estimating the amount of chloride release. The Erlenmeyer flasks (250 mL) containing 100 mL of NMS medium added with varying concentrations of lindane (10 mg L^{-1}, 20 mg L^{-1} 30 mg L^{-1} 40 mg L^{-1} 50 mg L^{-1} and 60 mg L^{-1}) as a sole carbon source were inoculated with control and SBRJS01, SBIJS02 and SBJS03 isolates. The flasks were incubated for 30 days at 30 ^{o}C. The free chlorine released during incubation was measured on 20th day using the procedure of Phillips et al. (2001). In brief, 0.2 mL of ammonium iron (III) sulphate (0.25 M) was dissolved in nitric acid (9 M). The reagent was added to 2 mL aliquot of the culture supernatant, followed by the addition of 0.2 mL saturated solution of mercury (II) thiocyanate dissolved in ethanol. The chloride content was estimated using UV–Visible spectrophotometer (SPECORD 210 Plus, Analytik Jena) at 460 nm, NaCl solution was used as standard for estimation of chlorine. All analytical measurements were completed in triplicate.

6.2.6. Assessment of lindane degradation potential of identified methanotrophic strains at different environmental conditions (pH and temperature)

The optimal growth and lindane degradation conditions of SBRJS01, SBIJS02, SBJS03 isolates at different environmental conditions (pH and temperature) was determined in NMS medium amended with lindane (40 mg L^{-1}). A wide range of pH (5.0, 6.0, 7.0, 8.0 and 9.0) and temperature (20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC) was used to study the impact of environmental parameters on degradation of lindane by bacterial strains SBRJS01, SBIJS02 SBJS03. The acidic and alkaline pH of the medium was maintained by using hydrochloric acid and sodium hydroxide. The optical density of culture was observed on 20th day of incubation Spectrophotometrically at 600 nm and after a particular time interval to determine optimum growth and maximum lindane degradation at particular pH and temperature condition.

6.2.7. GC-MS/MS analysis of end product during lindane degradation by methanotrophic isolates

The metabolic end production resulted after lindane degradation by most efficient lindane tolerant methanotrophic isolate SBJS03 was extracted in acetone, n-hexane, ethyl acetate, methanol, and isopropanol under acidic conditions as described by Bharagava and Chandra (2009). To extract the metabolites, 10 mL NMS broth having methanotrophic isolate SBJS03 was acidified with 35% hydrochloric acid (HCL) and then placed in a separating funnel, after which an equal volume of ethyl acetate was added and the mixture was shaken continuously for 5 hours on a shaker (New Brunswick scientific). The extraction was repeated successively three times to complete extraction of metabolites. The extract was kept on a rotatory evaporator (Rotavapor RE 120, Buchi, Flawil, Sweden) at ≤ 40 ºC. An aliquot of the concentrate was dissolved in 3 mL of methanol, filtered through 0.22 µm syringe filters (Millipore Ltd., Bedford, MA, USA) and used for further GC-MS/MS analysis.

The 100 µL of sample extract was taken into 1.5 mL Eppendorf tube, to this 80 µL of methoxyamine hydrochloride and 100 µL of BSTFA were added and then these samples were kept in shaker (BRTH-100 and BR Biochem) at 65 ºC and 1400 rpm speed for 1 h. Samples were filtered through 0.22 µm syringe filter after incubation. Then the derivatized phase was transferred into GC-MS/MS vial. The GC-MS/MS was used for the identification of different unknown components present in the sample fraction. The sample was injected on DB-5MS (30 m × 0.25 mm ID, 0.25 µm film thickness) column. The helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1.2 mL minute^{-1} with split less mode, and 1 µL of sample was injected in the GC-MS/MS system. The injector port temperature was maintained at 150 $^{\circ}$C and the mass transfer line temperature was maintained at 290°C. The oven temperature was programmed at 65°C for 2 minute, with an increase of 6 $^{\circ}$C minute^{-1} to 230 $^{\circ}$C, then 10 $^{\circ}$C minute^{-1} to 290 $^{\circ}$C, ending with a 20 minute^{-1} hold. The solvent delay was 6.5 minute and the total GC-MS/MS running time was 55 minute with full scan mode. Various components were identified by their retention time and based on MS library research (NIST) has derived mass fragmentation pattern of all the fractions. GC-MS/MS method was used to detect the chemical constituents present in all the unknown samples

6.2.8. Statistical analysis

All the experiment set up and data analysis were performed in triplicate (n=3) to reduce analytical errors and the results were expresses as mean. SPSS software SPSS Statistics Version 20.0 (IBM, Armonk, NY, USA).

6.3. Results and Discussion

6.3.1. Lindane tolerance potential of SBRJS01, SBIJS02 and SBJS03 isolates

After 20 day of incubation the three methanotrophic isolates (SBRJS01, SBIJS02, and SBJS03) were monitored for their potential to withstand at different lindane concentrations (10 mgL^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1}, 50 mg L^{-1} and 60 mg L^{-1}) by inoculating into NMS broth media. Across different doses of lindane compared to control (*Methylosinus trichosporium* URRH3) the SBRJS01, SBIJS02 and SBJS03 showed maximum growth in terms of turbidity (Figure 6.1 and Figure 6.2) at 40 mg L^{-1} lindane concentration. The growth of all methanotrophs isolates was found to be diminished at higher lindane concentrations (50 and 60 mg L^{-1}). Similarly after 12-15 days of incubation other microbial organism also showed similar type of lindane degradation results in *Pseudomonas paucimobiliis* (Senoo and wada, 1989), fungus *Conidiobolus* 03-1-56 (Nagpal et al., 2008) and yeast *Rhodotorula* sp. VITJzNo3

(Salam et al., 2013). Higher concentrations of toxic contaminants like cadmium have been reported to inhibit the growth of other microbes too (Kumar et al., 2010).

Figure 6.1 Serum bottle containing NMS broth media, turbidity showing growth of methanotrophs.

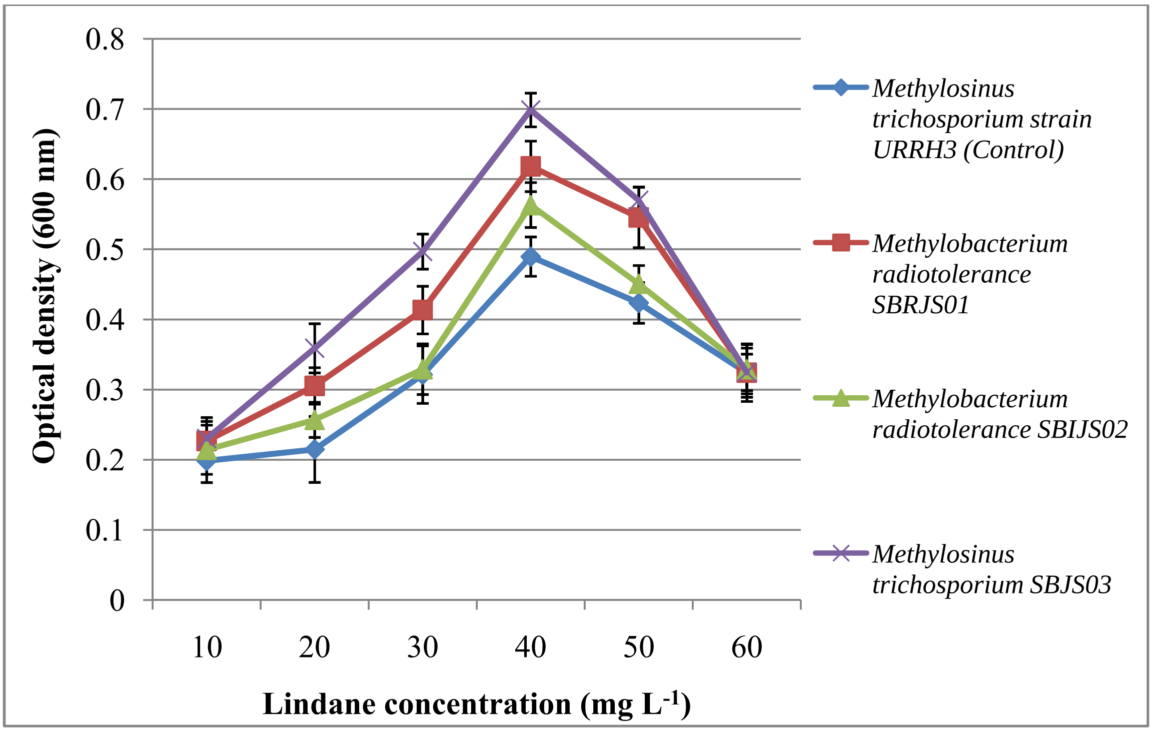


Figure 6.2 Methanotrophic isolates growth at different lindane concentrations.

6.3.2 16S rRNA identification of lindane tolerant SBRJS01, SBIJS02 and SBJS03 isolates

The methanotrophic isolates (SBRJS01, SBIJS02 and SBJS03) showing lindane (40 mg L^{-1}) degrading potential was subjected to 16S rDNA sequence analyses. The National centre of biotechnology information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used as basic local alignment search tool BLASTn to identify the most similar sequences. The comparative analysis of SBRJS01, SBIJS02 and SBJS03 DNA sequences showed close relationship to the known methanotrophs (already available NCBI database) *Methylobacterium radiotolerans, Methylobacterium radiotolerans* and *Methylosinus trichosporium* (**Figure 6.3**). The 16S rRNA sequences of SBRJS01, SBIJS02 and SBJS03 have been deposited in GenBank indicated SBRJS01 is a strain of *Methylobacterium* sp. (accession no. MK481040), SBIJS02 is a strain of *Methylobacterium radiotolerans* (accession no. MK481064) and SBJS03 is a strain of *Methylosinus trichosporium* (accession no. MK503991) (Table 6.1).

Table 6.1 The accession number of identified methanotrophic isolated based on 16S rRNA molecular analysis.

Methanotrophic isolates	Accession No.
Methylobacterium radiotolerans strain SBRJS01	MK481040
Methylobacterium radiotolerans strain SBIJS02	MK481064
Methylosinus trichosporium strain SBJS03	MK503991

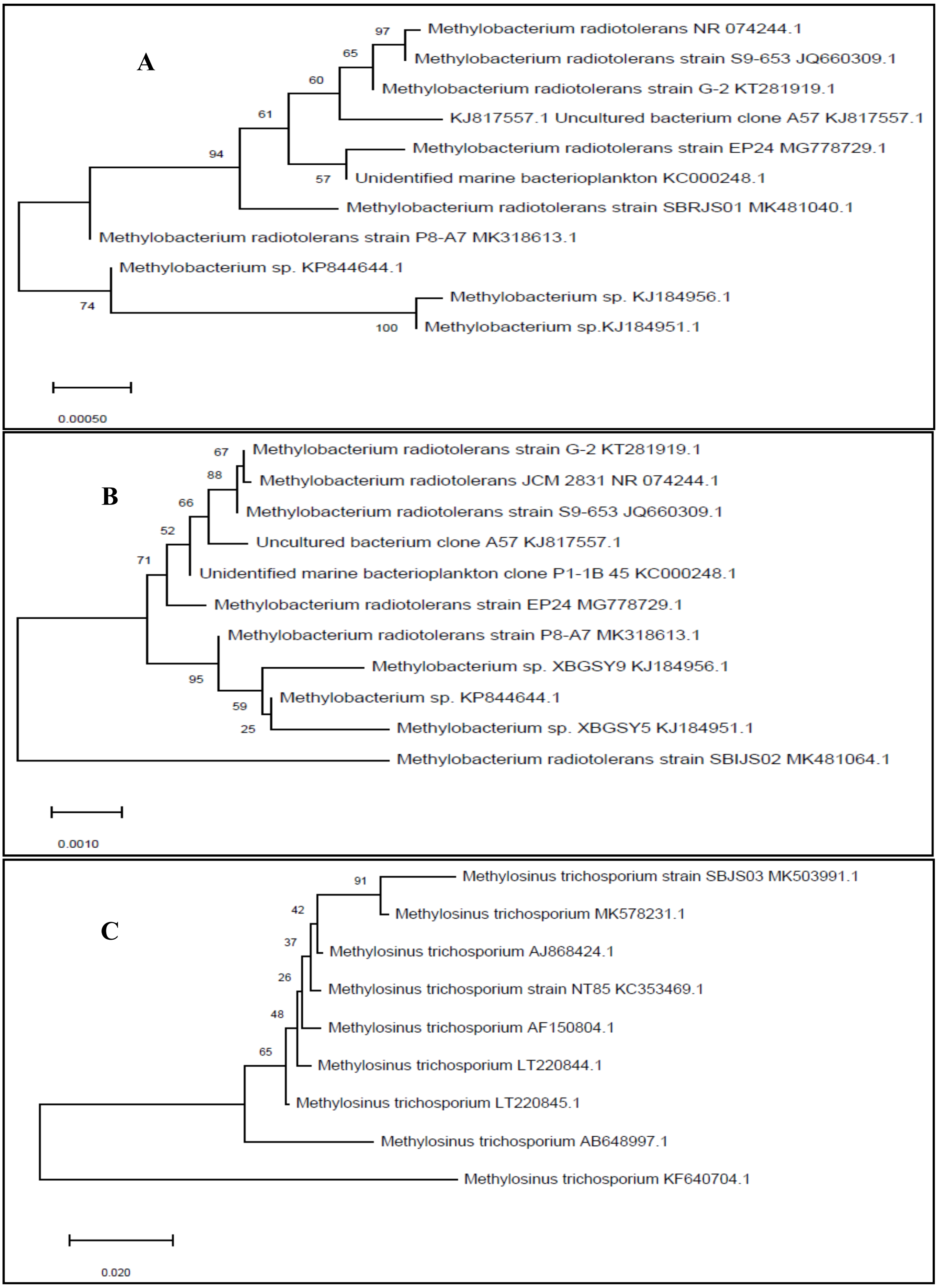


Figure 6.3 Phylogenetic tree of isolated bacteria strains and their related species based on 16S rDNA gene sequence (A): SBRJS01; (B): SBIJS02; (C): SBJS03

6.3.3 Dechlorination during lindane degradation by methanotrophic isolates

Dechlorination (removal of Cl^- atom from organic halogenic compounds) is the main reaction in microbial mediated degradation of lindane. In dechlorination, Cl^- atoms are substituted by hydrogen or hydroxyl group to the lindane (Camacho-Perez *et al*., 2012). In present study during lindane degradation, a decrease in pH was observed due to dechlorination of lindane. The coloured change in reaction mixture yellow to red was confirmed the release of Cl^- during lindane degradation process by the methanotrophic isolates (**Figure 6.4**).

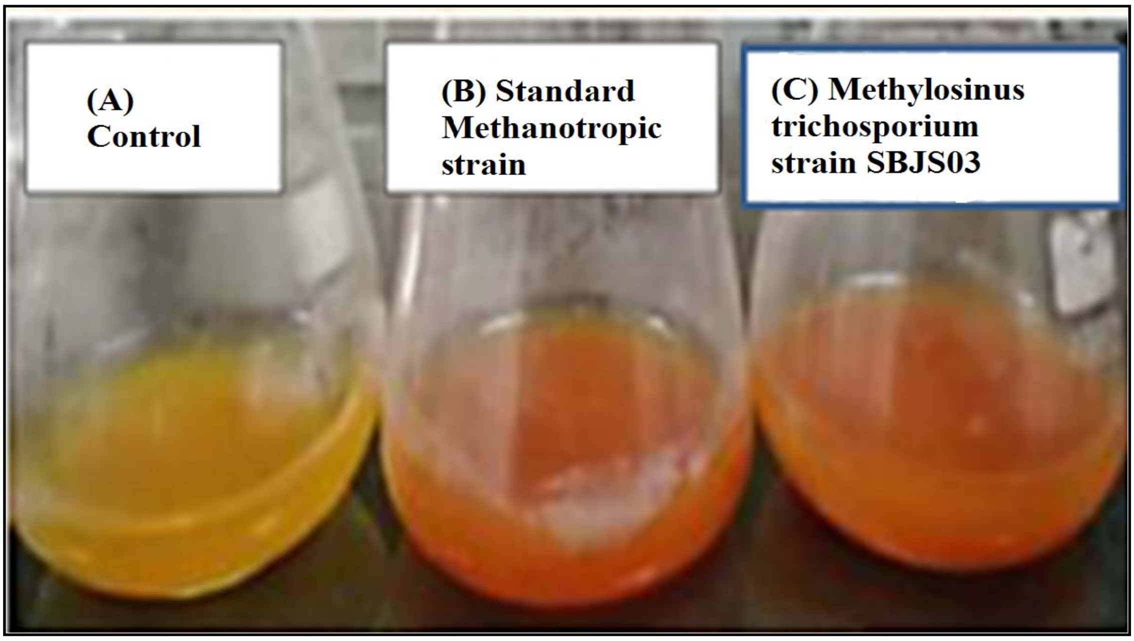


Figure 6.4 flask showing lindane degrading activity based on chlorine release. (A) showing negative while (B) and (C) showing positive result

The quantification of chloride ion concentration showed its gradual increase from 0 day to 20th day of incubation in experimental setup and no significant increase were observed in control. The maximum chlorine release and optical density (600 nm) was followed a trend as SBJS03 > SBRJS01 > SBIJS02 > Control URRH3 at 20 days of incubation (Figure 6.5 and Figure 6.6). These results depict that SBRJS01, SBIJS02 and SBJS03 isolated from lindane contaminated soil have potential to utilize lindane as source of carbon in place of CH_4 because during incubation no methane was added. Further, it was confirmed that the concentration of Cl ions in the NMS medium was measured highest at 20th days of incubation.

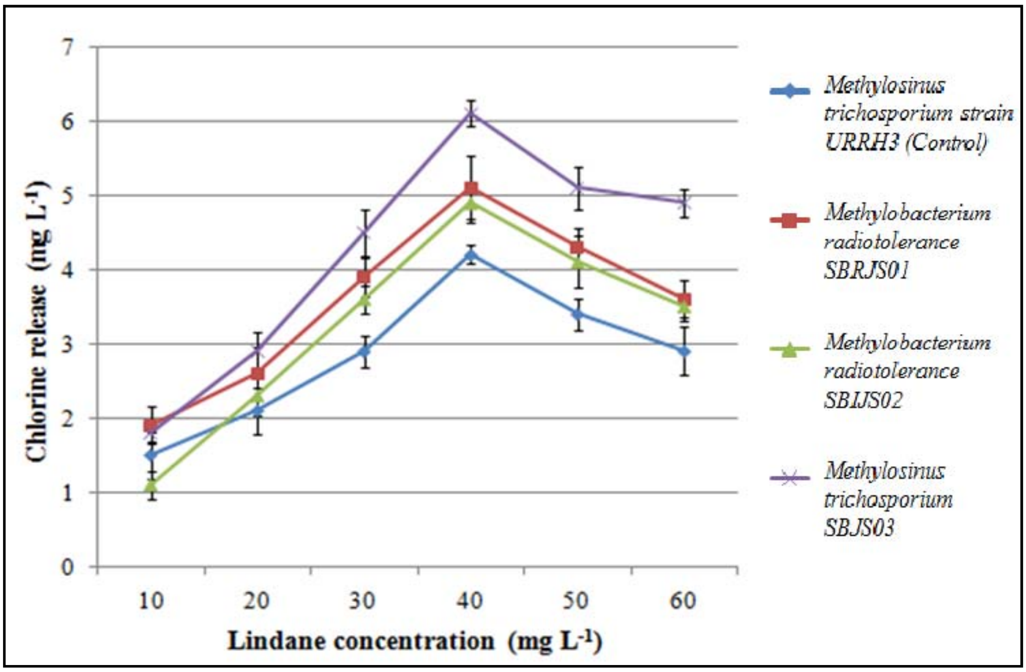


Figure 6.5 Graphical representation of chlorine release of different lindane concentrations

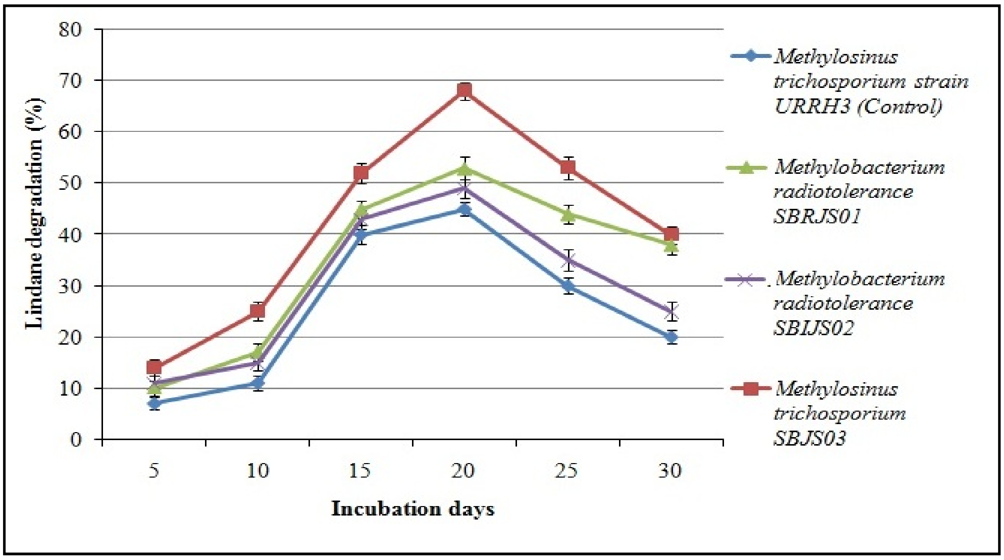


Figure 6.6 Lindane degradation by different methanotrophic isolates with 40 mg L^{-1} lindane on 20th day of incubation.

The maximum growth of methanotrophic isolates and lindane degradation potential on 20th day of incubation at 40 mg L^{-1} lindane concentration exhibited a linear relationship between lindane degradation and chlorine release (Figure 6.7).

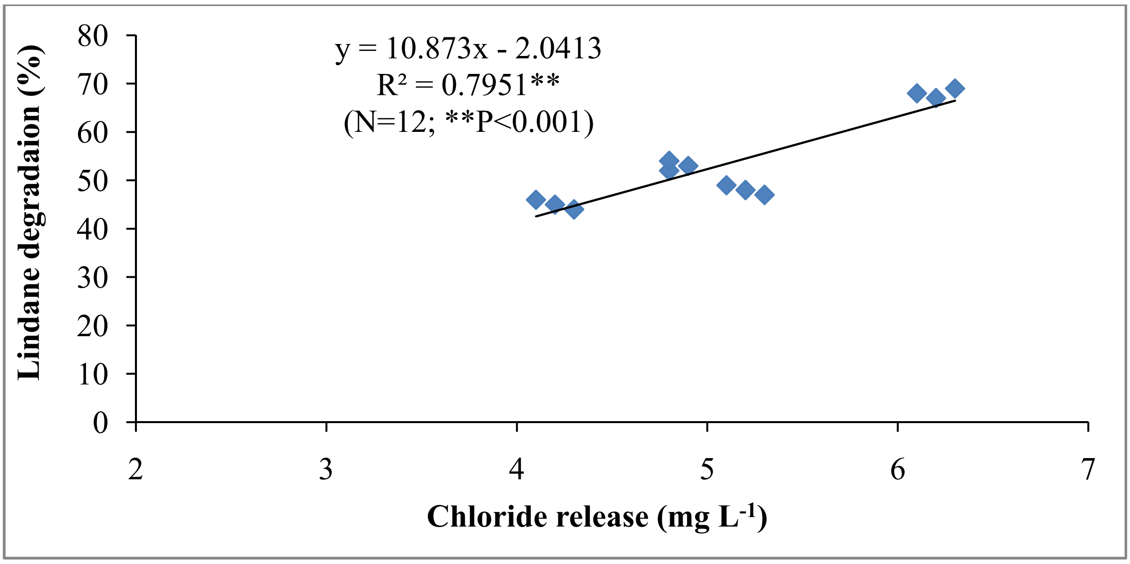


Figure 6.7 A linear relationship between lindane degradation and chlorine release by methanotrophic isolates with 40 mg L^{-1} lindane on 20th day of incubation.

The growth of nitrifying and denitrifying bacteria has been reported to be inhibited by the presence of lindane at varying concentrations (Saez et al., 2006). Severe toxicity of lindane on fishes and gilthead sea bream was demonstrated by Johnson and Finley (1980) and Oliva et al. (2008). The yeast strain (Candida VITJzN04), reported in a study was among the best alternative for lindane biodegradation with a half-life of 1.17 days (Salam and Das, 2014). Becerra-Castro et al. (2013) reported that after microbial inoculation decrease in HCH phytotoxicity was observed.

6.3.4 Lindane degradation potential of identified methanotrophic isolates at different environmental conditions (pH and temperature)

The pH and temperature has been found as important environmental parameters affecting the microbial growth, activity and degradation potential of toxins in laboratory and natural conditions. Effect of different environmental parameters like temperature (20–40°C) and pH (5–9) were studied to find out their effects on lindane degradation by methanotrophic isolates. The *Methylosinus trichosporium* strain URRH3 was used as reference control in all the experiments. The maximum degradation of lindane was obtained at neutral (pH 7.0) after 20 days of incubation time (Figure 6.8). But when the pH was increased the growth of methanotrophic isolates was decreased.

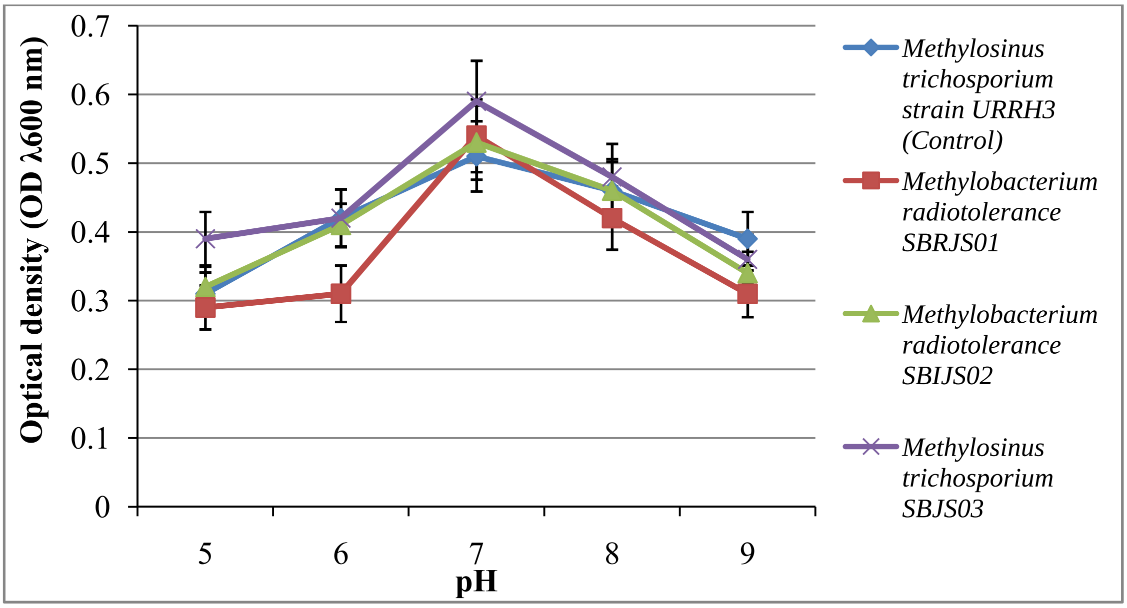


Figure 6.8 Effect of pH on growth of methanotrophic isolates with 40 mg L^{-1} lindane on 20th day of incubation.

When temperature was considered at environmental driver at 30 ºC maximum degradation of lindane was observed on 20th days of incubation time (Figure 6.9). However, when the temperature was increased the growth of different methanotrophic isolates was decreased significantly. Similar results were observed by Benemeli et al.

(2007) showing maximum lindane removal by *Streptomyces* sp. at pH 7 and temperature 30 ºC.

This suggested that as the pH and temperature increases from lower to higher values, the growth and lindane degradation potential of methanotrophic isolates were also decreases. The negative effect of higher pH and temperature on the lindane degradation activity of bacterial cultures was also demonstrated by several investigators (De Paolis et al., 2013; Salam and Das, 2014). The 30 ºC reported as optimum temperature for lindane reduction in present study is in conformity to the results of Quintero et al. (2005a). However, Nagata et al. (2007) reported 37 ºC temperature was optimum for lindane resistant *E. coli* strain. Further, the 30 ºC as incubation temperature has been found as optimum temperature for lindane degradation as reported by Zhang et al. (2010, 2012) and Salam et al. (2013).

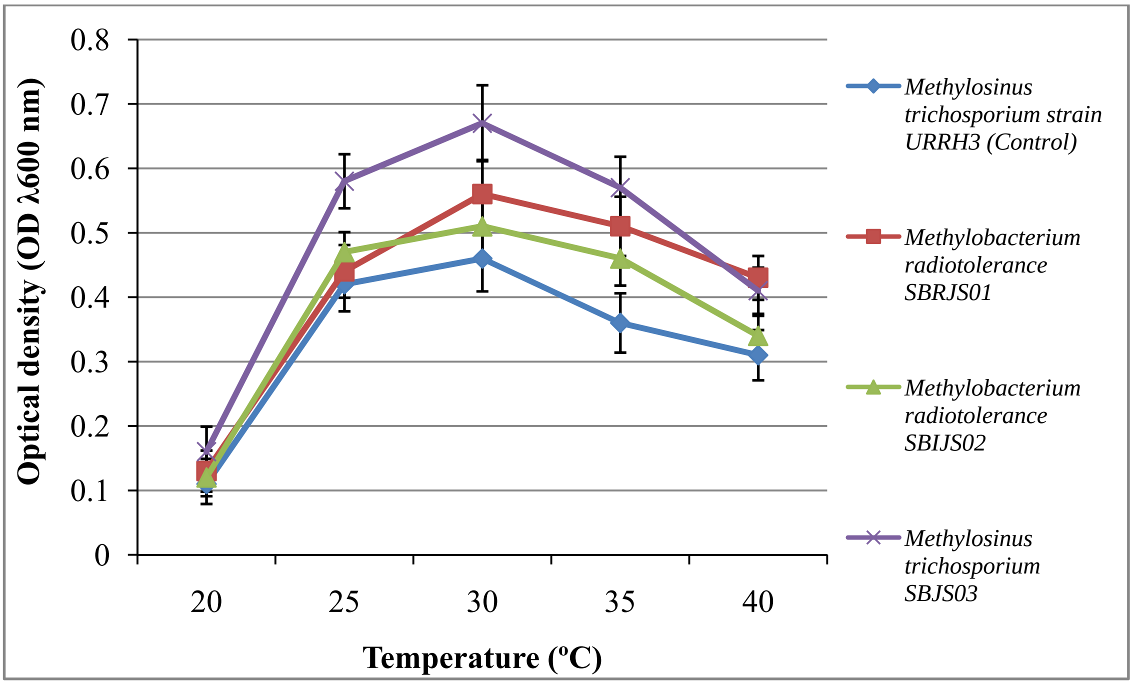


Figure 6.9 Effect of temperature on growth of methanotrophic isolates with 40 mg L^{-1} lindane on 20^{th} day of incubation.

6.3.5 GC-MS/MS Analysis

In order to investigate the end product during lindane degradation (after 20 days of incubation) by most tolerant methanotrophic isolate SBJS03, the resulted samples after degradation process were analyzed by GC-MS/MS technique. The results of identified compound present at different RT peaks as shown in Table 6.2.

Table 6.2 The results of identified compound present at different RT peaks by GC-MS/MS analyses

SNo	RT	Compounds identified by GC-MS/MS	Molecular formula
1.	6.17	1, 2-Bis (trimethylsiloxy) ethane	C8H22O2Si2
2.	9.72	N,O,O-tri (trimethylsilyl) -1–c (13)-N-carboxy-glycine	C8H21NO3Si2
3.	12.14	3,6,9-trioxa- 2, 10-disilaundecane, 2, 2, 10, 10-tetramethyl	C10H26O3Si2
4.	18.41	(+-)-5-Hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxynaph thoquinoneThoquinone,	C14H14O6
5.	25.08	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	C17H24O3
6.	27.31	Hexadecanoic acid, trimethylsilyl ester, Octadecanoic acid	C19H40O2Si
7.	30.21	Octadecanoic acid, trimethylsilyl ester	C21H44O2Si
8.	39.30	à-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic phenylboronate	C19H33BO6Si2
9.	48.13	D-Ribofuranose, 1,2,3-tris-O-(trimethylsilyl)-, bis(trimethylsilyl)	C26H14
10.	51.28	1-Demethoxy-1,2-dehydro-12,13-seco-16ápseudaconi n-3,14-dione	C24H33NO6

Unlike previous study (Mougin et al., 1996; Singh and Kuhad, 2000; Manickam et al., 2007), in present study there is no clear cut detection of end product after lindane degradation. Nagata et al. (2007) proposed degradation pathways of γ-HCH in *S. japonicum* UT26. Compounds: 1 γ-Hexachlorocyclohexane (γ-HCH), 2 pentachlorocyclohexene (γ-PCCH), 3 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN), 4 1,2,4-trichlorobenzene (1,2,4-TCB), 5 2,4,5-trichloro- 2,5-cyclohexadiene-1-ol (2,4,5-DNOL), 6 2,5-dichlorophenol (2,5- DCP), 7 2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2,5-DDOL), 8 2,5-dichlorohydroquinone (2,5-DCHQ), 9 chlorohydroquinone (CHQ), 10 hydroquinone (HQ), 11 acylchloride, 12 γ-hydroxymuconic semialdehyde, 13 maleylacetate (MA; 2-maleylacetate, 4-oxohex-2- enedioate), 14 β-ketoadipate (3-oxoadipate), 15 3-oxoadipyl-CoA, 16 succinyl-CoA, 17 acetyl-CoA, 18 2,6 dichlorohydroquinone (2,6- DCHQ), and 19 2-chloromaleylacetate (2-CMA). The TCA, citrate/tricarboxylic acid cycle; GSH, glutathione (reduced form); GS-SG, glutathione (oxidized form).

The *Methylosinus trichosporium* SBJS03 showed the lindane degradation potential which may be seen with clear peaks in GC-MS/MS chromatogram of cell extract as shown in Figure 6.10. The GC-MS/MS spectra showed that the lindane might be completely degraded therefore; final end product could not identified using the available library database. The presence of end product was observed in between 6.17 to 48.13 RT minutes. No any intermediate product was found as reported by in previous study. So, *Methylosinus trichosporium* SBJS03 was able to degrade lindane completely without forming any end product as per data available in The National Institute of Standards and Technology (NIST) library of high-quality database.

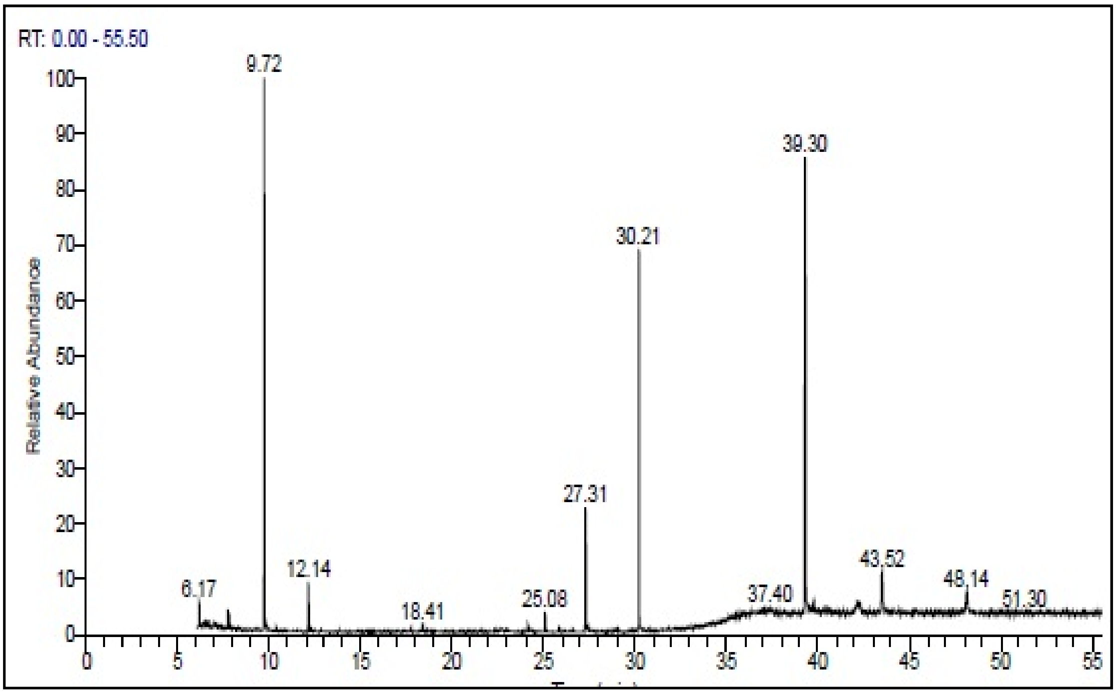


Figure 6.10. GC-MS chromatograms of the soil sample after degradation of lindane by methanotroph isolate SBJS03.

6.4 Conclusions

In present study the identified methanotrophic isolates SBRJS01, SBIJS02 and SBJS03 were tolerant to different lindane concentrations. Lindane concentration (60 mg L^{-1}) was toxic to bacteria because a significant decrease in growth rates was noted in almost all the methanotrophs isolates of present study. Among the methanotrophs isolates the maximum specific growth rate was noted in *Methylosinus trichosprium* SBJS03 strain at different environmental conditions and was positively correlated with maximum release of chlorine. So, *Methylosinus trichosprium* SBJS03 strain could be a good bioremediation tool for lindane residues and other related pesticides in soils. The methanotrophs strains investigated in the present study are highly recommended for bioremediation of soil systems contaminated by lindane and other chlorinated pesticides. The results from present investigation showed that across different study environmental parameters the pH (7.0) and temperature (30 ºC) was main factors affecting methanotrophs growth at 40 mg L^{-1} lindane concentration. Present study provided an example of a methanotrophs isolates that degrade lindane, thus extending the bioremediation potential of methanotrophs isolated from lindane contaminated soils.

CHAPTER-7

Correlation analysis between soil physico-chemical properties, lindane pesticides, methanotrophs diversity and soil microbial biomass

CHAPTER 7

CORRELATION ANALYSES BETWEEN SOIL PHYSICO-CHEMICAL PROPERTIES, LINDANE PESTICIDES, METHANOTROPHS DIVERSITY AND SOIL MICROBIAL BIOMASS

7.1 Introduction

As in introduction chapter it is already stated that the aim of this research work was to assess the status of soil physico-chemical soil properties, lindane residues concentrations, methanotrophs abundance and community composition and soil microbial biomass by selection of six different sites located in Lucknow and Renukoot region of Sonebhadra district of Uttar Pradesh. The results showed that above selected parameters varied significantly due to sites. The results in previous chapters showed that the main reasons for a significant variations in above study parameters at two different selected regions is due to variation in anthropogenic disturbances, soil organic matter composition and vegetation covers. The previous study showed that soil physico-chemical drivers and dominant vegetation covers at any sites significantly influence the microbial activity and their biomass in soils. However, reports on soil lindane residue concentrations and its relationship with methanotrophs diversity and soil microbial biomass are almost unknown. It is assumed that soil lindane resides levels and soil properties may influence the methanotrophs diversity/abundance and microbial biomass levels at different selected sites of Lucknow and Renukoot region. Therefore, this study also investigated correlation analyses between soil lindane pesticides concentrations, soil physico-chemical properties, methanotrophs diversity and soil microbial biomass levels.

7.2 Material and Methods

For correlation analyses total number of soil samples collected from Lucknow and Renukoot region was N=18 (6 sites × 3 replicates). The relationship between lindane and organochlorine pesticides, soil physico-chemical properties, SMB-C, -N, -P and methanotrophs were examined by Pearson correlation analyses. All the data in the experiment were processed and calculated using the SPSS version 20 (IBM, Armonk, NY, USA).

7.3 Results and Discussions

7.3.1 Correlation between organochlorine pesticide residues and soil physico-chemical properties

The results of correlation analyses between soil organochlorine pesticide residues and soil physico-chemical properties are shown in Table 7.1. The data showed negative relationship between pesticides concentrations and soil physico-chemical properties. But, no exact reason/information is available on how soil pollution/lindane residues specifically affect the soil physico-chemical properties. However, it is well reported that toxic soil pollutants may influence the beneficial soil microbial activity involved in decay decomposition and mineralization complex plant/animal residues and in turn may affect the soil nutrients organic-C, WHC, moisture contents, etc. it is also reported that soil having high organic contents may have greater WHC and moisture content. If the soils are polluted with toxic material like lindane pesticide, the microbial community involved in generation of organic matter contents may get adversely affected and in turn reduced WHC and moisture contents. It is suggested that the future research on soil physico-chemical properties as affected by the soil pollutants like lindane residues and its correlation with the soil functioning holds potential as the complementary criteria for evaluating the soil properties and pollutant relationships with rehabilitation progress in restoration ecology.

Table 7.1 Pearson correlation analyses (2-tailed) between organochlorine pesticides and soil physico-chemical parameters across different selected sites of Lucknow and Renukoot region.

Parameters ($\mu g\ g^{-1}$ soil)	α –HCH	β –HCH	γ –HCH	ALDRIN	PP-DDE	B-ENDO	PP-DDD	OP-DDT	PP-DDT	pH	Temp.	Moisture	EC	Total- C	Total-N	Total-P	NH_4-N
α –HCH	1.000																
β –HCH	0.724**	1.000															
γ –HCH	0.832**	0.359	1.000														
ALDRIN	-0.157	-0.255	0.057	1.000													
PP-DDE	-0.043	-0.221	-0.232	-0.452	1.000												
B-ENDO	0.625**	0.111	0.743**	-0.260	0.299	1.000											
PP-DDD	0.807**	0.217	0.937**	-0.077	0.045	0.841**	1.000										
OP-DDT	0.725	0.845	0.393	0.116	0.005	0.360	0.944	1.000									
PP-DDT	0.626**	0.194	0.711**	-0.427	0.271	0.935**	0.818**	0.192	1.000								
Soil pH	0.222	-0.026	0.438	0.066	-0.032	0.509*	0.482*	-0.251	0.537*	1.000							
Temperature (°C)	0.553*	0.548*	0.313	-0.089	-0.081	0.091	0.254	0.058	-0.003	-0.277	1.000						
Moisture content (%)	0.008	-0.259	0.359	-0.100	-0.110	0.585*	0.370	-0.200	0.579*	0.644**	-0.248	1.000					
EC ($\mu S\ cm^{-1}$)	-0.342	0.020	-0.435	0.527*	-0.485*	-0.839**	-0.597**	-0.313	-0.908**	-0.540*	0.197	-0.570*	1.000				
Total-C	-0.192	0.070	-0.300	0.533*	-0.472*	-0.707**	-0.442	-0.267	-0.770**	-0.477*	0.317	0.553*	0.925**	1.000			
Total-N	-0.376	-0.398	-0.182	0.558*	-0.230	-0.335	-0.219	-0.250	-0.404	-0.162	0.145	-0.056	0.371	0.322	1.000		
Total-P	0.257	-0.134	0.520*	0.123	0.022	0.574*	0.498*	0.076	0.527*	0.473*	-0.223	0.408	-0.536*	-0.440	-0.225	1.000	
NH_4-N	0.380	0.092	0.514*	-0.160	0.029	0.429	0.548*	-0.246	0.452	0.194	0.329	0.152	-0.300	-0.073	-0.020	0.187	1.000

*Significant at P<0.05; **Significant at P<0.01

7.3.2 Correlation between soil physico-chemical properties and soil microbial biomass

Across different study sites of Lucknow and Renukoot region correlation analysis between soil physico-chemical characteristics and SMB-C, -N and -P values shows positive correlation with total-C, -N and EC while negative with soil moisture content, pH, temperature, etc. (Table 7.2). A negative correlation between soil moisture and pH suggests that these soil factors have significant influence on the distribution SMB in soils of Lucknow and Renukoot region. Singh and Gupta (2018) reported that soil organic content is key environmental drivers that significantly influence the SMB in forest soils. Singh et al. (2010) noted that soil moisture content was negatively correlated with SMB values in soils of forest and agro-ecosystem of dry tropical region. Furthermore, the soil temperature and soil moisture contents are more crucial soil drivers that may impart effects on soil microbial population, activity and their biomass, therefore, in this study these soil drivers are negatively related with SMB. The soil moisture levels in any ecosystems may effectively enhance the nutrient and oxygen supply for the microbial growth, hence this can be considered as key controlling microbial activity in soil. Further the soil stresses and land degradation are directly correlated with loss of microbial diversity and abundance. Therefore, alleviating soil stresses on soil microbial communities due to ecological restoration may enhance the levels of SMB. It seems that the key ecological factors which stabilize the SMB levels may play an important role in the soil nutrient dynamics and ecosystem productivity. Because of the existing public concern about the deleterious impacts of soil pollution and degradation, there is an increasing interest in improving the understanding of soil microbial dynamics/diversity/biomass, and the way, it contributes in functioning and restoration of ecosystems.

Table 7.2 Pearson correlation (2-tailed) analyses between soil physico-chemical parameters and soil microbial biomass–C, –N and –P across different selected sites of Lucknow and Renukoot region.

Parameters ($\mu g\ g^{-1}$ soil)	Soil pH	Temperature	Moisture content (%)	EC ($\mu S\ cm^{-1}$)	Total-C	Total-N	Total-P	NH_4-N	SMB-C	SMB-N	SMB-P
Soil pH	1.000										
Temperature (°C)	-0.277	1.000									
Moisture content (%)	0.644**	-0.248	1.000								
EC ($\mu S\ cm^{-1}$)	-0.540*	0.197	-0.570*	1.000							
Total-C	-0.477*	0.317	-0.553*	0.925**	1.000						
Total-N	-0.162	0.145	-0.056	0.371	0.322	1.000					
Total-P	0.473*	-0.223	0.408	-0.536*	-0.440	-0.225	1.000				
NH_4-N	0.194	0.329	0.152	-0.300	-0.073	-0.020	0.187	1.000			
SMB-C	-0.251	-0.334	-0.105	0.740**	0.614**	0.464	-0.287	-0.282	1.000		
SMB-N	-0.445	-0.142	-0.373	0.867**	0.702**	0.326	-0.571*	-0.403	0.862**	1.000	
SMB-P	-0.408	-0.401	-0.351	0.597**	0.477*	0.204	-0.418	-0.345	0.802**	0.782**	1.000

*Significant at P<0.05; **Significant at P<0.01

7.3.3 Correlation between organochlorine pesticide residues and soil microbial biomass

In this study, a negative correlation between soil pesticides (HCH and OCPs) residues and SMB-C, -N and -P quantity was noted. Since the soil pesticides are lethal compounds and may adversely affects the microbial growth/biomass therefore, higher concentration of pesticides may show negative relationships with pesticides residues. The negative correlation between HCH and OCPs residues and SMB-C, -N and –P values indicates that an increase in the concentration of these soil pesticides resulted in a decrease in SMB values. However, the exact reason about negative relationship between HCH and OCPs residues with SMB-C, -N and –P is not known yet. Brantner and Senko, (2014) also reported that soil pesticide pollution negatively affect the microbial community and their activity in soil ecosystems. However, Sun et al. (2018) reported that OCPs soil pollution was not influential to soil functioning of agro-ecosystem in China. Saez et al., (2018) also demonstrated that microbial population was not affected by the soil lindane contamination. The presence of higher concentration of HCH and OCPs residues in soils may pose potential hazardous effects on soil microorganisms and can suppress the growth of microbes and their biomass. Martinez et al. (1993) reported that lindane significantly decreases the population of chemoautotrophic nitrifying bacteria, but it does not influence the population of heterotrophic bacteria. Rodriguz and Toranzos, (2003) reported a reduction in bacterial population and a reduced rate of substrate utilization when lindane (100 mg kg^{-1}) was applied artificially in soils. The negative relationship between HCH (α –HCH, β –HCH and γ –HCH) residues and SMB-C, -N and -P levels indicates that these HCH residues are potent pesticides and an increase in concentrations of these pesticides residues significantly reduces the soil microbial community and biomass.

Table 7.3. Pearson's correlation (2-tailed) between organochlorine pesticides and soil microbial biomass–C, –N and –P across different selected sites of Lucknow and Renukoot region.

Parameters ($\mu g\ g^{-1}$ soil)	α –HCH	β –HCH	γ –HCH	ALDRIN	PP-DDE	B-ENDO	PP-DDD	OP-DDT	PP-DDT	SMB-C	SMB-N	SMB-P
α –HCH	1.000											
β –HCH	0.724**	1.000										
γ –HCH	0.832**	0.359	1.000									
ALDRIN	-0.157	-0.255	0.570	1.000								
PP-DDE	-0.043	-0.221	-0.232	-0.452	1.000							
B-ENDO	0.625**	0.111	0.743**	-0.260	0.299	1.000						
PP-DDD	0.807**	0.217	0.937**	-0.077	0.450	0.841**	1.000					
OP-DDT	0.089	-0.050	-0.214	-0.384	0.629**	0.230	0.018	1.000				
PP-DDT	0.626	0.194	0.711**	-0.427	0.271	0.935**	0.818**	0.192	1.000			
SMB-C	-0.789**	-0.539**	-0.529*	0.550*	-0.449	-0.746**	-0.649**	-0.485*	-0.796**	1.000		
SMB-N	-0.664**	-0.158	-0.684*	0.364	-0.437	-0.913**	-0.830**	-0.374	-0.921**	0.862**	1.000	
SMB-P	-0.855**	-0.558*	-818**	0.141	0.006*	-0.805**	-0.800**	-0.079	-0.796**	0.802**	0.782**	1.000

*Significant at P<0.05; **Significant at P<0.01

7.4 Conclusions

The result shows that SMB-C, -N, and -P and soil physico-chemical properties are good indicators of the soil. Present study resulted that disturbances of soil properties in Lucknow have low SMB-C, -N, and -P, where as soil sample of Renukoot region have higher SMB comparatively to Lucknow soil because of forest area less disturbed soil. It is evident that over time recycling of inorganic and organic nutrient via litter returns significantly increases the soil organic matter content and improves the soil methanotrophs abundance and SMB status of undisturbed natural forest. Present study also indicate that soil methanotrophs abundance and SMB levels decreases with soil depth, which is likely linked to decreased soil organic/nutrient matter, soil moisture availability. Thus, it is clear that soil lindane residue concentrations, physico-chemical properties affect soil methanotrophs abundance/diversity and SMB values.

The results suggested that disturbance in soil physico-chemical condition due to forest cutting by anthropogenic activity may lead to disturbances in soil moisture, WHC, organic matter and consequently showed a negative relationship with the SMB values. It is clear that different plant species via their quality and quantity of litter inputs, strongly affect the soil methanotrophs community and SMB level can serve as potential key ecological indicators of soil quality. This research recommended a plantation with suitable naive broad -leaved species to restore the soil methanotroph community and SMB levels of degraded nutrient poor soils of Lucknow region and other parts of India. This study suggests that continuous routine assessment and monitoring of HCH, OCPs and other lethal pesticides in the Lucknow, Renukoot and other regions is essential for the control, prevention and to minimize contamination and health risk of concerned people. Further, the results of this study confirmed that variations in soil physico-chemical properties and HCH and OCPs residues may significantly affect the SMB-C, -N and –P compositions. Therefore, removal of lethal HCH and OCPs residues from soils via bioremediation/ phytoremediation can be a viable and eco-friendly soil management practice to improve the beneficial species of soil microbial communities and biomass which may consequently support the soil, agriculture and environmental sustainability.

CHAPTER-8
Summary

CHAPTER 8

SUMMARY

In present study, two study regions namely Lucknow (having IPL Chinhat, Chakhar village and Maati village) and Renukoot (having Adjacent to ABCIL, Sheo Park Bazar and Donki Nala) located in Uttar Pradesh state of India, were considered with following objectives:

1. To analyse the lindane residues in collected soil samples from lindane contaminated sites/area.
2. To study the soil methanotrophs compositions and microbial biomass-C, -N and -P from lindane contaminated sites.
3. To monitor the methanotrophic strains for lindane degradation tolerance level.
4. To assess the lindane degradation potential of identified methanotrophic strains at different environmental conditions.
5. To examine the end products during lindane degradation by methanotrophs in laboratory conditions.

Soil physico-chemical properties

The results revealed that soil samples collected from Lucknow and Renukoot regions are slightly acidic in nature with pH values ranged from 6.1 to 6.9. The moisture content (%) for both the study region varied from 2.77 to 6.90 %. The electrical conductivity (EC) was found between 305.2 to 676.6 µS cm^{-1}across the study sites. Across the study sites, the total-C was found to be maximum (11, 250.5 µg g^{-1} soil) in soil samples of Renukoot region and minimum (6,105.2 µg g^{-1} soil) in soils of Lucknow region. Similarly, the total-N was measured maximum (705.6 µg g^{-1} soil) at S5 (Renukoot site) and minimum (500.5 µg g^{-1} soil) at S1 (Lucknow site), respectively.

Across study sites the total-P was found minimum (567.5 μg g^{-1}) at S1 and maximum (766.2 μg g^{-1}) at S5 respectively, Lucknow and Renukoot sites. The soil NH_4-N content was found maximum in S6 (17.2 ± 1.9 μg g^{-1}) site of Renukoot region and minimum at S1 (6.8 ± 0.83 μg g^{-1}) site of Lucknow region. The higher total–C, –N and –P in the soils of Renukoot region compared to Lucknow region could be because soils of Renukoot region receives more plant litters via forest vegetation compared to Lucknow urban area.

Lindane residues

The GC and GC-MS/MS analysis of collected soil samples showed that the pesticides residues of HCH were found in collected soils of both the Lucknow and Renukoot region. The results confirm the presence of HCH isomers and OCPs such as α-HCH, β-HCH, γ-HCH, Aldrin, PP-DDE, β- Endo, PP-DDD, OP- DDT and PP- DDT in soil both the regions. The HCH residues in soil samples in Lucknow region were found comparatively higher concentration as compared to Renukoot region and varied significantly due to sites. Among the HCH isomers, the concentration of α-HCH and γ-HCH were found higher in soil samples of Lucknow region and varied from 309.97 and 182.12 μg g^{-1} soil, respectively. The β-HCH was highest (169.15 μg g^{-1} soil) at S4 while lowest (5.93 ± 1.05 μg g^{-1} soil) at S5 site. The Aldrin was highest (0.02 μg g^{-1} soil) at S6 and lowest (0.006 μg g^{-1} soil) in soil of S4 site. The low concentration of pesticide residues in soils of Renukoot region compared to Lucknow region could be low anthropogenic interference because of lesser dense human population and agriculture activities. The results also showed that Folpet (fungicide) was also detected in soil of Lucknow region. The Captan compound was also detected in soils of Renukoot region. In Lucknow region on RT- 21.81, α-lindane and on RT 28.18 Captan, on RT 28.35 Folpet was confirmed by GC-MS/MS and at Renukoot region on RT-

28.18 Captan was detected. All these results shows that the soil samples of sites S1 and S2, S3 were also contaminated with the persistent organic pollutants.

Methanotrophs community composition

The cream and pink colour colonies on NMS (nitrate mineral salt media) specific media for the isolation of methanotrophs confirmed the growth of aerobic methanotrophs. After several rounds of streaking from plate to plate, pure colonies were obtained on the plates. The colonies were observed to be cream or pink in colour, round and measuring about 1 mm in diameter. The cells were found to be motile and Gram-stain negative. Different morphology of methanotrophs was observed during cultivation period. After incubation period (2-3 weeks), the methanotrophic bacterial growth on Petri plates was observed which indicates the presence of methanotrophs in lindane contaminated site of Lucknow and Renukoot region. The result demonstrated that methanotrophic population in Renukoot region was significantly higher than Lucknow region due to large forest area and soil having higher amount of organic contents which contributes growth of methanotrophs in well aerated soil. Out of these 15 methanotrophic isolates only three (SBRJS01, SBIJS02 and SBJS03) potential isolates were selected for further study.

Metagenomics is used for the detection, identification and relative quantification of environmental microorganisms. The growing accessibility of next generation DNA sequencing (NGS) methods has greatly advanced our understanding of microbial diversity in medical and environmental science. In this study, high-throughput metagenomic sequencing provided a powerful strategy to investigate the methanotrophs community structure and functional potential associated in lindane contaminated soil. Alpha diversity is a measurement of richness and relative abundance of bacteria within the sample.

The contaminated soil contained 44,946 reads having 42,981 classified and 1,965 non-classified reads of bacterial population. The metagenomics of soil sample showed that the total number of methanotrophs present in selected site was 676. Further, the methanotrophic community composition in lindane contaminated Renukoot soil. Significant differences in methanotrophs community composition were found among soils exposed to distinct land utilization patterns and lindane contaminated sites of Lucknow and Renukoot region. The lindane concentration and soil organic matter concentrations seem to have major effects on methanotrophs community composition and abundance. The lower number of methanotrophs community in soil of Lucknow region compared to Renukoot region soil (Chapter 5; Table 5.8 and Figure 5.11) could be due to higher concentration of lindane residues at the soil of former site. Given the fact that the dense vegetation covers at Renukoot site could be a critical factor for the greater abundance and community composition of soil methanotrophs compared to Lucknow site. Similar to this study the investigation of Chen et al. (2008) also demonstrated that the abundance and community composition size of methanotroph was decreased because of removal of the standing vegetation cover. The high proportion of physiologically distinct methanotrophs abundance in forest uplands (Renukoot) versus disturbed urban (Lucknow) soils clearly indicated that land use changes and anthropogenic disturbances could particularly select for a certain community of methanotrophs, possibly by altering ecological niches such as nutrient concentration and chemical (lindane) status of targeted bacteria.

Soil microbial biomass

The data on SMB-C, -N, -P (μg g^{-1} soil) across different study sites of Lucknow and Renukoot region are presented in Table 5.9. ANOVA revealed significant variation ($P < 0.001$) in SMB-C, -N and –P values due to sites and being highest in soils of

Renukoot region. The SMB-C quantity across the sites ranged from 102.7 (S1) to 389.3 (S5) µg g^{-1} soil. Similarly the SMB-N ranged from 15.8 (S3) to 69.1 (S5) µg g^{-1} soil. The values of SMB-P ranged between 12.2 (S3) to 32.9 (S5) µg g^{-1} soil (Table 5.9). A significant variation in values of SMB-C, -N, -P in both the study region may be because the organic contents and quality due to the variation in vegetation compositions and anthropogenic activities (Singh et al., 2010). Since the six selected sites and regions of present study varied in terms of soil physico-chemical properties, HCH and OCPs residues concentrations and vegetation composition therefore, significant variations in SMB–C, –N and –P contents among sites may be also expected. It possible that across Lucknow and Renukoot region that high SMB-C, -N and -P at S4, S5 and S6 sites of Renukoot region may be because of more suitable soil conditions, sufficient soil moisture contents, organic matters via availability of sufficient quantity of plant litters. The unfavourable soil moisture condition and low organic matter contents at S1, S2 and S3 sites of Lucknow region could suppress the SMB quantity too.

The selected sites (S1 to S6) distributed in Lucknow and Renukoot region varied significantly in terms of soil physico-chemical properties (total-C, -N, -P, moisture contents, pH, etc.), pesticides (HCH and OCPs) residues and SMB-C -N and -P values. The soils of Lucknow region sites, having higher amount of pesticides residues, may reduces the SMB-C -N and -P quantity compared to sites of Renukoot region. A higher litters and plant residues available at sites of Renukoot region due to dense natural forest soil as compared to Lucknow urban region sites, may considerably enhances the favourable soil conditions and therefore, a higher SMB-C, -N and -P values at sites of Renukoot region might be expected. A negative correlation between pesticides (lindane residues) concentrations and SMB-C, -N and -P suggests that higher HCH and OCPs present in soil may adversely influence the growth and multiplication of microbial diversity/biomass (Chapter 5; Table 5.10). The results of this study strongly confirms that variations in soil physico-chemical properties and HCH and OCPs residues may significantly affects the SMB-C, -N and -P compositions. Therefore, removal of lethal HCH and OCPs residues from soils via bioremediation/ phytoremediation can be a viable and eco-friendly soil management practice to improve the beneficial species of soil microbial communities and biomass which may consequently support the soil, agriculture and environmental sustainability.

Tolerance level and degradation potential of methanotrophs at different environmental conditions

After 20 day of incubation the three methanotrophic isolates (SBRJS01, SBIJS02, and SBJS03) were monitored for their potential to withstand at different lindane concentrations (10 mgL^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1}, 50 mg L^{-1} and 60 mg L^{-1}) by inoculating into NMS broth media. Across different doses of lindane compared to control (*Methylosinus trichosporium* URRH3) the SBRJS01, SBIJS02 and SBJS03 showed maximum growth in terms of turbidity (Chapter 6; Figure 6.1 and Figure 6.2) at 40 mg L^{-1} lindane concentration. The growth of all methanotrophs isolates was found to be diminished at higher lindane concentrations (50 and 60 mg L^{-1}).

The methanotrophic isolates (SBRJS01, SBIJS02 and SBJS03) showing lindane (40 mg L^{-1}) degrading potential was subjected to 16S rDNA sequence analyses. The National centre of biotechnology information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used as basic local alignment search tool BLASTn to identify the most similar sequences. The comparative analysis of SBRJS01, SBIJS02 and SBJS03 DNA sequences showed close relationship to the known methanotrophs (already available NCBI database) *Methylobacterium radiotolerans, Methylobacterium radiotolerans* and *Methylosinus trichosporium* (Chapter 6; Figure 6.3). The 16S rRNA sequences of SBRJS01, SBIJS02 and SBJS03 have been deposited in GenBank indicated SBRJS01 is a strain of *Methylobacterium* sp. (accession no. MK481040), SBIJS02 is a strain of *Methylobacterium radiotolerans* (accession no. MK481064) and SBJS03 is a strain of *Methylosinus trichosporium* (accession no. MK503991).

Dechlorination (removal of Chlorine atom from organic halogenic compounds) is the main reaction in microbial mediated degradation of lindane. In dechlorination, Cl atoms are substituted by hydrogen or hydroxyl group to the lindane (Camacho-Perez *et al*., 2012). In present study during lindane degradation, a decrease in pH was observed due to dechlorination of lindane. The coloured change in reaction mixture red to yellow was confirmed the release of CL during lindane degradation process by the methanotrophic isolates (Chapter 6; Figure 6.4). The quantification of chloride ion concentration showed its gradual increase from 0 day to 20^{th} day of incubation in experimental setup and no significant increase were observed in control. The maximum chlorine release and optical density (600 nm) was followed a trend as SBJS03 > SBRJS01 > SBIJS02 > Control URRH3 at 20 days of incubation (Chapter 6; Figure 6.5 and 6.6). These results depict that SBRJS01, SBIJS02 and SBJS03 isolated from lindane contaminated soil have potential to utilize lindane as source of carbon in place of CH_4 because during incubation no methane was added. Further, it was confirmed that the concentration of Cl ions in the NMS medium was measured highest at 20^{th} days of incubation. The maximum growth of methanotrophic isolates and lindane degradation potential on 20^{th} day of incubation at 40 mg L^{-1} lindane concentration exhibited a linear relationship between lindane degradation and chlorine release (Chapter 6; Figure 6.7).

The pH and temperature has been found as important environmental parameters affecting the microbial growth, activity and degradation potential of toxins in laboratory and natural conditions. Effect of different environmental parameters like temperature (20–40°C) and pH (5–9) were studied to find out their effects on lindane degradation by methanotrophic isolates. The *Methylosinus trichosporium* strain URRH3 was used as reference control in all the experiments. The maximum degradation of lindane was obtained at neutral (pH 7.0) after 20 days of incubation time (Chapter 6; Figure 6.8**)**. But when the pH was increased the growth of methanotrophic isolates was decreased. When temperature was considered at environmental driver at 30 ºC maximum degradation of lindane was observed on 20th days of incubation time (Figure 6.9). However, when the temperature was increased the growth of different methanotrophic isolates was decreased significantly. Similar results were observed by Benemeli et al. (2007) showing maximum lindane removal by *Streptomyces* sp. at pH 7 and temperature 30 ºC. This suggested that as the pH and temperature increases from lower to higher values, the growth and lindane degradation potential of methanotrophic isolates were also decreases.

In order to investigate the end product during lindane degradation (after 20 days of incubation) by most tolerant methanotrophic isolate SBJS03, the resulted samples after degradation process were analyzed by GC-MS/MS technique. The results showed the presence of lindane isomers at different RT peaks as shown in Chapter 6 (Table 6.2). The *Methylosinus trichosporium* SBJS03 showed the lindane degradation potential which may be seen with clear peaks in GC-MS/MS chromatogram of cell extract as shown in Chapter 6 (Figure 6.10). The GC-MS/MS spectra showed that the lindane might be completely degraded therefore; final end product could not identified using the available library database. The presence of end product was observed in between 6.17

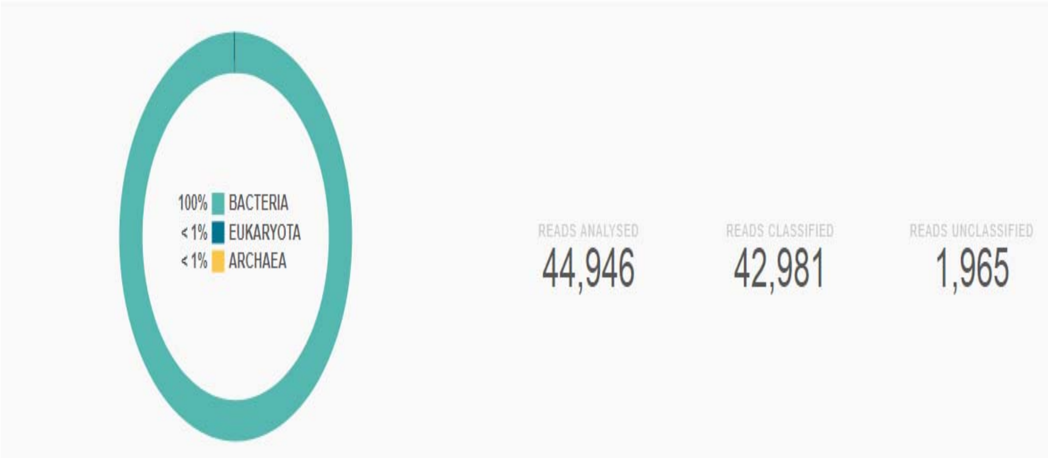
to 48.13 RT minutes. No any intermediate product was found as reported by in previous study. So, *Methylosinus trichosporium* SBJS03 was able to degrade lindane completely without forming any end product as per data available in The National Institute of Standards and Technology (NIST) library of high-quality database.

In present study the identified methanotrophic isolates SBRJS01, SBIJS02 and SBJS03 were tolerant to different lindane concentrations. Lindane concentration (60 mg L^{-1}) was toxic to bacteria because a significant decrease in growth rates was noted in almost all the methanotrophs isolates of present study. Among the methanotrophs isolates the maximum specific growth rate was noted in *Methylosinus trichosprium* SBJS03 strain at different environmental conditions and was positively correlated with maximum release of chlorine. So, *Methylosinus trichosprium* SBJS03 strain could be a good bioremediation tool for lindane residues and other related pesticides in soils. The methanotrophs strains investigated in the present study are highly recommended for bioremediation of soil systems contaminated by lindane and other chlorinated pesticides. The results from present investigation showed that across different study environmental parameters the pH (7.0) and temperature (30 ºC) was main factors affecting methanotrophs growth at 40 mg L^{-1} lindane concentration. Present study provided an example of a methanotrophs isolates that degrade lindane, thus extending the bioremediation potential of methanotrophs isolated from lindane contaminated soils.

Appendix

APPENDIX

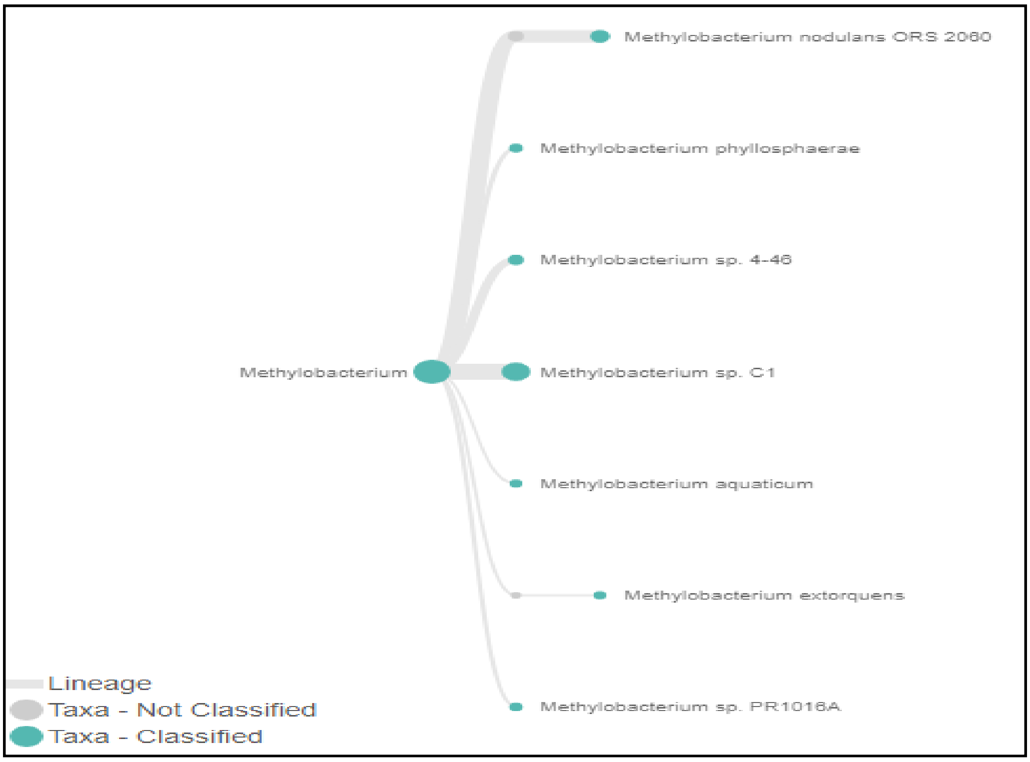
METHANOTROPHIC COMMUNITY STRUCTURE FROM LINDANE CONTAMINATED SITE

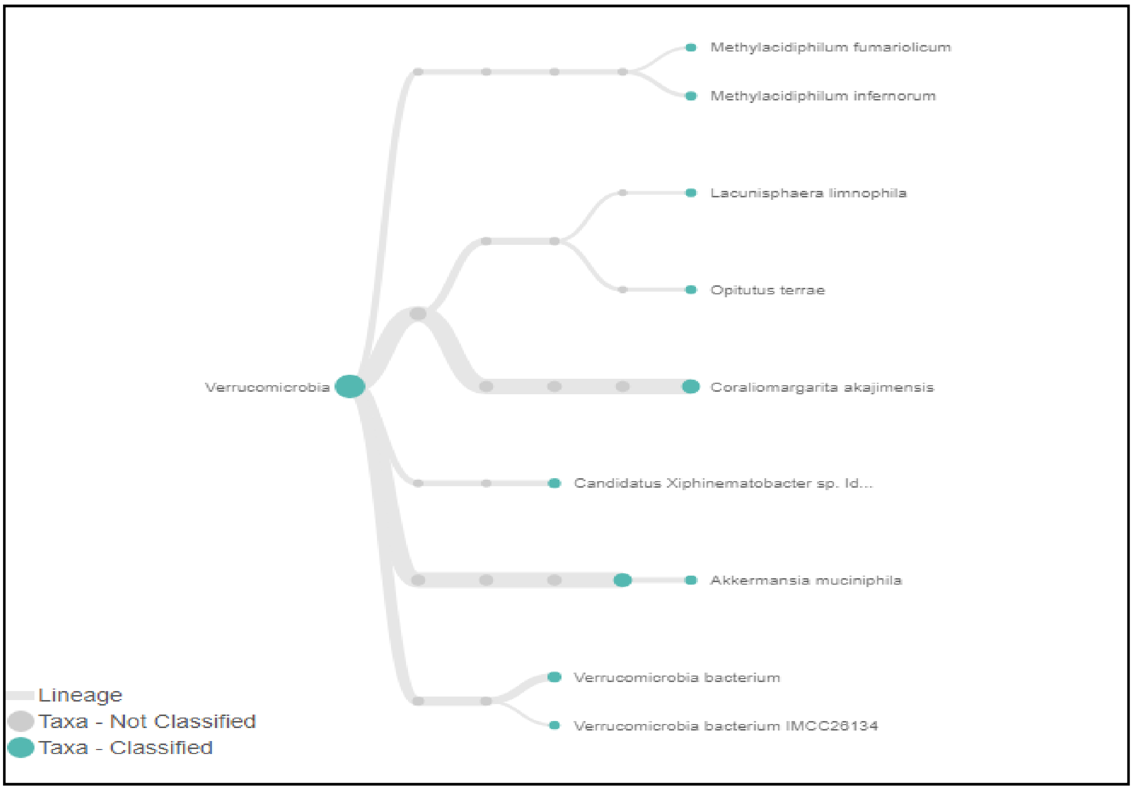


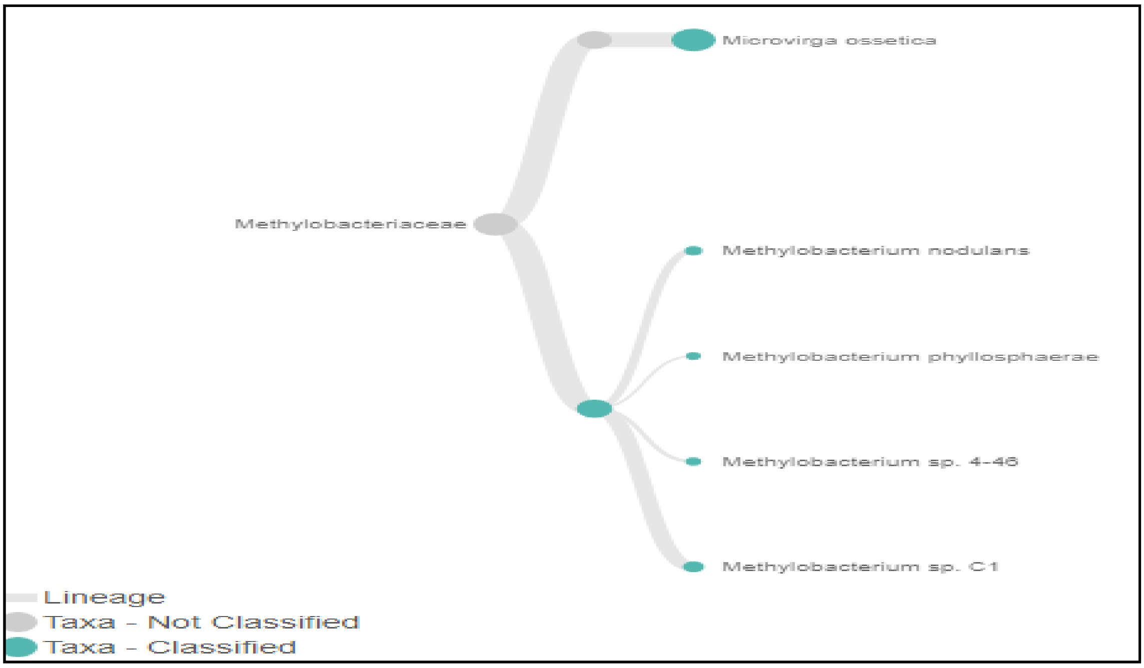
Taxon	Cumulative Reads
Methylophaga	676
Methylobacterium	380
Methylocystis	72
Methyloceanibacter	40
Methylosinus	31
Methylomonas	31
Methylomicrobium	19
Methylibium	18
Methylotenera	14
Methylococcus	13
Methylocella	11
Methylacidiphilum	8

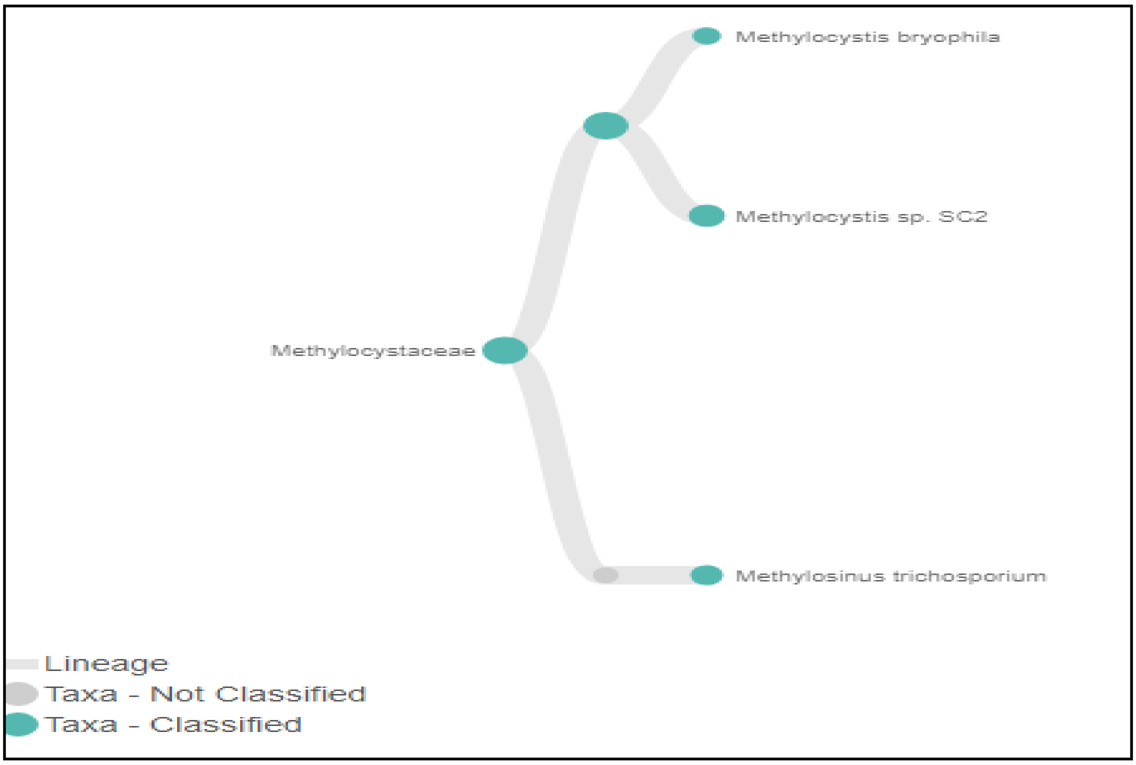
Taxon	Cumulative Reads
Methylophaga nitratireducenticrescens	367
Methylophaga frappieri	268
Methylobacterium sp. C1	123
Methylobacterium nodulans	57
Methyloceanibacter caenitepidi	40
Methylocystis sp. SC2	38
Methylosinus trichosporium	31
Methylobacterium sp. 4-46	26
Methylocystis bryophila	24
Methylomicrobium alcaliphilum	19
Methylococcus capsulatus	13
Methylomonas denitrificans	12

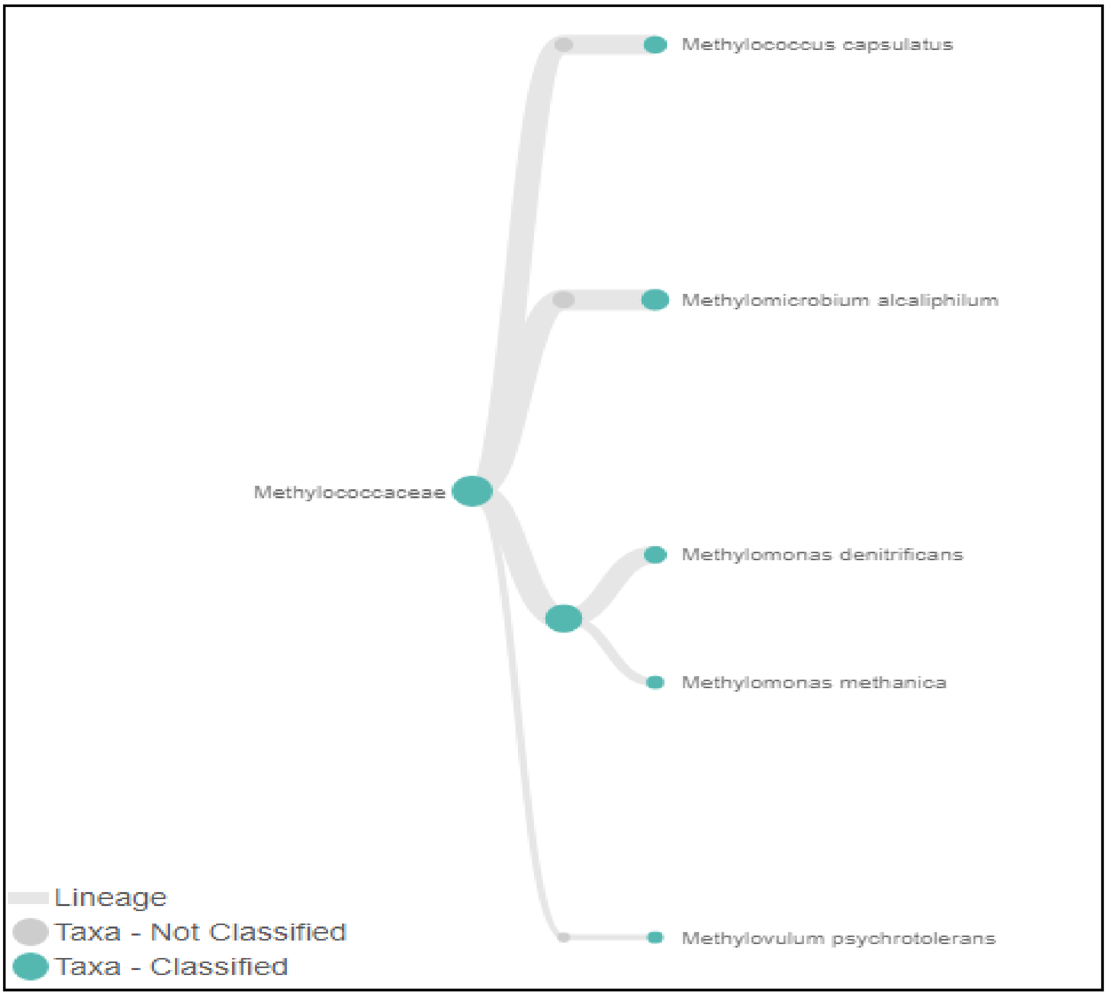
Phylogenetic Tree

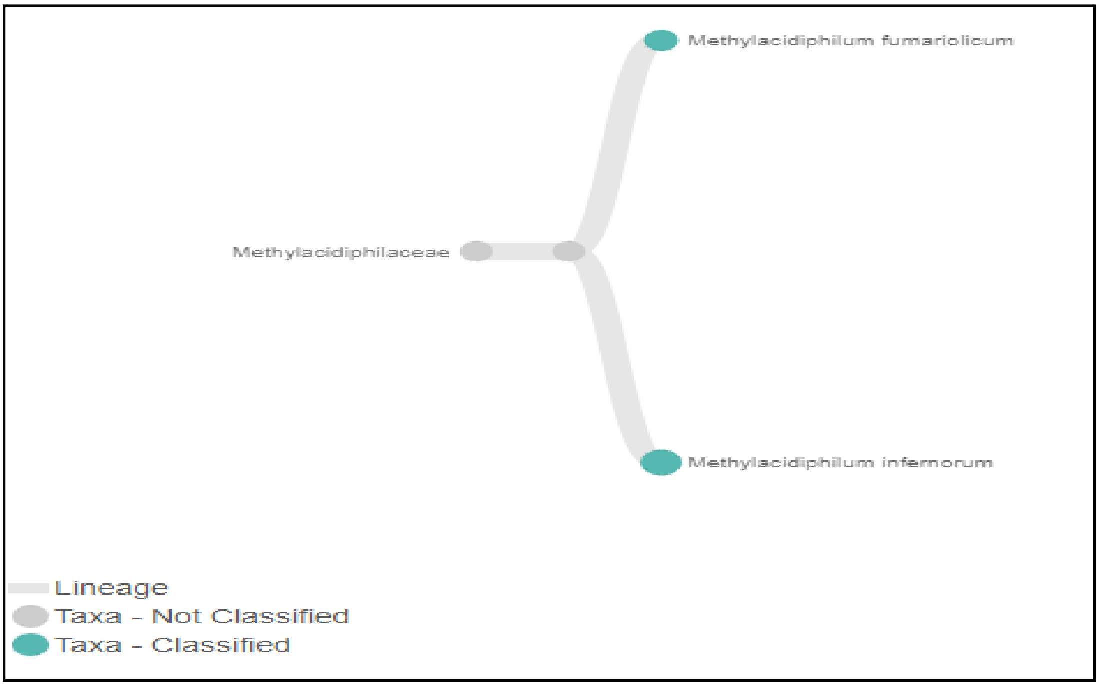





Microvirga ossetica
Methylobacteriaceae
Methylobacterium nodulans
Methylobacterium phyllosphaerae
Methylobacterium sp. 4-46
Methylobacterium sp. C1
Lineage
Taxa - Not Classified
Taxa - Classified


Methylocystis bryophila
Methylocystis sp. SC2
Methylocystaceae
Methylosinus trichosporium
Lineage
Taxa - Not Classified
Taxa - Classified


Methylococcus capsulatus
Methylomicrobium alcaliphilum
Methylococcaceae
Methylomonas denitrificans
Methylomonas methanica
Methylovulum psychrotolerans
Lineage
Taxa - Not Classified
Taxa - Classified


Methylacidiphilum fumariolicum
Methylacidiphilaceae
Methylacidiphilum infernorum
Lineage
Taxa - Not Classified
Taxa - Classified

Bibliography

BIBLIOGRAPHY

Abhilash, P.C., Singh, N., 2008. Influence of the application of sugarcane bagasse on Lindane mobility through soil columns: implication for biotreatment. Bioresour. Technol. 99, 8961-8966.

Abhilash, P.C., Singh, N., 2009. Pesticide use and application: an Indian scenario. J. Hazard. Mater. 165, 1-12.

Abhilash, P.C., Singh, N., 2009. Pesticide use and application: an Indian scenario. J. Hazard. Mater. 165, 1-12.

Abrusci, C., Pablos, J.L., Corrales, T., López-Marín, J., Marín, I., Catalina, F., 2011. Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives. Int. Biodeter. Biodegr. 65, 451-459.

Adhya, T.K., Apte, S.K., Raghu, K., Sethunathan, N., Murthy, N.B.K., 1996. Novel polypeptides induced by the insecticide lindane (gamma-hexachlo-rocyclohexane) are required for its biodegradation by a *Sphingobium paucimobilis* strain. Biochem. Biophy. Res. Comm. 221, 755-761.

Afful, S., Anim, A., Serfor-Armah, Y., 2010. Spectrum of organochlorine pesticide residues in fish samples from the Densu Basin. Res. J. Environ. Earth. Sci. 2, 133-138.

Agbeve, S.K., Osei-Fosu, P., Carboo, D., 2014. Levels of organochlorine pesticide residues in Mondiawhitei, a medicinal plant used in traditional medicine for erectile dysfunction in Ghana. Int. J. Adv. Agric. Res. 1, 9-16.

Ahire, D.V., Chaudhari, P.R., Ahire, V.D., Patil, A.A., 2013. Correlations of Electrical Conductivity and Dielectric Constant with Physico-Chemical Properties of Black Soils. Inter. J. Sci. Res. 3(2), 1-16.

Alexander, M., 1965. Biodegradation: problems of molecular recalcitrance and microbial fallibility. Adv. Appl. Microbiol. 7, 35-80.

Ali, U., Syed, J.H., Malik, R.N., Katsoyiannis, A., Li, J., Zhang, G., Jones, K.C., 2014. Organochlorine pesticides in South Asian Region: a review. Sci. Total Environ. 476-447.

Amador, J.A., 2012. Microbial ecology of tropical forest soils. Trop. Ecol. 53, 183-184.

Amaraneni, S.R., Pillala, R.R., 2001. Concentrations of pesticide residues in tissues of fish from Kolleru Lake in India. Environ. Toxicol. 16, 550-556.

Andrews, S., 2017. Fast QC A Quality tool for high throughput sequence data (Internet). http.//www.bioinformatics.babraham.ac.uk/projects/fastaqc/. Available from: http://www.bioinformaatics.babraham.ac.uk/projects/fastqc/.

Aspinwall, L.S., Bermudez, I., King, L.A., 1997. The interactions of hexachlorocyclohexane isomers with human gamma-aminobutyric acid(A) receptors expressed in *Xenopus oocytes.* J. Pharmacol. Experiment. Therapeut. 282, 1557-1564.

ATSDR, 1999. Toxicological profile for alpha-, beta-, gamma-, and delta hexachlorocyclohexane. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, GA.

ATSDR, 2005. Toxicological Profile for Alpha-, Beta-, Gamma-, and Delta Hexachlorocyclohexane. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/tp43.html.

Attia, A.M., Richardson, B.A., Rodriguez, C., 1991. Lindane may enhance nocturnal pineal N acetyltransferase activity via beta-adrenergic receptors. Brain Resour. 35, 427-439

Aulakh, R.S., Gill, J.P.S., Bedi, J.S., Sharma, J.K., Joia, B.S., Ockerman, H.W., 2006. Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. J. Sc. Food Agri. 86, 741–744.

Auman, A.J., Speake, C.C., Lid- strom, M.E., 2001. Nif H sequences and nitrogen fixation in type Iand typeII methanotrophs. Appl. Environ. Microbiol. 67, 4009-4016.

Babraham Bioinformatics–Trim Galore (Internet) 2017. Available from: http://www.bioinformaatics.babraham.ac.uk/projects/trim_galore/.

Bajaj, S., Singh, D., 2015. Biodegradation of persistent organic pollutants in soil, water and pristine sites by cold-adapted microorganisms: mini review. Int. Biodeterior. Biodegrad. 100, 98-105.

Bakke, B., Stewart, P.A., Waters, M.A., 2007. Uses of and exposure to trichloroethylene in US industry: a systematic literature review. J. Occup. Environ. Hyg. 4, 375-390.

Bala, K., Sharma, P., Lal, R., 2009. *Sphingobium quisquiliarum* sp. nov., a hexachlorocyclohexane (HCH) degrading bacterium isolated from HCH contaminated soil. Int. J. Syst. Evol. Microbiol. 60, 429-433.

Barczi, I., 1994. The greatest chlorinated hydrocarbon problem of Hungary, 3rd HCH and Halogenated Pesticides Forum, 19-20 September 1994, Bilbao. http://www.ihpa.info/library.htm 3rd Forum)

Barragan-Huerta, B.E., Costa-Perez, C., Peralta-Cruz, J., Barrera-Cortes, J., Esparza-Garcıa, F., Rodrıguez-Vazquez, R., 2007. Biodegradation of organochlorine pesticides by bacteria grown inmicroniches of the porous structure of green bean coffee. Int. Biodeterior. Biodegr. 59, 239–244.

Bastos, A.C., Magan, N., 2009. Trametes versicolor: potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions Int. Biodeter. Biodegr. 63, 389-394.

Becerra-Castro, C., Kidd, P.S., Rodríguez-Garrido, B., Monterroso, C., Santos-Ucha, P., Prieto-Fernandez, A., 2013. Phytoremediation of hexachlorocyclohexane (HCH) - contaminated soils using *Cytisus striatus* and bacterial inoculants in soils with distinct organic matter content. Environ. Pollut. 178, 202-210.

Benimeli, C. S., Castro, G. R., Chaile, A. P., Amoroso, M. J. 2007. Lindane uptake and degradation by aquatic *Streptomyces* sp. strain M7. Int. Biodeter. Biodegrad. 59, 148-155.

Bentz, R., 2004. Remediation of the Maag-site in Dielsdorf (Switzerland), Contaminated Soil, Proceedings of the Eighth International FZK/TNO Conference on contaminated Soil, 12-16 May, Gent, Belgium.SpS-3899.

Beyer, A., Matthies, M., 2001. Long-range transport potential of semivolatile organic chemicals in coupled air–water systems. Environ. Sci. Pollut. Res. 8, 173–179.

Bhuvan, S., Sahu, S.K., Adhya, T.K., Sethunathan, N., 1992. Accelerated aerobic degradation of T-hexachlorocyclohexane in suspensions of flooded and non-flooded soils pre-treated with hexachlorocyclohexane. Biol. Fertil. Soils 12, 279-284.

Bolt, H.M., 2005 Vinyl chloride - a classical industrial toxicant of new interest. Crit. Rev. Toxicol. 35, 307-323.

Boltner, D., Moreno-Morillas, S., Ramos, J.L., 2005. 16S rDNA phylogeny and distribution of *lin* genes in novel hexachlorocyclohexane degrading *Sphingomonas* strains. Environ. Microbiol. 7, 1329-1339.

Brantner, J.S., Senko, J.M., 2014. Response of soil-associated microbial communities to intrusion of coal mine-derived acid mine drainage. Environ. Sci. Technol. 48, 8556-8563.

Brigmon, R.L., Anderson, T.A., Fliermans, C.B., 1999. Methanotrophic bacteria in the rhizosphere of trichloroethylene-degrading plants. Int. J. Phytorem. 1, 241-253.

Brindha, R.K., Vasudevan, N., 2017. Methane oxidation capacity of Methanotrophs isolated from different soil ecosystem. Int. J. Environ. Sci. Technol. 1546-1555.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and release of soil N: a rapid direct extraction method to measure microbial biomass N soil. Soil Biol. Biochem. 17, 837-842.

Bumpus, J. A., Tien, M., Wright, D., Aust, S. D., 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science 228, 1434-1436.

Camacho-Perez, B., Rios-Leal, E., Rinderknecht-Seijas, N., Poggi-Varaldo, H.M., 2012. Enzymes involved in the biodegradation of hexachlorocyclohexane: a mini review. J. Environ. Manage. 95, 306-318.

Ceremonie, H., Boubakri, H., Mavingui, P., Simonet, P., Vogel, T. M., 2006. Plasmid-encoded γ-hexachlorocyclohexane degradation genes and insertion sequences in *Sphingobium francense* (ex-*Sphingomonas paucimobilis* Sp+). FEMS Microbiol. Ecol. 257, 243-252.

Chandra, P., Singh, D.P., 2014. Removal of Cr (VI) by a halotolerant bacterium *Halomonas* sp. CSB 5 isolated from Sambhar salt lake Rajasthan (India). Cell. Mol. Biol. 60 (5), 64-72.

Chaudhary, P., Kumar, M., Khangarot B.S., Kumar, A., 2006. Degradation and detoxification of hexachlorocyclohexane isomers by *Pseudomonas aeruginosa* ITRC-5. Int. Biodeterior. Biodegrad. 57,107-113.

ChemSources. 2009. http://www.chemsources.com/chemonline.html and search on lindane. chloropesticides banned by Stockholm convention. Int. J. Environ. Sci. Technol. 1-10.

Chen, Y., Dumont, M.G., Cebron, A., Murrell, J.C., 2007. Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. Environ. Microbiol. 9(11), 2855-2869.

Chen, Y., McNamara, N.P., Dumont, M.G., Bodrossy, L., Stralis- Pauese, N., et al., 2008. The impact of burning and Calluna removal on below-ground methanotroph diversity and activity in a peatland soil. Appl. Soil Ecol. 40(2), 291-298.

Choi, D.W., Do, Y.S., Zea, C.J., Mc Ellistrem, M.T., Lee, et al., 2006. Spectral and thermodynamic properties of Ag(I), Au(III), Cd(II), Co(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(IV), and Zn(II) binding by methanobactin from *Methylosinus trichosporium* OB3b. J. Inorg. Biochem. 100, 2150-2161.

Choi, D.W., Do, Y.S., Zea, C.J., McEllistrem, M.T., Lee et al. 2006. Spectral and thermodynamic properties of Ag(I), Au(III), Cd(II), Co(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(IV), and Zn(II) binding by methanobactin from *Methylosinus trichosporium* OB3b. J Inorg Biochem 100, 2150-2161.

Choudhary, A., Sharma, D.C., 2008. Pesticide residues in honey samples from Himachal Pradesh (India). Bull. Environ. Contam. Toxicol. 80, 417-422.

Coleman, N.V., Mattes, T.E., Gossett, J.M., Spain, J.C., 2002. Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. Appl. Environ. Microbiol. 68, 6162-6171.

Concha-Granam, E., Turnes-Carou, M.I., Muniategui-Lornzo, S., Lopez-mahia, P., Prada- Rodriguez, D., Fernandez, E., 2006. Evaluation of HCH isomers and metabolites in soils, lechates, river water and sediments of a highly contaminated area. Chemosphere 64, 588-595.

Cousins, I.T., Bondi, G., Jones, K.C., 1999. Measuring and modelling the vertical distribution of semi volatile organic compounds in soils I: PCB and PAH soil core data. Chemosphere 19, 2507-2518.

De Paolis, M.R., Lippi, D., Guerriero, E., Palcaro, C.M., Dunati, E., 2013. Biodegradation of alpha, beta, gamma-hexachlorocyclohexane by *Arthrobacter* *fluorescence* and *Arthrobacter giecomelloi*. Appl. Biochem. Biotechnol. 170(3), 514-524.

De Schrijver, A., De Mot, R., 1999. Degradation of pesticides by actinomycetes. Crit. Rev. Microbiol. 25, 85-119.

De, B.J., 1979. Final Report, Reduction of Chlordane, DDT, Heptachlor, Hexachlorobenzene and Hexachlorocyclohexane isomers, Prepared for the European Communities Environment and Consumer Protection Service, May 1979.

Dedysh, S.N., Liesack, W., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Semrau, J.D., Bares et al., 2000. *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. Int. J. System Evol. Microbiol. 50, 955-969.

DeSantis, T.Z, Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., krller, K., Huber, T., et al., 2006. Greengenes, a Chimera- Checked 16S rRNA Gene Database and workbench compatible with ARB . Appl. Environ. Microbiol.72 (7), 5069-5072.

Dhananjayan, V., Ravichandran, B., Rajmohan, H.R., 2012. Organochlorine pesticide residues in blood samples of agriculture and sheep wool workers in Bangalore (Rural), India. Bull. Environ. Contam. Toxicol. 88, 497-500.

Do Couto, G.M., Eisenhauer, N., De Oliveira, E.B., Cesarz, S., Feliciano, A.L.P., Marangon, L.C., 2016. Response of soil microbial biomass and activity in early restored lands in the north-eastern Brazilian Atlantic Forest. Restor. Ecol. 24, 609-616.

Doleman, P., Maanstra, L., Loonen, H., Vyas, A., 1990. Decomposition of α- and β-hexachlorocyclohexane in soil under field conditions in a temperate climate. Soil Biol. Biochem. 22, 629-634.

Dominguez, C.M., Parchao, J., Rodriguez, S., Lorenzo, D., Romero, A., Santos, A., 2016. Kinetics of lindane dechlorination by zero valent iron microparticles: effect of different salts and stability study. Ind. Eng. Chem. Res. 55(50), 12776-12785.

Doronina, N.V., Ezhov, V.A., Trotsenko,Yu.A., 2008. Growth of *Methylosinustrichosporium* OB3b on methane and poly beta hydroxybutyrate biosynthesis. Prikl. Biokhim. Mikrobiol. 44, 182-185.

Dritsa, V., Rigas, F., Doulia, D., Avramides, E.J., Hatzianestis, I., 2009. Optimization of culture conditions for the biodegradation of lindane by the polypore fungus *Ganoderma australe*. Water, Air, & Soil Pollut. 204, 19-27.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 20011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27(2), 194-200.

Elcey, C.D, Kunhi, A.A.M., 2010. Substantially enhanced degradation of hexachlorocyclohexane isomers by a microbial consortium on acclimation. J. Agric. Food. Chem. 58, 1046-1054.

Endo, R., Kamakura, M., Miyauchi, K., Fukuda, M., Ohtsubo, Y., Tsuda, M., Nagata, Y., 2005. Identification and characterization of genes involved in the downstream degradation pathway of γ-hexachlorocyclohexane in *Sphingomonas paucimobilis* UT26. J. Bacteriol.187, 847-853.

Erwin, D.P., Erickson, I.K., Delwiche, M.E., Colwell, F.S., Strap, J.L., Ronald, L.C., 2005. Diversity of oxygenase genes from methane- and ammonia-oxidizing bacteria in an Eastern Snake River Plain Aquifer. Appl. Environ. Microbiol. 71, 2016-2025.

FDA. 2006. Total Diet Study Market Baskets 1991-3 through 2003-2004.

FDA. 2009. The Electronic Orange Book. *U.S. Food and Drug Administration.* http://www.fda.gov/cder/ob/default.htm

Fernandes, E.C.M., Motavalli, P.P., Castilla, C., Mukurumbira, L., 1997. Management control of soil organic matter dynamics in tropical land use systems, Geoderma 79, 49-67.

Ferris, F.G., Konhauser, K.O., Lyven, B., Pedersen, K., 1999. Accumulation of metals by bacteriogenic iron oxides in a subterranean environment. Geomicrobiol. J. 16, 181-192.

Fitch, M.W.,Speitel, G.E., and Georgiou,G., 1996. Degradation of trichloroethylene by methanol grown cultures of *Methylosinustrichosporium* OB3b. Appl. Environ. Microbiol. 62, 1124-1128.

Fjellbirkeland, A., Torsvik, V., Ovreas, L., 2001. Methanotrophic diversity in an agricultural soil as evaluated by denaturing gradient gel electrophoresis profiles of *pmoA*, *mxaF* and 16S rDNA sequences. Antonie van Leeuwenhoek. 79(2), 209-217.

Francis, A.J., Spanggord, R.J., Ouchi, G.I., 1975. Degradation of lindane by *Escherichia coli*. Appl. Microbiol. 29, 567- 568.

Futamata, H., Harayama, S., Watanabe, K., 2001. Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria. Appl. Microbiol. Biotechnol. 55, 248-253.

Ge, X., Yang, L., Sheets, J.P., Yu, Z., Li, Y., 2014. Biological conversion of methane to liquid fuels: status and opportunities. Biotechnol. Adv. 32, 1460-1475.

Gulledge, J., Ahmad, A., Steudler, P.A., Pomerantz, W.J., Cavanaugh, C.M., 2001. Family- and genus-level 16S rRNA-targeted oligonucleotide probes for ecological studies of methanotrophic bacteria. Appl. Environ. Microbiol. 67(10), 4726-4733.

Gupta, A., Kaushik, C.P., Kaushik, A., 2000. Degradation of hexachlorocyclohexane (HCH; α-, β-, γ-and δ-) by *Bacillus circulans* and *Bacillus brevis* isolated from soil contaminated with HCH. Soil Biol Biochem. 32, 1803-1805

Gupta, P.K., 1986. Pesticides in the Indian environment. Interprint, New Delhi, 1-16.

Hakemian, A.S., Rosenzweig, A.C., 2007. The biochemistry of methane oxidation. Annu.Rev. Biochem. 76, 223-241.

Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiol. Rev. 60, 439-471.

Hanson, R.S., Tsien, H.C., Tsuji, K., Brusseau, G.A., Wackett, L.P., 1990. Biodegradation of low molecular-weight halogenated hydrocarbons by methanotrophic bacteria. FEMS Microbiol. Lett. 87, 273-278.

Hasin, A.A., Gurman, S.J., Murphy, L.M., Perry, A., Smith, T.J., Gardiner, P.E., 2010. Remediation of chromium (VI) by a methane-oxidizing bacterium. Environ. Sci. Technol. 44, 400-405.

Hasin, A.A., Gurman, S.J., Murphy, L.M., Perry, A., Smith, T.J., Gardiner, P.E., 2010. Remediation of chromium (VI) by a methane-oxidizing bacterium. Environ. Sci. Technol. 44, 400-405.

Hauzenberger, I., 2004. Reports on Substances Scheduled for Reassessments Under the UNECE POPs Protocol, Technical Review Report on Lindane, Federal Environment Agency, Wien, Austria.

Hazen, T.C., Chakraborty, R., Fleming, J.M., Gregory, I.R., Bowman, J.P., Jimenez, L., Zhang, D., Pfiffner, S.M., Brockman, F.J., Sayler, G.S., 2009. Use of gene probes to assess the impact and effectiveness of aerobic *in situ* bioremediation of TCE. Arch. Microbiol. 191, 221-232.

Henckel, T., Friedrich, M., Conrad, R., 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl Environ Microbiol. 65(5), 1980-1990.

Heritage, A.D., MacRae, I.C., 1977. Identification of intermediates formed during the degradation of hexachlorocyclohexanes by *Clostridium sphenoides*. Appl. Environ. Microbiol. 33, 1295-1297.

Hitch, R.K., Day, H.R., 1992. Unusual persistence of DDT in some western USA soils. Bull. Environ. Contam. Toxicol. 48, 259-264.

Holliger, C., Jackson, C., Oakeshott, J.G., 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol. Mol. Biol. Rev. 74, 58-80.

Horz, H.P., Yimga, M.T., Liesack, W., 2001. Detection of methanotroph diversity on roots of submerged rice plants by molecular retrieval of *pmoA*, *mmoX*, *mxaF*, and 16S rRNA and ribosomal DNA, including *pmoA*-based terminal restriction fragment length polymorphism profiling. Appl. Environ Microbiol. 67 (9), 4177-4185.

Hou, C.T., Laskin, A.I., Patel, R.N., 1978. Growth and polysaccharide production by Methylocystis parvus OBBP on methanol. Appl Environ Microbiol. 37(5), 800-804.

Hou, C.T., Patel, R., Laskin, A.I., Barnabe, N., 1979. Microbial oxidation of gaseous hydro- carbons: epoxidation of C2 to C4 n-alkenes by methylotrophic bacteria. Appl.Environ.Microbiol. 38, 127-134.

HSDB. 2009. Hazardous Substances Data Bank.National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search on CAS number.

IARC. 1998. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42, Supplement 7. Lyon, France.

IARC. 1979. Hexachlorocyclohexane (technical HCH and lindane). In Some Halogenated Hydrocarbons. IARC Monographs on the Evaluation of

Carcinogenic Risk of Chemicals to Humans, vol. 20. Lyon, France: International Agency for Research on Cancer. 195-239.

Im, J., Semrau, J.D., 2001. Pollutant degradation by a *Methylocystis* strain SB2 grown on ethanol: bioremediation via facultative methanotrophy. FEMS Microbiol Lett 318, 137-142.

Iwamoto, T., Tani, K., Nakamura, K., Suzuki, Y., Kitagawa, M., Eguchi, M., Nasu, M., 2000. Monitoring impact of in situ biostimulation treatment on groundwater bacterial community by DGGE. FEMS Microbiol Ecol 32, 129-141.

Iwasaki, I., Utsumi, S., Ozawa, T., 1952. New colorimetric determination of chloride using mercuric thiocyanate and ferric ion. B. Chem. Soc. Jpn. 25(3), 226-226.

Iwata, H., Tanabe, S., Sakai, N., Tatsukawa, R., 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean ontheir global transport and fate. Environ. Sci. Technol. 27, 1080-1098.

Jagnow, G., Haider, K., Ellwardt, P.C., 1977. Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers anaerobic and facultative anaerobic bacteria. Arch. Microbiol. 115, 285-292.

Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soils: Measurement and turnover. In: Paul, E.A., Ladd, J.N., (Eds), Soil biochemistry. New York: Marcel Decker, pp.415-417.

Jha, A.K., and Singh, J.S., 1991. Soil characteristics and vegetation development of an age series of mine spoilin a dry tropical environment. Vegetatio. 97, 63 -76.

Jiang, H., Chen, Y., Jiang, P.X., Zhang, C., Smith, T.J., Murrell, J.C., Xing, X.H., 2010. Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering. Biochem. Eng. J. 49, 277-288.

Jiang, H., Chen, Y., Jiang, P.X., Zhang, C., Smith, T.J., Murrell, J.C., Xing, X.H., 2010 Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering. Biochem Enginee J. 49, 277-288.

Jia-Ying, Xin., Ying-Xin, Zhang., Jing, Dong., Qi-Qiong, Zhou., Yan, Wang. Xiao-Dan, Zhang., Chun-Gu, Xia., 2010. Epoxypropane biosynthesis by whole cell suspension of methanol-growth Methylosinus trichosporium IMV 3011. World. J. Microbiol. Biotechnol. 26,701-708.

Johnson, W.W., Finley, M.T. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Resource publication 137. Washington DC: U.S. Department of interior fish and wild life service. pp. 6-56.

Johri, A.K., Dua, M., Tuteja, D., Saxena, R., Saxena, D.M., Lal, R., 1998. Degradation of alpha-,beta-, gamma- and delta- hexachlorocyclohexane by *Sphingobium paucimobilis*. Biotechnol. Lett. 20, 885-887.

Kalra, R.L., Singh, B., Battu, R.S., 2003. Organochlorine pesticide residues in human milk in Punjab, India. Environ. Pollut. 85, 47-151.

Kalyuzhnaya, M.G., Puri, A.W., Lidstrom, M.E. 2015. Metabolic engineering in methanotrophic bacteria. Metab. Eng. 29, 142-52.

Kashyap, S.K., Nigam, S.K., Gupta, R.C., Karnik, A.B., Chatterjee, S.K., 1979. Carcinogenicity of hexachlorocyclohexane (BHC) in pure inbred Swiss mice. J. Environ. Sci.Health.B14, 305-318.

Kaushik, C.P., Sharma, H.R., Jain, S., Dawra, J., Kaushik, A., 2007. Pesticide residues in river Yamuna and its canals in Haryana and Delhi, India. Environ. Monitor. Assess. 144, 329-340.

Khan, S., Han, C., Khan, H.M., Boccelli, D.L., Dionysiou, D.D., 2017. Efficient degradation of lindane by visible and simulated solar light-assisted S-TiO2/peroxymonosulfate process: kinetics and mechanistic investigations. J. MolCatal. A-Chem. 428, 9–6.

Kikuchi, T., Iwasaki, K., Nishihara, H., Takamura, Y., Yagi, O., 2002. Quantitative and rapid detection of the trichloroethylene-degrading bacterium *Methylocystis* sp. M in groundwater by real-time PCR. Appl Microbiol Biotechnol. 59, 731-736.

Kim, H.J., Graham, D.W., DiSpirito, A.A., Alterman, M.A., Galeva, N., Larive, C.K., Asunskis, D., Sherwood, P.M.A., 2004. Methanobactin, A copper-acquisition compound from methane-oxidizing bacteria. Science 305, 1612-1615.

Kizilova, A.K., Sukhacheva,M.V., Pimenov, N.V., Yurkov, A.M., Kravchenko, I.K., 2014. Methane oxidation activity and diversity of aerobic methanotrophs in pH-neutral and semi neutral thermal springsof the Kunashir Island, Russian Far East. Extremophiles.18 (2), 207-208.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids Res. 41, 1-11.

Knapp, C.W., Fowle, D.A., Kulczycki, E., Roberts, JA., Graham, D.W., 2007. Methane monooxygenase gene expression mediated by methanobactin in the presence of mineral copper sources. Proc. Nat. Acad. Sci. 104, 12040-12045.

Kumar, A., Cameotra, S.S., Gupta, S., 2010. Screening and characterization of potential cadminum biosorbant *Alcaligenes* strain from industrial effluent J. Basic. Microbiol. Methods 51,1-7.

Kumar, M., Chaudhary, P., Dwivedi, M., Kumar, R., Paul, D., Jain, R.K., Garg, S.K., Kumar, A., 2005. Enhanced biodegradation of β and δ -hexachlorocyclohexane in the presence of α- and γ-isomers in contaminated soils. Environ. Sci. Technol.39, 4005-4011.

Kumar, S., Stecher, G., Tamura, K., (2016) MEGA 7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol.Evol. 33, 1870-1874.

Lal, R., Saxena, D.M., 1982. Accumulation, Metabolism, and Effects of Organochlorine Insecticides on Microorganisms. Microbiol Rev. 46, 95-127.

Olsen, S., Cole, C., Watanabe, F., Dean, L., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., et al., 2013. Pridictive functional profiling of microbial communities using 16S rRNA marker gene sequence. Nat. Biotechnol. 8, 1-10.

Lau E, Ahmad A, Steudler P A, Cavanaugh C M, 2007. Molecular characterization of methanotrophic communities in forest soils that consume atmospheric methane. FEMS. Microbiol. Ecol, 60(3), 490-500.

Lee, SW., Keeney, D.R., Lim, D.H., Dispirito, A.A., Semrau, J.D., 2006. Mixed pollutant degradation by *Methylosinus trichosporium* OB3b expressing either soluble or particulate methane monooxygenase: can the tortoise beat the hare? Appl. Environ. Microbiol. 72, 7503-7509.

Li, Y. F., Zhulidov, A.V., Robarts, R.D., Korotova,L. G., 2005. Hexachlorocyclohexane Use in the Former Soviet Union, the Arch Environ Contam Toxicol. 48, 10-15.

Li, Y.F., 1999. Global Technical Hexachlorocyclohexane usage and its contamination consequences in environment: from 1948 to 1997. Sci. Total. Environ. 232, 123-160.

Lin, J.L., Radajewski, S., Eshinimaev, B.T., Trotsenko, Y.A., McDonald, I.R., et al., 2004. Molecular diversity of methanotrophs in Transbaikal soda lake sediments and identification of potentially active populations by stable isotope probing. Environ. Microbiol. 6(10), 1049-1060.

Liu, J., Hua, Z.S., Chen, L.X., Kuang, J.L., Li, S.J., Shu, W.S., Huang, L.N. 2012. Correlating microbial diversity patterns with geochemistry in an extreme and heterogenous environment of mine tailing, Appl. Environ. Microbiol. 80, 3677-3686.

Lodha, B., Bhat, P., Kumar, M.S., Vaidya, A.N., Mudliar, S., Killedar, D.J., Chakrabarti, T., 2007. Bioisomerization kinetics of γ -HCH and biokinetics of *Pseudomonas aeruginosa* degrading technical HCH. Biochem. Eng. J. 35, 12-19.

Lontoh, S., Semrau, J.D., 1998. Methane and trichloroethylene degradation by *Methylosinus trichosporium* OB3b expressing particulate methane monooxygenase. Appl. Environ. Microbiol. 64, 1106-1114.

Lontoh, S., Zahn, J.A., DiSpirito, A.A., Semrau, J.D., 2000. Identification of intermediates of in vivo trichloroethylene oxidation by the membrane-associated methane monooxygenase. FEMS. Microbiol. Lett. 186, 109–113.

Lopez, A.G., Deutcher, M.T., Aquino, W.F., Marker, A., 1998. HCH and pesticides contaminated sites in Brazil –Stat of the Art - 5th International HCH and Pesticides Forum, Bilbao, Basque Country, Forum Book, IHOBE.

Luizao, R.C.C., Bonde, T.A., Rosswall, T., 1992. Seasonal variation of soil microbial biomass- the effects of clearfelling a tropical rain forest and establishment of pasture in central Amazon. Soil. Biol. Biochem. 24, 805-813.

MacRae, I.C., Raghu, K., Bautista, E.M., 1969. Anaerobic degradation of the insecticide Lindane by *Clostridium* sp. Nature (London). 221, 859-860.

Madaj, R., Sobiecka, E., Kalinowska, H., 2017. Lindane, kepone and pentachlorobenzene:

Manickam, N., Mau, M., Schlomann, M., 2006. Characterization of the novel HCH-degrading strain, *Microbacterium* sp. ITRC1. Appl. Microbiol. Biotech. 69(5), 580-588.

Manickam, N., Pathak, A., Saini, H.S., Mayilraj, N., Shankar, R., 2010. Metabolic profiles and phylogenetic diversity of microbial communities from chlorinated pesticides contaminated sites of different geographical habitats of India. J. Appl. Microbiol. 109, 1458-1468.

Manickam, N., Reddy, M.K., Saini, H.S., Shanker, R., 2008. Isolation of hexachlorocyclohexane degrading Sphingomonas sp. by dehalogenase assay and characterization of genes involved in γ-HCH degradation. J. Appl. Microbiol. 104, 952-960.

Martínez-Toledo, M.V., Salmeron, V., Rodelas, B., Pozo, C., Gonzalez- Lopez, J., 1993. Studies on the effects of a chlorinated hydrocarbon insecticide, lindane, on soil microorganisms. Chemosphere. 27, 2261-2270.

Maymo-Gatell, X., Anguish, T., Zinder, S.H., 1999. Reductive dechlorination of chlorinated ethenes and 1, 2-dichloroethane by *Dehalococcoides ethenogenes* 195. Appl Environ Microbiol. 65, 3108-3113.

McCue, T., Hoxworth, S., Randall, A.A., 2002. Degradation of halogenated aliphatic compounds utilizing sequential anaerobic/aerobic treatments. Water. Sci. Technol. 47, 79-84.

McDonald, I. R., Kenna, E. M., Murrell, J. C., 1995. Detection of methanotrophic bacteria in environmental samples with the PCR. Appl Environ. Microbiol. 61(1), 116-121.

McErlean, C., Marchant, R., Banat, I.M., 2006. An evaluation of soil colonisation potential of selected fungi and their production of ligninolytic enzymes for use in soil bioremediation applications. Antonie Van Leeuwenhoek. 90, 147-158

McFarland, M. J.; Vogel, C. M.; Spain, J. C. (1992). Methanotrophic cometabolism of trichloroethylene (TCE) in a 2 stage bioreactor system. Water Res. 26, 259-265.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoSONE. 8(4), 612-617.

Megonigal, J.P., Guenther, A.B., 2008. Methane emissions from upland forest soils and vegetation, Tree Physiol. 28 (4), 49-498.

Mertens, B., Boon, N., Verstraete, W., 2005. Stereospecific effect of hexachlorocyclohexane on activity and structure of methanotrophic communities. Environ. Microbiol. 7, 660-669.

Mertens, B., Boon, N., Verstraete, W., 2006. Slow-release inoculation allows sustained biodegradation of g-hexachlorocyclohexane. Applied and Environmental Microbiology 72, 622-627.

Mishra, C., Gupta, S.K., 2001. Hybrid reactor for priority pollutant trichloroethylene removal. Water Res. 35, 160-166.

Miyauchi, K., Adachi, Y., Nagata, Y., Takagi, M., 1999. Cloning and sequencing of a novel metacleavage dioxygenase gene whose product is involved in degradation of γ-hexachlorocyclohexane in *Sphingomonas paucimobilis*. J. Bac. 181, 6712-6719.

Miyauchi, K., Lee, H.S., Fukuda, M., Takagi, M., Nagata, Y., 2002. Cloning and characterization of linR, involved in regulation of downstream pathway for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. Appl. Environ. Microbiol. 68, 1803-1807.

Mohn, W.W., Mertens, B., Neufeld, J.D., Verstraete, W., Lorenzo, V., 2006. Distribution of phylogeny of hexachlorocyclohexane-degrading bacteria in soils from Spain. Environ Microbiol. 8, 60-68.

Morgan, D.P., Roberts, R.J., Walter, A.W., Stockdale, E.M., 1980. Anemia associated with exposure to lindane. Arch. Environ. Health. 35, 307-310.

Mougin, C., Pericaud, C., Dubroca, J., Asther, M., 1997. Enhanced mineralization of lindane in soils supplemented with the white rot basidiomycete *Phanerochaete chrysosporium*. Soil Biol. Biochem. 29, 1321-1324.

Munir, K.M., Soman, C.S., Bhide, S.V., 1983. Hexachlorocyclohexane-induced tumorigenicity in mice under different experimental conditions. Tumorol. 69, 383-386.

Nagata, Y., Endo, R., Ito, M., Ohtsubo, Y., Tsudo, M., 2007. Aerobic degradation of lindane (γ-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. Appl. Microbiol Biotechnol. 76, 741-752

Nagata, Y., Miyauchi, K., Takagi, M., 1999. Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. J. Indus. Microbiol. Biotech. 23, 380-390.

Nagata, Y., Nariya, T., Ohtomo, R., Fukuda, M., Yano, K., Takagi, M., 1994. Cloning and sequencing of a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase gene involved in the degradation of γ-hexachlorocyclohexane in *Pseudomonas paucimobilis*. J. Bac.176, 3117-3125.

Nagata, Y., Prokop, Z., Sato, Y., Jerabek, P., Kumar A, et al. 2005. Degradation of β-hexachlorocyclohexane by Haloalkanedehalogenase *LinB* from *Sphingomonas paucimobilis* UT26. Appl. Environ. Microbiol. 71, 2183-2185.

Nagata, Y., Prokop, Z., Sato, Y., Jerabek, P., Kumar, A., Ohtsubo, Y., Tsuda, M., Damborsky, J., 2005. Degradation of β- hexachlorocyclohexane by haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. Appl. Environ. Microbiol.71, 2183-2185.

Nagpal, V., Srinivasan, M.C., Paknikar, K.M., 2008. Biodegradation of γ hexachlorocyclohexane (Lindane) by a non- white rot fungus *Conidiobolus* 03-1-56 isolated from litter. Ind. J. Microbiol. 48, 134-141.

Nagpal, V., Srinivasan, M.C., Parnikar, K.M., 2008. Biodegradation of γ-hexachlorocyclohexane (lindane) by a non- white rot fungus Conidiobolous 03-1-56 isolated from litter. Indian J. Microbiol. 48, 134-141.

Nantel, A.J., Ayotte, L., Beneditti, J.L., Savoie, J.Y., Tessier, L., Schwarz, T., Weber, J.P., 1977. A group of adults acutely poisoned by food contaminated with lindane. Acta. Pharmaco. Toxicol. 41(2), 250-256.

Nawab, A., Aleem, A., Malik, A., 2003. Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by *Pseudomonas* strains. Bioresour. Technol. 88, 41–46.

Nedkova-Bratanova, N., Ivanov, E., Savov, G., 1979. Effect of the pesticides phosalone and l lindane on the activity of some dipeptidases and disaccharidases in rat intestinal mucosa. Enzyme. 24(5), 281-284.

Newby, D.T., Reed, D.W., Petzke, L.M., Igoe, A.L., Delwiche, M.E., Roberto, F.F., McKinley, J.P., Whiticar, M.J., Colwell., F.S., 2004. Diversity of methanotroph communities in a basalt aquifer. FEMS. Microbiol. Ecol. 48, 333-344.

Nikiema, J., Bibeau, L., Lavoie, J., Brzezinski, R., Vigneux, J., Heitz, M., 2005. Biofiltration of methane: an experimental study. Chem Enginee J. 113, 111-117.

Oakley, C.J., Murrell, J.C., 1988. Nif H genes in the obligate methane oxidizing bacteria. FEMS Micriobiol. Lett. 49, 53-57.

Ohisa, N., M, Yamaguchi., 1978. Gamma-BHC degradation accompanied by the growth of *Clostridium rectum* isolated from paddy field soil. Agri. Biological. Chem.42, 1819-1823.

Okan, O.C., 2004. World Bank internal report POPS in Azerbaijan Brief Assessment.

Okeke, B.C., Siddique, T., Arbestain, M.C., Frankenberger, W.T., 2002. Biodegradation of c- hexachlorocyclohexane (Lindane) and γ-hexachlorocyclohexane in water and soil slurry by a *Pandoraea* species. J. Agric. Food Chem. 50, 2548-2555.

Okeke, B.C., Siddique, T., Camps Arbestain, M. and Frankenberger, W.T. 2002. Biodegradation of C-hexachlorocyclohexane (Lindane) and F-hexachlorocyclohexane in water and a soil slurry by a Pandoraea species. J. Agric. Food Chem. 50, 2548-2555.

Oldenhuis, R., Oedzes, J. Y., van der Waarde, J. J., and Janssen, D. B. 1991. Kinetics of chlorinated hydrocarbondegradation by *Methy- losinustrichosporium* OB3b and toxicity of trichloroethylene. Appl. Environ. Microbiol. 57, 7-14.

Oldenhuis, R., Vink, R.L.J.M., Janssen, D.B., Witholt, B., 1989. Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. 55, 2819-2826.

Oliva, M., Garrido, C., Sales, D., Canales, M.L.G. 2008. Lindane toxicity on early life stages of gilthead sea bream (*Sparus aurata*) with a note on its histopathological manifestations. Environ. Toxicol. Pharmacol., 25(1), 94-102.

Osterreicher-Cunha, P., Langenbach, T., Torres, J.P., Lima, A.L., de Campos, T.M. et al. 2003. HCH distribution and microbial parameters after liming of a heavily contaminated soil in Rio de Janeiro. Environ. Resour. 93, 316-327.

Overland, M., Tauson, A.H., Shearer, K., Skrede, A., 2010. Evaluation of methane-utilizing bacteria products as feed ingredients for monogastric animals. Arch. Animal Nutri. 64, 171-189.

Paknikar, K.M., Nagpal, V., Pethkar, A.V., Rajwade, J.M., 2005. Degradation of lindane from aqueous solutions using iron sulphide nanoparticles stabilized by biopolymers. Sci technol adv mat. 6, 370-374.

Pandey, V.C., Singh, J.S., Singh, D.P., Singh, R.P., 2014. Methanotrophs: promising bacteria for environmental remediation. Int. J. Environ. Sci. Technol. 11, 241-250.

Pannu, K., Kumar, D., 2014. Biodegradation study of γ-hexachlorocyclohexane using selected bacteria isolated from agriculture soil. AFR. J. Microbiol. Res. 8(36), 3335-3346.

Pathak, R., Suke, S.G., Ahmed, R.S., Tripathi, A.K., Guleria, K., Sharma, C.S., Makhijani, S.D., Mishra, M., Banerjee, B.D., 2008. Endosulfan and other organochlorine pesticide residues in maternal and cord blood in North Indian population. Bull. Environ. Conta. Toxico. 81, 216-219.

Pathak, R., Suke, S.G., Ahmed, R.S., Tripathi, A.K., Guleria, K., Sharma, C.S., Makhijani, S.D., Mishra, M., Banerjee, B.D., 2008. Endosulfan and Other Organochlorine Pesticide Residues in Maternal and Cord Blood in North Indian Population. Bull. Environ. Contam. Toxicol. 81, 216-219.

Paudel, S., Sah, J.P., 2003. Physiochemical characteristics of soil in tropical sal (Shorea robusta Gaertn.) forests in eastern Nepal. Himalayan J. Sci. 21(2), 107-110.

Pavilikova, N., Blahova, L., Klan, P., Reddy Bathula, S., Sklenar, V., Giesy, J.P., et al., 2012. Enantio selective effects of alpha-hexacyclohexane (HCH) isomers on androgen receptor activity in vitro. Chemosphere 86, 65–69.

Peng, L., Deng, D., Guan, M., Fang, X., Zhu, Q., 2015. Remediation HCHs POPs-contaminated soil by activated persulfate technologies: feasibility, impact of activation methods and mechanistic implications. Sep. Purif. Technol. 150, 215–222.

Pesticide properties database for environmental decision making. Rev. Environ. Contam. Toxicol. 123, 1-157.

Pfiffner, S.M., Palumbo, A.V., Phelps, T.J., Hazen, T.C., 1997. Effects of nutrient dosing on subsurface methanotrophic populations and trichloroethylene degradation. J. Ind. Microbiol. Biotechnol. 18, 204-212.

Pfiffner, S.M., Palumbo, A.V., Phelps, T.J., Hazen, T.C., 1997. Effects of nutrient dosing on subsurface methanotrophic populations and trichloroethylene degradation. J. Ind. Microbiol. Biotechnol. 18, 204-212.

Phillips, T.M., Lee, H., Trevors, J.T., Seech, A.G., 2006. Full-scale in situ bioremediation of hexachlorocyclohexane-contaminated soil. J. Chem. Technol. Biotech. 81, 289-298.

Phillips, T.M., Seech, A.G., Lee, H., Trevors, J.T., 2001. Colorimetric assay for Lindane dechlorination by bacteria. J Microbiol Meth. 47(2), 181-8.

Phillips, T.M., Seech, A.G., Lee, H., Trevors, J.T., 2005. Biodegradationof hexachlorocyclohexane (HCH) by microorganisms. Biodegradation. 16, 363-392.

Pomes, A., Rodriguez-Farre, E., Sunol, C., 1994. Effects of organochlorine pesticides on 36Cl-flux in primary neuronal cultures. Neurotoxicol. 15, 745-749.

Prakash, O., Suar, M., Raina, V., Dogra, C., Pal, R., 2004. Residues of hexachlorocyclohexane isomers in soil and water samples from Delhi and adjoining areas. Curr. Sci. 87, 73-77.

Questionnaire on HCH and Lindane. 2004. Submitted by Japanese Ministry of Agriculture.

Questionnaire on HCH and Lindane. 2004. Submitted by Lindita Tafaj, Albania.

Questionnaire", Executive Body for the Convention on Long-Range Transboundary Air Pollution, Available at http://www.unece.org/env/documents/2000/eb/eb.air.2000.1.q. 37-49.pdf.

Quintero,J.C., Moreira, M.T., Feijoo, G., Lema, J.M., 2005a. Effect of surfactants on the soil desorption of hexachlorocyclohexane (HCH) isomers and their anaerobic biodegradation. J. Chem. Tech. biotec. 80,1005-1015.

Quintero,J.C., Moreira, M.T., Feijoo, G., Lema, J.M., 2005b. Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. Chemosphere. 61, 528-536.

Qunitero, J.C., Lu-Chau, T.A., Moreira, M.T., Feijoo, G., Lema, J.M., 2007. Bioremediation of HCH present in soil by white rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. Int Biodeter Biodegrad. 60, 319-326.

Rai, P.K., Rai, A., Singh, S., 2018. Change in soil microbial biomass along a rural urban gradient in Varanasi (U.P., India). Geol. Ecol. Landsca. 2 (1), 15-21.

Rangel-Vasconcelos, L.G.T., Zarin, D.J., de Assis Oliveira, F., Vasconcelos, S.S., de Carvalho, C.J.R de Lourdes Silva Santos, M.M., 2015. Effect of water availability on the soil microbial biomass in secondary forest in eastern Amazonia. R. Bras. Ci. Solo 39, 377-384.

Rastogi, G., Ranade, D.R., Yeole, T.Y., Gupta, A.K., Patole, M.S., Shouche, Y.S., 2009. Novel methanotroph diversity evidenced by molecular characterization of particulate methane monooxygenase A (*pmoA*) genes in a biogas reactor. Microbiol Res. 164(5), 536-544.

Rigas, F., Dritsa, V., Marchant, R., Papadopouloua, K., Avramides, E.J., Hatzianestis I., 2005. Biodegradation of lindane by *Pleurotus ostreatus* via central composite design. Env. Int. 31, 191–196.

Rigas, F., Dritsa, V., Marchant, R., Papadopouloua, K., Avramides, E.J., Hatzianestis I., 2005. Biodegradation of lindane by *Pleurotus ostreatus* via central composite design. Environ Int. 31, 191-196.

Rigas, F., Papadopoulou, K., Dritsa, V., Doulia, D., 2007. Bioremediation of a soil contaminated by Lindane utilizing the fungus *Ganoderma australe* via response surface methodology. J Hazard. Mater. 140, 325-332.

Rijnaarts, H.M.M., Bachmann, A., Jumelet, J.C., Zehnder, A.J.B., 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of γ-hexachlorocyclohexane in a calcerous soil. Environ. Sci. Technol. 24, 1349-1354.

Rodriguez , R.A., Toranzos, G.A., 2003. Stability of bacterial populations in tropical soil upon exposure to lindane. Int. Microbiol. 6, 253-258.

Saez, F., Pozo, C., Gomez, M.A., Toledo, M.V.M., Rodelas, B., López, J.G. 2006. Growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064 in the presence of selected pesticides. Appl. Microbiol. Biotechnol., 71, 563-567.

Saez, J.M., Bigliardo, A.L., Raimondo, E.E., Briceno, G.E., Polti, M.A., 2018. Lindane dissipation in a biomixture: Effect of soil properties and bioaugmentation. Ecotox. Eviron. Safe. 156, 97-105.

Sagar, V., Singh, D.P., 2011. Biodegradation of lindane pesticide by non white-rots soil fungus *Fusarium* sp. World J. Microbiol. Biotechnol. 27, 1747-1754.

Sahu, S.K., Patnaik, K.K., Sharmila, M., Sethunathan, N., 1990. Degradation of alpha-, beta-, and gamma-hexachlorocyclohexane by a soil bacterium under aerobic conditions. Appl. Environ. Microbiol.56, 3620-3622.

Saitoh, S., Iwasaki, K., Yagi, O., 2002., Development of a new most probable number method for enumerating methanotrophs using 48-well micro-titre plates. Microb. Environ. 17, 191-196.

Salam, J.A., Das, N., 2013. Enhanced biodegradation of lindane using oil-in water bio-microemulsion stabilized by bio-surfactant produced by a new yeast strain *Pseudozyma* VITJzN01. J. Microbiol. Biotechnol. 23, 1598-1609.

Salam, J.A., Das, N., 2014. Lindane degradation by Candida VITJzN04, a newly isolated yeast strain from contaminated soil: kinetic study, enzyme analysis and biodegradation pathway. World J. Microbiol. Biotechnol. 30, 1301-1313.

Salam, J.A., Das, N., 2015. Degradation of lindane by a novel embedded bio-nano hybrid system in aqueous environment. Appl. Microbiol. Biotechnol. 99, 2351-2360.

Scheutz, C., Mosbaek, H., Kjeldsen, P., 2004. Attenuation of methane and volatile organic compounds in landfill soil covers. J. Environ. Qual. 33, 61-71.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. 2009. Introducing mothur : Open- Source, Platform-Independent, Community-Supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 37- 41.

Scott, CS., Chiu, W.A., 2006. Trichloroethylene cancer epidemiology: a consideration of select issues. Environ Health Perspect. 114, 1471-1478.

Semrau, J.D., Di Spirito, A.A., Yoon, S., 2010. Methanotrophs and copper. FEMS. Microbiol. Rev. 34, 496-531.

Senoo, K., Wada, H., 1989. Isolation and identification of an aerobic γ-HCH decomposing bacterium from soil. Soil Sci.Plant. nutr. 35, 79-87.

Sharma, B.M., Bharat, G.K., Tayal, S., Nizzetto, L., Čupr, P., Thorjørn, L., 2014. Environment and human exposure to persistent organic pollutants (POPs) in India: a systematic review of recent and historical data. Environ. Int. 66, 48-64.

Sharp, C.E., Smirnova, A.V., Graham, J.M., Stott, M.B., Khadka, R., Moore, T.R., Grasby, S.E., Strack, M., Dunfield, P.F., 2014. Distribution and diversity of Verrucomicrobia methanotrophs in geothermal and acidic environments. Environ Microbiol. 16(6), 1867-1878.

Sheng, J., Wang, X., Gong, P., Joswiak, D.R., Tian, L., Yao, T., Jones, K.C., 2013. Monsoon-driven transport of organochlorine pesticides and polychlorinated biphenyls to the Tibetan Plateau: three year atmospheric monitoring study. Environ. Sci. Technol. 47 (7), 3199-3208.

Sheomaker, K.T., Breisch, A.R., Jaycox, J.W., Gibbs, J.P., 2014.Disambiguating the minimum viable population concepts : Response to Read and McCoy. Conserv. Biol. 28(3), 871-873.

Shigematsu, T., Hanada, S., Eguchi, M., Kamagata, Y., Kanagawa, T., Kurane, R., 1999. Soluble methane monooxygenase gene clusters from trichloroethylene-degrading *Methylomonas* sp. strains and detection of methanotrophs during *in situ* bioremediation. Appl Environ Microbiol. 65, 5198-5206.

Shukla, A.K., Vishwakarma, P., Upadhyay, S.N., Tripathi, A.K., Prasana, H.C., Dubey, S.K., 2009. Biodegradation of trichloroethylene (TCE) by methanotrophic community. Biores Technol 100, 2469–2474.

Siddique, T., Okeke, B.C., Arshad, M., Frankenberger, W.T., 2002. Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry. J. Agric. Food Chem. 50, 5070-5076.

Sidi, Y., Kiltchevsky, E., Shaklai, M., Pinkhas, J., 1983. Acute myeloblastic leukemia and insecticide. N. Y. state j. med.83, 161-166.

Simonich, S.L., Hiteis, R.A., 1995. Organic pollutants accumulation in vegetation. Environ. Sci. Technol. 29, 2905-2914.

Singh, B.K., Kuhad, R.C., 1999. Biodegradation of lindane (γ-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. Lett. Appl. Microbiol. 28, 238-241.

Singh, B.K., Kuhad, R.C., 2000. Degradation of insecticide lindane (γ-HCH) by white-rot fungi *Cyathus bulleri* and *Phanerochaete sordida*. Pest. Manag. Sci. 56, 142-146.

Singh, G., Kathpal, T.S., Spencer, W.F., Dhankar, J.S., 1991. Dissipation of some organochlorine insecticides in cropped and uncropped soil. Environ. Pollut. 70, 219-239.

Singh, J.S., 2011. Methanotrophs: the potential biological sink to mitigate the global methane load. Curr. Sci. 100, 29-30.

Singh, J.S., 2014. Cyanobacteria: A vital bio-agent in eco-restoration of degraded lands and sustainable agriculture. Clim Change Environ Sustain. 2, 133-137.

Singh, J.S., 2015. Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. Agric Ecosyst Environ. 203, 80-82.

Singh, J.S., Gupta, V.K., 2018. Methanotrophs: Soil microbial biomass: A key soil driver in management of ecosystem functioning. Sci.Total Environ. 634, 497-500.

Singh, J.S., Kashyap, A.K., 2007. Seasonal variations in soil N-mineralization and nitrification in seasonally dry tropical forest and savanna ecosystem in Vindhyan region, India. Tropical Ecol. 48, 27-35.

Singh, J.S., Pandey, V., 2013. Fly ash application in nutrient poor agriculture soils: Impact on methanotrophs population dynamics and paddy yields. Ecotoxicol. Environ. Saf. 89, 43-51.

Singh, J.S., Raghubanshi, A.S., Singh, R.S., Srivastava, S.C., 1989. Microbial biomass acts as source of plant nutrients in dry tropical forest and savana. Nature. 388, 499-500.

Singh, J.S., Singh, D.P., 2012. Reforestation: a potential approach to mitigate the excess CH_4 build-up. Ecol. Manage. Restor. 13, 245-248.

Singh, J.S., Singh, D.P., Kashyap, A.K., 2009. A comparative account of the microbial biomass-N and N-mineralization of soils under natural forest, grassland and crop field from dry tropical region, India. Plant. Soil. Environ. 55 (6), 223-230.

Singh, J.S., Singh, D.P., Kashyap, A.K., 2010. Microbial Biomass C, N and P in disturbed dry tropical forest soils, India. Pedosphere. 20, 780-788.

Singh, J.S., Strong, P.J., 2016. Biologically derived fertilizer: A multifaceted bio-tool in methane mitigation. Ecotoxicol Environ Saf. 124, 267-276.

Singh. C., Tiwari, S., Gupta, V.K., Singh, J.S., 2018. The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils. Catena 171, 485-493.

Sparling, G.P., Whale, K.N., Ramsa, A.J., 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air dried soils. Aust. J. Soil Res 23, 613-621.

SRI. 2009. Directory of Chemical Producers. Menlo Park, CA: SRI Consulting.

Srivastava, S.C., Singh, J.S., 1988. Carbon and Phosphorous in the soil biomass of some tropical soils of India. Soil. Biol. Biochem. 20, 743-747.

Strong, P.J., Xie, S., Clarke, W.P., 2015. Methane as a resource: can the methanotrophs add value? Environ. Sci. Technol. 49, 4001-4018.

Subbaiah, B.V., and Asija, G.L., 1956. A rapid procedure for the estimation of available nitrogen in soil. Curr. Sci. 25, 259-260.

Sumithra, S., Ankalaiah, C., Rao, D., Yamuna, R.T., 2013. A case study on physico-chemical characteristics of soil around industrial and agricultural area of yerraguntla, kadapa district, A. P, india. Int. J. Geo. Earth and Environ. Sci. 3(2), 28-34.

Sun, J., Pan, L., C.W., Tsang, D., Li, Z., et al., 2018. Phthalate esters and organochlorine pesticides in agricultural soils and vegetables from fast-growing regions: a case study from eastern China. Environ Sci Pollut Res. 25, 34-42.

Szabo, D.T., Loccisano, A.E., 2012. POPs and human health risk assessment. In: Schecter, A. (Ed.), Dioxins and Health Including Other Persistent Organic Pollutants and Endocrine Disruptors, third ed. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Takeuchi, M., Nanba, K., Iwamoto, H., Nirei, H., Kusuda, T., Kazaoka, O., Owaki, M., Furuya, K., 2005. *In situ* bioremediation of a cis-dichloroethylene-contaminated aquifer utilizing methane-rich groundwater from an uncontaminated aquifer. Water Res. 39, 2438–2444.

Tekere, M., Ncube, I., Read, J.S., Zvauya, R., 2001. Biodegradation of the organochlorine pesticide, lindane by a subtropical white rot fungus in batch and packed bed bioreactor system. Environ. Technol. 23, 199–206.

Tekere, M., Ncube, I., Read, J.S., Zvauya, R., 2001. Biodegradation of the organochlorine pesticide, lindane by a subtropical white rot fungus in batch and packed bed bioreactor system. Environ. Technol. 23, 199-206.

Tekere, M., Ncube, I., Read, J.S., Zvauya, R., 2002. Biodegradation of the organochlorine pesticide, lindane by a sub-tropical white rot fungus in batch and packed bed bioreactor systems. Environ. Technol. 23, 199-206.

Thakur, M.P., Milcu, A., Manning, P., Niklaus, P.A., Roscher, C., Power, S., Reich, P.B., Scheu, S., Tilman, D., Ai, F., Guo, H., Ji, R., Pierce, S., Ramirez, N.G., Richter, A.N., Steinauer, K., Strecker, T., Vogel, A., Eisenhauer, N., 2015. Plant devirsity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors. Glob. Change. Biol. 21, 4076-4085.

Trotsenko, Y.A., Murrell, J.C., 2008. Metabolic aspects of aerobic obligate methanotrophy. Adv in Appl Microbiol. 63, 183-229.

Tsuji, K., Tsien, H.C., Hanson, R.S., Depalma, S.R., Scholtz, R. and Laroche, S. 1990. 16S ribosomal-RNA sequence-analysis for determination of phylogenetic relationship among methylotrophs. J. Gen. Microbiol. 136, 1-10.

Turkman, A., Nuri, A., Nermin, A., 1993. An Overview on HCH application in Turkey, A Situation Report, HCH and Halogenated Pesticides –State of the Art for Risk Assessment and technology Development. (See http://www.ihpa.info/library.htm 1st & 2nd Forum)

U.S. Environmental Protection Agency (USEPA) 2006. Lindane and Other HCH Isomers-EPA Risk Assessment Fact Sheet. www.epa.gov/oppsrrd1/REDs/factsheets/lindane_isomers_fs.htm.

U.S. Environmental Protection Agency (USEPA). 2012. Water QualityCriteria.www.epa.gov/waterscience/criteria/wqcriteria.html.

U.S. Food and Drug Administration (FDA). 2003a. Center for Drug Evaluation and Research. Lindane Lotion USP, 1% RX Only.

UNECE. 2000. "The 2000 Review on Strategies and Policies for air Pollution Abatement, the 1998 Protocol on Persistent Organic Pollutants, Replies to Questions 37-49 of the 2000.

UNEP. 2002. Sub-Saharan Africa, Regional Report, Regional Based Assessment of Persistent Toxic Substances.

USITC. 2009. USITC Interactive Tariff and Trade DataWeb. United States International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp and search on HTS no. 290351.

Van der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority : soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296-310.

Van Hylckama, V.J.E.T., Janssen, D.B., 2001. Formation and detoxification of reactive intermediates in the metabolism of chlorinated ethenes. J. Biotechnol. 85, 81–102.

Vance, E.D., Brookes,P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Vega, M., Romano, D., Uotila, E., 2016. Lindane (persistent organic pollutant) in the EU. Directorate General for Internal Policies. Policy Department C: Citizens' Rights and Constitutional Affairs. Petitions (PETI). PE 571–398.

Verce, M.F., Ulrich, R.L., Freedman, D.L., 2000. Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. Appl. Environ. Microbiol. 66, 3535-3542.

Vijgen, J., Abhilash, P.C., Li, Y., Lal, R., Forter, M., Torres, J., 2011. Hexachlorocyclohexane (HCH) as new Stockholm convention POPs-a global perspective on the management of lindane and its waste isomers. Environ. Sci. Pollut. Res. 18, 152-162.

Vijgen, J., Yi, L.F., Forter, M., Lal, R., Weber, R., 2006. The legacy of lindane and technical HCH production. Organohalo. Compd. 68, 899-904.

Vimal, S.R., Singh, J.S., Arora N.K., Singh, S., 2017. Soil-plant-microbe intractions in stressed agricultural management: A review. Pedosphere. 27(2), 177-192.

Waliszewski, S.M., 1993. Residues of lindane, HCH isomers and HCB in the soil after lindane application. Environ. Pollut. 82, 289-293.

Walker, K.,Vallero, D.A., Lewis, R.G.,1999. Factors influencing the distribution of lindane and other hexachlorocyclohexanes in the environment. Environ. Sci. Technol. 33, 4373-4378.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

Wang, Z., Peng, P., Huang, W., 2009. Dechlorination of gammahexachlorocyclohexane by zerovalent metallic iron. J. Hazard. Mater. 166, 992–997.

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soils. Biol. Rev. 67, 321-358.

Wardle, D.A., 1998. Controls of temporal variability of the soil microbial biomass : a global scale synthesis. Soil Biol. Biochem. 30 (13), 1627-1637.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between above ground and below ground biota. Science 304, 1629-1633.

Wauchope, R.D., Buttler, T.M., G., H.A., Beckers, A. and Burt, J.P. (1992) SCS/ARS/CES Pesticide properties database for environmental decision making. Rev. Environ. Contam. Toxicol. 123, 1-157.

Whittenbury, R., Phillips, K.C., Wilkinson, J.F., 1970. Enrichment isolation and some properties of methane-utilizing bacteria. J.Gen. Microbiol. 61, 202-218.

Willett, K.L., Ulrich, E.M., Hites, R.A., 1998. Differential toxicity and environmental fate of hexachlorocyclohexane isomers. Environ. Sci. Techno. 32, 2197-2207.

Williams, A.M., 1988. The Biochemistry and Physiology of Poly- beta Hydroxybutyrate Metabolismin Methylosinus trichosporium OB3b. Ph.D.thesis, Biotechnology Centre, Cranfield Institute of Technology, Cranfield.

Wilson, J.T., Wilson, B.H., 1985. Biotransformation of trichloroethylene in soil. Appl Environ Microbiol. 49, 242-243.

Wise, M.G., McArthur, J.V., Shimkets, L.J., 1999. Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture independent 16S ribosomal DNA analysis. Appl Environ Microbiol. 65(11), 4887-4897.

Yang, J., Kang, Y., Sakurai, K., Siriguleng, 2016. Change of soil microbial biomass c,n between long-time free grazing and exclosure pasture in semiarid ecosystem in Tongliao and Chiefeng of Inner Mongolia J. Bioremed. Biodegrad. 7, 34-43.

Yang, L.H., Bastow, J.L.,Spence, K.O., Wright, A.N., 2008. What can be learn from resource pulses. Ecology. 89, 621-634.

Zayed, A.M., Terry, N., 2003. Chromium in the environment: Factors affecting biological remediation. Plant Soil. 249, 139–156.

Zhang, H., Hu, C., Jia, X., Xu, Y., Wu, C., Chen, L., Wang, F., 2012. Characteristics of chexachlorocyclohexane biodegradation by a nitrogen-fixing Cyanobacterium-Anabaena azotica. J Appl Phycol. 24, 221-225.

Zhang, H., Wan, H., Song, L., Jiang, H., Wang, H., Qiao, C., 2010. Development of an auto fluorescent Pseudomonas nitroreducens with dehydrochlorinase activity for efficient mineralization of gamma-hexachlorocyclohexane (gamma-HCH). J Biotechnol. 146(3), 114-119.

Zhang, M., Wu, J., Yang, F., Lei Y., Zang, Q., Cheng, X., 2016. Alterations in soil microbial community and composition and biomass following agricultural Land use change. Sci. Rep. 6, 365-387.

Zhang, W.J., Jiang, F.B., Ou, J.F., 2011. Global pesticide consumption and pollution: with China as a focus. Proc. Int. Acad. Ecol. Environ. Sci. 1, 125-144.

Zhu, Y., Liu, H., Xi, Z., Cheng, H., Xu, X., 2005. Organochlorine pesticides (DDTs and HCHs) in soils from the outskirts of Beijing, China. Chemosphere. 60, 770–778.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V., Mataix-Beneyto, J., 2009. Changes in soil microbial community structure following the abandonment of agricultural traces in mountainous areas of eastern Spain. Appl. Soil Ecol. 42, 315-323.

Scientific publications and achievements

SCIENTIFIC PUBLICATIONS AND ACHIEVEMENTS

WORKSHOP AND CONFERENCE ATTENDED

1. Participated in the workshop "Hands-On-Training for SEM, FTIR, FPLC and held Ion Chromatography" on February 18-20, 2015, USIC, BBAU, Lucknow.
2. International workshop on Bridging Development Divide for Inclusive Growth through Science, Technology and Innovation (BRIDGES) on Jan 16-17, 2015
3. Seven days workshop on "Gene Cloning & Its Expression, To produce genetically modified organisms" on November 20-26, 2017.
4. National Conference on "Recent Trends in Applied Microbiology, Human Health and Human Health" on March 27-28, 2015 in BU, Jhansi.
5. National seminar on University partnership programme: A march towards sustainable growth (AMTSG- 2015) on March 12-13, 2015 in CIIPP, BBAU, Lucknow, pp- 66.
6. 3rd Lucknow Science Congress. A National Conference. (LUSCON-2015) held in BBAU, Lucknow.
7. National Conference on "Science for Society an Interdisciplinary Approach" on Oct 31- Nov 02, 2015 in BBAU, Lucknow.
8. 4th Lucknow Science Congress. A National Conference. March, 2017. held in BBAU, Lucknow.
9. "103rd Indian Science Congress" held at University of Mysore, Mysore on January 03-07, 2016
10. "104th Indian Science Congress" held at Sri vankateswara University, Tirupati, Andra Pradesh held on January 03-07, 2017

11. 58th Annual Conference of Association of Microbiologists of India (AMI-2017) & International Symposium on “Microbes for Sustainable Development: Scope & Applications” (MSDSA-2017) from November 16-19, 2017.

Professional Memberships

1. Lifetime membership of **Indian Science Congress Association (ISCA)** with Membership No. L27407.
2. Lifetime membership of **Association of Mircrobiologists of India (**AMI**)** with Membership No. 4621.

Poster Presentation

1. **Siddharth Boudh**, Shashank Tiwari, Jay Shankar Singh. Methanotrophs: A sustainable tool for lindane remediation. National Conference on “Recent Trends in Applied Microbiology, Human Health and Human Health” on March 27-28, 2015 in BU, Jhansi. **Poster Presentation**
2. **Siddharth Boudh,** Jay Shankar Singh. “Methanotrophs: A key bioagent in bioremediation of Organochlorine pesticides”. 4rd Lucknow Science Congress (LUSCON-2017) & National Conference on “Science Technology & Innovations for Sustainable Development” on 3rd and 4th March, 2017 in BBAU, Lucknow. **Poster Presentation**
3. **Siddharth Boudh**, Jay Shankar Singh. Methanotrophs: An emerging and ecofriendly tool in bioremediation of organochlorine pesticides. 58th Annual Conference of Association of Microbiologists of India (AMI-2017) & International Symposium on “Microbes for Sustainable Development: Scope & Applications” (MSDSA-2017) from November 16-19, 2017. **Poster Presentation**

Boudh et al., Expert Opin Environ Biol 2019, 8:1
DOI: 10.4172/2325-9655.1000162

Expert Opinion on Environmental Biology

Research Article

A SCITECHNOL JOURNAL

An Assessment of Hexachlorocyclohexane Residues Contamination and Soil Microbial Biomass Level in Soil of Northern India

Siddharth Boudh[1], Jay Shankar Singh[1*], Satyanarayana GNV[2,3] and Patel DK[2]

Abstract

In the present study, soil physico-chemical properties, hexachlorocyclohexane (HCH) isomers and soil microbial biomass (SMB) –C, –N and –P were investigated by selecting 6 study sites in Lucknow and Renukoot region of Uttar Pradesh of Northern India. The ANOVA revealed significant variations soil physico-chemical properties (P<0.02-<0.001), HCH residues concentrations (P<0.005–<0.001), and SMB-C, -N and –P (P<0.001), across different study sites. The GC and GC-MS/MS analysis showed that soil samples were contaminated with HCH isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH) and organochlorine pesticides (OCPs) like aldrin, β-endosulphan and dichlorodiphenyltrichloroethane (DDT). The concentration of HCH isomers ranged between 0.43–310.97 μg g^{-1} soils in the samples of Lucknow region, whereas in the Renukoot region the values ranged between 0.12–194.44 μg g^{-1} soils. The SMB–C, –N and –P values were recorded maximum (365.2, 65.5 and 26.8 μg g^{-1} soil, respectively) in soils of Renukoot region and minimum (99.7, 15.8 and 12.2 μg g^{-1} soil, respectively) at Lucknow sites. Across different study sites the SMB–C, –N and –P values were negatively correlated with HCH residues concentrations and key soil drivers such as total-C, -N and electrical conductivity. The result suggests that presence of lethal HCH and OCPs residues persisting in soils negatively impacts the soil microbial diversity and their biomass. Therefore, on-site monitoring and bioremediation of HCH and OCPs might be more helpful for prevention and reduction of toxic pollutants in the soil and its bad impacts on beneficial microbes and living beings.

Keywords

HCH; GC-MS/MS; Lindane; Organochlorine pesticides; POPs

Introduction

Lindane, a highly debated common insecticide, severely restricted under the international protocol, is mainly manufactured in India and Romania. During 1 tonnes of lindane (γ-hexachlorocyclohexane-HCH) manufacturing about 9 tonnes of waste-a complex "muck" of HCH residues are generated [1]. The γ-HCH or lindane is purified from rest of the HCH isomers and rest becomes "muck" which becomes an environmental problem due to highly persistence nature [2]. At the same time as the application of the lindane is matter of discussion in the west and international forums, the spontaneous production of lindane in a developing country like India, with negligent environmental protection laws and occupational safety regulations, leads to generation of dangerous dumping sites (stockpiles). The dumping sites contains highly persistent toxic wastes and are illegally disposed in the country sides, water reservoirs, agricultural fields and most likely in the bodies of people and cattle residing nearby the dumping sites located close to lindane producing industries.

The organochlorine pesticides (OCPs) have long residual action and are persistent in the environment without losing their toxicity [3]. The soils contaminated with organochlorine pesticides are turning into gradual re-emission sources, which can cause lasting damage to the environment [4]. Most of the countries have banned the use of xenobiotic compounds, but some are still using Lindane (γ-HCH) for economic reasons and new sites are consequently being contaminated with pesticides. In addition, the HCH isomers are commonly used against lice infestations [5]. In 2009, lindane together with α- and β-HCH was added to the list of POPs under the Stockholm Convention, which signified that HCH contamination has been a matter of global concern [6]. The use of HCHs (a mixture of α, β, γ, δ, and ε HCH isomers) and the purified lindane (γ-HCH, the only isomer with insecticidal properties) in the agricultural sector and, in a less extent, for public health purposes [7, 8] has resulted into serious contamination problem globally due to their occurrence in aquatic, soil and air environment [9-12]. As a result it has been estimated that there are huge accumulations of HCHs in different environments worldwide [13]. Organochlorine pesticides (OCPs) have been commonly used across the world to control agricultural pests and vector born diseases [14]. For many years, humans have enjoyed the benefits of using pesticides to control weeds, insects, pathogenic fungi, parasites and rodent pests in crops. Pesticides are the only effective means of controlling pathogens, weeds or insect pests in many circumstances [15]. It has been estimated that about 10 million tons of the technical HCH have been used globally from 1948 to 1997 [16, 17].

In the production of lindane, waste of HCH isomers also generated with no insecticidal properties (commonly called as 'muck'; 65–70% of α-HCH, 7–10% of ß-HCH, 75% of δ-HCH, 1–2% of ε-HCH, <2% of η-and θ-HCH) [18]. Though the use of pesticides has offered considerable economic benefits by enhancing the production and yield of crops but at the same time these persistent organic pollutants (POPs) causes severe environmental hazards and threat to living beings. It is not known, how the HCH isomers quantities impact the microbial community and biomass in the soils. Therefore, identification and quantification of these chemical pesticides in soils may provide important information regarding their correlation with microbial community and removal from the soil and environment.

The soil microbial biomass (SMB) has been considered as an important source of plant nutrients in nutrient poor soils and agriculture and natural forest. Land-use changes and deforestation have important effect not only for availability of inorganic nutrients but also for SMB levels. Hence, any disturbances in soil SMB pools could be one of the crucial aspects which may naturally affect the

*Corresponding author: Jay Shankar Singh, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow 226025, Uttar Pradesh, India, Tel: 915222440822; E-mail: jayshankar_1@yahoo.co.in

Received: April 25, 2019 Accepted: May 17, 2019 Published: May 27, 2019

Journal of Environmental Management 242 (2019) 1–10

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Land use change: A key ecological disturbance declines soil microbial biomass in dry tropical uplands

Shashank Tiwari[a], Chhatarpal Singh[a], Siddharth Boudh[a], Pradeep Kumar Rai[b], Vijai Kumar Gupta[c], Jay Shankar Singh[a,*]

[a] Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Raebarelly Road, Lucknow, 226025, Uttar Pradesh, India
[b] Department of Botany, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India
[c] Department of Chemistry and Biotechnology, ERA Chair of Green Chemistry, School of Science, Tallinn University of Technology, Akadeemia Tee 15, 12618, Tallinn, Estonia

ARTICLE INFO

Keywords:
Land use changes
Soil microbial biomass-C
Soil microbial biomass-N
Soil microbial biomass-P

ABSTRACT

Land use changes such as transformation of natural landscapes, forest degradation and increase in croplands due to human activities are considered amongst the most influential ecological disturbances affecting soil, ecosystems and environmental sustainability. The previous works from India are limited to show that soil disturbances influence abiotic and biotic factors along a rural–urban gradient. However, variations in soil microbial biomass (SMB) –C, –N and –P quantity due to land use changes at different soil depths across different land use types remain poorly understood on comparative ground. We investigated the impact of land use types on soil properties and SMB –C, –N and –P levels across different soil depths (0–10, 10–20 and 20–30 cm) in dry tropical uplands. Four land use types/covers (natural forest, mixed forest, savanna and agriculture land) were selected. The present study is based on two hypotheses: i) different land use types affect SMB levels in top surface soil (0–10 cm), but have less effects in deeper soil profiles (20–30 cm); and ii) SMB levels in top surface soil are highest in natural forest, followed by mixed forest and then savanna and agriculture lands. ANOVA showed significant differences in SMB values due to land use covers ($P < 0.001$), soil depths ($P < 0.001$) and land use types × soil depths interaction ($P < 0.001$). Although, there had no effect of land use types on SMB levels in deeper soil profiles (20–30 cm) but soil parameters (soil pH, soil moisture, soil temperature, total-N, C/N ratio and organic-C) significantly affect SMB levels in top surface (0–10 cm) soil. The study suggests that SMB may be considered as a key indicator of soil fertility index, while land use practices are a major cause for loss of microbial community composition/biomass in dry tropical upland soil.

1. Introduction

Land-use change (LUC) is one of the main global environmental disturbances, greatly contributing to climate change, loss of ecosystem services and microbial species diversity (Maharjan et al., 2017; Leeuwen et al., 2017). Land-use conversion has been considered as an intensive anthropogenic activity which strongly alters the soil ecosystem functioning (Pabst et al., 2013; Cao et al., 2017) - specifically the tropical soils, since they are nutrient-limited and highly weathered (Tilman et al., 2001; Singh and Gupta, 2018). The land use management practices may influence the functional roles of soil microorganisms through modification of the quantity and quality of organic matter inputs (Steenwerth et al., 2002; Jin et al., 2010). The degradation rate of natural tropical forest, which covers 7% of the earth's surface, is ~15.4 million ha year^{-1} (Parrotta et al., 1997; Kumar and Ghoshal, 2014). Conversion of forest to agriculture and intensive farming practices contribute to the loss of soil organic matter (Lagomarsino et al., 2011), alters microbial activities and ultimately affect soil quality (Schloter et al., 2003; Kumar and Ghoshal, 2017). Nowadays, to satisfy the human food demands, better land use management practices such as reduced inputs of synthetic fertilizers, promotion of organic farming and bio-fertilizer technology, restoration of degraded land, afforestation, etc. may be crucial for the improvement of soil and environmental quality management (Singh, 2015a, b).

The rapid growth rate of population caused by urbanization in northern India requires additional farmlands for the production of crops. One way is to expand the tillable area by clearing the forests and converting pastures into farming land (L. Deng et al., 2014).

* Corresponding author.
E-mail address: jayshankar_1@yahoo.co.in (J.S. Singh).

https://doi.org/10.1016/j.jenvman.2019.04.052
Received 21 August 2018; Received in revised form 14 April 2019; Accepted 15 April 2019
0301-4797/

Climate Change and Environmental Sustainability (October 2016) 4(2): 237-242
DOI: 10.5958/2320-642X.2016.00026.0

OPINION

Climate Change Resilient Crops to Sustain Indian Agriculture

Jay Shankar Singh[1]* • Siddharth Boudh[2]

Abstract The agriculture sector in India is susceptible to the frequent and erratic climatic irregularities. Anticipated changes in the climate due to intensive anthropogenic activities may cause worst conditions. Models of crop production, considered together with global climate models, indicated that global warming would increase the exposure of major crops to temperature stress, leading ultimately to lower crop yields. Such decrease in yields varied significantly across different states of the country, and there remained significant uncertainties about their magnitude. Various studies also indicated that climatic variability alone had the potential to decrease yields to an extent comparable with or greater than the decrease in yields expected due to rising mean temperatures. The climatic changes led to increase in temperature and water stress, which caused heavy losses in yield of any crop. It may be a big challenge for the agriculture scientists and country leaders to feed the coming future Indian population sufficiently. Development of new climate resilient crop varieties, generation of renewable energy resources and use of green technologies might be helpful for mitigating the impact of climate change and sustained agriculture productivity in near future. To cope with intense changes as projected in climate, new research paradigm of bringing all stake holders is urgently required to establish suitable long-term strategies for addressing the Indian agriculture sustainability.

Keywords Agriculture, Climate change, Drought, Floods, GHGs.

1. Introduction

Agriculture is one of the most vulnerable sectors to the climate change (Singh *et al.*, 2011a,b). The anticipated changes in climate as well as their associated impacts are all likely to affect substantially the potential of future agriculture in India. Despite the technological advances in the Green Revolution during 1967–1968 onwards, weather and climate are the basic deciding factors in the agricultural productivity. Agriculture in India is extremely diverse in the range of crops grown and livestock raised. Climate change will increase productivity of certain crops and regions with inferior quality in short–term but ultimately it will affect negatively over the longer period. Crop responses in a variable climate reflect the interplay among three main factors: (i) increasing temperature (ii) changing water resources and (iii) increasing carbon dioxide (CO_2) concentration. In this communication, various issues related to the impact of climate change on agriculture production in the country and future research directions have been discussed.

One of the potential threats to the Indian agriculture is the disturbances in rainfall pattern due to climate change. The shifting in onset monsoon is causing the disturbances in crop cultivation, and consequently, reduced agriculture production and its sustainability. Thus, India is one of the most vulnerable countries to climate change that is affecting agricultural yields. For the last one decade, India has been witnessing too many climatic shifts and natural calamities. The country has been frequently facing severe natural disasters such as floods, droughts, whereas Uttar Pradesh (UP) and Gujarat region has faced one of the worst situations of the last decade. The impact of climate change has adversely affected agricultural production resulting in huge loss of paddy and other crops in eastern districts and regional crops in the country (Singh, 2013a,b). Climatic disturbances have brought prevalent unhappiness and huge economic losses to the nation, adversely affecting public health, food security, water resources and biodiversity in the country. Floods are the most common annual occurrences in most of the states particularly Bihar, Assam, Jammu Kashmir, Uttar Pradesh; the most affected being the districts of the eastern UP and Terai region. Agriculture in India is very much weather-dependent (monsoon) and therefore, it may be said that Indian agriculture is nothing but gamble of the monsoon. It has been frequently observing that reduced amount of

[1]Assistant Professor, [2]Research Scholar, Department of Environmental Microbiology, B.B. Ambedkar (Central) University, Lucknow-226025, Uttar Pradesh, India
*Corresponding author E-mail id: jayshankar_1@yahoo.co.in

c0019 Chapter 19

Microbial resources mediated bioremediation of persistent organic pollutants

Siddharth Boudh[1], Jay Shankar Singh[1] and Preeti Chaturvedi[2]

[1]*Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, India,* [2]*Aquatic Toxicology Laboratory, Environmental Toxicology Division, CSIR—Indian Institute of Toxicology Research, Lucknow, India*

Chapter Outline

s0010 19.1 Introduction

p0255 Persistent organic pollutants (POPs) are bioaccumulative, toxic chemicals persistent in nature that do not break down easily in the environment. The POPs are synthetic chemicals that can cause harmful effects on human health and animals. POPs are chemically stable and do not readily degrade in the environment. POPs are lipophilic (have an affinity for fats) and easily soluble in fat and biomagnified as they move up through the food chain. They can travel long distances on wind and water and can be found in regions far away from their points of origin or use. These compounds are semivolatile in nature and because of their physicochemical property they can occur either in vapor phase or can be absorbed in atmospheric particles. These properties help them in their wide ranging transportation in the atmosphere. POPs have become widespread environmental contaminants and a global problem, and due to rapid industrial development and human activities POPs have become lethal to mankind. Some major organic chemical compounds that cause contamination in environment are organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, and dibenzofurans (Ersekova et al., 2014).

p0260 Various remediation technologies are being developed to treat these pollutants. These compounds stay intact within the environment massively for a long time period because of their nonbiodegradable nature, that is, they resist photolytic, chemical, and biological degradation. In addition, these compounds are of great concern because of long-range transport, persistent nature, and bioaccumulation tendency; once they enter into the food chain they get accumulated in the fatty tissue of the human body and pose a risk to cause adverse effects to the environment, wildlife, and human

New and Future Developments in Microbial Biotechnology and Bioengineering. DOI: https://doi.org/10.1016/B978-0-12-818258-1.00019-4

Singh-NFDMBB-1631790 978-0-12-818258-1 00019

Chapter 12
Pesticide Contamination: Environmental Problems and Remediation Strategies

Siddharth Boudh and Jay Shankar Singh

Abstract Pesticides are the chemicals used in the control of weeds and pests. The larger inputs of pesticides and fertilisers contaminate food commodities with trace amounts of chemical pesticides and its invasion in crops causes diseases, which is a growing source of concern for the universal population and environment in today's world. The extensive utilisation of pesticides possibly enhances their accumulation in the agricultural fields and environmental components, such as enlarged farms, field sizes, loss of landscape elements etc. Nevertheless, their low biodegradability has classified these chemical substances as a persistent toxic element. Furthermore, organo-chlorine pesticides have caused multiple problems of health hazards, such as acute and chronic effects including developmental effects and neurological disruptors in humans and animals. The biological stability of pesticides and the higher content of lipophilicity in food products create a significant effect on the physical condition of human beings and animals. As the bio-accumulation and bio-magnification of lethal pesticides are the main cause of the loss of plants, microbes and animal biodiversity, therefore, microbially based bioremediation of toxic pollutants from the polluted sites has been proposed to be a safe and sustainable means of decontaminating the environment. In this communication, we have tried to explain the source of environmental pollution by pesticides, its hazardous effects on living beings and remediation strategies.

Keywords Fertilisers · Pesticide · Bioremediation technologies · Composting

1 Introduction

Environmental exposure to toxic chemicals such as pesticides is a significant health risk to humans and other animals (Azmi et al. 2006; Kiefer and Firestone 2007; Rothlein et al. 2006; Singh et al. 2011). Use of organochlorine pesticides (OCPs) to

---

S. Boudh · J. S. Singh (✉)
Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@yahoo.co.in

R. N. Bharagava, P. Chowdhary (eds.), *Emerging and Eco-Friendly Approaches for Waste Management*, https://doi.org/10.1007/978-981-10-8669-4_12

Chapter 11
Microbial-Mediated Lindane Bioremediation

Siddharth Boudh, Shashank Tiwari, and Jay Shankar Singh

Abstract Hexachlorocyclohexane (HCH), commonly known as lindane, is the term which collectively identifies the eight isomers of the HCH. The lindane has only insecticidal properties and also considered as most toxic isomer of the HCH. The lindane has been listed as a persistent organic pollutant (POPs) under Stockholm Convention on June 29, 2005. More than 52 countries not only globally banned for its formulation but also use of lindane in any form. After knowing facts regarding its toxicity, persistence nature, and bioaccumulation, some countries are still producing and exporting the lindane on large scale. The countries involved in lindane formulation are creating dumping sites which are the major source of lindane contamination to the adjoining area. The lindane deposited in the cultivated soils is also affecting to the non-target organisms. Apart from this, scientists start working on its degradation and find out that bioremediation is the easiest, cheapest, and safest way to remove the lindane from contaminated sites. Bioremediation by the microalgae could help in decontaminating polluted aquatic ecosystems and in cleaning the effluents before they are discharged into aquatic systems. Many microorganisms show tremendous potential in lindane degradation. The present review article describes about all known possible lindane-degrading microorganisms used for its bioremediation and also concise advanced techniques used for this purpose.

Keywords Bioremediation • Lindane • Microbes • Nanoparticles • Persistent organic pollutant

S. Boudh • S. Tiwari
Department of Environmental Microbiology, School for Environmental Sciences,
B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_11

Chapter 7
Biochar Application in Management of Paddy Crop Production and Methane Mitigation

Chhatarpal Singh, Shashank Tiwari, Siddharth Boudh, and Jay Shankar Singh

Abstract Paddy agriculture is one of the major anthropogenic sources of methane (CH_4) emission at global level. A decrease in CH_4 release in the atmosphere from paddy fields can add significantly to the management of global warming and climate change. Biochar production and application in agriculture prepared from crop straw has been proposed as one of the effective countermeasure to mitigate the greenhouse gas emissions (GHGs) during farming. Biochar, a co-product of a controlled pyrolysis process, can be used as a tool to offset GHGs emissions and as a soil conditioner. Biochar application increased rice productivity, soil pH, soil organic carbon, total N but decreased soil bulk density in the long term. Recent studies have confirmed that the use of biochar in paddy agriculture has the capability to minimise the CH_4 production, but its essential mechanism has yet to be clarified. The additions of biochar to the agriculture soil showed higher CH_4 consumption because it improves soil aeration and porosity and enhances methanotrophs performance. However, further investigations are needed to evaluate the effect of biochar addition on net CH_4 emissions and consumptions, respectively, by methanogens and methanotrophs. Long-term experiments should be conducted to monitor any changes over the years on the influence of biochar amendments on soil–methanotrophs–paddy systems.

Keywords Biochar • GHGs • Methane • Methanotrophs • Paddy

C. Singh • S. Tiwari • S. Boudh
Department of Environmental Microbiology, School for Environmental Sciences, B.B. Ambedkar (Central) University, Raibarelly Road, Lucknow, Uttar Pradesh 226025, India

J.S. Singh (✉)
Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India
e-mail: jayshankar_1@bbau.ac.in

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_7