OPTIMIZATION OF LINDANE DEGRADING POTENTIAL OF MICROBES ISOLATED FROM CONTAMINATED SOILS OF PALAKKAD DISTRICT, KERALA, INDIA

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By

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This is to certify that the thesis entitled, **"Optimization of Lindane Degrading Potential of Microbes Isolated from Contaminated Soils of Palakkad District, Kerala, India"** is an authentic record of the research work carried out by Mrs. Nisa K G (Reg. No. 5034), under my scientific supervision and guidance in the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for award of the degree of Doctor of Philosophy of Cochin University of Science and Technology and that no part thereof has been presented before for the award of any other degree, diploma or associateship in any University/Institute. All the relevant corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral Committee have been incorporated in the thesis

Kochi
September 2019

Dr. C H Sujatha
(Supervising Guide)

Declaration

I hereby declare that the thesis entitled, "**Optimization of Lindane Degrading Potential of Microbes Isolated from Contaminated Soils of Palakkad District, Kerala, India**" is an authentic record of the research work conducted by me under the supervision and guidance of Dr. C H Sujatha, Professor, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology and no part of this has previously formed the basis of the award of any degree, diploma, associateship, fellowship or any other similar title or recognition from any University/Institution.

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Dedicated to dear and near ones…

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Preface

Environmental pollution is a universal problem, resulting serious negative impacts on entire living organisms, including human beings. Pollutants from various sources such as domestic and industrial outputs could contaminate soil and could find their way, along with water into the livestock production system and ultimately hinder the food chain. Similarly, the accumulation of such components can reduce crop yield and degrade the quality of the soil as well as agricultural products, finally effecting the balance of the ecosystem. In recent years, high levels of heavy metals, organochlorine insecticide residues including other agro-chemicals as different soil constituents has been detected in various locations of India and the reports on these were published. The toxic effects of such persistent pollutants from the environment needs to be globally portrayed. These pollutants are hydrophobic in nature and always tend to associate with soil bound carbonaceous matter and also associated to biological tissues with lipid profiles rather than dissolving in aqueous phase. By the development of novel technologies by the researchers all over the world, constantly identified the capability of microorganisms for removing xenobiotic such as pesticides and heavy metals from the entire system. A wide range of such microorganisms were rapidly screened, identified and characterized from variant sources such as soil, industrial effluent, polluted sites, sediment and water. The efficiency for restoration and clean-up the contaminated sites were studied, were successfully deployed in remediation programmes. The proper understanding of the current status of the environment pollution by monitoring and assessing the physico-chemical parameters possibly help us to achieve the goal in this direction. Following to this, the thesis entitled

"Optimization of Lindane Degrading Potential of Microbes Isolated from Contaminated Soils of Palakkad District, Kerala, India" truly confirms these descriptions of the xenobiotics such as heavy metals, organochlorine insecticide residues in the soils of Palakkad district in Kerala. The region is particularly known as the granary of Kerala there the misuse and malpractice of insecticides used for various crops is very common. Further, the main purpose of the investigation is to provide a footstep towards the modelling clean-up strategy of lindane contaminated sites. This is achieved by utilizing the capacity of *potential lindane degrading microbial strains*. In order to fulfil the requirements of the research work, the thesis is divided into six chapters and the details are furnished below:

Chapter 1 - *Introduction*

This chapter deals with the general introduction of the research work and highlights the importance of implementing the term bioremediation for the beneficial utilization of soil microbial strains by metabolizing toxic chemicals of the contaminated sites into non-toxic by-products. Similarly, the chapter discussed about the harmful effects of environmental pollution particularly organochlorine insecticides and heavy metals. Furthermore, the chapter comprise of review of literature, significance, main objectives and scope the study.

Chapter 2 - *Description of the Study Area*

The second chapter comprises of the details of study area and sampling strategies.

Chapter 3 - *Soil Properties and Metal Distribution*

The basic soil characteristics such as general parameters including pH, textural characteristics and organic matter content of the study area (spatio-temporal) are described in chapter 3. Heavy metal accumulation/ contamination of the study sites is evaluated using the various pollution indices viz. enrichment factor (EF), contamination factors (CF) geoaccumulation index (Igeo) and pollution load index (PLI). The statistical analyses, correlation and PCA were adopted for determining the sources, were portrayed in this chapter.

Chapter 4 - *Spatio-Temporal Distribution Pattern of Organochlorine Insecticides*

This chapter encloses the spatio-temporal distribution pattern of organochlorine insecticides (OCIs) in the study area. The analyzed OCIs were α - BHC, β - BHC, γ - BHC, heptachlor, aldrin, heptachlor epoxide (B), 4,4'- DDE, dieldrin, endrin, 2,4'- DDD, 4,4'- DDD, 2,4'- DDT, 4,4'- DDT, α - endosulphan, β - endosulphan. The results were discussed in detail and the relationships between the general parameters were evaluated statistically.

Chapter 5 - *Isolation and Characterization of OCI Degrading Microbes Specific to Lindane*

Persistence of OCIs and trace metals in the surface soils of the study area has triggered the need of bioremediation. Thus, chapter 5 highlights the importance of executing an eco-friendly and cost effective microbial biodegradation method to eradicate the OCIs. This chapter provide a clear background information on the bacterial population in the study area.

Secondly, the isolation, screening and developing microbial cultures from lindane treated soil samples from the study area for offering and adopting bioremediation strategies which is the main objective of the investigation. This chapter describes in detail the identification of isolated cultures and their deposition to NCBI Gen- Bank for the accession numbers.

Chapter 6 - ***Lindane Degrading Soil Microbes and their Potential in Lindane Biodegradation***

This chapter discussed the ability of isolated microbes to degrade the toxic chlorine compound, lindane i.e., the ability of these bacterial isolates to utilise the carbon source for their metabolism. The degradation rate was measured by quantitatively by estimating the amount of chlorine ions released in to the system. Additionally, this chapter also unravels the effect of different controlling parameters of the environmental niche which focus on varying pH and temperature and finally to determine the optimum temperature and pH for the lindane degradation.

Chapter 7 – ***Summary and Conclusions***

Finally this chapter provides the salient findings of the investigation in a nutshell and scope of future studies.

References are provided at the end of each chapter.

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Abbreviations

OCI	-	Organochlorine Insecticides
HCH	-	Hexachlorocyclohexane
DDT	-	Dichlorodiphenyltrichloroethane
DDE	-	Dichlorodiphenyldichloroethylene
DDD	-	Dichlorodiphenyldichloroethane
γ -TCCH	-	γ - 3,4,5,6-tetrachloro-1-cyclohexene
EF	-	Enrichment Factor
CF	-	Contamination Factors
Igeo	-	Geoaccumulation Index
PLI	-	Pollution Load Index
TOC	-	Total Organic Carbon
AAS	-	Atomic Adsorption spectroscopy
PCA	-	Principal Component Analysis
SPSS	-	Statistical Program for Social Sciences
CHO	-	Carbohydrate
LIP	-	Lipid

PRT	-	Protein
TL	-	Tannin/Lignin
ND	-	Non Detectable
LADD	-	Lifetime Average Daily Dose
ILCR	-	Incremental Lifetime Cancer Risk
HQ	-	Hazard Quotient
PCR	-	Polymerase Chain Reaction
BLAST	-	Basic Local Alignment Search Tool
NCBI	-	National Centre of Biotechnology Information
RNA	-	Ribonucleic Acid
MMMM	-	Modified Minimal Mineral Medium
MC	-	Mean Concentration
SD	-	Standard Deviation
Mini	-	Minimum
Max	-	Maximum

Chapter

1

INTRODUCTION

Contents

1. 1 General Introduction

Good establishment of new technologies in the field of agricultural research, such as the high-yielding variety of seeds and fertilizers has undoubtedly increased crop productivity. However, the productivity in the present scenario has been stagnant, which directly affect the earnings of peasants and farmers leading to threat in their living hood. It is believed that the decline in the productivity in agriculture sector generally be due to the scarcity of resources such as good quality (non-contaminated) cultivable land and the use of unpolluted water for the irrigation purpose. Furthermore, the involvement of using new types and variety of insecticides and metal containing fertilizers has led to an undesirable impact on the environment to

create a depletion and deterioration of the soil realm including the water column and by contaminating groundwater and soil surface.

In various case studies, certain heavily contaminated locations were identified and reported their lethal effects on human health in all over the world, especially in developing nations like India (Bhardwaj and Sharma, 2013). Considering these facts, the accumulating frequencies of xenobiotics such as metals and insecticides specifically organochlorine insecticides (OCIs) and their transformation products has led to a remarkable effort in the research field to implement new technologies to eradicate them or minimize their toxic effects on the environment. In order to remove the hazardous chemicals and to maintain the quality of soil environment, habitual techniques such as photocatalysis, recycling, remediation by nanoparticles, land-filling, activated carbon, incineration, pyrolysis, phytoremediation, biosorption, biocatalytic dechlorination and microbial degradation were adopted (Paul et al., 2005).

Among various techniques, bioremediation, a biological process of utilizing the capacity of soil microbes by metabolizing these toxic chemicals into beneficial products, is an efficacious and cost-effective method to eliminate the pollutants from the contaminated environment (Heitzer and Sayler, 1993; Gheewala and Annachatre, 1997; Gadd, 2000; Finley et al., 2010; Dell'Anno et al., 2012; Singh et al., 2013; Montagnolli et al., 2015). The schematic representation of biodegradation of xenobiotic is shown in the Fig. 1.1.

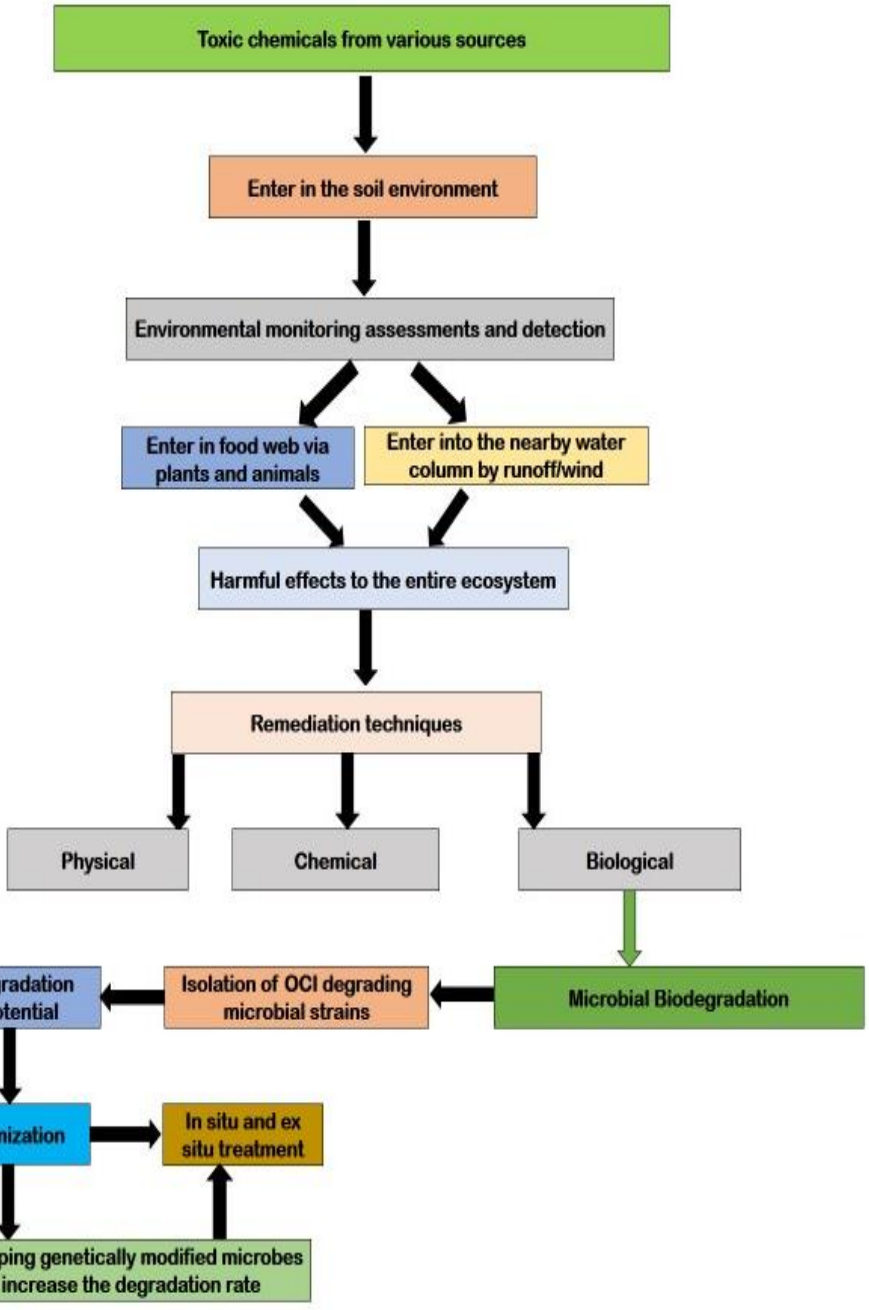

Fig. 1.1 Schematic representation of biodegradation of OCIs

1.2 A peep into the glimpse of Indian soil

India is the seventh-largest country in the world based on its geographical area. It has unique and specific geographical features comprising mountains, plateaus, coasts, desert and islands as well as varied environmental conditions such as climate, vegetation and has a wide variety of soil classes which exhibits all over the country. It is bounded by the Indian Ocean on the south, the Arabian Sea on the southwest, and the Bay of Bengal on the southeast. It shares land borders with Pakistan to the west; China, Nepal, and Bhutan to the northeast; and Myanmar and Bangladesh to the east. Recently, ICAR has classified Indian soil (Table 1.1) according to the texture, structure, colour, pH and porosity as following groups (Husain, 2014).

Table 1.1 ICAR classification of Indian soil

SL. No	Soil	Percentage (%)
1.	Alluvial Soils	43.3
2.	Red Soils	18.49
3.	Regur (Black - Earth) Soils	15.09
4.	Desert Soils	4.42
5.	Laterite Soils	3.70
6.	Mountain Soils	5.51
7.	Red and Black soils	5.40
8.	Grey and Brown Soils	1.09
9.	Submonate Soils	1.73
10.	Snowfields	1.2
11.	Others (Peaty & Marshy soils, Saline & Alkaline Soils and Karewa Soils)	0.07

Alluvial soils are most predominant in the northern part of India and vary in texture from sandy to silty loams which are suitable to cultivate wheat, rice, maize, sugarcane, pulses, oilseeds, vegetables and fruits. While in South India, were found to be thick and dark coloured fertile soils which are enriched with plant nutrients implies them very suitable for agriculture. Indian soil typically composed of inorganic matter (40%), organic matter (10%), soil water (25%) and soil air (25%). These also include the organisms such as protozoans, mites, nematodes, rotifers, blue-green or green, soil bacteria, fungi arthropods like mite, myriapods, spiders, insect larvae, collembolan, etc. (Siddiqui and Fatima, 2017). The nature of the soil in a particular place is primarily depending upon the climate, natural vegetation and rocks.

1.3 Metal (Inorganic) contamination

Over the past decades, the contamination due to trace metals and its health hazards to the living system is a subject of concern and performed a detailed examination and research in their perspective field. Metals have been widely distributed in the soil as a result of geologic (like minerals, chemical weathering, soil leaching, etc.) and anthropogenic activities (such as industrial and domestic effluents, usage of agricultural organic insecticides and fertilizers, sewage sludge, mining, burning of fossil fuels, smelting of metals, etc.). Metals such as Mg, Cd, Pb, Cu, Mn, and Zn were important components in phosphatic fertilizers as well as pest control agent such as Bordeaux mixture (copper sulphate), copper oxychloride, lead arsenate, etc. subsequently applied to the agricultural soils for the complete requirements of crops (Jones and Jarvis, 1981; Raven et al., 1998). Thus the

continuous use of such chemicals in the soil leads to the accumulation of these metals create hazardous effects on the environment. Moreover the application of various manures and wastewater irrigation (livestock, poultry, cattle, and pig manures, composts and municipal sewage sludge) into the arable fields could also enhance the accumulation of Cd, Cu, Pb, Ni and Zn in the environment (Reed et al., 1995; Chaney and Oliver, 1996; Canet et al., 1998; Sumner, 2000; Basta et al., 2005; Bjuhr, 2007). Biosolids (sewage sludge) were considered as fertilizers in various countries nowadays since these are primary organic biproduct of wastewater treatment plants (US EPA, 1994; Silveira et al., 2003). Pb and Cd accumulation possibly derive from airborne depositions due to their ability to volatilize at a higher temperature during various practices such as fuel burning, metal processing, etc. These eventually transform into metal oxides and condense to minute particles, ultimately reach the soil surface along with the raindrops. Residues of Zn and Cd might present in the roadside soil surfaces which could be attributed by tyres and lubricant oils used in motor vehicles. Other sources of these metals could be derived from various small-scale industries such as textile and tanning industries, consumption of petroleum derivatives, photographic waste materials and pharmaceutical amenities (US EPA, 1996; Smith et al., 2011). Pollution due to aforementioned metals could leach downwards through the soil profile and reach the groundwater column thereby contaminating the system (McLaren et al., 2005).

Due to the toxicity of non-essential and essential heavy metals beyond the permissible levels, leads to certain health hazards in humans as well as the ecosystem (Zarazua et al., 2006; Chibuike and Obiora, 2014; Kankia and Abdulhamid, 2014; Shahid et al., 2015; Adimalla et al., 2019; Wang et al.,

2019). The harmful effects certain metals in humans are depicted in Table 1.2.

Table 1.2 Harmful effects of certain trace metals (Li et al., 2019)

Trace metals	Harmful effects
Cu	Abdominal pain, anemia, diarrhea, headache, liver and kidney damage, metabolic disorders, nausea, vomiting
Cd	Bone disease, coughing, emphysema, headache, hypertension, itai–itai, kidney diseases, lung and prostate cancer, lymphocytosis, microcytic hypochromic anemia, testicular atrophy, vomiting
Ni	Cardiovascular diseases, chest pain, dermatitis, dizziness, dry cough and shortness of breath, headache, kidney diseases, lung and nasal cancer, nausea
Zn	Ataxia, depression, gastrointestinal irritation, hematuria, icterus, impotence, kidney and liver failure, lethargy, macular degeneration, metal fume fever, prostate cancer, seizures, vomiting
Pb	Anorexia, chronic nephropathy, damage to neurons, high blood pressure, hyperactivity, insomnia, learning deficits, reduced fertility, renal system damage, risk factor for Alzheimer's disease, shortened attention span

Subsequently, several soil contamination studies, revealed metals like Pb, Cd, Cu, Zn, Ni, Mn, Co and Mg as the most hazardous inorganic pollutants encountered in various soil environments of the world (Rajaganapathy et al., 2011; Tchounwou et al., 2012). Similar reports were published in India, China, Nigeria, Norway, USA, Italy, Iran, etc. were depicted in Table 1.3. As aforementioned, the primary source of metal pollution has considerably derived from crop yielding products applied into the farmlands for agricultural deeds (Hu et al., 2017). Since these metals are not able to endure further degradation by the means of chemical and

biological technologies and remain in the soil niche for decades (Tchounwou et al., 2012). The accumulation of these toxic metals in the ecosystem may affect the biodiversity of microorganisms and eventually rely on different controlling factors like temperature, pH, clay minerals, organic matter, inorganic anions and cations, and various chemical moieties of metal residing in the soil.

Table 1.3 The concentrations of some metals in soils around the world (mg kg^{-1})

Study area	Cu	Pb	Ni	Zn	Reference
India	159.78	77.25	120.43	437.44	Adimalla, 2019
China	40.77	50.13	31.14	155.33	Pan et al., 2018
Nigeria	516	568	16.5	93.5	Odewande and Abimbola, 2008; Nwachukwu et al., 2010
Norway	32	32	43	80	Andersson et al, 2010, Lu et al., 2013
USA	59	198	31	235	Cannon and Horton, 2009
Italy	74	141	89	158	Cicchella et al., 2008
Iran	111	93.18	105.21	133.86	Jamshidi-Zanjani and Saeedi, 2013; Salehi et al., 2014; Modabberi et al., 2018

Some microorganisms are highly adaptive to these worst environmental conditions and are capable of dislocating these inorganic pollutants and later, oxidize/reduce them to non-poisonous/less poisonous forms (Friedlová, 2010; Chibuike and Obiora, 2014). *Sporosarcina ginsengisoli* (Achal et al., 2012), *Pseudomonas putida* (Balamurugan et al., 2014), *Bacillus subtilis* (Imam et al., 2016), etc. were such prominent

microbial strains reported capable of degrading trace metals. Mixtures of bacterial strains *Viridibacillus arenosi B - 21, Sporosarcina soli B - 22*, *Enterobacter cloacae KJ - 46*, and *E. cloacae KJ - 47* proved their capability to remediate Cd, Cu, and Pb from contaminated soils (Kang et al., 2016).

1.4 Insecticide (Organic) contamination

Needless to say, contamination due to insecticides in the environment is a major global concern, mainly involved in the eradication of insects, weeds, fungi, bacteria, etc. and their accumulation in the soil leads to severe health hazards to the living organisms belonging to the ecosystem. To improve the crop production and to have high a yield in this sector, the addition of chemical agents namely, Organochlorine Insecticides (OCIs) are common practice in the country due to their effectiveness and low cost (Anonymous, 1989). As a result of continuous uncontrolled usage of these chemicals has led to the accumulation of chemical toxicants in the soil environment causing innumerable pollution effects. The leaching properties of the soil translocate them into the deeper layers of the Earth surface and also transfer these chemicals into adjacent areas by means of wind and dispersion through showers. Ultimately, they mixed with groundwater and neighbouring rivers and finally find their ways into the sea and polluting the whole marine environment. This specific group of insecticides is considered particularly hazardous because of their ability to bioaccumulate into the food chain, to remain stable for many years and finally move into the environment in every potential way (air, water, soil, biota).

In India, according to the statistics of Rajendran (2002), every year a huge amount of money-loss occurred in various issues related to the pests and for the spreading of diseases and the degree of impact increases consequently. Thus the pest control in every aspect is the requirement in the Indian agricultural economy; as a result, the practice of insecticides and their usage is an essential component in various fields. Therefore, aforementioned factors such as anthropogenic as well as geographical processes are the causatives for the existence of these xenobiotics throughout the soil environment (Trapp and Matthies, 1995; Daam et al., 2019). In this regard, researchers all over the world are working for developing and suggesting new strategies for eradicating these pollutants from contaminated locations.

1.5 Organochlorine insecticides (OCIs)

Organochlorine insecticides are halogenated synthetic compounds, extensively used for the pest control by disrupting the endocrine system of the target creature. These compounds specifically act as a vital agent for prohibiting the attack of vectors and insects that spreading vector-borne diseases to humans. In India, the diseases such as malaria, filariasis, dengue, Japanese encephalitis, cholera and louse-borne typhus are still controlled by the usage of these compounds (Yadav et al., 2015). Even though these toxic organic pollutants are specific to the pests and pathogens, these chemicals affect an extensive collection of non-target living organisms in the environment.

These cluster of organic pollutants are categorized into, the classes of hexachlorocyclohexane (α-HCH, β-HCH, γ-HCH, δ-HCH),

hexachlorobenzene (HCB), the cyclodiene compounds (aldrin, dieldrin, endrin, chlordane, nonachlor, heptachlor and heptachlor-epoxide), chlorinated hydrocarbon compounds (dodecachlorine, toxaphene, and chlordecone) and chlorinated diphenyl ethane compounds (dichlorodiphenyltrichloroethane-DDT, their derivatives - DDD, DDE and methoxychlor) (Menone et al., 2001; Patnaik, 2007).

As mentioned above, OCIs reaches the soil environment from various point sources including the atmospheric deposition, effluents flowing out from industrial and waste discharges from municipal sewage as well as agricultural and urban non-point source run-off. This could also have attributed by the input of waste deposition from the pharmaceutical and cosmetic industry (Anonymous, 1989). According to statistics, about 25,000 MT of OCIs were utilized every year in India (Mathur, 1993). OCI residues are an important potential component of chemical pollutants used extensively for agriculture and sanitation purposes in India as these are comparatively cheap and effective.

In general, these deadly and carcinogenic compounds persist in the soil once enter into the soil for many years and leads to their distribution throughout the environment, thus accumulates in the soil and undergoes chemical and biological degradation and transformation. Here in these groups of chemicals in the soil translocated into other environments by runoff/wind, thereby enter into the ecological system, initially absorbed by plants through vascular tissues and thereby transferred to the higher organisms and eventually to entering the food web and resulting in harmful health hazards in biota including humans and ultimately sweep into Oceans,

adversely affecting the marine ecosystem (GESAMP, 1989; Trapp and Matthies, 1995; Kumar et al., 2006a; Pandit et al., 2001). These organic pollutants have developed an interesting objective of study all over the realm and are monitored continuously because of their proven toxicity to human beings and other animal and plant life (Pandit et al., 2001; Kumar et al., 2006b; Devi et al., 2011, 2013). The mild poisoning of OCIs in humans causes headaches, dizziness, gastrointestinal disturbances, numbness and weakness of the extremities, apprehension and hyperirritability. These compounds are readily absorbed into fatty tissues and concentrate without undergoing metabolism. Few OCIs acts as endocrine-disrupting compounds leading to severe health issues such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities, cardio-vascular, gastrointestinal, renal, hepatic, respiratory effects and cancer. OCI poisoning is one of the major concerns noticed in India. As well, national information and poison control centers regarding these issues were lacking in India (Zheng et al., 2008; Fujiwara et al., 2012; Yadav et al., 2015).

The distributional patterns of certain OCIs in soils from various parts of India were detected and reported in the beginning of early 70's especially in agricultural prominent areas (Nath et al., 1974; Kathpal and Dewan, 1976; Yadav et al., 1981; Ramesh et al., 1991; Nair et al., 1991; Kumar et al., 2018). DDT and HCH contamination in soils were reported in northern India including Uttar Pradesh, Punjab, Haryana and Delhi ranging from 0.675 and 0.032 μg g^{-1} (Anonymous, 1988; Kumari et al., 1996). The fate and the translocation of endosulfan isomers after their application under the sub-tropical conditions of northern India were investigated by Kathpal and his teammates (1997). They suggested that major portion of these isomers

are highly persistent in the upper layer of soil especially the β isomer, undergoes photodegradation in high temperate zones and converted into endosulfan sulfate and diol in sandy loam soil. The soil samples from the urban and suburban region of Agra city were analyzed for the OCIs and were found to be relatively high (Singh, 2001). In another case study in agriculture soil samples of Kanpur, DDT were detected and ranged from 0.05 - 0.98 mg/kg (Sanghi and Sasi, 2001).

The aerial spraying of endosulfan on the cashew cultivating zones of about 4500 hectares in Kasargode district of Kerala by the Plantation Corporation of Kerala (PCK) for the control of pests resulted in the long term accumulation of endosulfan residues in the soil and leaves (Ramesh and Vijayalakshmi, 2002). From the agriculture soil samples collected from Grand Trunk Road, Aligarh, UP, residues of OCIs such as DDT, DDD, DDE, HCH derivatives and Aldrin were detected. Levels of HCH derivatives significantly reported as α - HCH, 38.81 ppb; γ - HCH, 47.35 ppb; β - HCH, 1.79 ppb, respectively (Nawab et al., 2003). The total OCIs levels in the Gangetic alluvium geo-region in the northern part of India ranged from 0.36 - 104.50 ng g^{-1} and β - and δ - isomers of HCH were detected most frequently in soil samples (Singh et al., 2007). However, the ranges of OCIs in the soils of Keoladeo National Park, Bharatpur, Rajasthan, India were found 0.1173 (dieldrin) to 7.54 ppm (γ - HCH) (Bhadouria et al., 2012). OCI contamination in Assam, Tripura and Manipur were investigated and were found to be higher with trace amounts of DDT (49.6 - 5068 pgg $^{-1}$), HCH (9.5 - 2850 pgg $^{-1}$) and endosulfan (26.9 - 1,990 pgg $^{-1}$) (Devi et al., 2013). The ∑OCI level in surface soil of Indian Himalayan Region ranged from 0.28 to 2143.96 ng/g and the most dominant

among them was DDT (216.6 ng/g) (Devi et al., 2015). In another case study, quantification of OCIs along urban, suburban, rural transects from New Delhi and Agra in the north, Kolkata in the east, Mumbai and Goa in the west and Chennai and Bangalore in the southern part of India were conducted and ∑OCIs were found with ranges from 2 to 410 ng g^{-1} dry weight (Chakraborty et al., 2015). The traces of OCIs were found in the paddy fields of Kuttanad agroecosystem (KAE), highlighting the recent application of banned OCIs which implies a serious threat to the human health (Sruthi et al., 2017). OCI concentrations in central India, Gwalior city, Madhya Pradesh, observed < 0.01 and 29.5 µg kg^{-1} with an average and median value of 10.3 and 7.7 µg kg^{-1} (SD ± 8.2) (Kumar et al., 2018).

All these public issues are very much concerned in Kerala, inspired protests, and several harmful insecticides were banned in Kerala as early as 2001, following a report by the National Institute of Occupational Health. The levels of OCIs were detected in various parts of Kerala, Kasargod District and Idukki district and were published. Several reports related to the health issues were discussed (Frederick, 2007; Uppinangady, 2009; Akhil and Sujatha, 2012; Jyothish and Sujatha, 2013; Susan and Resmi, 2015).

1.6 Lindane (γ - HCH)

Lindane is commonly known as gamma hexachlorocyclohexane (γ - HCH/BHC/Hexachlorocyclohexane/Gammaxene/Gammallin) and is for its prominent due to its insecticidal properties decades back. This ubiquitous compound is extensively used due to its potent action and cost-effectiveness in crop production. Therefore, in some tropical countries like India, to control the mosquito-borne vector diseases as well as enhancing the

agricultural productivity and maintaining the livestock, it is still used (Anupama and Paul, 2009). Literally, technical grade HCH are composed of different isomeric forms according to the orientation of chlorine atoms positioned around the cyclohexane ring and the major compounds are α - HCH (53 to 70%), β - HCH (3 to 14%), γ - HCH (11 to 18%), ∞ - HCH (6 to 10%) and ε - HCH (3 to 5%) (Olivero et al., 2011) (Fig. 1.1). Among them, γ – HCH exhibits greatest insecticidal action (Kutz et al., 1991; Lal et al., 2008). Lindane is a colourless crystalline solid, having a molecular weight of 290.85 and melting point, approximately 113 °C, one of the most extensively used broad spectrum of OCIs in India (Murthy and Manonmani, 2007). It is reported as a group B2/C, potential carcinogen according to Environmental Protection Agency (US EPA, 2000).

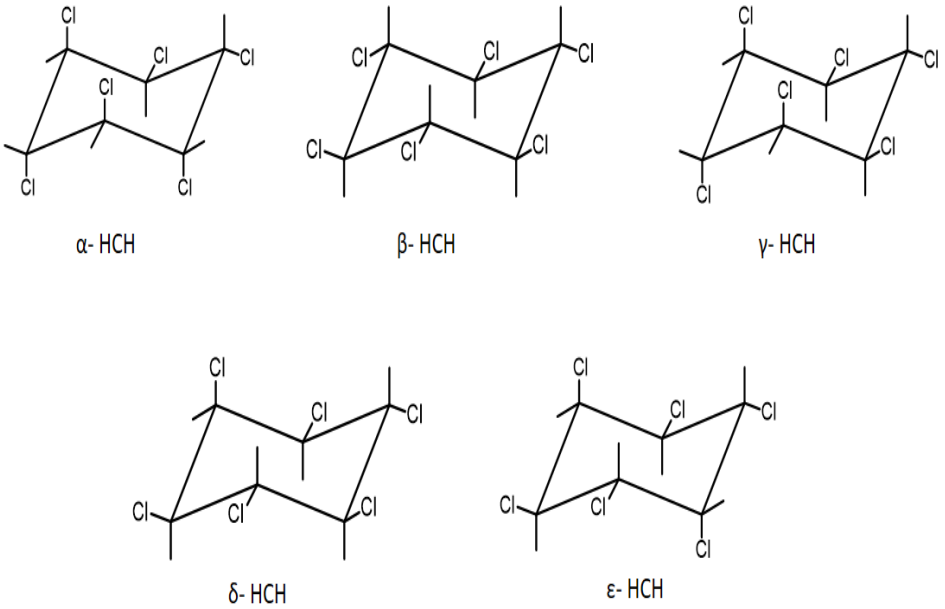

Fig. 1.2 Structure of HCH isomers (chair conformations - axial (a) and equatorial (e) positions of the chlorine atoms of α - HCH as aaaaee, β - HCH as eeeeee, γ - HCH as aaaeee, δ - HCH as aeeeee and ε - HCH: aeeaee; Lal et al., 2008)

The insecticidal properties of technical HCH were first described in the 1940s and the active gamma-isomer was named lindane after Dr. Teunis van der Linden, discovered the alpha and gamma-isomers (Hardie, 1964). Hence these properties of lindane have unlocked its exclusive range of application in various fields such as domestic purposes (medical, sanitary and veterinary adducts) and agricultural sector (Golow and Godzi, 1994; Martinez and Martinez-Conde, 1995; Hirthe et al., 2001). High liposolublity of lindane has led to the biomagnification and associated health hazards leading to its ban in developing countries like India (Misra et al., 2007). Lindane once enters the soil, it directly sorbed into the soil organic particles present thereby immobilizing them finally accumulate for decades in the soil segment. Thus their accumulation in the soil particles and organic matters accelerates their translocation to plant tissues through roots and also the volatilized form by aerial plant parts (Pereira et al., 2010). Additionally, along with the rainwater, the soil particles i.e., lindane sorbed onto organic matter as well as unbounded lindane present in the soil leach into the nearby water column and groundwater (Wauchope et al., 1992).

1.7 Hexachlorocyclohexane (HCH/Lindane) Accumulation Scenario

The accumulation behaviour of the pesticide, HCH in living organisms such as planktons, fish, benthos, clam, frogs, milk and human blood were reported in the newspaper daily. And these levels of accumulation among fish, sediment, samples of human blood were ranged from 0.20 to 9.58 ppm, 2.13 to 12.24 ppm, 0.005 to 0.15 ppm respectively. Total mean lindane concentration levels in the water and milk samples (ppm) of Palakkad district, Kerala were $1.935 \times 10^{-4} \pm 6.779 \times 10^{-5}$ and

$2.600 \times 10^{-3} \pm 7.734 \times 10^{-4}$ respectively. According to FAO/WHO the maximum residual limits (MRL) of lindane in milk is 0.01ppm (Dhanya et al., 2012).

A study carried out at Regional Cancer Centre, Thiruvananthapuram, revealed that the average BHC levels in the breast adipose tissues of the patients were as high as 3,119 ng/g of tissue. Similar levels of pesticide were found in blood samples also the findings of the International Agency for Research on Cancer (IARC) under the WHO reported that Lindane, used for agricultural purposes as a substitute for DDT could cause cancer, non-Hodgkin Lymphoma.

It is also reported that the use of organochlorine insecticides in the mango plantations in Palakkad district, though the traders and farmers vehemently deny it. Informal interaction with the labourers working in the farms and some of the local people and a few respondents confirm the use of these insecticides in these areas (Devi, 2010).

1.8 Microbial Biodegradation

Owing to the harmful effects of the persistence of the toxic pollutants in the soil, the researchers and scientists all over the world are aiming to determine novel techniques for their removal from the soil system. Microbial bioremediation is such a cost-effective innovative method in which the technique relayed on the beneficial utilization of the capability of soil microorganism to consume the carbon source from these toxic pollutants thereby detoxifying them (Salam and Das, 2012). The detoxification process of contaminants, microorganisms gain some amount of energy as a result of the breakdown of chemical bonds. Thus these can be

summarized as oxidation-reduction reaction being toxic contaminants as the electron donors. These can take place either in the presence of oxygen or in their absence. In the presence of oxygen, the microbes utilize it and transform into the water as a result of the reduction reaction. Further, the carbon present in the contaminants undergoes oxidation to carbon dioxide and is known as aerobic respiration.

Additionally, anaerobic respiration is another mechanism in which microorganisms can survive without the presence of oxygen, replacing them by inorganic substances such as nitrate (NO_3^-), sulfate (SO_4^{2-}), iron (Fe^{3+}) and manganese (Mn^{4+}), CO_2, etc. and thereby reduce to nitrogen gas (N_2), hydrogen sulfide (H_2S), reduced forms of metals, and methane (CH_4) etc. as secondary adducts. In an alternate anaerobic metabolic process, fermentation, microorganisms utilize organic compounds as both electron acceptor and electron donor thereby splitting the carbon-containing pollutant to alcohol or acids, finally undergoes degradable to CO_2 and water (NRC, 1993).

Reductive dehalogenation is another microbial catalyzed method in which the halogen group, mostly chlorine is removed from the substrate by substituting hydrogen atom, thereby detoxifying them. In this process, toxicity is exterminated from the system but energy generation is not fully known; however, the investigations are emerging in this regard (NRC, 1993). Cometabolism is an enzyme-mediated mechanism to detoxify the contaminant, also known as secondary utilization, cometabolism or gratuitous metabolism which yields no benefit to the organism.

Bioremediation can be grouped into two intrinsic and engineered: the former is utilizing the capacity of a microorganism to degrade the contaminants, while later deals with the nutrimental requirements are supplied externally to enhance the microbial growth and activity. The process, bioremediation mainly depends on the nature of the contaminant, pH, temperature, moisture content, oxygen content, redox potential, nutritional requirements and lastly the nature of the soil (MacRae et al., 1969; Castro and Yoshida 1974; Sethunathan et al., 1982; MacRae et al., 1984; Sharma, 2012). Even though bioremediation holding certain limitations, for instance, high specificity and time consuming microbial procedure, is a promising asset for conserving the environmental concerns.

1.9 Microbial Biodegradation of Lindane

Considering the facts of lindane, as a potential carcinogen, their accumulation and persistence in the soil environment has been studied and then focused on remediation. The remediation of lindane, since it possess six chlorine atom per molecule, involves the removal of chlorine atom i.e., dechlorination and thereby replacing it by hydrogen atoms (Nagata et al., 2007; Camacho-Pérez et al., 2012). The deletion of the halogen group from the OCI, lindane, decreases the risk of formation of toxic intermediates during the remedial process.

Several case studies have been observed that γ - TCCH , 5,6-dichlorocyclohexa-1,2-diene, monochlorobenzene, trichlorinated benzenes and benzene as the major intermediates in anaerobic degradation process (Beland et al., 1976; Heritage and MacRae, 1977; Jagnow et al., 1977; Ohisa et al., 1978; Boyle et al., 1999; Phillips et al., 2005). In aerobic

degradation of lindane, 1,3,4,6 - tetrachloro-1,4-cyclohexadiene (1,3,4,6 - TCDN), γ - pentachlorocyclohexene (γ - PCCH), 2,5 - dichloro-2,5-cyclohexadiene-1,4-diol (2,5 - DDOL), 2,4,5 - trichloro-2,5 - cyclohexadiene-1-ol (2,4,5 - DNOL), 2,5 - dichlorohydroquinone (2,5 - DCHQ), etc. were the main transformed products (Nagata et al., 1993a, 1993b, 2007).

From the beginning of the 1960s, the anaerobic microbial degradation of HCH were firstly reported, however, immediately after the aerobic degrading bacteria were also found. Biodegradation of lindane was firstly reported in by white-rot fungi *Phanerochaete chrysosporium* (Bumpus and Aust, 1987). Senoo and Wada, (1989), first published the degradation potential of aerobic bacteria, *Pseudomonas paucimobilis SS86*. Following this, in India, another bacterial strain, *Pseudomonas sp.* was isolated from sugarcane fields by Sahu et al. (1990). The degradation pathway of lindane degradation by the microbial isolates of *Pseudomonas paucimobilis* were studied and reported 1,4-TCDN and 1,2,4-TCB as the major transformable products formed (Nagata et al., 1993a,b).

In another case study, the microbial cultures of *actinomycetes Streptomyces* M7 strains were reported to possess lindane degrading capacity by utilizing the carbon content. Dechlorinase is the major enzyme involved in the degradation process, accordingly these microbial strains grown in lindane containing medium as a sole carbon source (Cuozzo et al., 2009). Bacterial strains of *Cyanobacteria, Anabaena sp*. strain PCC7120 and *Nostoc ellipsosporum* another group of microorganisms to degrade lindane. Further, these are the first published report to transform lindane

with the presence of nitrate as a growth substrate, to mixture of less toxic chemicals, γ- pentachlorocyclohexene and chlorobenzenes by Kuritz et al. (1995). Nalin et al. (1999), isolated strains of *Rhodanobacter lindanclasticus* under aerobic condition, for lindane degradation. The lindane degradation ability of the liquid cultures of *Trametus hirsutus,* a white-rot fungus, was investigated (Singh and Kuhad, 1999). The culture of *Bacillus brevis* and *Bacillus circulans* grown in a medium containing 5 µg/mL of HCH were studied for lindane biodegradation and observed 80 % of degradation within 8 days of incubation (Gupta et al., 2000). The aerobic bacterial isolates of *Arthrobacter citreus BI-100,* were investigated for the growth rate and degradation pattern in mineral salts medium supplemented with γ-HCH (100 mg/L) published by Datta et al. (2000).

Cultures of *Microbacterium sp. strain ITRC1* were isolated and identified Manickam et al., 2006). Bacterial strains of *Alkaligens faecalis*, isolated from agricultural fields were found to be potent lindane degraders (Gupta et al., 2001). The degradation capacity of *Bjerkandera adusta* a white-rot fungus were conducted by Quintero et al. (2007). Isolates of *Xanthomonas sp.* were isolated from contaminated soil samples were studied for biodegradation studies and found capable of removing chlorine atoms from lindane (Manickam et al., 2007). Similarly, Boltner et al. (2008), carried out two-step enrichment method for the isolation of lindane degrading bacteria *Sphingomonas* for identifying effective methods in soil remediation.

Biodegradation of lindane and growth rates of bacterial strains *Pseudomonas aeruginosa* were examined under aerobic condition by Lodha

et al. (2007). Bioremediation of lindane and effects on Zea mays growth of bacterial strains of *Streptomyces sp. M7* were investigated (Benimeli et al., 2008). *Sphingobium quisquiliarum, P25 (T),* a yellow-pigmented lindane degrading bacterial strain, was isolated from an HCH contaminated area of the northern part of India (Bala et al., 2010). Similarly, another yellow-pigmented bacterium, *Sphingobium chinhatense* were isolated (Dadhwal et al., 2009). From Argentina soils Fuentes et al. (2010a), isolated strains of *Actinomycete*. In another case study, consortium of four *Actinomycete* isolates (A2, A5, A8 and A11), *Streptomyces sp. M7 (M7)* and *Streptomyces coelicolor A3 (ScA3)* were cultivated for lindane degradation (Fuentes et al., 2010b). Later, remediation studies were conducted by Abhilash et al. (2011), on soil isolated bacterial strains viz. *Staphylococcus cohnii, Staphylococcus equorum, Kocuria rhizophilla* and *Microbacterium resistens*.

1.10 Aim and Objectives

The increased demand for food leads to the large scale application of insecticides and fertilizers in farmlands. As a result of providing the supplementary requirements such as chemical fertilizers and insecticides, the yield of food crops progressively improved. Consequently, the accumulation of these chemical moieties in the soil gradually affect the quality of the soil environment. Subsequently, the quantification of these xenobiotics has acquired its importance in this particular perspective. Furthermore, to reinstitute the quality of the soil environment, the aforementioned toxic chemicals should be removed by adopting newly executed methods, for example, microbial bioremediation. Microbial bioremediation includes the

isolation of microbes capable of degrading lindane, a known carcinogen, all-purpose insecticide and their degradation analysis.

Considering the vast area, high population rate, climatic alternations, soil characteristics, the need of costly sophisticated instruments for measuring the concentrations in nanogram level, the studies related to the adsorption and distributional patterns of OCIs and related xenobiotics in Indian environment are scarce. Like other agricultural zones of India, Palakkad district of Kerala is known for its agricultural activities. The district is particularly focused on paddy cultivation and hence the use of insecticides is very common for increasing the production of high yield variety of rice paddy. The study of organochlorine insecticides and heavy metals acquired greater relevance due to their toxic effects and biomagnification. Isolation of diverse microbial species proficient of degrading the isomers of lindane, an important OCI, will help in large scale bioremediation in various parts of the world. Available reports are scanty on remediation technologies for the field-scale treatment of soil and water contaminated with lindane. Furthermore, in lindane remediation studies there is a requirement of detailed information regarding the possible mechanisms, regulating factors, interactions and the nature of the 'potential lindane degrading microorganisms' with the soil environment. Therefore, the present study focused on the remediation of the persistent and toxic environmental pollutants by microbes isolated from contaminated soils from, Palakkad district.

The present investigation made an attempt to evaluate the content of metals and organochlorine insecticides in the soils of Palakkad district, in

order to estimate their level and degree of contamination. The studies on bioremediation of agricultural soils contaminated insecticides are scarce in the State of Kerala. The present study employ soil microorganisms as monitors and degraders of organochlorine insecticides specifically, lindane and hence highly relevant in the current scenario.

Therefore the objectives of the research work are as follows:

- To determine the soil characteristics of the selected study sites.
- The distribution pattern of heavy metals in the assigned area for evaluating the extent of contamination/accumulation.
- Quantification of organochlorine insecticides in the study area for evaluating the contamination status.
- Isolation of microbial strains capable of degrading lindane from the selected study site for formulating remedial strategies.
- Biochemical and molecular level identification of isolated microbial strains.
- Degradation and optimization analysis of lindane using isolated microbial strains.

Isolation and optimization of lindane degrading microbes from Palakkad zones will aid in developing ecofriendly and cost effective method for remediating and regulating the lindane levels in the agricultural fields.

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Chapter

2

DESCRIPTION OF THE STUDY AREA

Contents

2.1 Introduction

The widespread farming activities in the Palakkad region have resulted in the indiscriminate and unscientific application of insecticides for enhancing productivity. The insecticides applied in the agricultural soils also contain toxic trace metals. Both organochlorine insecticides and heavy metals are nonbiodegradable, persistent contaminants that are capable of undergoing biomagnification. The soil quality of the region has been deteriorated due to long term use of pesticides and fertilizers and consequently such practices have affected the health of environment. If proper measures are not taken, quality of the soils will be completely lost leading economic loss and food security of the state. In order to develop a strategy preliminary investigation of these zones are required, which include general geochemistry, heavy metals and pesticide levels. Hence, this chapter will cover justification for selecting the study area.

So far there has been no elaborated study regarding the general geology, natural metal availability and OCIs in the study area or adjacent locations. Reports from the study area will be the preliminary reports that can be used in formulating strategies by regulatory bodies.

2.2 Study area

Palakkad is one of the most agrarian districts in Kerala and it is also known as the rice bowl of the State. It is located between north latitude $10^{o}20'$ and $11^{o}14'$ and east $70^{o}02'$ and $76^{o}34'$, which is bounded by Malappuram and Nilgiri districts on the North, Coimbatore district on the east, Trichur district on the South and Malappuram and Trichur districts on the west. One of the magnificent vantage points in the district is the Western Ghats, which stretch over a 1000 km; break their continuity at the Palakkad Gap with a width of 40 km. The sides of the gap are sandwiched by the giant Nilgiri hills and Anamalai hills. This act as a vibrant pathway for transportation between the two states Kerala and Tamil Nadu. Additionally, in the east and west coastlines of Peninsular India, the gap provides an imperative part for trades and marketing. Palakkad gap is responsible for the variant climate in the district which receives proper seasonal rainfall and oppressive temperature yearly. The prevailing seasons can be divided into four according to the climate perceived in the district such as the hot season from March to May, the southwest monsoon from June to September (approximately, 71 % of rainfall received) and then, from October to November (approximately, 18 %) northeast monsoon season. Finally, the rest of the period from December to February is generally dry. Therefore, the district experiences a dry climate exhibits the characteristic of cracking

during summer and extreme temperature (41 °C) were recorded in earlier reports. According to the Soil Survey Unit of the Department of Agriculture in Kerala State, the soils of Palakkad were categorized into laterite soils, virgin forest soils, black cotton soils, and alluvial soils are based on the physico-chemical properties and morphology. Moreover, periodically changing humid climate prevailing in the low land and midland of this district, could reduce the adsorption potential of their laterite rich soils. Also, the textural characteristics of the soil determine the adsorption behaviour of labile organic matter into the soil matrix (Prakash, 2008; DSR, 2016).

Table 2.1 Geographical location of the sampling sites

Sl. No	Sampling site	Latitude & Longitude
1.	Kanjikode I (K I)	10°47.7581N 76°43.762 E
2.	Kanjikode II (K II)	10°47.4141N 76°45.170 E
3.	Kanjikode III (K III)	10°47.5651N 76°46.791 E
4.	Chittur I (C I)	10°41.754N 76°46.791 E
5.	Chittur II (C II)	10°42.069N 76°45.206 E
6.	Chittur III (CIII)	10°44.245N 76°43.989 E

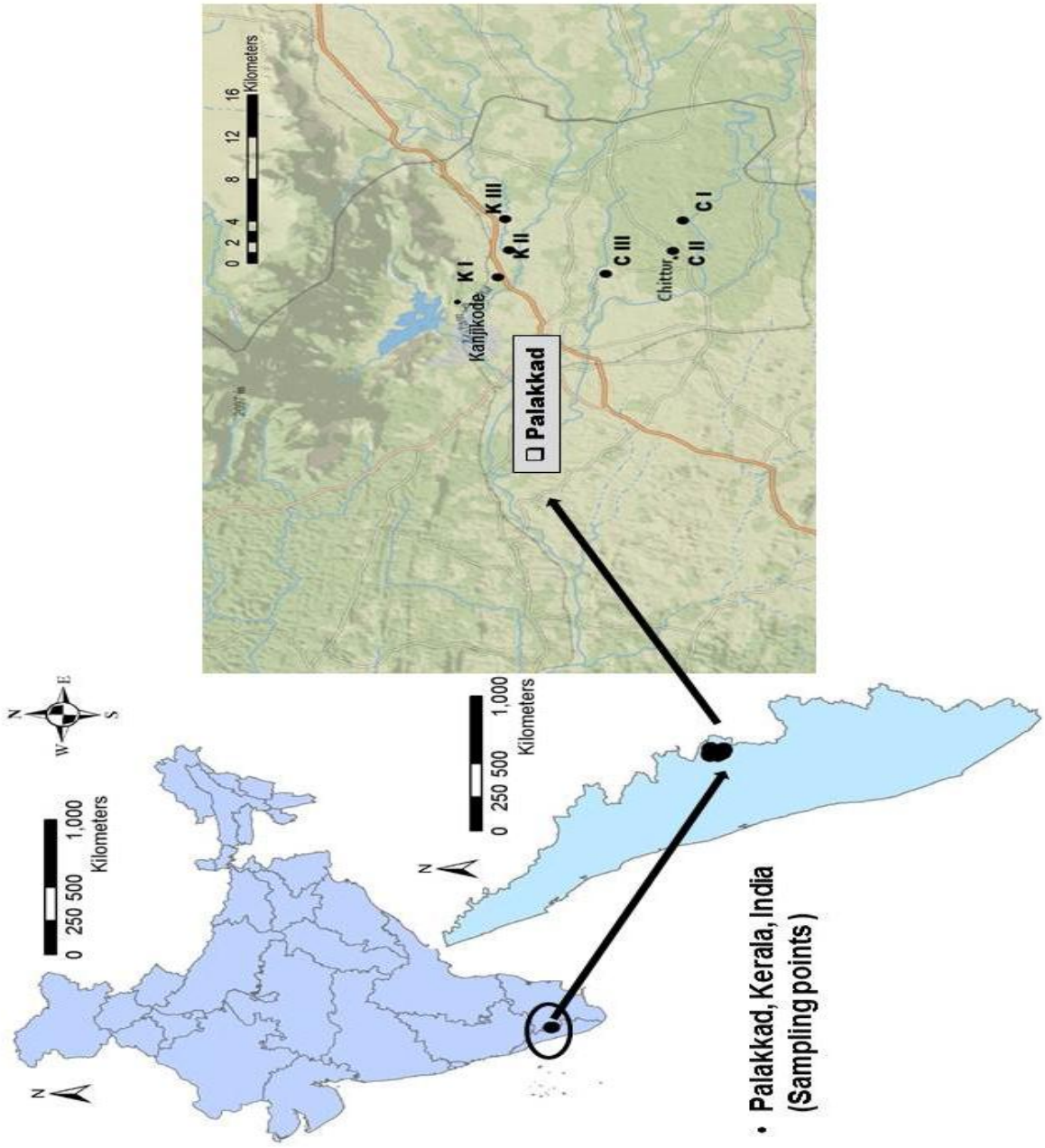

Fig. 2.1 Location map of the study area

Based on the geographical, morphological and virtuous significance on agriculture, the sampling sites were selected and soil samples were collected from six prominent locations (Fig. 2.1 and Table 2.1) of Palakkad district, Kerala, in which three of them from Chittur (CI, CII, CIII) and the other three from Kanjikode (KI, KII, KIII). Six consecutive samplings were carried out between June 2013 to May 2015, for monitoring the chemical constituent status and to infer the pollution density (Estefan et al., 2013). Stations can be classified into three zones. Zones - I (CI, CII, CIII and KI) exclusively belonging to agricultural area, Zone - II (KII) which has the influences urban activities and Zone - III (KIII) which are adjacent to Industrial area.

2.3 Sampling and Storage

Kerala lies in the tropical region, south-west part of India on the shores of the Arabian Sea, with equable climate throughout the year. Most part of Kerala experiences a humid tropical wet climate, however, the eastern borders experiences drier tropical wet and dry climate. Due to its diverse geographical features, the seasons of the state can be classified accordingly as: monsoon (June-September), post-monsoon (October-January) and pre-monsoon (February-May). Seasonal changes usually influence the physicochemical characteristics of the soil. Hence in order to account for the temporal variability, seasonal samplings were adopted in the present study.

Soil samples were collected from six stations along the Chittur and Kanjikode of Palakkad district, Kerala, in six sampling campaigns, accordingly, June 2013 (monsoon 2013: MONJN13), September 2013

(monsoon 2013: MONSP13), March 2014 (pre-monsoon 2014: PRMMH14), September 2014 (monsoon 2014: MONSP14), February 2015 (pre-monsoon 2015: PRMF15) and May 2015 (pre-monsoon 2015: PRMMY15). The surface soil samples were collected using plastic scoop and were immediately transferred to polythene bags, according to standard protocol (US EPA, 1997). The samples were kept in ice box and transported to the laboratory where they were placed in deeper freezer at -20 °C till analysis. Prior to the analysis, soil samples were freeze-dried and ground to a fine powder by agate mortar. All the parameters were analyzed in triplicate and estimated as their mean values ± standard deviation.

2.4 Reference

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Chapter

3

SOIL PROPERTIES AND METAL DISTRIBUTION

Contents

3.1 Introduction

Almost all the living organisms are composed of organomineral compounds, minerals and chemical compounds specifically elements such as C, H, O, N, K, Na, P, Ca, Mg, S, Fe, etc. in the required proportion. Mother Nature is composed of all these requirements, for the biochemical and metabolic functioning of the living forms. Living beings had to adapt eminent techniques to select the essential chemical compositions required for their metabolism and eliminate the harmful elements that persist in the biosphere. Thus living organisms are highly dependent on the geochemistry and adversely affected the drastic circumstances in the environment.

Soil, a complex component, acts as the outer layer of the Earth's surface constitutes minerals, organic matter, gases, liquids, and organisms, altogether supports the life of the universe. The chemical and physical

characteristics of the soil differ with respect to the locality and thereby determines the rate of productivity. In this particular context, the nature of the soil environment and their trace metal composition is a crucial part of the survival of the living organisms and are the major concern in recent research field. The nature of the soil can be determined by the presence of organic constituents and its textural characteristics which in turn govern the water intake rates (infiltration); water movement through soil (hydraulic conductivity); permeability; soil water holding capacity; the ease of tilling the soil; and the amount of aeration (which is vital to root growth), finally the structure. The organic matter comprises of all the known classes of biochemical components such as carbohydrates, lipids, proteins and tannin/lignin, were generally originated by the partial decay and decomposition of dead organisms. Soil texture is a vital soil property, which were determined by the relative proportion by weight percentage of sand, silt, and clay and has a marked influence on soil fertility. The major biomolecules and the soil particles altogether function as the nutrient reservoir of the environment in the form of organomineral compounds i.e., nitrogen, sulphur, etc. for the plants.

Metals originate from the diverse sources, enter the soil niche by various pathways, and finally adsorbs onto the interior soil. The solubility, availability and mobility of these metals mainly depends upon the chemical properties of the soil (McBride, 1994). This process relatively depend upon the pH, degradation of organic matter and textural characteristics (Jacob and Joseph, 1994; Alloway, 1996; Evanko and Dzombak, 1997; Álvarez-Ayuso and García-Sánchez, 2003; Bradl, 2004). The adsorption and precipitation

processes restrict the downward movement of the metals in the soil surface until it forms complexes with other components present in the soil.

The human being's thirst for invasions of synthetic chemicals in the field of development led to noticeable changes in the vulnerable ecosystem. Over the last decades, these metals introduced in to the biosphere from of anthropogenic sources such as power generation, smelting, combusting, incineration, motor vehicle emissions, mining, industrial as well as agricultural activities (Kabata-Pendias and Pendias, 2001; Wang et al., 2004; Qin et al., 2012). Artificial chemical input has led to the exposure of a large amount of toxic substances in to the environment, thereby accumulating these xenobiotics into the soil causing environmental pollution. Among these chemical compounds, trace elements are known as the most hazardous belongings and their excessive accumulations and persistence in the environment have acquired serious health significance (Rieuwerts et al., 1999; Martley et al., 2004; Chen et al., 2008; Deng et al., 2011; Lu et al., 2012).

The purpose of the document is to introduce the general parameters including pH, textural characteristics and organic matter content of the Palakkad soils. Further, to determine the metal concentration emphasizing the various pollution indicators viz. enrichment factor (EF), contamination factors (CF) geoaccumulation index (Igeo) and pollution load index (PLI). These indices often used to assess the occurrence and intensity of anthropogenic pollutant deposition on the soil. The common hazardous metals investigated in this research work include Cd, Cu, Co, Pb, Mn, Mg, Zn, Ni and Fe.

3.2 Materials and Methods

3.2.1 Quantification of General Parameters and Analysis of Metals in the Soil

3.2.1.1 Textural Characteristics and Quantification of Total Organic Carbon

Textural characteristics (sand, silt, and clay) and quantification of OM (protein, lipid and carbohydrate, tannin/lignin) were carried out by standard protocols. The texture of the soil samples were determined by using pipette analysis (Krumbein and Pettijohn, 1938; Lewis, 1984), as per Stoke's law after removing inorganic carbonates and organic matter by treatment with 10 % HCl and 15 % H_2O_2 respectively. After the processing, soil samples were treated with sodium hexametaphosphate for overnight followed by sieving through a 63 μm sieve, and the sand fraction was carefully washed and collected. This fraction was then dried in a hot air oven maintained at 150 °C. The rest of the fractions were partitioned into silt and clay by gravimetric extraction of dispersed sediments at fixed time intervals (Folk, 1974).

Sample preparation for total organic carbon (TOC) analysis was done by treating the samples with 1 M HCl to remove the carbonates and repeated two/three times to ensure the complete exclusion of carbonates. Samples were washed with Milli-Q water to remove salts and finally freeze-dried and the total organic carbon was determined using TOC analyzer (Elementar Vario Select, Germany).

3.2.1.2 Quantification of Biochemical Compositions

The biochemical composition of soil samples were determined by using standard spectrophotometric methods. Total proteins were determined

using the method of Lowry et al. (1951), albumin was used as the standard reference compound. Total carbohydrate estimation was done by the phenol-sulphuric acid method with glucose as the calibration standard (Dubois et al., 1956). Content of total lipid was estimated by the Sulpho-phosphovanillin method using cholesterol as standard material (Barnes and Blackstock, 1973). Tannin and lignin in the soil samples were spectrophotometrically after extracting with 0.05 M NaOH at 60 °C for 90 minutes and estimated using sodium tungstate-phosphomolybdic acid method (Nair et al., 1989).

3.2.1.3 Analysis of Metals and Quality Control

For the estimation of metals (Cu, Cd, Pb, Mn, Ni, Zn, Fe, Mg and Co) the dried soil samples were finely powdered. 1gm was accurately weighed and digested with 1:5 mixture of $HClO_4$ - HNO_3 using Microwave digestion system (Anton Paar Multiwave 3000). All reagents used for the analysis of the samples were A.R Select grade or Suprapur grade (Merck). The samples were evaporated to dryness at moderate temperature 65 - 70 º C. Finally, the samples were filtered and diluted to 25 mL with Milli Q water. A blank is also prepared simultaneously for the analysis (APHA, 1995). Heavy metals in the soil samples were analyzed in AAS (Perkin Elmer AAS 3110). Detection limits for AAS (model: Perkin Elmer AAS 3110) were 0.028 mg kg^{-1} for Cd, 0.12 mg kg^{-1} for Co, 0.45 mg kg^{-1} for Pb, 0.077 mg kg^{-1} for Cu, 0.018 mg kg^{-1} for Zn, 0.052 mg kg^{-1} for Mn, and 0.0078 mg kg^{-1} for Mg. All the analysis were carried out in triplicate analysis. All the materials used in metal analysis were acid cleaned (dil. HNO3) and washed several times with deionized water (Milli-Q). To ensure non-contamination of samples during analysis, a series of analytical blanks were measured. These blanks were treated in the same way as the samples and the results were blank corrected. Accuracy of the metal analysis was checked using standard reference material BCSS-I (National Research Council of Canada). Triplicate analysis of quality control samples showed a good accuracy and the recovery rate ranged between 91.2% for Mn and 103.8% for Zn (Table A1.14).

3.2.2 Pollution Indices

The degrees of pollution in the soil were calculated by certain calculation methods such as EF, CF, Igeo and PLI, etc. Generally, Fe is used as reference metal due to its wide distribution on Earth's crust and less interaction property with other metals (Wedepohl, 1995; Al-Wabel et al., 2017).

3.2.2.1 Enrichment Factor

EF is calculated by the formula,

$$EF = \frac{(M/X)\ Sample}{M/X\ Background}$$

M→ Metal

X→ Selected reference metal

(M/X) sample and (M/X) background → Ratios between metal and the reference metal (Covelli and Fontolan, 1997; Simeonov et al., 2000; Zhang et al., 2007)

3.2.2.2 Contamination Factor

The CF is given by,

$$CF = \frac{metal\ concentration\ in\ sediment}{background\ value\ of\ metal}$$

3.2.2.3 Geoaccumulation Index

The contamination according to I_{geo} is calculated by (Müller, 1969),

$$I_{geo} = \log_2 \frac{Cn}{1.5 \times Bn}$$

C_n→ Metal total metal concentration

B_n → Background concentration of the metal

1.5→ Correction in factor

3.2.2.4 Pollution Load Index

The contamination assessment of metals on behalf of PLI can be measured by the formula (Tomlinson et al., 1980),

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{1/n}$$

CF→ Contamination factor

n→ Number of metals studied.

3.2.3 Heavy Metal Pollution Source Assessment

Multivariate statistical analyses, PCA and Pearson correlation analysis were determined concurrently for investigating the relationship between the metals and physico-chemical parameters (Facchinelli et al., 2001; Ma et al., 2015) and thereby provide information regarding their source. All the statistical analyses, correlation and PCA in the study, were computed by the software Statistical Program for Social Sciences (SPSS version 13.0).

3.3 Results and Discussion

3.3.1 General Soil Parameters

An extensive variation in the soil parameters were observed in all the stations. The physico-chemical parameters including the textural characteristics and TOC were represented in Tables 3.1 - 3.4. Soil texture in the study area revealed significant seasonal and temporal variations in the sand content ranging from 17.1 ± 0.01 % (C III; MONJN13) to 91.1 ± 0.02 % (K III; MONJN13). Additionally, the clay fractions varied from 1.3 ± 0.01 % (C II; MONJN13) to 55.2 ± 0.11 % (K II; MONJN13) and that of silt fluctuated between 3.4 ± 0.01 % (K I; MONJN13) and 71.2 ± 0.02 % (C III; MONJN13). The pH of the scrutinized soil samples exhibited slight alkaline nature and ranged from 7.6 ± 0.12 (K II; MONJN13) to 8.2 ± 0.12 (C I; PRMF15) respectively.

The biochemical characteristics of the soil samples displayed significant spatial and temporal variations and were represented in Table 3.3. Total carbohydrate (CHO) content in the soil samples ranged from 656.7 ± 201 µg/g (C III; MONSP14) to 47463.3 ± 422 µg/g (K II; PRMMY15). Higher values were observed during PRMM15 and

PRMMY15 in all the stations. Concentration of total protein (PRT) content in the soil samples varied from 275.9 ± 221 μg/g (K I; PRMM15) to 7693.3 ± 216 μg/g (C II; MONSP14). Meanwhile, concentration of total lipid (LPD) in soils ranged from 105.3 ± 140 μg/g (K I; PRMMH14) 2483.8 ± 124 μg/g (K II: PRMMY15). Tannin and lignin (TL) varied from 273.052 ± 542 μg/g (C I; MONSP14) and 7475.8 ± 246 μg/g (K III; PRMMH14) respectively. However, total organic carbon (TOC) in the study area varied from 0.03 ± 0.11 % (K II; MONSP14) to 2.32 ± 0.22 % (K III; PRMMH14) (Table 3.4).

3.3.2 Heavy Metal Distribution

Heavy metal (Cu, Cd, Mn, Mg, Co, Zn, Ni, Pb, Fe) distribution along the study area varied significantly and exhibited a random behaviour during the study period. The values were compared with permissible level by Indian Standard, (Awashthi, 2000), Lindsay, (1979) and US EPA, (1983) (Table 3.5). The range of analyzed metals throughout the sampling period were observed as follows: Cu, 1.64 - 90.03 mg kg $^{-1}$, Cd, ND - 8.56 mg kg $^{-1}$, Mn, ND - 733.33 mg kg $^{-1}$, Mg, 183.68 - 5100.00 mg kg $^{-1}$, Co, ND - 29.20 mg kg $^{-1}$, Zn, 0.42 - 455.63 mg kg $^{-1}$, Ni, ND - 89.68 mg kg $^{-1}$, Pb, ND - 112.84 mg kg $^{-1}$, Fe, 1478.14 - 123500.00 mg kg $^{-1}$ (Fig. 3.1 - 3.9). The stations under investigation are situated on the deposition fringes of rainwater runoff remaining, hectic and roadside regions as well as domestic waste dumping proximities. These factors could determine the deposition and accumulation of these metals in the scrutinized area.

Table 3.1 Values of Temperature and pH in soils

Seasons	MONJN13		MONSP13		PRMMH14		MONSP14		PRMF15		PRMMY15	
Stations	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH
KI	29.80	7.9 ± 0.02	29.7	7.8 ± 0.06	37.1	7.15 ± 0.14	30.1	7.85 ± 0.13	29.9	7.7 ± 0.15	35.4	7.35 ± 0.15
KII	30.20	7.6 ± 0.12	28.2	8.0 ± 0.13	35.3	8.1 ± 0.15	29.9	7.54 ± 0.03	39.2	7.94 ± 0.07	35.2	8.13 ± 0.2
KIII	30.70	7.9 ± 0.05	28.6	8.1 ± 0.02	33.2	7.9 ± 0.01	29.8	7.84 ± 0.2	29.3	7.26 ± 0.04	34.2	7.88 ± 0.12
CI	29.70	7.8 ± 0.11	29.2	8.2 ± 0.08	35.5	7.8 ± 0.2	29.9	7.77 ± 0.15	30.4	8.2 ± 0.12	37.2	7.52 ± 0.9
CII	29.10	7.7 ± 0.16	27.4	7.7 ± 0.27	36.6	7.86 ± 0.11	29	7.74 ± 0.22	28.9	7.37 ± 0.08	36.4	7.74 ± 0.07
CIII	27.70	7.8 ± 0.07	27.7	7.9 ± 0.15	37.3	7.52 ±0.1	28.5	7.54 ± 0.08	29.1	8.11 ± 0.22	36.8	7.71 ± 0.21

Table 3.2 Textural characteristics of soils (%)

Stations	MONJN13			MONSP13			PRMMH14			MONSP14			PRMF15			PRMMY15		
	Sand	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt
KI	90.2 ± 0.01	6.4 ± 0.21	3.4 ± 0.01	70.9 ± 2.1	21.0 ± 1.5	8.1 ±0.1	64.9 ± 2.3	27.8 ± 0.1	7.4 ± 0.1	87.5 ± 2.5	8.1 ± 2.1	4.4 ± 0.1	61.6 ± 2.1	25.9 ± 0.5	14.8 ± 1.4	64.7 ± 1.2	25.6 ± 1.1	9.7 ± 0.2
KII	22.2 ± 0.01	55.2 ± 0.11	22.6 ± 2.1	52.3 ± 1.2	38.6 ± 3.4	9.1 ± 0.2	65.9 ± 1.4	28.1 ± 1.1	6.0 ± 0.2	49.1 ± 1.2	45.2 ± 1.2	5.8 ± 0.1	59.1 ± 1.2	25.1 ± 2.1	16.8 ± 1.1	65.7 ± 2.2	21.6 ± 1.01	12.7 ± 0.3
KIII	91.1 ± 0.02	4.2 ± 1.1	4.7 ± 0.1	83.8 ± 1.01	2.5 ± 0.4	13.7 ± 1.1	67.8 ± 1.1	28.6 ± 1.2	3.6 ±1.1	74.7 ± 1.2	13.7 ± 1.1	11.5 ± 1.2	60.1 ± 2.1	26.7 ± 1.5	14.0 ± 0.4	62.1 ± 2.0	26.4 ± 1.2	12.6 ± 1.1
CI	52.5 ± 0.21	36.9 ±1.01	10.7 ± 1.1	73.8 ± 2.2	17.4 ± 1.1	8.8 ± 0.3	62.3 ± 1.1	28.3 ± 0.2	9.4 ± 1.02	70.3 ± 2.1	18.6 ± 1.2	11.1 ± 2.1	60.6 ± 1.2	26.1 ± 1.1	13.2 ± 0.3	66.2 ± 2.1	24.8 ± 1.5	9.0 ± 0.2
CII	85.5 ± 0.10	1.3 ± 0.01	13.2 ± 2.1	72.8 ± 1.1	13.3 ± 3.1	13.9 ± 1.1	68.3 ± 1.1	14.5 ± 1.2	17.2 ± 0.1	73.1 ± 1.4	15.9 ± 1.4	11.0 ± 1.4	61.2 ± 1.4	25.4 ± 1.2	13.0 ± 0.6	66.3 ± 2.3	22.5 ± 1.2	11.3 ± 1.1
CIII	17.1 ± 0.01	11.7 ±1.1	71.2 ± 0.02	72.2 ± 2.1	16.4 ± 1.2	11.4 ± 1.1	69.4 ± 1.2	25.3 ± 1.3	5.3 ± 1.02	73.1 ± 2.1	11.4 ± 0.5	15.4 ± 0.9	62.8 ± 1.2	24.6 ± 1.06	12.4 ± 1.1	66.4 ± 2.1	23.8 ± 0.2	9.8 ± 0.5

Table 3.3 Biochemical composition of soils (μg/g)

Stations	MONJN13		MONSP13		PRMMH14		MONSP14		PRMF15		PRMMY15	
	CHO	LIP	CHO	LIP	CHO	LIP	CHO	LIP	CHO	LIP	CHO	LIP
KI	1717.9 ± 116	494.5 ± 151	1433.82 ± 104	402.3 ± 241	6773.4 ± 186	105.3 ± 140	808.4 ± 214	303.6 ± 275	14669.1 ± 124	203.7 ± 145	29338.3 ±451	406.8 ± 124
KII	3007.5 ± 145	209.1 ± 102	2082.61 ± 113	795.5 ± 215	3452.9 ± 140	111.2 ± 134	979.8 ± 123	156.2 ± 241	18553.0 ± 215	169.8 ± 112	47463.3 ± 422	241.0 ± 111
KIII	2294.7 ± 124	489.9 ± 142	1130.1 ± 141	316.8 ± 308	6774.7 ± 171	196.4 ± 123	684.3 ± 241	114.0 ± 154	15059.7 ± 224	154.2 ± 146	6315.0 ± 433	264.0 ± 103
CI	2052.0 ± 231	477.7 ± 121	1195.13 ± 117	471.6 ± 421	8615.1 ± 164	173.2 ± 151	1160.9 ± 125	652.4 ± 147	15986.7 ± 213	231.6 ± 147	39390.0 ± 215	1088.8 ± 134
CII	1947.9 ± 214	413.7 ± 101	3624.4 ± 143	443.6 ± 124	3270.4 ± 210	118.3 ± 147	1162.7 ± 315	989.5 ± 256	17102.3 ± 241	386.6 ± 157	46104.3 ± 245	2483.8 ± 124
CIII	2430.4 ± 271	249.3 ± 213	4412.89 ± 110	584.4 ± 412	6206.3 ± 113	197.1 ± 131	656.7 ± 201	150.6 ± 412	16463.8 ± 335	345.5 ± 134	24413.0 ± 341	144.3 ± 110
	PRT	TL	PRT	TL	PRT	TL	PRT	TL	PRT	TL	PRT	TL
KI	1330 ± 240	2305.9 ± 134	1540.4 ± 310	3670.2 ± 421	3740.4 ± 143	1719.9 ± 257	1929.1 ± 421	1444.79 ± 254	275.9 ± 221	2729.1 ± 214	551.8 ± 142	3945.5 ± 124
KII	2579.8 ± 140	3256.1 ± 113	2547.6 ± 152	743.5 ± 451	3060.8 ± 301	7453.1 ± 547	2864.0 ± 214	2376.17 ± 642	304.2 ± 245	3706.4 ± 142	683.8 ± 112	2331.1 ± 241
KIII	3141.6 ± 310	1808.6 ± 210	3419.4 ± 214	5804.7 ± 342	3868.8 ± 110	7475.8 ± 246	2998.8 ± 521	425.795 ± 471	386.8 ± 124	1469.7 ± 214	4580.0 ± 132	6249.1 ± 234
CI	5602.1 ± 142	1809.5 ± 131	2452.8 ± 254	1691.3 ± 142	3711.7 ± 214	1829.5 ± 236	3219.2 ± 145	273.052 ± 542	672.2 ± 114	1378.2 ± 221	3627.3 ± 124	3805.6 ± 214
CII	1239.4 ± 146	1851.6 ± 172	3801.8 ± 321	2302.7 ± 514	3290.5 ± 154	1713.3 ± 457	7693.3 ± 216	978.239 ± 754	831.3 ± 154	1240.0 ± 121	3360.3 ± 142	2661.4 ± 224
CIII	4904.8 ± 214	641.7 ± 204	3534.3 ± 175	925.1 ± 314	3849.4 ± 114	1405.2 ± 457	2383.1 ± 221	574.661± 543	774.8 ± 112	1120.7 ± 124	798.8 ± 115	1955.5 ± 145

Table 3.4 Total organic carbon content (TOC %) in soils of the study area

Stations	KI	KII	KIII	CI	CII	CIII
MONJN13	0.28 ± 0.10	1.14 ± 0.12	1.03 ± 0.20	1.46 ± 0.14	1.77 ± 0.21	1.92 ± 0.14
MONSP13	0.89 ± 0.30	0.68 ± 0.12	0.61 ± 0.10	0.57 ± 0.11	1.83 ± 1.0	1.16 ± 0.01
PRMMH14	0.36 ± 0.11	0.67 ± 0.10	2.32 ± 0.22	0.37 ± 0.13	0.74 ± 0.02	0.44 ± 0.13
MONSP14	0.09 ± 0.30	0.03 ± 0.11	0.15 ± 0.10	0.12 ± 0.02	0.24 ± 0.10	0.11 ± 0.11
PRMM15	0.89 ± 0.10	0.63 ± 0.01	0.88 ± 0.11	0.94 ± 0.10	1.31 ± 0.20	1.28 ± 0.10
PRMMY15	0.7 ± 0.40	0.65 ± 0.20	0.93 ± 0.12	0.86 ± 0.11	1.66 ± 0.11	1.17 ± 0.12

Table 3.5 The mean concentration (mg kg $^{-1}$) of heavy metals in soils from the study area with published threshold guidelines

Metals	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb	Fe
Indian Standard (Awashthi 2000)	135 - 270	3 - 6	*	*	*	300 - 600	75 - 150	250 - 500	*
Lindsay (1979)	*	*	600	5000	8	50	40	10	38000
US EPA (1983)	*	0.06	600	5000	8	50	40	10	*
MC MONJN13	6.48	1.85	78.51	446.85	1.9	7.06	0.93	2.73	9642.02
MC MONSP13	9.04	0.96	84.61	345.02	4.32	11.23	0.96	3.2	18138.38
MC PRMMH14	27.67	2.10	246.63	573.45	12.83	24.01	ND	2.18	10752.86
MC MONSP14	35.98	0.36	403.69	2766.67	18.46	136.45	36.20	35.40	25750
MC PRMF15	26.97	0.16	382.68	1750	19.30	123.28	44.49	16.33	44983.33
MC PRMMY15	5.09	3.4	249.65	512.94	15.25	20.74	18.24	5.29	67283.33

*Data not available
*MC= Mean concentration
*ND= Non detectable

Peak concentrations of Cu (Fig. 3.1) were observed during MONSP14, PRMMH14 and PRMM15, in almost all the stations, specifically at K II (90.03 mg kg $^{-1}$). Sharp variations were observed in other seasons could be attributed by the influence of climatic fluctuations

experienced in the sampling locations. Moreover, certain contributing factors including temperature, pH, organic content and the nature of soil fractions also significantly regulate the rate of accumulation of Cu in the soil. According to Indian Standard (Awashthi, 2000), the levels of Cu remain within the permissible range in all the stations throughout the sampling period. The elevated levels of Cu during MONSP14 could be attributed to the rainwater runoff along with the soil particles and finally, deposit in the sampling sites.

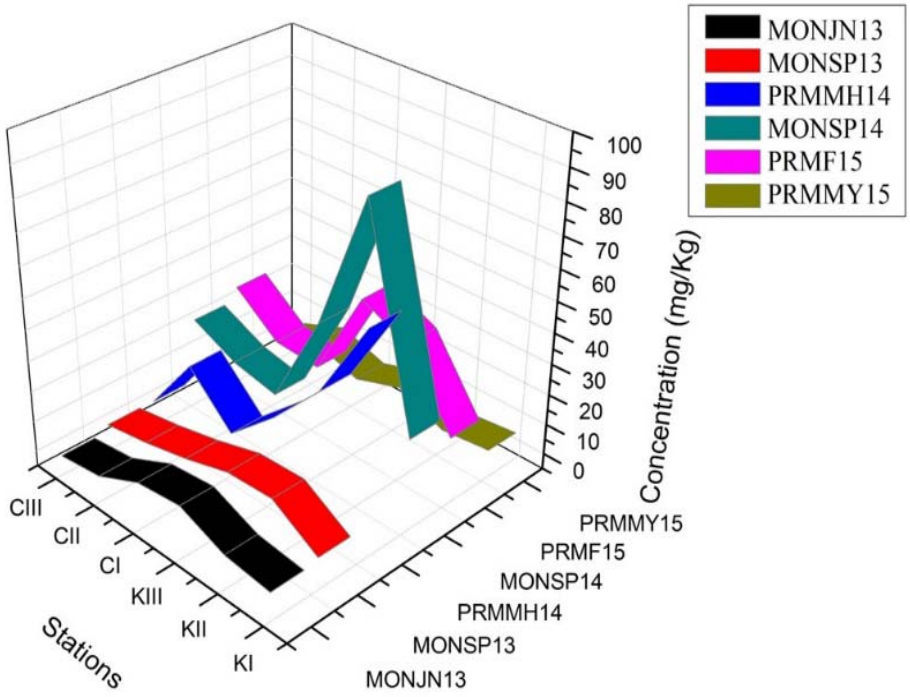

Fig. 3.1 Distribution pattern of Cu in soils of the study area

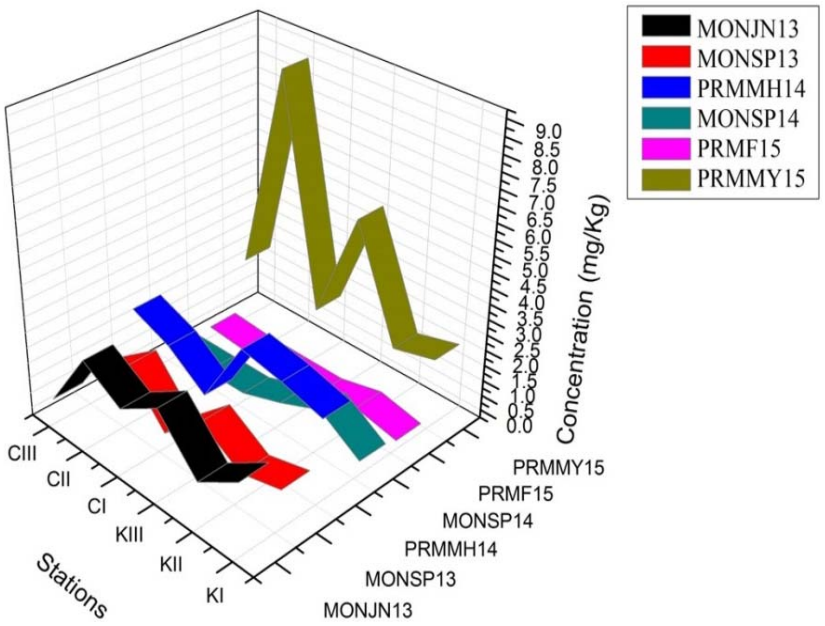

Fig. 3.2 Distribution pattern of Cd in soils of the study area

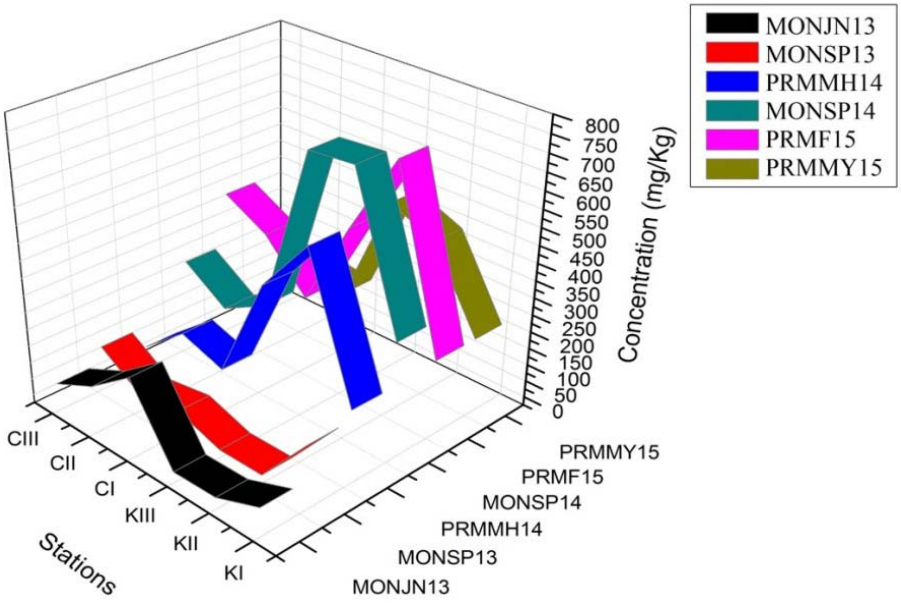

Fig. 3.3 Distribution pattern of Mn in soils of the study area

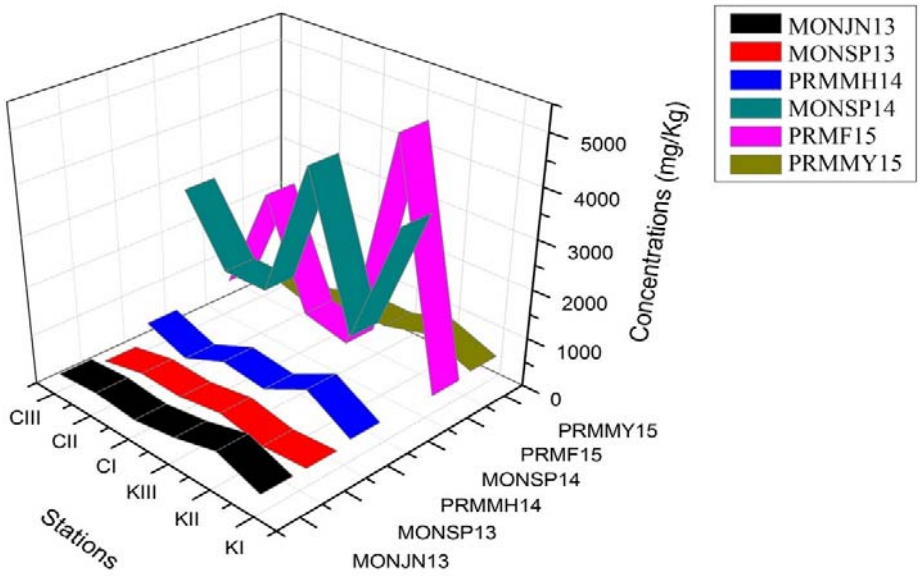

Fig. 3.4 Distribution pattern of Mg in soils of the study area

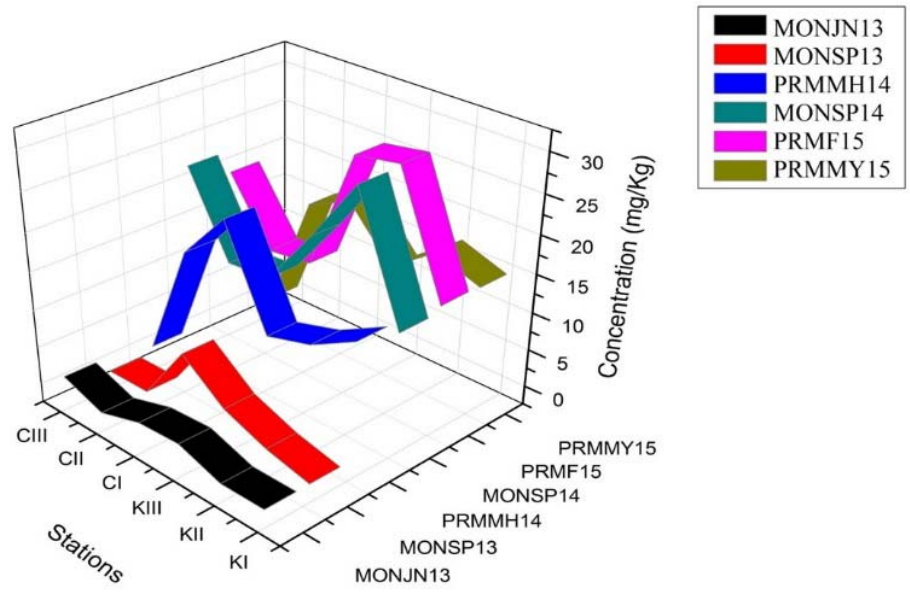

Fig. 3.5 Distribution pattern of Co in soils of the study area

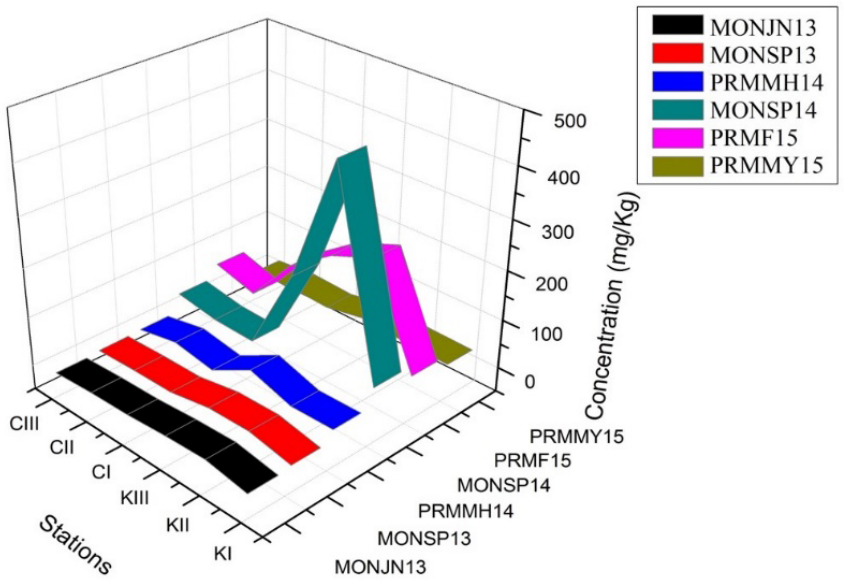

Fig. 3.6 Distribution pattern of Zn in soils of the study area

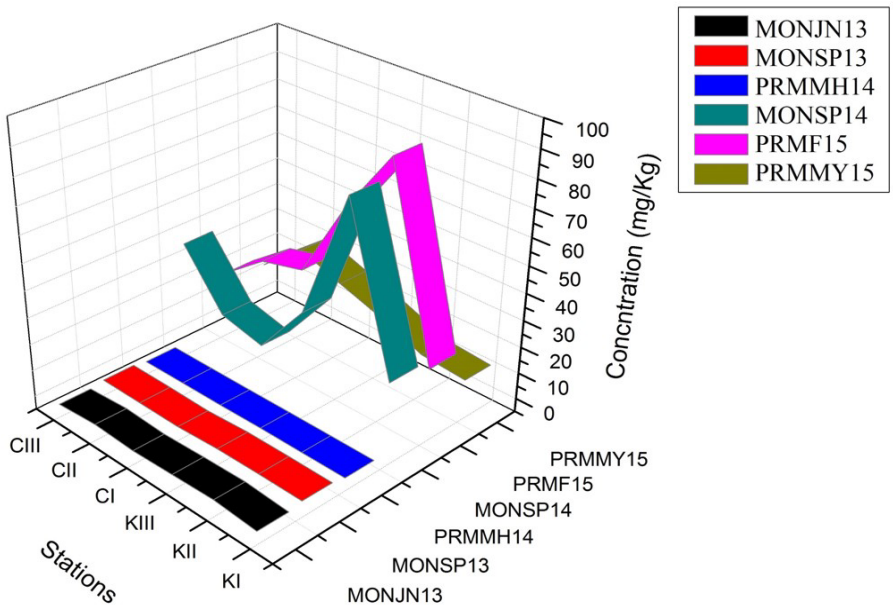

Fig. 3.7 Distribution pattern of Ni in soils of the study area

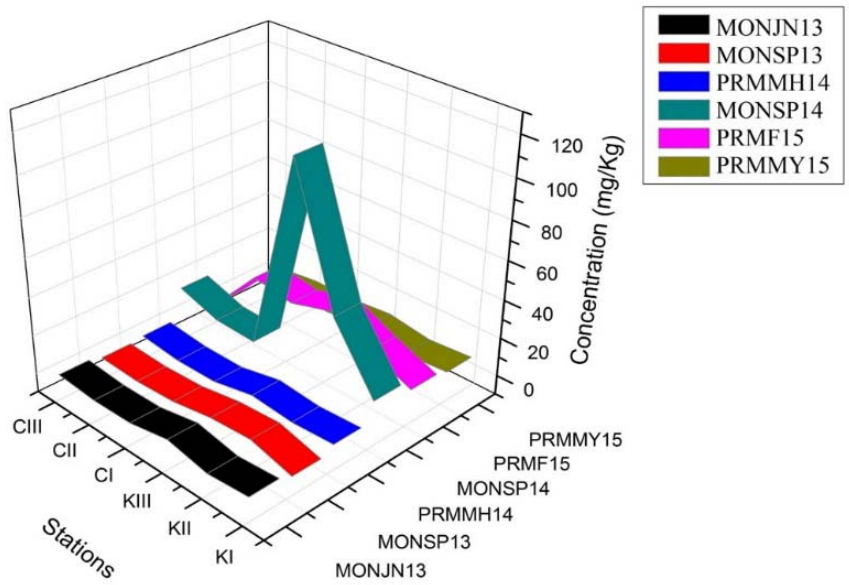

Fig. 3.8 Distribution pattern of Pb in soils of the study area

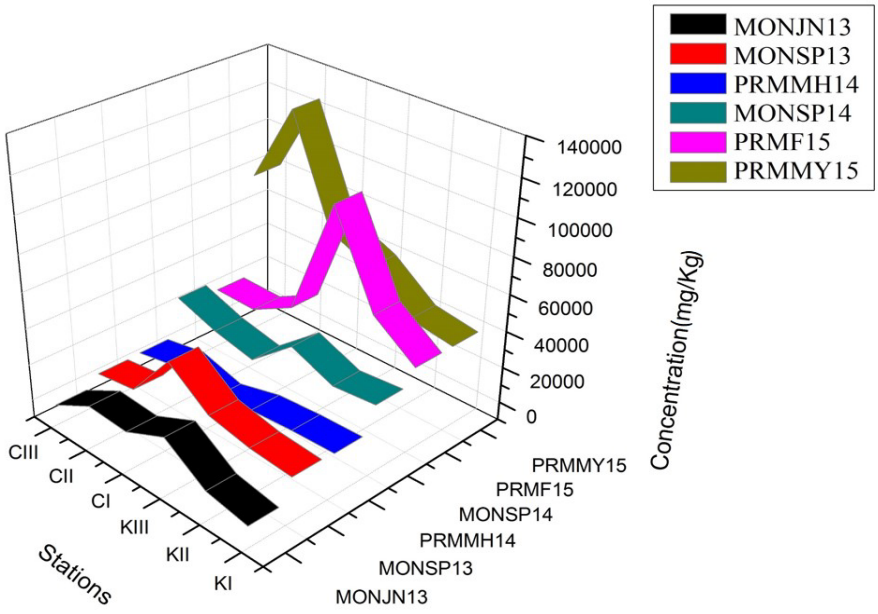

Fig. 3.9 Distribution pattern of Fe in soils of the study area

Cd is one of the toxic heavy metal known, and their residual levels were detected in the sampling region. The highest concentrations were observed at C II (8.56 mg kg $^{-1}$) during PRMMY15. The content of Cd (Fig. 3.2) exceeded the permissible levels proposed by US EPA (1983) in most of the sites throughout the sampling seasons while, the values were observed below the prescribed level based on the Indian Standard (Awashthi, 2000), except at C II (PRMMY15). The residual levels of Cd in the study area could be due to the broad spectrum of utilization of potassium fertilizers into the arable soils (Akhil and Sujatha, 2012) as well as the application of organic farmland compost or sewage sludge (Jones et al., 1981).

The seasonal and spatial variations of Mn (Fig. 3.3) in the study region were observed in an asymmetrical pattern, in which the levels were increasing yearly and the highest values were observed in MONSP14, at stations K II (733.33 mg kg $^{-1}$) and K III (722.9 mg kg $^{-1}$) and PRMF15 at K II (700.99 mg kg $^{-1}$). According to the guidelines proposed by the US EPA (1983) and Lindsay (1979), levels of Mn were found below the permissible levels except for the station, K II (MONSP14, PRMF15) and K III (MONSP14). The higher concentration of Mn along the sampling sites were primarily associated with traffic linked sources, for example, metallurgic corrosion, the dust particles generated from construction department and roads in addition to the disintegration of the tyre and engine parts (Fergusson and Kim, 1991). Similarly, Mg (Fig. 3.4) is one of the essential nutrients for plant growth and widely distributed in soil. The highest concentrations were observed at the station K II (5100 mg kg $^{-1}$) during PRMF15. However, in the study region, all the stations recorded below the

permissible levels proposed by US EPA (1983) and Lindsay (1979) except K II, (PRMF15).

Co is one of the metals, occurring on the Earth's surface, as associated with other metals such as copper, nickel, manganese etc. Random behavioural patterns were observed throughout the study, enhanced levels were observed during PRMMH14, MONSP14, PRMF15 and PRMMY15 specifically at the stations C I (22.56 mg kg $^{-1}$), K II (28.86 mg kg $^{-1}$), K II and K III (29.2 mg kg $^{-1}$ and 28.25 mg kg $^{-1}$) and C I (18.29 mg kg $^{-1}$), respectively. According to US EPA (1983) and Lindsay (1979) the concentrations of Co exceeded permissible levels in majority of the seasons at all the stations (PRMMH14: All stations except C III, MONSP14: All stations, PRMF15: All stations and PRMMY15: All stations except C III) (Fig. 3.5). The fundamental mainspring of these soil impurities could be derived from wastewater supplies, sewage sludge, the establishment of industrialization and possibly the atmospheric depositions (Fergusson and Kim, 1991).

Like other metals, Zn also exists in the soil environment naturally, holding a vital role in biological sustainability, and in the present study, the levels displayed an irregular pattern (Fig. 3.6). The highest values were recorded at K II (455.63 mg kg $^{-1}$) during MONSP14. According to US EPA (1983) and Lindsay (1979), the observed concentration exceeded during the season PRMF15 (K II, K III, C I, C II) and MONSP14 (K I, K II, K III), however, on comparing with proposed Indian Standard (Awashthi, 2000), all the values were found within the permissible levels. The elevated ranges of Zn in the region could arise mainly as part of leaching process (Jung,

2008) and dumping of domestic as well as industrial wastes such as paint, steel and automotive parts, etc. wherein Zn is an essential constituent in their production (Alloway, 2005). Additionally, the intensive usages of phosphatic fertilizers possibly mark the reason for the elevated levels of Zn in the arable fields (Preston et al., 2016).

Generally, Ni in soil environments were found to bind with iron and cobalt and their movement mainly influenced by the nature of the soil fractions (Kabata-Pendias and Pendias, 2001). At the station K II (89.68 mg kg $^{-1}$) during PRMF15, highest concentration was observed. Based on the US EPA (1983) and Lindsay (1979) permissible values, the stations KII and K III (MONSP14, PRMF15) (Fig. 3.7) were found to steep beyond the permissible levels.

Concentration of the metal Pb, recorded a zigzag manner in all the stations (Fig. 3.8) throughout the sampling seasons and the maximum was observed at the station K III (112.84 mg kg $^{-1}$) during MONSP14. On comparing the guideline values of Indian Standard (Awashthi 2000), the Pb content was found within the limit at all the stations. Fe is the fourth most abundant metal on Earth and an essential element for plant growth and development. On comparing with the Lindsay (1979) guideline values, the observed levels exceeded the permissible range during MONSP13 (K II, K III, C I, C II, C III), PRMF15 (K II, K III) and PRMM15 (C I) (Fig. 3.9) and the maximum content was recorded at C II (123500 mg kg $^{-1}$: PRMMY15). The distribution of Fe in the surface soils in the study area could be inferred as the contribution of natural sources (Lv et al., 2014).

However, from the data obtained, the concentration of most of the analyzed metals exceeded the allowed limit according to the standards available, in most of the stations during the sampling period. Generally, the concentrations of metals were found higher in the dry season, while, in wet season these could be washed and leached out by the rainwater. In addition to this, certain metals were found in higher concentrations during the wet season in the present study. Usually, most of the metals in trace amounts in the soil is an essential component for the proper growth of plants and other living organisms, however, their higher levels lead to toxicity and gradually results in the adverse effect on their growth and metabolism (Mitch, 2002). Heavy metal contamination in agricultural soil leads to stunned growth, chlorosis and necrosis in leaves (Pandey, 2006). The main sources of these metals include geogenic input, various anthropogenic and agricultural activities, domestic waste, airborne deposition, etc. Additionally, the metals derived from various anthropogenic sources translocate one place to another than the pedogenic or lithogenic origin (Kuo et al., 1983; Kaasalainen and Yli-Halla, 2003).

3.3.3 Pollution Indices

The pollution assessments were done by the application of Enrichment Factor, Contamination Factor, Geoaccumulation Index and Pollution Load Index.

3.3.3.1 Enrichment Factor

Estimated EF values suggested that most of the stations were enriched by the analyzed metals. Spatio-temporal variations in EF values represented in Fig. 3.10 and Table 3.6.

Table 3.6 Values of enrichment factor for heavy metals in the soils

Season		Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	Mean	2.06	99.77	0.93	0.23	0.71	0.46	0.10	1.29
	SD	1.25	93.14	0.90	0.21	1.44	0.49	0.14	1.60
	Mini	0.58	36.62	0.02	0.05	0	0.17	0	0.20
	Max	4.25	280.60	2.32	0.55	3.62	1.45	0.35	4.08
MONSP13	Mean	1.18	22.17	0.28	0.05	0.20	0.40	0.12	0.23
	SD	0.48	15.89	0.37	0.02	0.25	0.27	0.06	0.26
	Mini	0.55	1.98	0	0.02	0	0.1	0.02	0
	Max	1.93	48.41	0.83	0.08	0.56	0.84	0.21	0.65
PRMMH14	Mean	4.99	58.84	1.27	0.14	3.70	1.25	0.35	4.99
	SD	3.18	17.82	0.87	0.08	3.60	0.80	0.29	3.18
	Mini	0.93	39.34	0.31	0.05	0.25	0.45	0	0.93
	Max	10.37	79.32	2.56	0.26	10.69	2.78	0.72	10.37
MONSP14	Mean	2.97	3.27	0.90	0.24	1.94	3.01	2.32	2.21
	SD	2.31	4.84	0.47	0.07	0.61	3.85	1.54	1.97
	Mini	1.76	0.00	0.46	0.14	1.31	0.81	1.48	0.92
	Max	7.63	12.59	1.69	0.34	3.01	10.61	5.42	5.82
PRMF15	Mean	2.57	1.42	1.00	0.07	2.26	1.73	1.42	0.06
	SD	1.45	0.62	0.60	0.11	1.31	1.66	1.75	0.76
	Mini	0.62	0.83	0.32	0.11	0.54	0.78	0.72	0.67
	Max	0.98	0.00	0.27	0.01	0.75	0.71	1.06	0.06
PRMMY15	Mean	0.18	14.92	0.29	0.02	0.63	0.23	0.47	0.17
	SD	0.10	8.03	0.24	0.02	0.42	0.14	0.09	0.10
	Mini	0.07	6.42	0.04	0.00	0.00	0.09	0.34	0.06
	Max	0.33	26.70	0.63	0.05	1.09	0.37	0.58	0.31

According to Al-Wabel et al. (2017), the values were grouped into deficiently to minimal enrichment (< 2), moderate enrichment ($2 \leq EF < 5$), significant enrichment ($5 \leq EF < 20$), very high enrichment ($20 \leq EF < 40$)

and extremely high enrichment (EF ≥ 40). The values of Cd were found greater in all the stations during the sampling period and higher values were found during MONJN13 at the station K I (280.60). However, high and low range values were observed during the sampling periods along the stations and are consequently grouped into deficiently to extremely high-enriched status.

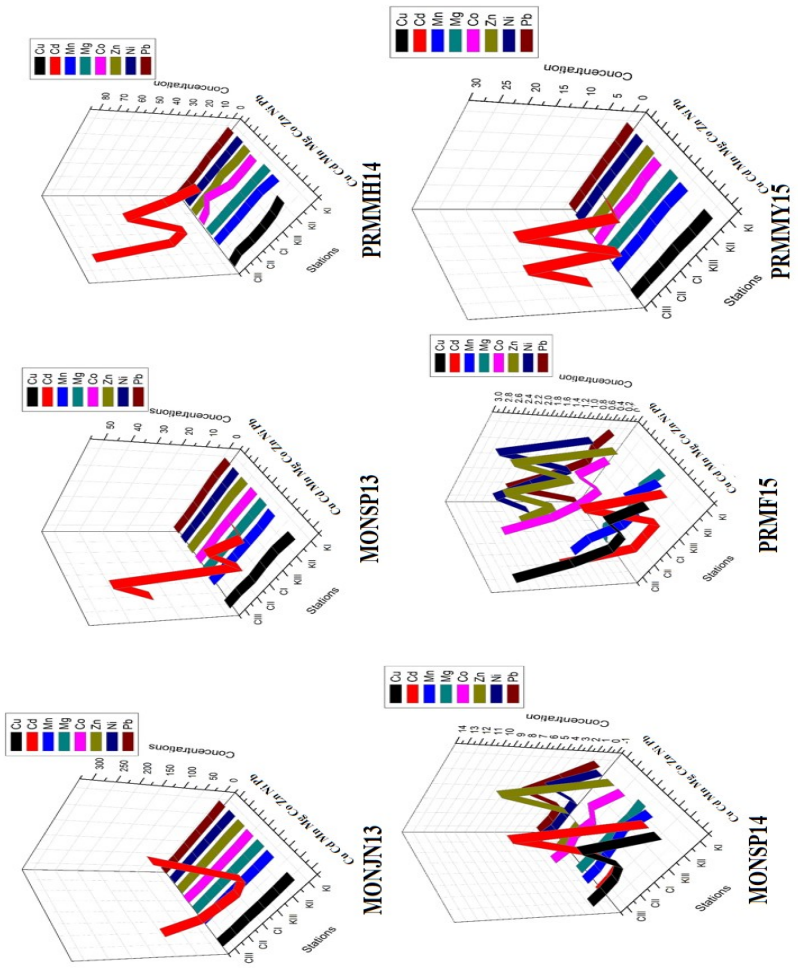

Fig. 3.10 Distribution pattern of EF in soils of the study area

Metals such as Cu, Co, Zn, Ni and Pb were grouped to deficiently to significantly enriched throughout the sampling period in all the stations and high values were observed at K I (10.37; PRMMH14), C I (10.69; PRMMH14), K II (10.61; MONSP14), K II (5.42; MONSP14) and K III (5.82; MONSP14) respectively. The values of Mn (except at K II; PRMMH14, K III; PRMMH14, C III; MONJN13) and Mg were observed < 2 in all the stations throughout the sampling period and grouped into deficiently to minimal enrichment.

3.2.3.2 Contamination Factor

Based on the classification of Al-Wabel et al. (2017), contamination factor can be grouped as low contamination (< 1), moderate contamination ($1 \leq CF \leq 3$), indicating as considerable contamination ($3 \leq CF \leq 6$), and very high contamination (> 6). The spatio-temporal variation in estimated EF values is depicted in Fig. 3.11 and Table 3.7.

Consequently, pollution can be grouped moderate to very high contamination and highest values were recorded at the station CII during PRMMY15. CF for Cu were recorded values > 1 in all the stations during PRMM15, MONSP14, PRMMH14 (Except C I and C III) and MONSP13 (Only in K II and K III) and falls under the category of moderate to very high contamination. CF values for Cu in other sampling seasons were observed as low contamination. CF values for Cu in other sampling seasons were observed as low contamination. Similarly in the CF values for Co were found < 1 in most of the station throughout the sampling period and were classified into moderate contamination sites. In the case of metals such as Zn, Ni and Pb in most of the stations throughout the sampling sections, the CF values were found < 1 and categorize as moderate to very high contamination. CF values for Mn > 1, observed only in PRMMH14 (K II), MONSP14 (K II and K III and PRMM15 (K II) and can be inferred as moderate contamination.

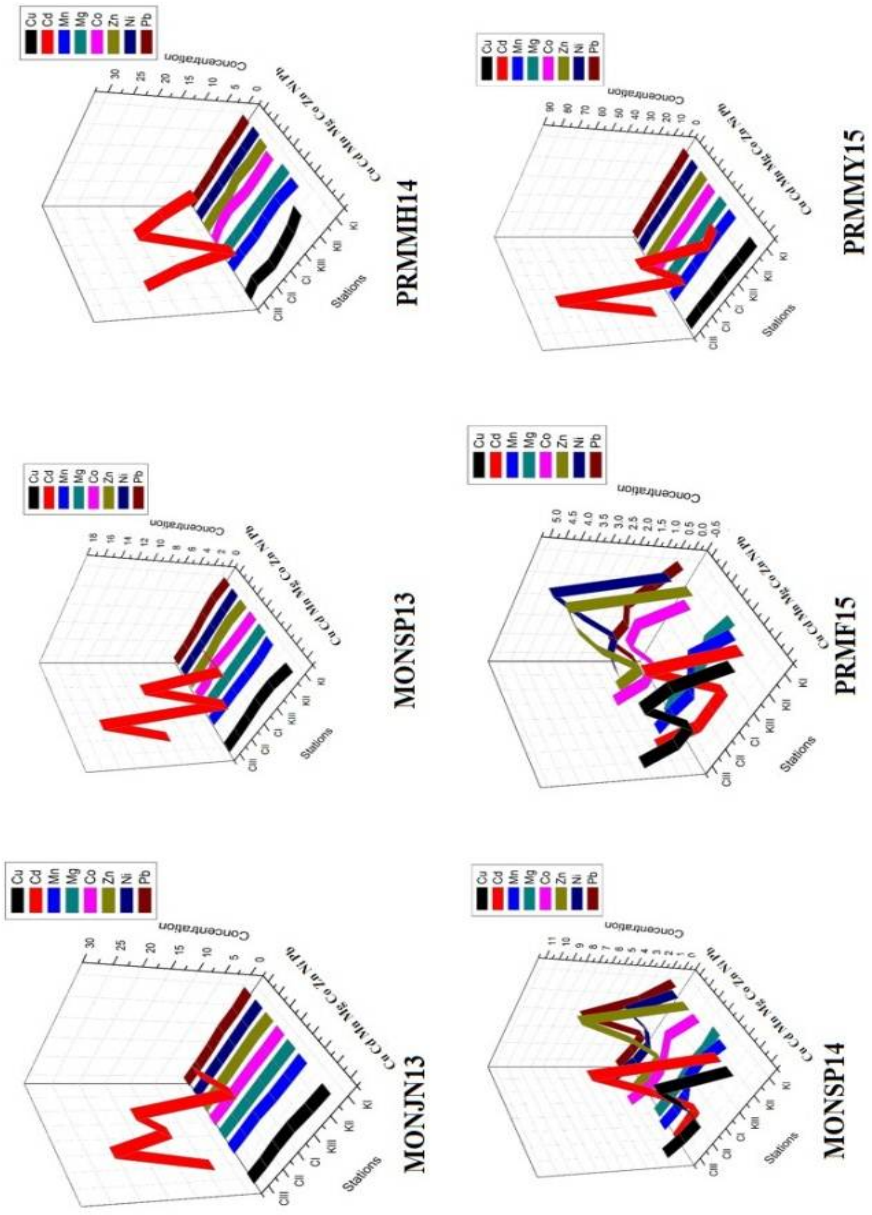

Fig. 3.11 Distribution pattern of CF in soils of the study area

Table 3.7 Values of contamination factor for heavy metals in soils of the study area

Season		Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	Mean	0.45	18.09	0.15	0.03	0.08	0.14	0.03	0.16
	SD	0.29	8.02	0.15	0.01	0.09	0.12	0.04	0.10
	Mini	0.11	5.69	0.02	0.02	0	0.01	0	0.07
	Max	0.86	26.27	0.41	0.05	0.17	0.31	0.08	0.34
MONSP13	Mean	0.63	9.44	0.11	0.03	0.19	0.22	0.05	0.13
	SD	0.33	5.30	0.12	0.01	0.28	0.17	0.02	0.15
	Mini	0.26	2.45	0	0.01	0	0.07	0.02	0
	Max	1.03	16.67	0.31	0.04	0.69	0.45	0.07	0.35
PRMMH14	Mean	1.94	20.62	0.47	0.04	1.10	0.46	0	0.13
	SD	1.60	8.09	0.39	0.02	0.63	0.33	0	0.11
	Mini	0.24	7.16	0.08	0.02	0.06	0.12	0	0
	Max	4.65	29.41	1.1	0.07	1.94	1.04	0	0.27
MONSP14	Mean	2.52	2.91	0.76	0.21	1.59	2.62	1.95	2.08
	SD	2.00	4.10	0.49	0.09	0.58	3.26	1.33	2.36
	Mini	1	0	0.31	0.12	1.07	0.5	0.8	0.62
	Max	6.3	10.39	1.39	0.33	2.49	8.76	4.47	6.64
PRMF15	Mean	1.89	0.80	0.73	0.13	1.67	2.38	2.39	0.96
	SD	0.90	1.27	0.36	0.14	0.68	1.62	1.48	0.59
	Mini	1.1	0	0.38	0.03	1.01	0.82	1.08	0.05
	Max	3.2	3.24	1.33	0.38	2.52	4.56	4.82	1.59
PRMMY15	Mean	0.36	33.33	0.47	0.04	1.10	0.40	0.98	0.31
	SD	0.20	28.15	0.32	0.01	0.57	0.14	0.39	0.10
	Mini	0.17	14.02	0.11	0.02	0	0.22	0.55	0.17
	Max	0.61	83.92	0.91	0.06	1.58	0.62	1.58	0.45

3.2.3.3 Geoaccumulation Index

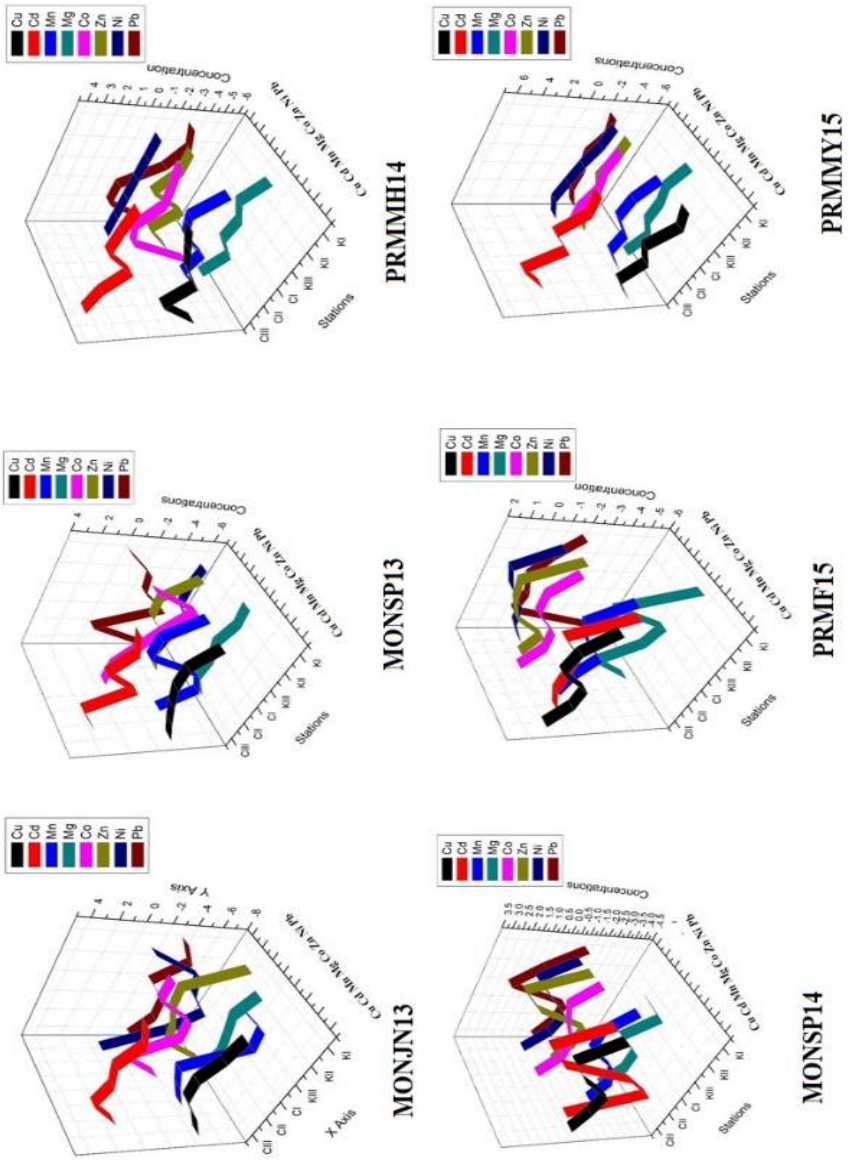

Fig. 3.12 Distribution pattern of Igeo in soils of the study area

The pollution rate is determined based on the Igeo values obtained and grouped into strongly, moderately and non-polluted. Igeo < 0 indicates pollution free, 0 ≤ Igeo < 1 indicates pollution free to moderately polluted, 1 ≤ Igeo <2 indicates moderately polluted, 2 ≤ Igeo <3 indicates moderately to strongly polluted, 3 ≤ Igeo < 4 means strongly polluted, 4 ≤ Igeo < 5 indicates strongly to much strongly polluted and Igeo ≥ 5 indicates very strongly polluted (Müller, 1969). The Igeo values for the studied metals throughout the sampling period were : -3.71 to 2.07 for Cu, -3.94 to 5.81 for Cd, -6.64 to 0 for Mn, -6.79 to -1.99 for Mg, -4.54 to 0.75 for Co, -7.54 to 2.55 for Zn, -6.69 to 1.68 for Ni and -5.01 to 2.15 for Pb (Fig. 3.12).

Based on the classification of Müller, (1969), most of the sites were free from pollution due to metals such as Cu, Mn, Mg, Co, Zn, Ni and Pb, during MONJN13 and MONSP13, however, these stations remain moderately to strongly polluted sites during rest of the seasons. Igeo for Cd recorded positive values throughout the seasons in all the stations indicating very strong pollution (except in K I & C III during PRMF15; C I & C III during MONSP14).

3.2.3.4 Pollution Load Index

PLI for the analyzed metals were depicted in Fig. 3.13 and were found in the range from 0 to 423.65 for all the metals. Pollution assessment of the region proposed by Tomlinson and his teammates (1980) were resolved based on the PLI values and are grouped into PLI < 1 defines a perfect region, PLI = 1 indicates the presence of the pollutants whereas PLI > 1 denoted high pollution indicating decline of the soil quality of the region. According to PLI classification, all the stations were found to be free from pollution during MONJN13, MONSP13 and PRMMH14. However, all the

stations were found extremely polluted during MONSP14, PRMF15 and PRMMY15 indicating an input of anthropogenic activities.

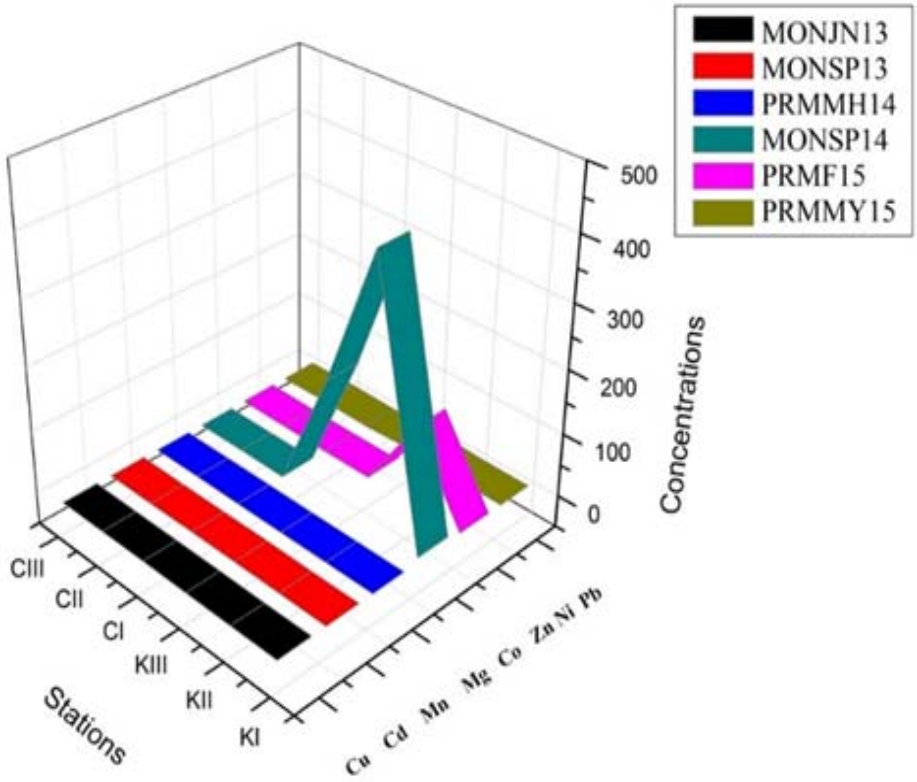

Fig. 3.13 Distribution pattern of PLI in soils of the study area

3.2.4 Source Assessment of Heavy Metal Pollution

Statistical tools such as Pearson correlation and Principal component analysis (PCA) was adopted for the determination of relationships between the environmental parameters and the origin of metals (Raju, 2006; Verma and Singh, 2013; Tiwari and Singh, 2014). All data procedures were carried out with SPSS Statistics 13, with the statistical significance level, $P < 0.05$.

3.2.4.1 Pearson Correlation

Pearson correlation analyses were performed to determine the relationships among the analyzed metals and the resolved soil parameters (Garcia et al., 1996; Facchinelli et al., 2001; Lucho-Constantino et al., 2005) and are depicted in Table 3.8. Most of the metals exhibited positive correlation with each other.

Strongest correlations were observed between the elements Cu with Mn, Co, Zn, Ni, Pb; Mn with Mg, Co, Zn, Ni, Pb; Mg with Co, Zn, Ni, Pb; Co with Zn, Ni, Pb, Fe; Zn with Ni and Pb; Ni with Pb and Fe; Cd with TL and LPD; Fe with CHO and LPD; CHO with LPD. A significant positive correlation noted among the analyzed metals, which implied the contamination possibly derived from the same source (Bai et al., 2015; Nazzal et al., 2016; Lu et al., 2009). Similarly, significant correlations were observed between Cd, Fe and TOC; Co and CHO; Silt and TOC. The data provides a piece of evidence for the metal binding capacity of organic fractions particularly CHO and TOC (Zimdahl and Skogerboe, 1977; Li et al., 2001; Quenea et al., 2009; Mohammad, 2015).

The organic materials in the soil are negatively charged moieties capable of adsorbing the positively charged cations thereby accelerating the formation of metal complexes and finally proficient in exchanging them with varying pH (McLaren et al., 1981; Rieuwerts et al., 1998). Negative correlations were exhibited among Cu with TOC; Cd with Mg; Mg with TOC; Ni with PRT; Pb with TOC; CHO with LPD.

Table 3.8 Pearson correlation coefficients (r) between analyzed trace metals and physical parameters in the soils

	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb	Fe	TOC	Sand	Clay	Silt	PRT	CHO	LPD	T/L
Cu	1																
Cd	-0.146	1															
Mn	***.679***	-0.047	1														
Mg	***.383****	***-.352****	***.600***	1													
Co	***.632***	-0.154	***.712***	***.490***	1												
Zn	***.793***	-0.221	***.737***	***.472***	***.655***	1											
Ni	***.594***	-0.2	***.697***	***.615***	***.771***	***.855***	1										
Pb	***.514***	-0.214	***.612***	***.624***	***.435***	***.639***	***.521***	1									
Fe	0.056	***.393****	0.24	0.061	***.514***	0.223	***.511***	0.159	1								
TOC	-0.076	0.107	-0.068	0.114	-0.064	-0.166	-0.124	0.03	0.034	1							
Sand	0.285	0.004	0.234	-0.106	0.203	0.312	0.212	0.005	0.069	***-.693***	1						
Clay	-0.187	-0.151	-0.14	-0.047	-0.113	-0.088	-0.037	-0.047	-0.106	***-.663***	-0.079	1					
Silt	-0.205	***.345****	0.107	-0.097	.354(*)	-0.036	0.222	-0.106	***.670***	-0.077	0.15	-0.046	1				
PRT	-0.289	***.559***	-0.249	-0.191	-0.103	-0.229	-0.037	-0.159	***.474***	0.104	-0.062	-0.084	***.437***	1			
CHO	-0.045	0.22	-0.193	-0.165	-0.26	-0.227	***-.350****	-0.074	-0.269	-0.101	-0.015	0.146	***-.356****	0.253	1		
LPD	0.013	***.441***	0.231	-0.172	-0.026	-0.045	-0.104	-0.232	0.07	0.035	0.16	-0.205	0.109	-0.075	0.036	1	
T/L	***-.387****	***.363****	-0.22	***-.421****	***-.394****	-0.305	-0.281	***-.342****	0.063	-0.268	0.019	***.349****	0.187	0.199	0.039	0.242	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

3.2.4.2 Principal Component Analysis

Principal component analysis was accomplished for the source identification of the metals, thereby distinguishing them as natural or anthropogenic derived pollutants (Garcia et al., 1996; Facchinelli et al., 2001; Lucho- Constantino et al., 2005). The PCA was obtained by taking the eigenvalues and the sources were classified by their factor loadings (Zhou et al., 2014; Li et al., 2015). Since the obtained eigenvalues are greater than unity, the developed components were assumed to be significant. Two components were obtained from the present dataset with 72.1 % of the total variance (Table 3.9). The first PC accounts for a total variance of 54.9 % with eigenvalues 4.95 while the second PC explained the total variance of 17.2 % with eigenvalues of 1.54 and the loadings were greater than 0.7 which are represented in italics.

PC 1 includes the Cu, Mn, Mg, Co, Zn, Ni and Pb and can be defined as the anthropogenic derived based on the airborne deposition including constructive field, dumping of waste products from various origins such as domestic as well as industries and vehicle emissions through burning fuels (Singh et al., 2015), agro-based chemicals, liming and domestic wastes (Reimann and De Caritat, 2005) as well as geological matrix and wastewater effluent (Spahić et al., 2018). Pb is mainly combined with gasoline products for decreasing various engine problems and for better performance (Yao et al., 2015). The present data provide evidence for the adsorption affinity between the elements Mn and Pb, which were simultaneously derived from mineralization of birnessite ($Na_4Mn_{14}O_{27}.9H_2O$) (Rieuwerts et al., 1998).

Table 3.9 Principal components estimated for metals in soils of the study area

Rotated Component Matrix		
Parameters	**Component**	
	PC I	PC II
Cu	***0.785***	-0.042
Cd	-0.27	***0.81***
Mn	***0.863***	0.112
Mg	***0.72***	-0.288
Co	***0.827***	0.239
Zn	***0.898***	0.001
Ni	***0.899***	0.179
Pb	***0.745***	-0.155
Fe	0.366	***0.825***
Eigenvalues	4.947	1.538
% of variance	54.854	17.195
Cumulative %	54.854	72.049

PC 2 comprises Cd and Fe which can be identified as the anthropogenic inputs from agricultural sources such as phosphatic fertilizers, ferrous ammonium sulfate $(NH_4)_2(FeSO_4)_2.6H_2O$), ferrous sulfate ($FeSO_4.H_2O$), organic fertilizers, nitrogen fertilizers, pesticides, germicides which are frequently applied in the arable fields (Singh et al., 2015). Several metals particularly, Cd were identified as the most important constituent in the production of synthetic organochlorine/phosphate pesticides and fertilizers (Chen et al., 2008). The presence of Fe could arise from the dumping of iron-steel alloys in the sampling site (Singh et al., 2015).

3.4 Conclusion

The present study investigate the metal contamination along the selected sites revealed a random behavioural pattern in all the stations throughout the sampling period. However, the results pointed out the fact that most of the elements exceeded the permissible levels based on the available standards. Estimated EF values indicated that almost all the stations were deficiently to extremely high enriched with heavy metals. According to CF values of the examined metals, most of them classified into moderate to very high contamination in most of the sampling sites throughout the sampling period. Igeo values suggested that all of the investigated regions remains moderately to strongly polluted sites, except for Cd which indicated very strong pollution in most of the stations. The PLI values implied deterioration of all the stations during MONSP14, PRMF15 and PRMMY15 especially the stations K II and K III. The major contribution of the pollution in these sites derived from the metals Cd, Cu, Co, Zn and Pb. The statistical analysis revealed that the metals could derive from the possible anthropogenic inputs from agricultural, pharmaceutical, domestic utilizes, random atmospheric deposition, geogenic inputs, constructive fields, etc. as well as naturally occurring processes. In addition to this, the organic matter in the soil significantly contributes to the adsorption capacity of the metals in the soil thereby increasing the concentration. As a result of contamination of soils due to these metals, immediate measures should be implemented to retain the environmental sustainability through organizing awareness program sections, discovering innovated technologies in the sphere of remediation, etc. Additionally, continuous monitoring endeavour should be executed in these areas for the

assessment of pollution rate thereby formulating remediation strategies later enhancing the quality of the soil.

3.5 References

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Chapter

4

SPATIO- TEMPORAL DISTRIBUTION PATTERN OF ORGANOCHLORINE INSECTCIDES

Contents

4.1 Introduction

Organochlorine insecticides are synthetic chlorinated organic compounds, has been exclusively and widely used as an insecticide in arable fields and in industries for controlling insect all over the world from the beginning of the 1940's. They are also a potent insect control for both malaria causing mosquitoes and typhus vectors (Aktar et al., 2009). Moreover, OCIs are present in considerable levels in certain cosmetic products such as shampoo due to its insecticidal property (Hall and Hall, 1999; Schachner and Hansen, 2011; Landrigan and Etzel, 2013; Anand et al., 2015; Yadav et al., 2015; Ghosh et al., 2018). Owing to their persistent nature, these compounds remain in the environment for a long period after its application in different environment segments. Since they are fat-soluble

in nature, they are readily absorbed into the body fat of the target insect and thereby threatening the proper functioning of the internal organs of the species. OCIs directly act on both the central and peripheral nervous system of the target organism by destructing the mechanism of Na/K pump for providing a potential gradient in the nerve cell membrane functioning for generating the necessary energy. These toxic compounds are absorbed immediately after consuming into the alimentary tract or articulately absorbed through the skin of the target insects and arthropods. This chemical compound is also known as endocrine disrupting insecticides (Pleština, 2003; Victoria et al., 2013). These water-insoluble compounds find their way of transferring and translocating from one place to another through land leaching and runoff and they persist for decades in the environment devoid of degradation.

Despite the fact that the extensive use of these multipurpose insecticides, leads to their accumulation in the soil system, groundwater column, and finally ends up in the ocean, thereby causing toxicity to the benthic communities (Reichenberger et al., 2007). Subsequently, these activities facilitate their exposure to human beings, other higher organisms and aquatic organisms, triggering nerve-related health issues (Paul and Balasubramaniam, 1997). These toxic pollutants enter into the life cycle of living organism thereby transferring them from the parental body to fetuses through the placenta or by lactation. Hence, the harmful effects will carry through generations to generations, resulting a community of handicapped society, causing permanent neurobehavioral impairment (Paul and Balasubramaniam, 1997; Metcalf, 1997; Jurewicz and Hanke, 2008; Guo et al., 2014; Genius et al., 2016). The toxicity of these classes of persistent

insecticides and its harmful effect on humanity and non-target organisms led to the prohibition of their production, sale and application/usage in several countries (EPA, 2009).

Considering these facts, this chapter provides knowledge on the distribution pattern of organochlorine insecticides namely, α - HCH, β - HCH, γ - HCH, heptachlor, aldrin, heptachlor epoxide (B), 4,4'-DDE, dieldrin, endrin, 2,4' - DDD, 4,4' - DDD, 2,4' - DDT, 4,4' - DDT, α - endosulphan, β - endosulphan in the selected soils of Palakkad district, Kerala, India. Besides, the findings highlight the necessity of periodic review monitoring of soil quality in the study area.

4.2 Materials and Methods

4.2.1 Extraction and Quantification of Organochlorine Insecticides in the Soil

4.2.1.1 Chemicals

The OCIs standards, α - hexachlorobenzene (HCH), β - HCH, γ - HCH, heptachlor, aldrin, heptachlor epoxide (B), 4,4'- DDE, dieldrin, endrin, 2,4'- DDD, 4,4'- DDD, 2,4'- DDT, 4,4'- DDT, α - endosulphan, β - endosulphan were purchased from EPA (USA) and Supelco (USA).

4.2.1.2 Procedure and Quality control

5 g of the powdered soil samples were accurately weighed and extracted twice with hexane: acetone (1:1) mixture and was subjected to a cleanup procedure involving elution through florisil column (60 cm $^{-1}$ 22 mm i.d.) with hexane: acetone (1:1). The extracts were concentrated to about 5 - 6 mL by means of the rotary evaporator at 50 - 60 °C for further

analysis using gas chromatograph (GC) with electron capture detector. All the procedures were subjected to strict quality control protocols. For every set of samples and procedural blanks with standards were used to check interference, cross-contamination and instrument performance. The detection limit of the method ranged from 0.01 to 0.08 ng g^{-1}. The instrumental detection limit values were calculated from the lowest standards with lowest concentration and extrapolating to the corresponding amount of analytes that generate a signal-to-noise ratio of 3:1. The detection limit was lowest for aldrin and highest for endrin. The quality of the data was assessed by the certified reference material (CRM) 804-050 soils (Sigma-Aldrich). The average recoveries (n = 3) for OCIs in the soil revealed an efficiency of 87 - 103 % (Table A1:13), showing that the analytical protocols is effective for the determination of OCIs in soil. The relative standard deviations (RSD) were below 5.0 % and fall within the requirement criteria of US-EPA (Recovery: 70 - 130 %, RSD is < 30 %).

4.2.1.3 Chromatographic Systems and Conditions

Quantification of the insecticides were carried out using GC (model 7890A, Agilent, Waldbronn, Germany) equipped with a split/splitless injector, an electron capture detector and a 30 m × 0.320 mm i.d., HP-35 capillary column with 0.5 mm film (35% - (phenyl) - methyl polysiloxane phase). The injector port temperature was maintained at 250 °C and the detector temperature at 350 °C. The GC oven temperature was programmed from 110 °C at 5 °C min^{-1} to 190 °C at 5 °C min^{-1} and held for another 2 min, then to 280 °C at a heating rate of 15 °C min^{-1}. This temperature was held constant for 10 min. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL min^{-1} with the split ratio 1:50.

4.2.2 Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 20.0 was used to generate the means of the results of physico-chemical parameters of the soil samples (Table 3.2 - 3.4; already discussed in Chapter 3) and the OCIs.

4.2.3 Health Risk Assessment

Health risk assessment in human populations were calculated using the obtained OCI data in terms of lifetime average daily dose (LADD), incremental lifetime cancer risk (ILCR) and hazard quotient (HQ) (EPA, 1989; Huang et al., 2014; Kumar et al., 2014a). The following equations were adopted to calculate health risk in adults and children:

$$\mathrm{LADD} = \frac{CS \times IR \times CF \times EF \times ED}{BW \times AT}$$

$$\mathrm{ILCR} = \frac{\mathrm{LADD}}{CSF}$$

$$\mathrm{HQ} = \frac{\mathrm{LADD}}{RfD}$$

[CS - Concentration of pesticide in the soil (ng/g), IR - Rate of soil, which comes to contact with the human body (Adult - 100 mg/day; Children - 200 mg/day), CF - Unit conversion factor (10^{-6} kg/mg), EF - Exposure frequency of pesticides (365 days/year), ED - Lifetime exposure duration of pesticides (Adult - 70 years; Children - 12 years), BW - Weight of the human body (Adult - 70 kg; Children - 27 kg), AT - Averaging time for carcinogens i.e., (EF × ED days), CSF - Cancer oral slope factor, RfD - Reference dose (mg kg^{-1} day^{-1})]

4.3 Results and Discussion

4.3.1 Distribution Pattern of Organochlorine Insecticides

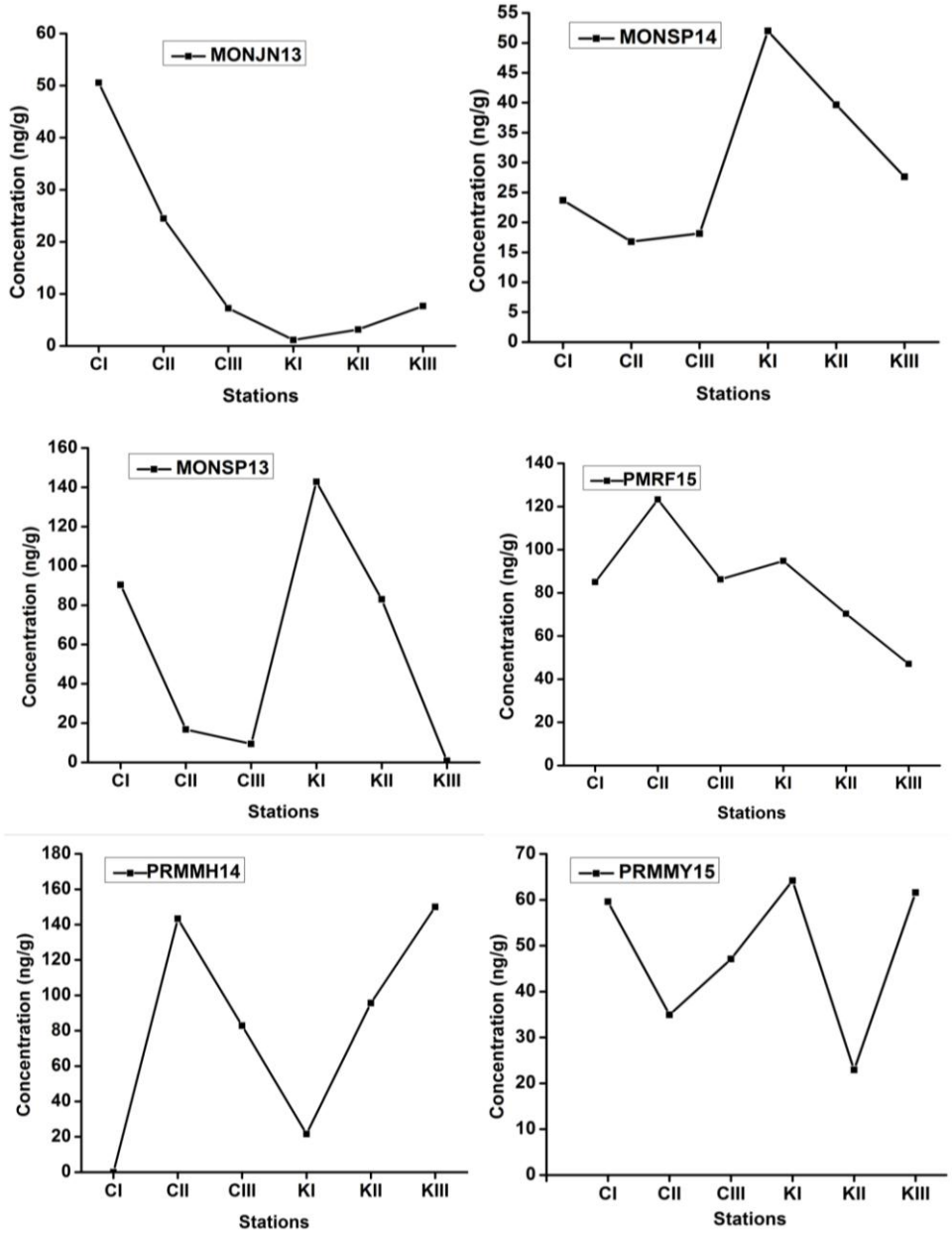

Fig. 4.1 Distribution pattern of ∑OCIs in the study area

Table 4.1 Concentration of OCIs (ng g^{-1}) in the surface soil during MONJN13

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
MONJN13	CI	1.92 ± 0.6	9.68 ± 6.3	15.88 ± 3.4	*	20.34 ± 11.2	4.66 ± 4.22	*	*	*	*	*	*	*	*	*
	CII	0.99 ± 0.2	7.28 ± 4.5	1.05 ± 1.5	2.43 ± 1.2	1.62 ± 1.5	7.52 ± 0.41	*	*	4.58 ± 0.3	*	*	*	*	*	*
	CIII	0.76 ± 0.2	6.64 ± 4.3	0.58 ± 1.11	*	*	*	*	*	*	*	*	*	*	*	*
	KI	1.14 ± 1.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	KII	*	*	0.88 ± 2.17	1.13 ± 1.24	*	1.13 ± 2.2	*	*	*	*	*	*	*	*	*
	KIII	*	*	0.67 ± 1.12	0.90 ± 1.1	1.90 ± 1.8	4.19 ± 1.1	*	*	*	*	*	*	*	*	*

* Non detectable level

Table 4.2 Concentration of OCIs (ng g^{-1}) in the surface soil during MONSP13

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
MONSP13	CI	0.79 ± 0.1	6.81 ± 0.3	0.72 ± 0.11	0.4 ± 0.14	1.25 ± 0.11	4.33 ± 0.2	5.73 ± 0.4	7.37 ± 3.1	63.74 ± 2.8	*	*	*	*	*	*
	CII	1.03 ± 0.2	6.93 ± 0.1	0.99 ± 0.1	0.79 ± 0.2	*	*	*	8.03 ±2.1	*	*	*	*	*	*	*
	CIII	1.37 ± 0.1	7.44 ± 0.13	*	0.53 ± 0.1	1.45 ± 0.2	*	*	*	*	*	*	*	*	*	*
	KI	1.24 ± 0.1	7.04 ± 1.1	0.62 ± 0.1	0.43 ± 0.11	1.45 ± 0.5	4.18 ± 0.1	5.49 ± 1.1	*	63.9 ± 5.4	59.71 ± 6.1	*	*	*	*	*
	KII	1.06 ± 0.11	6.98 ± 0.2	0.62 ± 0.2	0.6 ± 0.1	*	4.5 ± 2.1	5.51 ± 0.1	*	64.79 ± 4.3	*	*	*	*	*	*
	KIII	0.83 ± 0.21	*	0.82 ± 0.1	*	*	*	*	*	*	*	*	*	*	*	*

* Non detectable level

Table 4.3 Concentration of OCIs (ng g^{-1}) in the surface soil during PRMMH14

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
PRMMH14	CI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	CII	0.82 ± 0.3	0.67 ± 0.5	0.60 ± 1.6	0.40 ± 1.1	1.31 ± 0.1	4.77 ± 2.1	7.41 ± 1.32	63.85 ± 18.1	5.42 ± 0.36	58.96 ± 22.2	*	*	*	*	*
	CIII	0.79 ± 0.6	6.80 ± 4.2	0.66 ± 1.1	0.73 ± 0.1	1.39 ± 1.2	0.41 ± 1.1	7.73 ± 3.1	65.12 ± 15.4	*	*	*	*	*	*	*
	KI	*	6.65 ± 3.6	0.58 ± 0.1	*	1.31 ± 1.1	7.63 ± 2.31	*	*	5.43 ± 2.41	*	*	*	*	*	*
	KII	0.76 ± 1.4	6.65 ± 2.1	0.60 ± 0.6	*	13.22 ± 12.2	4.08 ± 1.1	7.33 ± 2.33	63.77 ± 22.3	*	*	*	*	*	*	*
	KIII	0.85 ± 1.07	6.78 ± 5.11	0.62 ± 1.3	0.62±0.4	1.37 ± 0.3	4.05 ± 1.21	7.51 ± 1.13	63.75 ± 11.1	5.49 ± 0.21	59.00 ± 14.6	*	*	*	*	*

* Non detectable level

Table 4.4 Concentration of OCIs (ng g^{-1}) in the surface soil during MONSP14

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
MONSP14	CI	0.94 ± 0.1	7.14 ± 2.1	11.04 ± 1.1	2.03 ± 0.1	2.54 ± 0.1	*	*	*	*	*	*	*	*	*	*
	CII	0.84 ± 0.2	10.02 ± 1.2	2.75 ± 2.1	1.6 ± 0.1	1.58 ± 0.2	*	*	*	*	*	*	*	*	*	*
	CIII	0.85 ± 0.1	6.83 ± 2.1	1.57 ± 3.1	0.98 ± 0.3	3.32 ± 0.12	4.61 ± 2.1	*	*	*	*	*	*	*	*	*
	KI	3.51 ± 1.1	24.94 ± 2.1	8.7 ± 0.2	5.69 ± 1.1	1.46 ± 0.1	*	7.71 ± 1.1	*	*	*	*	*	*	*	*
	KII	1.38 ± 1.2	16.46 ± 2.2	6.6 ± 1.2	5.34 ± 1.2	2.33 ± 1.1	7.55 ± 1.2	*	*	*	*	*	*	*	*	*
	KIII	0.94 ± 0.1	7.16 ± 2.1	6.89 ± 1.2	4.29 ± 0.1	2.15 ± 1.1	6.2 ± 1.2	*	*	*	*	*	*	*	*	*

* Non detectable level

Table 4.5 Concentration of OCIs (ng g^{-1}) in the surface soil during PRMF15

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
PRMF15	CI	5.38 ± 2.1	31.11 ± 2.1	8.53 ± 2.1	*	15.28 ± 1.1	24.73 ± 2.4	*	*	*	*	*	*	*	*	*
	CII	6.38 ± 1.2	31.15 ± 4.5	8.53 ± 1.1	*	15.27 ± 1.4	24.66 ± 2.1	*	37.28 ± 2.1	*	*	*	*	*	*	*
	CIII	6.52 ± 0.1	31.13 ± 3.1	8.53± 2.1	*	15.27 ± 1.2	24.82 ± 3.1	*	*	*	*	*	*	*	*	*
	KI	6.72 ± 0.2	31.12 ± 1.2	9.01 ± 2.4	7.88 ± 1.2	15.27 ± 4.1	24.79 ± 1.4	*	*	*	*	*	*	*	*	*
	KII	6.83 ± 1.1	31.15 ± 2.1	9.12 ± 2.1	7.96 ± 2.4	15.28 ± 2.6	*	*	*	*	*	*	*	*	*	*
	KIII	6.83 ± 2.2	31.16 ± 1.2	9.08 ± 2.1	*	*	*	*	*	*	*	*	*	*	*	*

* Non detectable level

Table 4.6 Concentration of OCIs (ng g^{-1}) in the surface soil during PRMMY15

Season	Stations	α-HCH	β-HCH	γ-HCH	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE (B)	DIELDRIN	ENDRIN	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	α -ENDO	β -ENDO
PRMMY15	CI	7.9 ± 2.1	19.9 ± 2.5	8.51 ± 3.2	7.97 ± 4.1	15.34 ± 4.2	*	*	*	*	*	*	*	*	*	*
	CII	*	*	18.76 ± 3.1	*	16.2 ± 0.1	*	*	*	*	*	*	*	*	*	*
	CIII	7.69 ± 1.1	31.08 ± 2.1	8.32 ± 2.2	*	*	*	*	*	*	*	*	*	*	*	*
	KI	8.46 ± 2.1	31.85 ± 2.3	8.25 ± 1.1	*	15.64 ± 2.1	*	*	*	*	*	*	*	*	*	*
	KII	*	*	7.68 ± 0.2	*	15.25 ± 1.2	*	*	*	*	*	*	*	*	*	*
	KIII	7.67 ± 2.1	31.01 ± 4.5	7.7 ± 1.1	*	15.22 ± 2.2	*	*	*	*	*	*	*	*	*	*

* Non detectable level

The use of OCIs in the soil for different purpose contributes to severe contamination concerns in the soil environment and responsible for certain human as well as animal health threats. The results of the four different OCI groups [first group - DDT; second group - cyclodiene (aldrin, dieldrin, endrin, chlordane, nonachlor, heptachlor and heptachlor-epoxide); third group - benzene hexachloride (BHC/HCH/lindane); and fourth group - endosulfan] examined during the MONJN13, MONSP13, PRMMH14, MONSP14, PRMF15 and PRMMY15 along the stations were exhibited a zig zag nature (Table 4.1 - 4.6). The ∑OCIs in the selected stations ranged from ND - 150 ng g^{-1} (Fig. 4.1). Peak concentrations of OCIs were observed during PRMMH14 at the station K III.

Among the four groups of OCIs investigated, cyclodiene compounds, HCBs and chlorinated derivative of diphenyl ethane were observed in the soil samples, whereas endosulfan was not detected in the study area. Among the quantified OCIs, HCH and its metabolites were detected in most of the stations throughout the sampling period ranged from ND - 31.85 ± 2.3 ng g^{-1}. β isomer is the highest observed derivative among the HCH forms and were found at the station K I during PRMMY15. The presence of residual levels of these particular OCIs may be due to their incessant application into the environment (Leena et al., 2012). Similar studies had reported that these persistent compounds were derived from direct application in various sectors such as vector control as well as agriculture purpose (Sankararamakrishnan et al., 2005).

Similar observations were reported in several soil environments all over the world such as in Korea, < 3 ng g^{-1} (Kim and Smith, 2001),

greenhouse soils from Beijing suburbs, 11.64 - 29.8 ng g^{-1} (Ma et al., 2003), Redon soil in Europe, 0.08 - 0.49 ng g^{-1} (Grimalt et al., 2004), Shanghai, ND to 10.38 ng g g^{-1} (Jiang et al., 2009), rural soil of Hong Kong, 6.19 ± 1.31 ng g^{-1} (Zhang et al., 2006), Delhi, 3.59 to 157.02 ng g^{-1} (Kumar et al., 2011), Korba 0.9 - 20 µg kg^{-1} (Kumar et al., 2014b) and Kuttanad agro ecosystem ND to 9.55 ng g^{-1} (Sruthi et al., 2017), respectively. Likewise, the ranges of HCH derivatives observed in different soils of India were tabulated in Table 4.7. According to the statistical reports available, India is the third largest consumer of HCH's in the world (Kutz, 1991; Iwata et al., 1993). Approximately 6840 tonnes of lindane were consumed during the year 1990-2004 (Vijgen, 2006). In the present study, as aforementioned, β-HCH was observed as the dominant isomer among the analyzed HCH derivatives in most of the stations throughout the sampling period and can be inferred as their recent application into the soil environment (Stewart and Chisholm, 1971; Andreu and Picó, 2004).

Moreover, among the HCH derivatives, β forms are the most stable compounds as they are devoid of microbial and photolytic degradation. As a result of enhanced stability, these compounds readily adsorb onto the soil particles along with the organic matter moieties thereby persist in the soil environment for decades (Kalbitz et al., 1997; Mackay et al., 1997). The presence of β isomer in the soil may be due to the usual conversion of α and γ-HCH isomers to β-HCH (Willett et al., 1998). In addition to these findings, the present study also highlights that the concentrations of HCH derivatives were observed in higher amounts in dry seasons than the wet seasons, contrary to previous reports (Ramesh et al., 1991; Takeoka et al., 1991).

Table 4.7 HCH derivatives (ng g^{-1}) in soils from various parts of India

Sl. No	Location and Type of Soil	HCH Derivatives	References
1.	Kuttanad Agro ecosystem	ND – 9.55	Sruthi et al., 2017
2.	Hyderabad Surface soil	75.89 ± 1.32	Kata et al., 2015
3.	Korba Residential soil	0.9 – 20	Kumar et al., 2014b
4.	Assam Forest soil	0.007 – 0.025	Devi et al., 2013
5.	Tripura Forest soil	0.003 – 0.049	Devi et al., 2013
6.	Manipur Forest soil	ND – 1.15	Devi et al., 2013
7.	Kurukshetra Surface soil	0.6 – 8.5	Kumar et al., 2013
8.	Keoladeo National Park Sediment	9039.9	Bhadouria et al., 2012
9.	Delhi Surface soil	3.59 to 157.02	Kumar et al., 2011
10.	Sunderban Wetland soil	0.05 – 12.4	Sarkar et al., 2008
11.	Hissar Agricultural soil	2 – 51	Kumari et al., 2008
12.	Unnao Surface soil	0.1 – 7.3	Singh et al., 2007
13.	Thiruvallur Surface soil	75.3	Jayashree and Vasudevan, 2006
14.	Dehradun Surface soil	326	Babu et al., 2003
15.	Aligarh Agricultural soil	88.9	Nawab et al., 2003
16.	Kasimedu Agricultural soil	0.1	Senthilkumar et al., 2001
17.	Ennore Agricultural soil	2.1	Senthilkumar et al., 2001
18.	Cochin Agricultural soil	4.8	Senthilkumar et al., 2001
19.	Visakapattnam Agricultural soil	0.21	Senthilkumar et al., 2001
20.	Agra Agricultural soil	0.50	Singh, 2001
21.	Farrukhabad Agricultural soil	158	Agnihotri et al., 1996
22.	Haridwar	61.12	Dua et al., 1996

*ND - Non detectable level

According to the reports of Chakraborty and his teammates (2015), the dumping of municipal wastes around the sampling sites possibly attribute the higher levels of HCH's. Considering the lethal effects in the environment as well as to mankind, these prominent OCIs were banned in India in 2013 (Chakraborty et al., 2015).

Besides HCH derivatives, the presence of representatives of cyclodiene compounds i.e., aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide (B) were detected in the study area. Endrin is the most conspicuous OCI detected in the studied soil samples, and the highest concentrations were observed during PRMMH14 at the station C III, which ranged from ND - 65.12 ± 15.4 ng g^{-1} throughout all the seasons. The presence of these perennial classes implied long term accumulation as a result prolonged discharge from industries and direct application to soil and crops. The nature of the soil matrix could exert a direct impact on the retention, transport, or degradation phenomena as well as their persistence in the environment (Donoso et al., 1979). According to the reports of Nash and Woolson (1967), the degradation rates of these class of OCI were relatively very slow process and could detect about 41 % even after 14 years subsequently after their application into the soil. Additionally, the fate and transport of endrin could also be influenced by certain agents mainly by volatilization, leaching, wind erosion, surface run-off, and crop uptake (Harris et al., 1966). Several studies were reported for the transport of endrin in certain food crops in different parts of the world such as in Togo, West Africa, they were observed in cowpea (Mawussi et al., 2009), cucumbers in Tokyo and winter squash in the USA (Jorgenson, 2001; Hashimoto, 2005). Another analyzed cyclodiene compound, aldrin, a

potential carcinogen, widely used for the removal of termites were detected in the range, ND - 20.34 ± 11.2 ng g $^{-1}$ and the highest concentration were observed during MONJN13 at the station C I.

Conversely, dieldrin was not detected during MONJN13, PRMF15 and PRMMY15, however, these compounds ranged from ND - 7.73 ± 3.1 ng g $^{-1}$ during the rest of the season and highest concentrations were detected at the station K I during PRMMH14. Even though the production, sale and import of these xenobiotics have been banned in India since 2003, trace amounts were detected in certain stations indicated their unauthorized practice in the scrutinized area leading to lethal effects on the environment (Acton, 2012; Subramani et al., 2014). Generally, aldrin is rarely detected than dieldrin in environmental monitoring studies, due to their rapid breakdown to dieldrin more stable form which is resistant to further degradation (Miglioranza et al., 2003; Akhil and Sujatha, 2014). However, aldrin is frequently distributed in some of the study sites in the present investigation along with dieldrin, which is an evidentiary involvement of these contaminants as a recent application due to their cost-effectiveness and their well acceptance (Akhil and Sujatha, 2014).

On the other hand, heptachlor and heptachlor epoxide (B) were detected in the present study ranging from ND - 7.97 ± 4.1 ng g $^{-1}$ (maximum concentration at the station C I during PRMMY15) and ND - 24.82 ± 3.1 ng g $^{-1}$ (maximum concentration at the station C III during PRMF15) respectively, possibly be originated directly from agricultural input. Several studies indicate the presence of these compound could enter into the food web causing harmful impacts to the biota (EPA, 1989; Keith, 1998; Singh et al., 2007).

Another prominent class of OCIs, DDT derivatives were not detected in any stations during MONSP14, PRMF15 and PRMMY15, however, 4,4'-DDE and 2,4'-DDD were the major members observed during rest of the seasons in the range ND - 64.79 ± 4.3 ng g^{-1}. The higher concentrations of 4,4'-DDE and 2,4'-DDD were observed at the station K II and K I, both during MONSP13, respectively. Generally, DDT and its metabolites were extensively used for the enhanced production of agricultural crops. However, statistical reports indicate 21,462 tonnes of DDT were consumed during the year 2000 - 2006, as vector control even after their ban for agricultural usage in India (PPQS, 2013).

The presence of these metabolites were detected only in certain stations in trace amounts implied the historic and wide usage of DDT (Bossi et al., 1992) as well as confirms the indirect evidence for the application of dicofol. These observation verified the extensive usage of dicofol in the study area in different brand names since DDT is the major intermediate compound in their production (Qiu et al., 2005). Furthermore, DDT can undergo degradation gradually into DDD and DDE metabolites by means of aerobic and anaerobic conditions. Additionally, their accumulation in the study area could probably mobilize along with the rainwater into adjacent water reservoirs and aquifers raising harmful threat to the human population (Kumar et al., 2013).

Likewise, the presence of DDT metabolites were reported in the groundwater column in Kasargod district, Kerala, India, showcasing their persistent nature (Akhil and Sujatha, 2012). These observations emphasized that the quality of the soil have affected adversely due to the agricultural activities i.e., direct or indirect application of these chemicals and their

degradation products, within the vicinity of the study area. Both the industrial and domestic usage of organic pollutants also contributes to a certain extent to the entry of such menacing substances into the soil and these findings are well supported by earlier reports (Subramani et al., 2014). The accumulation in the soil system, followed by groundwater column, and finally ends up in the ocean, thereby causing toxicity to the benthic communities (Reichenberger et al., 2007). Finally, these persistent organic pollutants transferred into the food web, ultimately reach into the humans causing severe health issues (Metcalf, 1997; Paul and Balasubramaniam, 1997)

4.3.2 Statistical Analysis

The statistical correlations between OCIs and the physico-chemical parameters of the soils in the study area is furnished in Table 3.8. Generally, these hydrophobic, persistent and bioaccumulable OCIs and their transformable products were directly bound to the organic content in the soil (Aktar et al., 2009). The data provides a noticeable correlation among the parameters in which significant positive correlations between α-HCH, β-HCH, γ-HCH and aldrin with that of carbohydrate content while negatively related to protein.

Additionally, heptachlor epoxide (B) exhibited significant negative correlation with lipid content. TOC and the carbohydrate content revealed a relevant correlation. Apparently, the persistent OCIs display significant correlations among them which illustrates their probable common source. These findings in the present study indicated the labile organic matter in the soils of the scrutinized area, likely influence the fate and distribution of these hydrophobic persistent contaminants in the soil. Thus the nature of the

soil environment and their physico-chemical characteristics is the main factor which defines the adsorption of OCIs into the soil particle moieties (Schnitzer and Khan, 1972; Ahmad et al., 2001; Škrbić et al., 2017).

Furthermore, the high organic, as well as TOC content in the study area, provides a platform for the microbial degradation of OCIs present, by contributing carbon source for their metabolism. There was no direct correlation between the TOC, soil fractions and analyzed OCIs, however, observed strong correlation with other organic contents signifying their substantial backing (Kalbitz et al., 1997; Jiang et al., 2009). The significant positive correlation of carbohydrate content towards the OCIs and clay fractions indicated the sorption of aliphatic compounds into the soil with high stability (Skjemstad et al., 1986). The hydrophobic active sites were responsible for the strong and energetic binding on the surface of organic matter present and likely immobilize and persist in the soil for long term (Murphy and Zachara, 1995). The negative correlation among the OCIs, protein and lipid could be justified by the activity of supplementary charged particles bound to the active sites of the molecule. Similar effects were reported in the case of atrazine in which the active sites were blocked by the ether soluble particles of the organic content in the soil specifically fats, waxes, lipids and proteinaceous materials (Dunigan and McIntosh, 1971). Similarly, the soil fraction can more easily bind to the atrazine according to the studies of Barriuso and Koskinen (1996). However, in the present study, such interactions were not observed. Differences in the sorption capabilities might be the irregular distribution pattern of organic content and the nature of the soil of the study area.

The structural elucidation as well as the chemical composition of the organic content, could provide more information regarding such interactions, however, no data were presented here. Since, this particular study only investigates the dependence of OCI adsorption on the nature of the organic matrix and their significance while assessing the fate and transport in soil, particularly in Palakkad. Generally, the sorption of hydrophobic compounds into the soil matrix were observed less significant for aliphatic carbon content than proteinaceous materials due to their reactive functional groups (Dunigan and McIntosh, 1971; Piccolo et al., 1998; Ahmad et al., 2001; Chaplin et al., 2015). Contradictory to these facts, the present investigation recorded significant positive correlation with polysaccharide enriched organic content than proteinaceous moieties. As mentioned earlier, these could be due to their lack of available active sites on protein moieties.

4.3.3 Health Risk Assessment

Health risk assessment of OCIs in humans were evaluated by the lifetime average daily dose (LADD), incremental lifetime cancer risk (ILCR) and hazard quotient (HQ) with respect to the OCI concentrations obtained from the study region (EPA, 1989; Kumar et al., 2014). Table 4.9 represents the LADD values and the trends of analyzed OCIs in increasing order 4,4'-DDE < heptachlor < dieldrin < α - HCH < heptachlor epoxide (B) < γ - HCH < 2,4'-DDD < aldrin < endrin < β - HCH.

Table 4.8 Pearson correlation coefficients (r) between the soil parameters and OCIs

	α-HCH	β-HCH	γ-HCH	HPT	aldrin	HPTE (B)	4,4'-DDE	dieldrin	Endrin	2,4'-DDD	TOC	Sand	Clay	Silt	PRT	CHO	LPD	T/L
α-HCH	1																	
β-HCH	**.835****	1																
γ-HCH	**.588****	**.512****	1															
HPT	0.194	0.282	0.246	1														
aldrin	**.428****	**.370***	**.657****	0.11	1													
HPTE (B)	0.1	0.251	0.05	0.05	0.307	1												
4,4'-DDE	-0.2	-0.012	**-.338***	0.06	-0.282	0.236	1											
dieldrin	-0.04	0.036	-0.232	-0.19	0.018	0.24	0.186	1										
Endrin	-0.23	-0.095	**-.457****	-0.15	-0.16	0.187	**.573****	**.544****	1									
2,4'-DDD	-0.13	-0.13	-0.284	-0.08	-0.132	0.166	**.554****	0.302	**.613****	1								
TOC	0.03	-0.215	-0.218	-0.23	-0.086	-0.097	-0.105	0.04	0.17	-0.13	1							
Sand	0.08	0.137	0.145	-0.1	0.028	-0.167	-0.063	0.021	0.16	0.09	-0.29	1						
Clay	0.26	0.207	0.111	0.03	0.132	0.088	0.186	0.212	0.07	0.1	-0.01	**-.445****	1					
Silt	0.041	-0.077	-0.008	0.11	0.095	0.294	-0.089	-0.155	-0.25	-0.26	0.32	**-.668****	0.012	1				
PRT	**-.547****	**-.421***	**-.575****	-0.04	**-.594****	-0.329	0.235	-0.072	0.23	0.15	-0.05	-0.08	-0.07	-0.1	1			
CHO	**.425****	0.171	**.432****	-0.21	**.541****	-0.034	-0.213	0.022	-0.15	-0.11	**.417***	-0.06	**.390***	0.11	**-.506****	1		
LPD	0.01	-0.071	0.17	0.09	-0.036	**-.358***	-0.033	-0.22	-0.17	-0.14	0.222	0.15	-0.15	-0.07	0.228	0.076	1	
T/L	0.12	0.026	-0.084	-0.33	0.12	-0.036	0.014	0.272	0.3	0.136	0.313	0.084	0.095	-0.27	-0.108	**.344***	-0.1	1

Correlation is significant at the 0.01 level (2-tailed). Correlation is significant at the 0.05 level (2-tailed).

Table 4.9 LADD values of OCIs in human population (mg/kg/day)

OCIs	LADD values for adult		LADD values for children	
	Average value	STD	Average value	STD
α-HCH	3.69858×10^{-6}	4.14568×10^{-6}	1.91778×10^{-5}	2.14961×10^{-5}
β-HCH	1.82352×10^{-5}	1.71612×10^{-5}	9.46×10^{-5}	8.89841×10^{-5}
γ-HCH	6.96248×10^{-6}	7.03252×10^{-6}	3.61017×10^{-5}	3.64649×10^{-5}
Heptachlor	2.09032×10^{-6}	3.49416×10^{-6}	1.08387×10^{-5}	1.81179×10^{-5}
Aldrin	8.29029×10^{-6}	9.87057×10^{-6}	4.29867×10^{-5}	5.11807×10^{-5}
Heptachlor epoxide (B)	6.04436×10^{-6}	9.79792×10^{-6}	3.13411×10^{-5}	5.0804×10^{-5}
4,4'-DDE	1.80035×10^{-6}	3.46312×10^{-6}	9.33514×10^{-6}	1.79569×10^{-5}
Dieldrin	3.28039×10^{-6}	9.48652×10^{-6}	1.70094×10^{-5}	4.91894×10^{-5}
Endrin	1.78145×10^{-5}	3.67756×10^{-5}	9.23717×10^{-5}	1.90689×10^{-4}
2,4'-DDD	7.05027×10^{-6}	2.37152×10^{-5}	3.6557×10^{-5}	1.22968×10^{-4}
4,4'-DDD	0	0	0	0
2,4'-DDT	0	0	0	0
4,4'-DDT	0	0	0	0
α - endosulphan	0	0	0	0
β - endosulphan	0	0	0	0

Table 4.10 ILCR values of OCIs in human population

OCIs	ILCR values for adult		ILCR values for children	
	Average value	STD	Average value	STD
α - HCH	5.87077×10^{-7}	6.58044×10^{-7}	3.0441×10^{-6}	3.41208×10^{-6}
β - HCH	1.01306×10^{-5}	9.53401×10^{-6}	5.25293×10^{-5}	4.94356×10^{-5}
γ - HCH	6.32953×10^{-6}	6.3932×10^{-6}	3.28198×10^{-5}	3.31499×10^{-5}
Heptachlor	4.64515×10^{-7}	7.76481×10^{-7}	3.69858×10^{-6}	4.0262×10^{-6}
Aldrin	4.87664×10^{-6}	5.80622×10^{-6}	3.69858×10^{-6}	3.01063×10^{-5}
Heptachlor epoxide (B)	6.64215×10^{-7}	1.07669×10^{-6}	3.69858×10^{-6}	5.58286×10^{-6}
4,4'- DDE	5.29514×10^{-6}	1.01856×10^{-6}	3.69858×10^{-6}	5.28144×10^{-5}
Dieldrin	2.05024×10^{-7}	5.92908×10^{-7}	3.69858×10^{-6}	3.07434×10^{-6}
Endrin	-	-	-	-
2,4'-DDD	2.93761×10^{-5}	9.88134×10^{-5}	3.69858×10^{-6}	5.12366×10^{-4}
4,4'- DDD	0	0	0	0
2,4'- DDT	0	0	0	0
4,4'- DDT	0	0	0	0
α - endosulphan	0	0	0	0
β - endosulphan	0	0	0	0

'-' indicates the uncalculated value (CSF and RfD values of the concerned pesticides are not available

Table 4.11 HQ values of OCIs in human population

OCIs	HQ values for adult		HQ values for children	
	Average value	STD	Average value	STD
α-HCH	0.000462323	0.00051821	0.00239723	0.002687013
β-HCH	-	-	-	-
γ-HCH	0.023208258	0.023441734	0.120339118	0.12154973
Heptachlor	0.004180637	0.006988329	0.021677376	0.036235782
Aldrin	0.276343125	0.329019097	1.432890279	1.706024948
Heptachlor epoxide (B)	0.120887166	0.195958403	0.626822343	1.016080606
4,4'-DDE	-	-	-	-
Dieldrin	0.065607714	0.189730427	0.340188148	0.983787397
Endrin	-	-	-	-
2,4'-DDD	0.059381799	0.122585498	0.307905624	0.635628506
4,4'-DDD	0	0	0	0
2,4'-DDT	0	0	0	0
4,4'-DDT	0	0	0	0
α - endosulphan	0	0	0	0
β – endosulphan	0	0	0	0

*'-' indicates the uncalculated value (CSF and RfD values of the concerned pesticides are not available

The ILCR results were depicted in Table 4.10. On comparing with permitted ILCR values which lies in between 10^{-6} and 10^{-4} as it indicates no potential risk (Chen and Liao, 2006), the present data indicates no significant cancer risk as the range falls around 10^{-7} and 10^{-5} for adults and

infants (range - 10^{-6} and 10^{-4}) (Qu et al., 2015; Sruthi et al., 2017). The highest value of ILCR obtained for 2,4'-DDD and the trends in the increasing order via dieldrin < heptachlor < α - HCH < heptachlor epoxide (B) < aldrin < 4,4'-DDE < γ - HCH < β - HCH < 2,4'-DDD. Hazard quotient is defined as the risk of humans to be effected on exposure to harmful chemicals over an average exposure period. The acceptable risk level of HQ ≤ 1, however for both HQ values of adults and children falls with the limit and were represented in the Table 4.11 (EPA 1989; Kumar et al., 2014a). However, the study area were found to be contaminated, the ILCR and HQ values imply low health risk concerns.

OCI distribution pattern in the study area further highlights the urgent need for devising apt measures to reclaim these locations. If remediation measures are not taken, adjacent groundwater and other ecologically significant zones will be prone to get contaminated. The present condition of the study zones is suitable for discovering microbes or consortium of microbes that are capable of degrading OCIs. Hence, soil and water from the zones can be utilized as a primary source for isolating novel OCI degrading strains that can be used for reclaiming study area and similar sites.

4.4. Conclusion

Several case studies in India have focused on the monitoring of persistent OCIs including hexachlorocyclohexane, DDTs, endosulfan, etc. present in the soil environment. In the present study, soils from regions of Palakkad, were monitored for OCIs namely α - HCH, β - HCH, γ - HCH, heptachlor, aldrin, heptachlor epoxide (B), 4,4'-DDE, dieldrin, endrin, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, α – endosulphan and β -

endosulphan. Most of the OCIs were observed in residual levels except 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, α - endosulphan and β - endosulphan. The trends of the distribution pattern of OCIs were observed in a zig-zag nature throughout the six sampling period. The six stations namely C I, C II, C III, K I, K II and K III were observed to be slightly contaminated within one or in another season. The highest concentrations were observed during PRMMH14 at the station K III. The elevated levels of OCIs in the study region could be due to their uncontrolled and unscientific application in agricultural fields as well as in various sectors thereby causing contamination. Subsequently, these led to the accumulation in different phases of the environment thereby translocate into other regions and enter into the water bodies and ultimately find their sink into the aquatic system. Consequently, these toxic substances consumed by the aquatic organisms and enter into the food chain and translocate and accumulate in the lipid profile of the organisms. Thus, prolonged accumulation would result in severe health hazards and menace in the aquatic realm. Hence the application, manufacture and storage of these chemical toxicants should be carefully taken care of and use judiciously. The relevant and concurrent health issues are reckoned by educating agricultural practitioners, civilians and the local farmers. However the present study area is slightly contaminated with residual levels of OCIs, the health risk evaluation indicated a low cancer risk health issues in humans. Hitherto, a complete awareness and training program should be scheduled to conduct continuous monitoring along with practicing innovated eco-friendly methods such as biofertilizeres and bioinsecticides to avoid the severe health risk issues as well as the soil quality concerns in future in these locations. The study area

is a hot spot for discovering novel strains that can be used to degrade OCIs through cheap and eco-friendly manner.

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Chapter

5

ISOLATION AND CHARACTERIZATION OF OCI DEGRADING MICROBES SPECIFIC TO LINDANE

Contents

5.1 Introduction

Residual levels of both inorganic and organic xenobiotics are increasing exponentially in soil, groundwater and marine environment. Studies have shown that the presence of hazardous and banned chemicals are needed to be remediated from the system to prevent their leaching in the aquatic realm, specifically to the drinking water and thereafter causing accumulation into the food chain. Various physico-chemical methods are in practice, for removing or remediating these contaminants. However, high cost ensued, in this regard is a major drawback and discourage farmers from such practices. Considering the necessity and need for reclaiming the agrarian fields, to restore the quality, and to prevent the transport of contaminants to other ecosystem, researchers in this field are keen for developing methods that are cheaper and feasible for practicing. In this

regard biological method based on microbe remediation strategy is gaining popularity.

Earlier studies have been reported where different microorganisms isolated from various sources including polluted soils, water, plants, animal tissues, etc. which are capable of swallowing the toxic pollutants as growth substrate (Qiu et al., 2007). Microbes including, *Arthrobacter* (Jain et al., 1994; Chauhan et al., 2000), *Bacillus* (Kadiyala and Spain, 1998), *Burkholderia* (Bhushan et al., 2000; Chauhan et al., 2000), *Pseudomonas* (Prakash et al., 1996; Zaidi et al., 1996; Liu et al., 2005; Kulkarni and Chaudhari, 2006), and *Rhodobacter* (Roldan et al., 1997) were characterized as potential degraders of various toxic organic pollutants.

Recently, the distribution of ΣOCIs in the realm of Palakkad district was reported from the range ND to 150 ng g^{-1} and one of the isomer of HCH, lindane was about ND to 15.9 ± 3.4 ng g^{-1} (Gopalan and Chenicherry, 2018). Substantial problems of lindane toxicity and potential risk acquired the importance of bioremediation concept using microbes for estimating the residues of OCI in the soil segment. This chapter substantiates some of the salient features focused on the isolation and characterization of microbes from contaminated soil samples of Palakkad district, Kerala. Isolating lindane degrading strains from lindane (selected as a model OCI compound) treated soil samples remains as a crucial phase in biodegradation perspective. Since these organochlorine insecticides were used exclusively in India, their presence ranged from the character of toxic to highly toxic in various environmental segments of one trophic level to another and extensive reports were published (Kata et al., 2015; Sruthi et al.,

2017). The toxicity and the harmful effects of lindane, a potential carcinogen, were well described and well known for the past several decades. Lindane has a wide range of application in agricultural, medical and veterinary products as a very effective systemic insecticide (Golow and Godzi, 1994; Martinez and Martinez-Conde, 1995; Hirthe et al., 2001). High liposolublity of lindane has led to the biomagnification and associated health hazards leading to its ban in several developing countries. However, due to the economic reason such as low cost and effectiveness, the developing nations continue to use the dreadful insecticide lindane (Anupama and Paul, 2009).

The concerns regarding the persistence and toxicity of lindane were reported instantly worldwide (Chakraborty et al., 2015), efforts are being organized for the elimination of these pollutants from the environment (Pannu and Kumar, 2017). Accordingly, isolating such lindane degrading bacteria from lindane enriched soil remains the key motif in lindane biodegradation. The primary reaction involved in the microbial degradation of lindane is the dehalogenation (removal of chlorine atom) (Camacho-Pérez et al., 2012) under aerobic and anaerobic conditions (Quintero et al., 2005; Nagata et al., 2007) and finally into non-toxic byproducts. *Bacillus* sp. (Gupta et al., 2000), *Clostridium* sp. (MacRae et al., 1969; Heritage and MacRae, 1977; Ohisa et al., 1978; Zheng et al., 2011), *Pseudomonas* sp. (Senoo and Wada, 1989), *Azotobacter* sp. (Hardisson et al., 1969) etc. were the major microbial genera reported that are capable to detoxify lindane.

The persistence of xenobiotics specifically, OCIs in the Palakkad soil samples has triggered (Gopalan and Chenicherry, 2018) the need for

bioremediation in the area and initiated to control the menace for the cultivation of microorganisms. The process has acquired its importance by reducing the impact on the soil environment and the ability to degrade the compound and remove the harmful moieties from the biota without affecting the soil structure. Thus the present study highlights an eco-friendly and cost-effective biotreatment initiating the utility and the capability of microbes to reduce the concentration level and control this noxious chemical, lindane from soils of Palakkad district.

The objectives of the study were assessed with regard to the aim of the bioremediation aspects of lindane by using native microbes are to determine the bacterial population density in the selected soil samples, then to isolate, screen and develop microbial cultures for bioremediation of lindane-contaminated soil and finally, characterization and identification of bacterial isolates.

5.2 Materials and Methods

5.2.1 Chemicals and Soil Sampling

Lindane (γ-HCH, > 99.9 % purity) was purchased from Sigma Aldrich, USA. Soil samplings were divided into two sections for total bacterial count and isolation procedures from the six stations of Chittur and Kanjikode of Palakkad district, (Fig. 2.1; Chapter 2). The first section includes June 2013 (monsoon 2013: MONJN13), September 2013 (monsoon 2013: MONSP13), March 2014 (pre-monsoon 2014: PRMMH14), September 2014 (monsoon 2014: MONSP14), February 2015 (pre-monsoon 2014: PRMM15) and May 2015 (pre-monsoon 2014: PRMMY15). Secondly, the soil samples collected from each station, May

2015 (pre-monsoon 2014: PRMMY15), were mixed well. Later, these were subjected to lindane treatment with a concentration of 10 ppm and kept for two weeks. All the samples were tested for the total bacterial population in terms of colony forming units per gram of the soil.

5.2.2 Isolation of Lindane Degrading Bacteria and Biochemical Characterization

One gram of the homogenized lindane treated soil was transferred into a conical flask containing 100 mL sterile distilled water to perform serial dilution in order to isolate lindane tolerant microbial species. Subsequently, the homogenized sample was taken and pipetted out into a test tube containing 9 mL of sterile diluent using sterile pipettes, which represents a dilution of 10^{-2}. All the experiment was repeated using sterile pipettes for each dilution up to 10^{-9}. From 10^{-5} & 10^{-6} dilution one mL was pipetted out into sterile petriplates in triplicates, followed by pouring the media (Nutrient Agar) and thoroughly mixed by rotating the plate which is then allowed to solidify. After 24 hours incubation at 30 °C, the plates were examined for the presence of individual colonies growing throughout the solid medium (Yu et al., 2006; Salam et al., 2014; Braide et al., 2017). The prominent colonies/strains were selected considering the shape, size and colour, and transferred to agar plates for obtaining the pure culture. Biochemical characterization such as indole, methyl red, Vogues- Proskeur and citrate were performed using standard protocols (Cappuccino and Sherman, 1996).

5.2.3 Molecular Characterization of Isolates

Bacterial genomic RNA was extracted from the isolates followed by polymerase chain reaction (PCR) amplification for targeting the 16S rRNA. The PCR products obtained were separated on 1% agarose gel by electrophoresis. Later, these were imagined using gel documentation system and ethidium bromide was used for staining (Pannu and Kumar, 2017). Afterwards, these were sent to Support AgriGenome, Kochi for sequencing. The obtained sequences were checked for online bioinformatics tool, BLAST N, using NCBI database and deposited to NCBI Gen-Bank with accession numbers MG581163- MG581166, respectively. The phylogenetic tree was constructed for partially amplified sequence of 16S rRNA sequence using the MEGA 6.0 software.

5.3 Results and Discussion

5.3.1 Isolation of Lindane Degrading Bacteria

Soil microorganisms primarily occur in the soil surface, immediately next to the plant roots i.e., the rhizosphere. They hold a major role in maintaining the soil structure by maintaining the fertility and quality as well as in the plant growth. Generally, microorganisms are considered as the pathogens which affect the health of both human beings and the living system. However, soil microbes provide significant contributions towards the conservation of soil ecosystem as organic matter decomposers, nitrogen fixers, detoxification agent (mineralization), effective disease manager, etc.

Considering these facts, for the proper management of the soil ecosystem, the scientists all over the world have developed bio pesticides

and marketed nowadays such as *Trichoderma fungi*, *Bacillus subtilis*, etc. which promotes the plant growth and function as anti-fungal and anti-bacterial agents. However, for decades, the practice for the application of persistent synthetic chemicals for the crop enhancement and soil quality were increased tremendously. This situation arose mostly in developing nations and deplete the soil environment. These critical conditions were studied, discussed and reported as a threat to the living system. As a part of these investigations, microbes degrading toxic chemical pesticides were identified and led to revolutionary impact in the remediation aspects of persistent organic as well as inorganic pollutants in the soil. Similar observations were perceived in the present study which exposes new strategies in remediation studies of toxic persistent organochlorine insecticide, specifically lindane extensively using all over the world.

Table 5.1 Total bacterial count in the soil samples of Palakkad district

STATIONS	MONJN13	MONSP13	PRMMH14	MONSP14	PRMM15	PRMMY15
KI	32×10^6	35×10^6	30×10^6	35×10^6	25×10^6	33×10^6
KII	38×10^6	43×10^6	39×10^6	34×10^6	33×10^6	24×10^6
KIII	30×10^6	28×10^6	33×10^6	30×10^6	38×10^6	26×10^6
CI	25×10^6	30×10^6	28×10^6	31×10^6	34×10^6	35×10^6
CII	40×10^6	44×10^6	35×10^6	38×10^6	33×10^6	34×10^6
CIII	34×10^6	43×10^6	36×10^6	35×10^6	34×10^6	30×10^6

In the present study, the total microbial counts were determined by the plate count method and were represented in Table 5.1. Microbial population in the sampling sites were varied spatially and temporally and the maximum numbers of microbes were observed during MONSP13 at C II (44 $\times 10^{6}$). Besides, the total number of colonies appeared in the dilutions ranging from 10^{-5} to 10^{-6} of homogenized lindane treated soil samples were 38 x 10^{-5} and 10 x 10^{-6} CFU/gram respectively (Fig. 5.1).

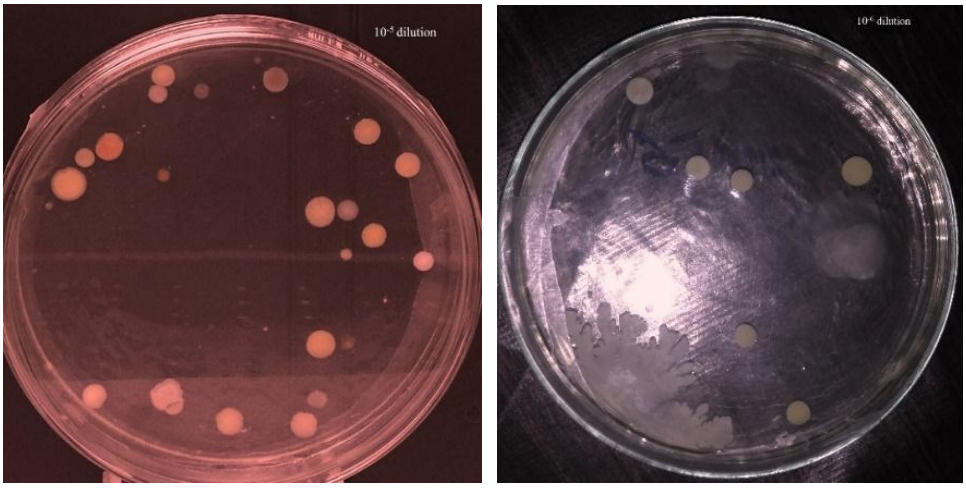

Fig. 5.1 Bacterial colonies growing on agar plates in the dilutions ranging from 10^{-5} to 10^{-6} of homogenized lindane treated soil samples

These colonies were considered as potential lindane degraders and four prominent colonies were selected based on the colony morphology, which were continuously subcultured to obtain pure cultures. Finally, the pure isolates namely COD NIS-24, COD NIS-25, COD NIS-26a and COD NIS-26b were preserved for further analysis under -20 °C in water, nutrient agar and glycerol stock. Morphological and biochemical observation results were depicted in Table 5.2.

Table 5.2 Biochemical characterization of the isolates

Microorganisms Test	*Bacillus drentensis COD NIS-24*	*Bacillus subtilis COD NIS-25*	*Bacillus cereus COD NIS-26*	*Lysinibacillus sphaericus COD NIS-26*
Gram +/-	**+ rods**	**+rods**	**+ rods**	**+ rods**
Shape	**Slightly convex**	**Slightly curved**	**Waxy, round-ended**	**Irregular**
Indole	**-**	**-**	**-**	**-**
Methyl Red	**-**	**+**	**+**	**-**
Vogues-Proskeur	**-**	**+**	**+**	**+**
Citrate	**-**	**-**	**-**	**-**

- Negative, + Positive

5.3.2 Molecular Characterization of Isolates

16s rRNA gene sequencing analysis was performed for the genus identification of the four pure isolates, COD NIS-24, COD NIS-25, COD NIS-26a and COD NIS-26b and the partial sequencing of the 16S rRNA gene produced 898, 703, 951 and 882 base pairs respectively.

The trimmed sequences of the four microbes are shown below:

1. COD NIS-24 (898bp)

GTCGAGCGAATCACTGGGAGCTTGCTCCCGGTGGTTAGCGGCGGACGGGTGAGTAACACG
TGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAAT
TTCTTCCCTCGCATGAGGGAAGGTTGAAAGTCGGTTTCGGCTGACACTTACAGATGGGCC
CGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGAC
CTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGCGATGA
AGGCCTTCGGGTCGTAAAGCTCTGTTGTCAGGGAAGAACAAGTATCGGAGTAACTGCCGG
TACCTTGACGGTACCTGACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTT
AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTG
AGTGCAGAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGG
AACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGG

GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT
AGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC
GGCCGCAAGGCTGAAACTCAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT

2. COD NIS-25 (703 bp)

CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCG
AAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTATCTAGTTG
GTGAGGTAACGGCTCACCAAGGCAACGATGCGTATCCGACCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAAC
TCTGTTGTTAGGGAAAAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAAC
CAAAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCC
ACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTG
GAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCG
ACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAG

3. COD NIS-26a (951 bp)

TAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCC
CATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCA
TGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAG
CTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTC
GTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGT

ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTG
AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAG
GAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGC
GAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCG
CCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGGAG
CAACGCGAAGACCCTTACCAGGTCTTGACATCCTCTGAAACCCTAGAGATA

4. COD NIS-26b (882 bp)

GTCGAGCGAACAGAGAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACA
CGTGGGCAACCTACCTTATAGTTTGGGATAACTCCGGGAAACCGGGGCTAATACCGAATA
ATCTATTGTCCCTCATGGGACAATACTGAAAGACGGTTTCGGCTGTCGCTATAGGATGGG
CCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCG
ACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAA
GAAGGATTTCGGTTCGTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGC
TGTACCTTGACGGTACCTTATTAAAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTC
TTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACT
TGAGTGCAGAAGAAGATAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGA
GGAACACCAGTGGCGAAGGCGACTATCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
TTAGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTTCCGCCTGGGGAGT
ACGGTCGCAGACTGAAACTCAAAGGAATTGACGGGGGCCCGC

Later, on evaluating the similarity of the organisms using BLAST, the isolates were consequently denoted as *Bacillus drentensis COD NIS-24* (Accession No. MG581163), *Bacillus subtilis COD NIS-25* (Accession No. MG581164), *Bacillus cereus COD NIS-26* (Accession No. MG581165) and *Lysinibacillus sphaericus COD NIS-26* (Accession No. MG581166). The phylogenetic trees were created using the sequence by MEGA 6.0 software. (Fig. 5.1 - 5.4).

The mineralization of lindane is mediated by several microorganisms and some of them were summarized in Table 5.3. Several case studies all over the world, previously reported a greater variety of microbes could degrade lindane such as *Streptomyces, Sphingobium, Fusarium, Pseudomonas* (Salam et al., 2014), and *Staphylococcus equorium, Staphylococcous cohnii subsp. urelyticus, K. rhizophila, Microbacterium resistens,* (Abhilash et al., 2011), *Bacillus sp. Cal-6F*, *Bacillus sp. Lad-2A*

and *Bacillus sp. Ym-7A* (Pannu and Kumar, 2017). Similarly, *Bacillus brevis* and *Bacillus circulans* (Gupta et al., 2000), *Bacillus cereus* and *Bacillus subtilis Strain HUK15* (Ehrhardt et al., 2010) were also reported to possess notable efficiency in lindane biodegradation. According to the reports of Francis et al. (1975) and Matsumura et al. (1976), aerobic lindane degradation was firstly documented in *Escherichia coli* and *Pseudomonas* strain. Likewise, *Sphingomonas paucimobilis* strains, capable of lindane degradation were isolated from French soil (Thomas et al., 1996). Similarly, isolates of *P. paucimobilis* were isolated for the aid of lindane degradation (Sahu et al., 1990). In another case study, it was reported that *S. paucimobilis* could degrade about 98 % of lindane aerobically within 12 days of incubation (Johri et al., 1998). Further, *Microbacterium sp*. strain, ITRC1 (Manickam et al., 2006), *Pseudoarthrobacter sp*., *Pseudomonas sp*. and *Klebsiella sp*. (Nagpal and Paknikar, 2006) and *Streptomyces sp. M7* (Benimeli et al., 2008) were another class of microbial strains reported to possess greater lindane degrading efficiency. Recently, in India, lindane degrading microbial cultures of *Sphingobium ummariense sp. nov* (Singh and Lal, 2009) and *Paracoccus sp. NITDBR1* (Sahoo et al., 2019) were isolated, respectively.

Table 5.3 List of previous reports on lindane degrading microorganisms

Sl No.	Microorganisms	Reference
1.	*Bacillus sp.*	Yule et al., 1967
2.	*Clostridium sp.*	MacRae et al., 1969
3.	*Pseudomonas putida*	Benezet and Matsumura, 1973
4.	*E.coli*	Francis et al., 1975
5.	*Pseudomonas sp.*	Tu, 1976; Sahu et al., 1990, 1995; Nawab et al., 2003
6.	*Citrobacter freundii*	Jagnow et al., 1977
7.	*Clostridium rectum S-17*	Ohisa and Yamaguchi, 1978
8.	*Pseudomonas vesicularis P59*	Huntjens et al., 19 Table Cont.
9.	*Sphingomonas paucimobilis UT26*	Imai et al., 1989
10.	*Sphingobium japonicum*	Senoo and Wada, 1989; Nagata et al., 2005
11.	*Sphingobium indicum B90A*	Sahu et al., 1990; Pal et al., 2005; Kumari et al., 2002
12.	*Cyanobacteria: Anabaena sp. and Nostoc ellipsosporum*	Kuritz and Wolk, 1995
13.	*Rhodanobacter lindaniclasticus*	Thomas et al., 1996; Nalin et al., 1999
14.	*Sphingomonas paucimobilis*	Miyauchi et al., 1998
15.	*Bacillus brevis and Bacillus circulans*	Gupta et al., 2000
16.	*Alkaligens faecalis*	Gupta et al., 2001
17.	*Sphingobium francense sp.*	Kumari et al., 2002; Cérémonie et al., 2006
18.	*Sphingomonas sp. γ1-2, Sphingomonas sp. γ4-2, Sphingomonas sp. DS2-2, Sphingomonas sp. DS3-1*	Böltner et al., 2005

Table Cont.

19.	*Pseudomonas aeruginosa ITRC-5*	Kumar et al., 2005
20.	*Microbacterium sp. strain ITRC1, Xanthomonas sp. ICH12*	Manickam et al., 2006
21.	*Sphingomonas sp. γ4-5, Sphingomonas sp. γ16-10, Sphingomonas sp. γ16-12*	Mohn et al., 2006
22.	*Pseudomonas aerogenosa*	Lodha et al., 2007
23.	*Sphingomonas sp. BHC-A*	Wu et al., 2007a, b
24.	*Sphingobium sp. MI1205*	Ito et al., 2007
25.	*Burkholderia pseudomallei*	Murthy and Manonmani, 2007
26.	*Lindane acclimated inocula*	Robles-Gonzalez et al., 2008
27.	*Pleuro tusostreatus, Trametes versicolor, Hypoxylon fragiforme*	Rigas et al., 2009
28.	*Azotobacter chroococcum JL102*	Anupama and Paul, 2010
29.	*Actinobacteria sp. and Streptomyces sp.*	Fuentes et al., 2010, 2011
30.	*Kocuria rhizophila, Microbacterium resistens, Staphylococcus equorum, Staphylococcus cohnii subsp. Ureolyticus*	Abhilash et al., 2011
31.	*Anabaena azotica*	Salam and Das, 2012
32.	*Fusarium verticillioides AT-100*	Guillén-Jiménez et al., 2012
33.	*Sphingomonas baderi*	Kaur et al., 2013
34.	*Arthrobacter forescens and Arthrobacter giacomelloi*	De Paolis et al., 2013
35.	*Rhodotorula sp. VITJzN03*	Salam et al., 2013
36.	*Candida VITJzN04*	Salam and Das, 2014
37.	*Clarias gariepinus*	Barnhoorn et al., 2015
38.	*Penicillium griseofulvum*	Ceci et al., 2015
39.	*Marinobacter, Sphingomonads, Chromohalobacter*	Lal et al., 2015

Table Cont.

40.	*Pleurotus forida*	Mohapatra and Pandey, 2015
41.	*Streptomyces sp. M7*	Sineli et al., 2016
42.	*Kocuria sp. DAB-1Y, Staphylococcus sp. DAB-1 W*	Kumar et al., 2016
43.	*Ganoderma lucidum GL-2*	Kaur et al., 2016
44.	*Chromohalobacter sp. LD2*	Bajaj et al., 2017
45.	*Bacillus sp. Lad-2a, Bacillus sp. Cal-6f and Bacillus sp. Ym-7e*	Pannu and Kumar, 2017
46.	*Paracoccus sp. NITDBR1*	Sahoo et al., 2019
47.	*Achromobacter sp. A3*	Singh and Singh, 2019

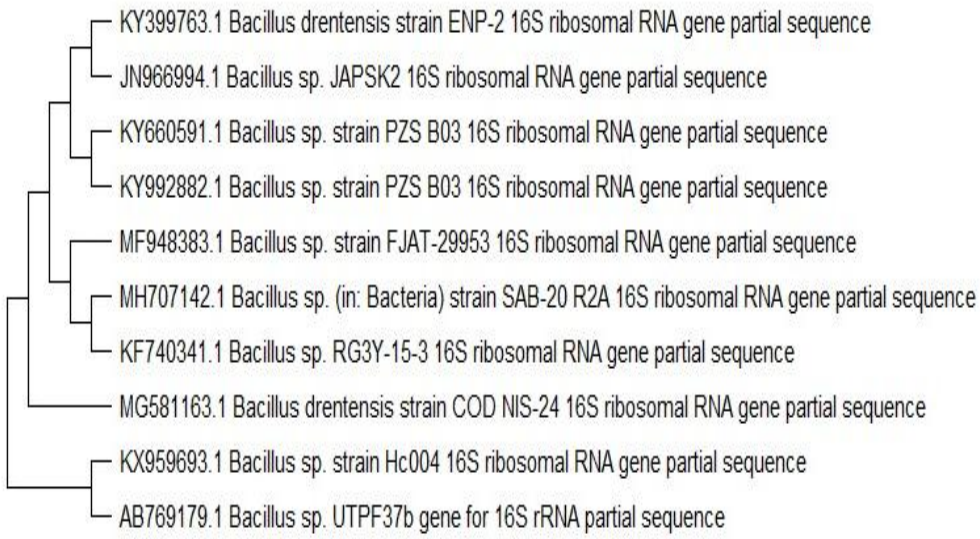

Fig. 5.2 Phylogenetic tree of *Bacillus drentensis COD NIS-24* (Accession No. MG581163) with closely related strains

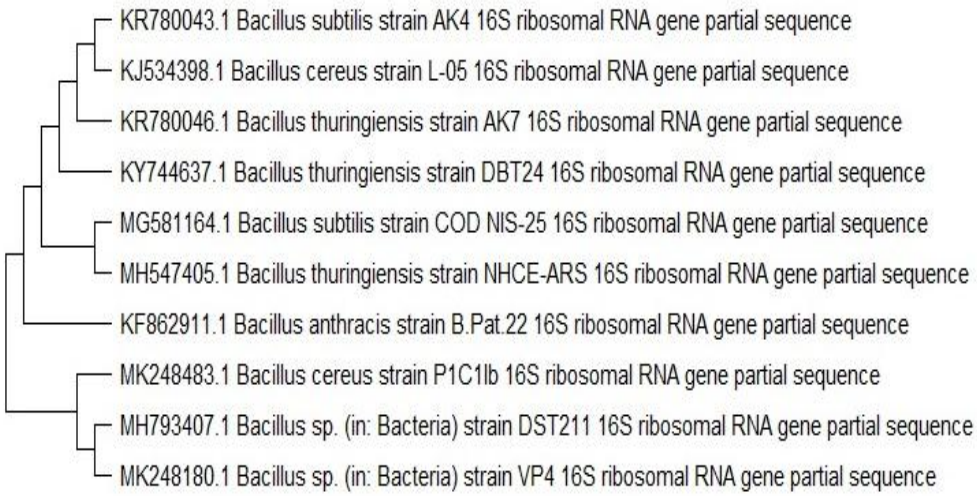

Fig. 5.3 Phylogenetic tree of *Bacillus subtilis COD NIS-25* (Accession No. MG581164*)* with closely related strains

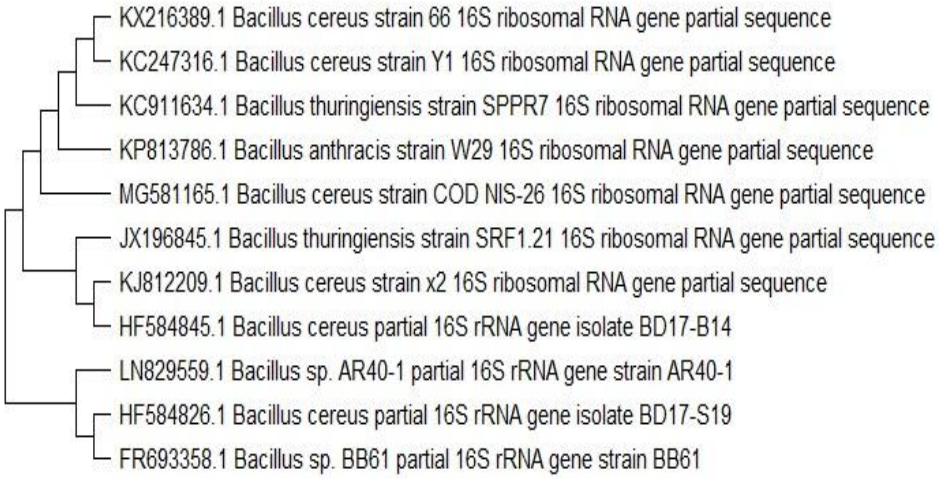

Fig. 5.4 Phylogenetic tree of *Bacillus cereus COD NIS-26* (Accession No. MG581165) with closely related strains

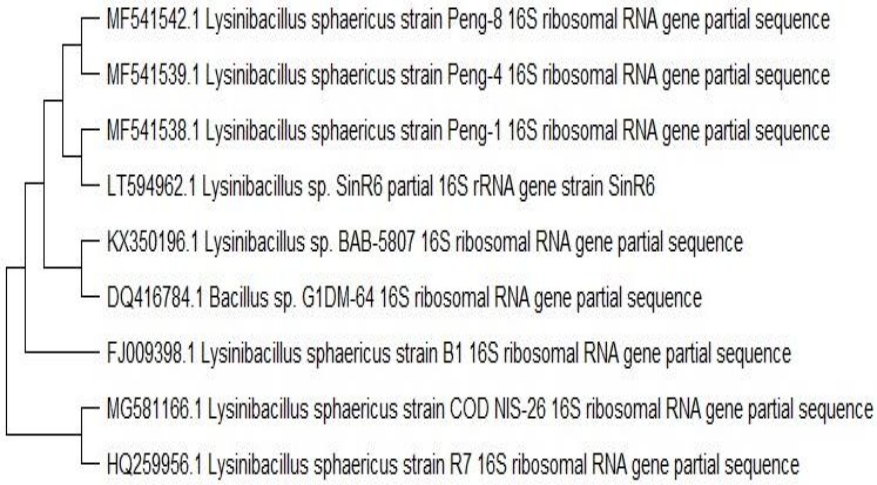

Fig. 5.5 Phylogenetic tree of *Lysinibacillus sphaericus COD NIS-26* (Accession No. MG581166) with closely related strains

5.4 Conclusion

Bacterial isolates COD NIS-24, COD NIS-25, COD NIS-26a and COD NIS-26b isolated from the selected soil samples of Palakkad district after homogenizing with 10 ppm lindane were observed to possess greater lindane resistant capacity thereby degrading them into non-toxic compounds. 16s rRNA gene sequencing analysis of these isolates proposed as *Bacillus drentensis COD NIS-24*, *Bacillus subtilis COD NIS-25*, *Bacillus cereus COD NIS-26* and *Lysinibacillus sphaericus COD NIS-26*. Later, the PCR products amplified by the 16S rRNA universal primers in this research endeavor were deposited to NCBI Gen- Bank with accession numbers MG581163 - MG581166, respectively.

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Chapter

6

LINDANE DEGRADING SOIL MICROBES AND THEIR POTENTIAL IN LINDANE BIODEGRADATION

Contents

6.1 Introduction

Lindane is considered as the most toxic organochlorine insecticide widely used in indoor and outdoor around the world to eradicate pests as well as disease causing vectors from the environment. These were synthesized by photochemical chlorination of benzene by activating with UV light. It contains a mixture of HCH isomers (α, β, γ, δ) and were considered as potential carcinogens and impart highly impact in nature (Waliszewski, 1993; Deo et al., 1994; Willett et al., 1998; Walker et al., 1999; Phillips et al., 2005). These chlorinated compounds persist in the environment for decades eventually enter the food chain causing severe health problems to the living system (Bidlan et al., 2004; Phillips et al., 2005; Lal et al., 2006; Pesce et al., 2008). Even in its restricted use these synthetic compounds were detected in various segments of the ecosystem

such as soil, water, animal and human tissues, milk, etc. in several countries (Herrero et al., 2010; Pannu and Kumar, 2017; Gopalan and Chenicherry, 2018).

With regard to the introduction of innovated technologies and methods, various bioremediation techniques were successfully adopted. Several case studies conducted by the researchers and scientists all over the world, indicating that microbial biodegradation is as an effective remediation technique by the ways of isolating capable pesticide degrading soil microbes (Kumar et al., 2016; Li et al., 2016; Pannu and Kumar, 2017). Lindane degrading microbes isolated from soil lindane enriched environment opened novel strategies in the remediation technologies. Cultures of *Clostridium* spp., *Pseudomonas*, *Escherichia coli*, *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Cyathus bulleri*, *Pleurotus ostreatus*, *sajor-caju*, *Sphingobacterium spiritivorum*, *Ochrobactrum anthropi*, *Bosea thiooxidans*, *Sphingomonas paucimobilis*, etc. were proved to possess lindane degrading capacity (Singh and Kuhad, 2000; Pesce and Wunderlin, 2004; Rigas et al., 2009). These organisms revealed greater potential to eliminate chlorine ions from lindane aerobically or anaerobically by consuming the carbon source thereby wiping out the residues of these toxic chemicals from soil environment (Sahu et al., 1995; Nagata et al., 1999; Kumari et al., 2002; Nagata et al., 2005; Murthy and Manomani, 2007; Elcey and Kunhi, 2010). Since both experimental approaches i.e., aerobically or anaerobically were performed in lindane degradation studies, it was effectively mineralized aerobically (Phillips et al., 2005). Biodegradation studies were firstly carried out in *Escherichia coli* (Matsumura et al., 1976) and *Pseudomonas* isolates (Francis et al., 1975).

As discussed detail in the fourth chapter, the need of remediation in Palakkad soils (Gopalan and Chenicherry, 2018) due to the presence of toxic insecticides has triggered the isolation of the potent microbes namely, *Bacillus drentensis COD NIS-24*, *Bacillus subtilis COD NIS-25*, *Bacillus cereus COD NIS-26* and *Lysinibacillus sphaericus COD NIS-26*. The focus of this chapter was the biodegradation studies i.e., the ability of the microorganisms to utilize the carbon source for their metabolism from the toxic chlorine compound, lindane. The degradation rate was measured by estimating quantitatively, the amount of chlorine ions released into the system. Additionally, the effects of different physiological parameters mainly at varying pH and temperature were determined for optimizing the biodegradation rates. The present study highlights an eco-friendly and cost-effective biotreatment to control the residues of the prementioned pollutants from Palakkad district, Kerala, India. Thus the main aim and objective of the chapter were to provide an efficient method for the bioremediation of lindane from the soil environment by utilizing ecofriendly capable microorganisms.

The objectives of the study were assessed with regard to the aim of the bioremediation of lindane by using native microbes are to determine the degradation potential by the quantitative estimation of released chlorine ions (mineralization) in the medium (Argentometric titration), to determine the potential bacterial isolate having a maximum capacity to degrade lindane and to determine the rate of degradation with varying pH and temperature (Optimization).

6.2 Materials and Methods

These are six chlorine atoms per lindane molecule and the dehalogenation phase is the most substantial step in biodegradation (Nagata et al., 1997). The dehalogenation of lindane is mediated by the isolated degrading microbes specifically, *Bacillus drentensis* COD NIS-24, *Bacillus subtilis COD NIS-25*, *Bacillus cereus COD NIS-26* and *Lysinibacillus sphaericus COD NIS-26*, from insecticide contaminated soils considering the environmental factors. The rates of degradation were studied accordingly to layout the framework for the remediation schemes.

6.2.1 Biodegradation Studies

The ability of the strains to utilize the carbon source from the chlorinated compound lindane was studied in modified minimal mineral medium (MMMM), (Hopwood et al., 1985) and pH was maintained at 7. The potent isolates were inoculated into the autoclaved (at 121 °C for 20 min) MMMM supplemented with γ-HCH (10 ppm) as carbon source and kept on a rotary shaker for 24 hours at 30 °C to obtain the degree of degradation quantitatively. The degradation rate of all the four potential bacterial isolates were determined by the amount of chlorine ions released under different time intervals (0, 1, 2, 5 and 6 days) with reference to the argentometric method postulated by Greenberg et al. (1992). All the experiments were carried out in triplicates.

$$\%\ \text{of degradation of lindanen} = \%\ \text{chlorine released}$$

6.2.1.1 Analysis of Chloride Released

Chlorine ion released can be measured by the argentometric method (Greenberg et al., 1992), to determine the degradation rate of lindane in the medium inoculated with isolated microbes. The release of the chlorine ions

into the medium is believed to be the action of dechlorinase enzyme of bacterial strains (Kumar et al., 2016). The isolated microbes, *Bacillus drentensis COD NIS-24*, *Bacillus subtilis COD NIS-25*, *Bacillus cereus COD NIS-26* and *Lysinibacillus sphaericus COD NIS-26* were inoculated in MMMM supplemented with 10 ppm lindane and incubated at 30 ºC, at pH 7. Afterward, 1 mL of the culture was withdrawn from each culture at different intervals viz 0, 1, 5 and 6 days to estimate chlorine ions and the percentage were calculated.

$$\% \text{ Chlorine released} = \frac{C_t}{C_o} \times 100$$

C_t → Concentration of Chlorine ions at time t

C_0 → Initial concentration of Chlorine ions.

All the experiments were carried out under sterilized conditions and in triplicate and were expressed as the mean of the results. Inoculated MMMM without HCH isomers (designed as biotic control) and non-inoculated MMMM with HCH isomers (designed as abiotic control) were also included.

6.2.2 Optimization of Various Physiological Parameters for Lindane Biodegradation

Effect of various physiological parameters on degradation rate were studied with isolated microbes, *Bacillus drentensis* COD NIS-24, *Bacillus subtilis* COD NIS-25, *Bacillus cereus* COD NIS-26 and *Lysinibacillus sphaericus* COD NIS-26 (Pannu and Kumar, 2017). To determine the effect of temperature, the tubes were inoculated with these microbes in the MMMM and were incubated at different temperatures, 25 ºC, 30 ºC and 35 ºC for 5 days. Finally, the chlorine ions released were determined.

To determine the effect of pH, the tubes were inoculated with these microbes in the MMMM and thereafter were incubated at different pH 5, 7 and 9 for 5 days. Later the released chlorine ions were determined. The uninoculated sterile MMMM broth tubes containing the same lindane concentration were also prepared for the analysis. All the sets were carried out in triplicates.

6.3 Results and Discussion

6.3.1 Biodegradation Studies

Table 6.1 Chlorine release of the bacterial isolates

Organism	Chlorine atom released (mg/mL)			
	1st Day	2nd Day	5th Day	6th Day
Bacillus drentensis COD NIS-24	1.07	2.13	2.84	2.84
Bacillus subtilis COD NIS-25	0.71	0.71	1.78	1.78
Bacillus cereus COD NIS-26	0.36	0.71	1.42	1.42
Lysinibacillus sphaericus COD NIS-26	0.71	0.71	1.78	1.78

The microbes isolated from the polluted area were considered to have a strong capacity to degrade the substrate lindane, thereby reduce the concentration from the soil environment. In several case studies, the degradation of chemical compounds especially the chlorinated ones have proven potential on their supplementation to medium as the carbon source for the isolated microbes (Manonmani et al., 2000; Bidlan et al., 2004; Nagpal and Paknikar, 2006; Deepthi et al., 2007; Murthy and Manonmani, 2007; Pannu and Kumar, 2014). When the soil bacterial isolates, *Bacillus drentensis COD NIS-24*, *Bacillus subtilis COD NIS-25*, *Bacillus cereus*

COD NIS-26 and *Lysinibacillus sphaericus COD NIS-26* utilize the carbon source, lindane (10 ppm) in the MMMM maintained at pH 7 and temperature 30 °C. Subsequently, the growth were distinguished by the turbidity attained by the medium.

Table 6.2 The lindane degradation rate of the bacterial isolates

Organism	% of lindane degradation			
	1st Day	2nd Day	5th Day	6th Day
Bacillus drentensis COD NIS-24	30.43	60.86	81.14	81.14
Bacillus subtilis COD NIS-25	20.29	20.29	50.71	50.71
Bacillus cereus COD NIS-26	10.14	20.29	40.57	40.57
Lysinibacillus sphaericus COD NIS-26	20.29	20.29	50.71	50.71

The ranges of degradation were obtained by quantitatively estimating the chlorine ions released into the medium and were represented in Table 6.1. The maximum concentration of chlorine ions obtained in the medium was 2.84 mg/mL, 1.78 mg/mL, 1.42 mg/mL and 1.78 mg/mL for *Bacillus drentensis COD NIS-24*, *Bacillus subtilis COD NIS-25*, *Bacillus cereus COD NIS-26* and *Lysinibacillus sphaericus COD NIS-26* respectively. The release of chlorine ions into the medium was mediated by the action of dechlorinase enzyme present in these microbes and similar reports found earlier (Alvarez et al., 2012). The concentration of chlorine ions in the medium was observed as increasing from 1 to 5 days of incubation periods thereby increasing the degree of degradation. The efficiency of lindane

degradation of *Bacillus drentensis COD NIS - 24* is found to be greater and a gradual increase were observed from 30.43 to 81.14 % with the increase in incubation time from day 1 to 5 days. The lindane degrading capacity of *Bacillus subtilis COD NIS - 25*, *Bacillus cereus COD NIS - 26* and *Lysinibacillus sphaericus COD NIS - 26* were found to be 20.29 to 50.71 %, 10.14 to 40.57 % and 20.29 to 50.71 % with the increase in incubation time from 1st day to 5th day respectively (Table 6.2).

Subsequently, the release of chlorine into the medium is merely ceased for the next day indicating declined growth of the isolates i.e. experiencing a death phase. Hence, in the lindane degradation process, these observations illustrate a direct connection amongst the chlorine release and bacterial growth (Pannu and Kumar, 2017). Recently, cultures of *Achromobacter sp. A3* isolated from roots of wetland plant *Acorus calamus* exhibited maximum degradation of 88.7 ± 1.24 % for 50 mg/l of lindane (Singh and Singh, 2019). Similarly, strains of *Paracoccus sp. NITDBR1* were isolated from agricultural fields in Manipur, India, significantly degrade 90 % of 100 mg/L of lindane within 8 days of incubation on culturing in liquid medium (Sahoo et al., 2019). Previously, after 8 weeks of aerobic incubation, liquid microbial cultures of *Pandoraea sp.* significantly degrade 89.9 % of lindane with a concentration of 10 to 200 mg/l (Okeke et al., 2002). In another case study, strains of *Sphingobacterium spiritivorum*, *Ochrobactrum anthropi*, *Bosea thiooxidans* and *S. paucimobilis*, were isolated from sediments of Suquia River, Cordoba, Argentina, and degradation rates were examined and pure isolates of *Bosea thiooxidans* and *S. paucimobilis* could rapidly degrade lindane after 3 days of incubation (Pesce and Wunderlin, 2004). Similarly, a consortium of bacterial isolates

including, 7 *Pseudomonas sp*. one of each Flavobacterium, Vibrio and Burkholderia were studied, and substantially degrade about 90 % of 25 ppm of lindane within 72 h of incubation (Mohammed et al., 2005). Further, cultures of *P. aeruginosa* ITRC-5, isolated from rhizosphere soil of lindane polluted region, were observed as potent microbes in their biodegradation and > 80% degradation reported after an incubation period of 24 days (Chaudhary et al., 2006).

Cultures procured from lindane containing soils collected from Gujarat, India, by enrichment culture method namely, *Pseudoarthrobacter sp*., *Pseudomonas sp*. and *Klebsiella sp*. showed ~50 % of lindane degradation capacity (Nagpal and Paknikar, 2006). Isolates of *Streptomyces sp. M7* (isolated from Tucuman, Argentina) grown in sterile soil at different concentrations of lindane viz. 100, 150, 200, and 300 µg/kg, exhibited a degradation at a rate of 29.1, 78.03, 38.81 and 14.42 %, respectively (Benimeli et al., 2003, 2008). Anupama and Paul, (2009), studied lindane degradation, by chloride estimation method, using bacterial isolates *Azotobacter chroococcum JL 102*, obtained previously from long term lindane treated farmlands of Indian Agricultural Research Institute, New Delhi. Later, these were reported to possess greater degradation rate at 8^{th} week of incubation at a 10 ppm concentration. Certain bacterial strains, *cyanobacteria Anabeana sp. PCC7120* and *Nostoc ellipsosporum*, were reported to metabolize lindane into 1,2,4- and 1,2,3-trichlorobenzenes (Kuritz and Wolk, 1995). Biodegradation of lindane by cynobacterial species such as *Qaroun* and *Mariut* (*Qaroun sp.:- Synechococcus sp., Oscillatoria sp. 12, Cyanothece sp., Nodularia sp., Oscillatoria sp. 13, Nostoc sp.,* and *Synechococcus sp.*; *Mariut sp.:- Microcystis aeruginosa*

MA1, *Anabaena cylindrica*, *Microcystis aeruginosa MA15*, *Anabaena spiroides* and *Aphanizomenon flosaquae*), were studied and reported as potential degraders both as individuals and as mixtures (ElBestawy et al., 2007). These authors reported the lindane degradation capacity of *Qaroun* recorded a range of 71.6 and 99.6 % and that of *Mariut* ranged between 45.23 to 100 %. Nagpal et al. (2008), isolated fungal strains namely, *Conidiobolus 03-1-56* procured from litter and recorded 100 % of degradation efficiency within 5 days of incubation. Reports of Quintero and his teammates (2007), indicates *Bjerkandera adusta*, a white-rot fungus significantly degrade lindane at a maximum of 94.5 % in 30 days. Similarly, white rot fungus such as *Pleurotus ostreatus*, *Plerotus sajorcaju* and *Trametus hirsutus*, were also reported as efficient lindane degraders at a rate of 85 to 95% (Arisoy and Kolankaya, 1997; Singh and Kuhad, 2000; Papadopoulou et al., 2006).

6.3.2 Optimization of Various Physiological Parameters for Lindane Biodegradation

The growth of bacterial isolates in lindane degradation mainly depends upon certain physiological factors such as pH and temperature. Extreme conditions (low-high and pH-temperature) could affect the growth rate of microbial cultures possibly result in death. The variations of these factors have a significant influence on the release of chlorine ions as well as the lindane degradation competence in the medium (Kodama et al., 2001).

Table 6.3 Percentage of degradation of lindane with respect to changing Temperature and pH

Organism	Percentage of degradation (%) with respect to Temperature			Percentage of degradation (%) with respect to pH		
Temperature	25	30	35	5	7	9
Bacillus drentensis COD NIS-24	37.19 ± 5.86	77.76 ± 5.86	71.00 ± 10.14	30.43 ± 10.14	77.76± 5.86	60.86 ± 10.14
Bacillus subtilis COD NIS-25	30.43± 10.14	50.71± 10.14	37.19± 5.86	16.90 ± 11.71	50.71± 10.14	20.29 ± 10.14
Bacillus cereus COD NIS-26	30.43± 10.14	43.95 ± 15.49	23.67 ± 5.86	16.90 ± 5.86	43.95 ± 15.49	20.29 ± 10.14
Lysinibacillus sphaericus COD NIS-26	40.57± 10.14	47.33 ± 5.86	30.43 ± 10.14	13.52 ± 5.86	47.33 ± 5.86	40.57 ± 10.14

In the present study, bacterial isolates were incubated for a period of 5 days was found to optimize for lindane mineralization and the growth rates gradually reduced and ceased after 5 days for all the microbes. In the case of temperature, the dechlorinase enzyme activity was maximum at 30 ºC and considered as the optimum temperature for all the four microbial strains (Fig. 6.1). Maximum degradation and mineralization were observed for the organism *Bacillus drentensis COD NIS-24* at 30 ºC (Table 6.3). Similar optimum conditions were observed for several microbes such as *Bacillus sp. Cal-6F*, *Bacillus sp. Lad-2A*, *Bacillus sp. Ym-7A*, *Pseudomonas aeruginosa*, *Rhodotorula sp. VITJzN03*, *actinobacteria Streptomyces sp. M7*, etc. (Manonmani et al., 2000; Bidlan et al., 2004; Nagpal and Paknikar, 2006; Deepthi et al., 2007; Murthy and Manonmani, 2007; Zheng et al., 2011; Salam et al., 2013; Pannu and Kumar, 2014; Sineli et al., 2016). The growth

rate and mineralization of lindane were observed better at 35 ºC than 20 ºC for all the organisms except for *Bacillus cereus COD NIS-26*, could be inferred as all the cultures possess different characteristics at varying temperature (Kumar et al., 2016). The variation in temperature exerts marked influence on microbial cultures in the degradation rate of lindane (Phillips et al., 2005). Studies of Elcey and Kunhi, (2010), revealed the fact that the removal of chloride ions is significantly influenced by the variation in temperature. They further observed a degradation frequency pattern at 5 to 60 ºC at a rate of 8 and 18%, respectively and recorded 30 - 35 ºC as optimum temperature. In another lindane degradation study, the optimum temperature were noticed for *Streptomyces sp. M7* at 30 ºC (Benimeli et al., 2008). Liquid cultures of *Ganoderma australe*, bracket-like polypore fungus exhibited a maximum lindane degradation efficiency at an incubation period of 5 days at 18 ºC containing 7 ppm of lindane (Dritsa et al., 2009).

Subsequently, the optimum pH was observed as 7 for all the four isolates (Fig. 6.2). Maximum growth rate and mineralization were recorded for *Bacillus drentensis COD NIS-24* at the given pH and the degradation rates were decreased after 5 days. Similar observations were noted for *Bacillus sp. Cal-6F*, Bacillus sp. Lad-2A, *Bacillus sp. Ym-7A* (Pannu and Kumar, 2014) and *Candida VITJzN04* (Salam and Das, 2014), etc. and for lindane degradation, neutral pH were reported as suitable (Siddique et al., 2002; Elcey and Kunhi, 2010). Generally, certain microbes, cease the degradation rate at low pH, as their considerable adverse influence on survival capacity. Thus the gradual increase in the pH towards neutral enhances the survival capacity of the microorganisms and similar study of Murthy and Manonmani, (2007) also supports these inference. Likewise,

Okeke et al. (2002) studied the optimum pH for the growth of *Pandoraea sp.* and lindane biodegradation in soil slurries was 9. Similar previous studies were reported in *Streptomyces sp. M7* (Benimeli et al., 2008) and in *Rhodotorula sp.VITJzN03* (Salam et al., 2013) and optimum lindane degrading efficiency were determined at pH 7 and 6, respectively.

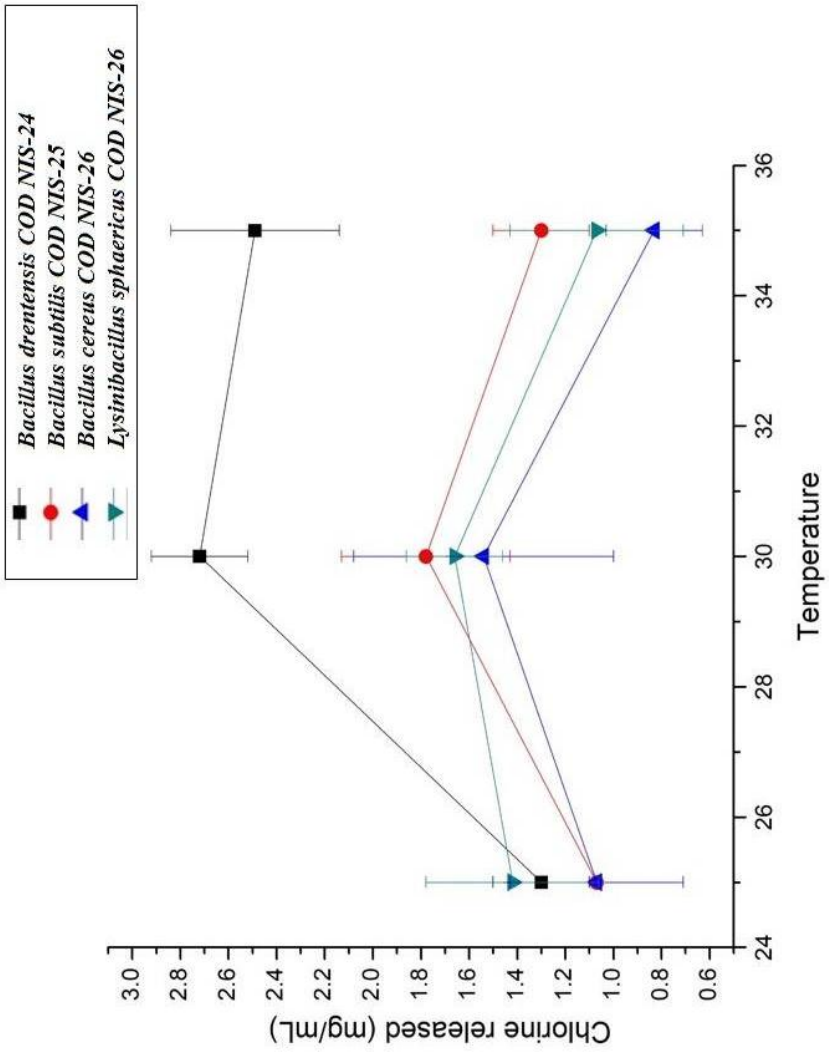

Fig. 6.1 Effect of temperature on chlorine release

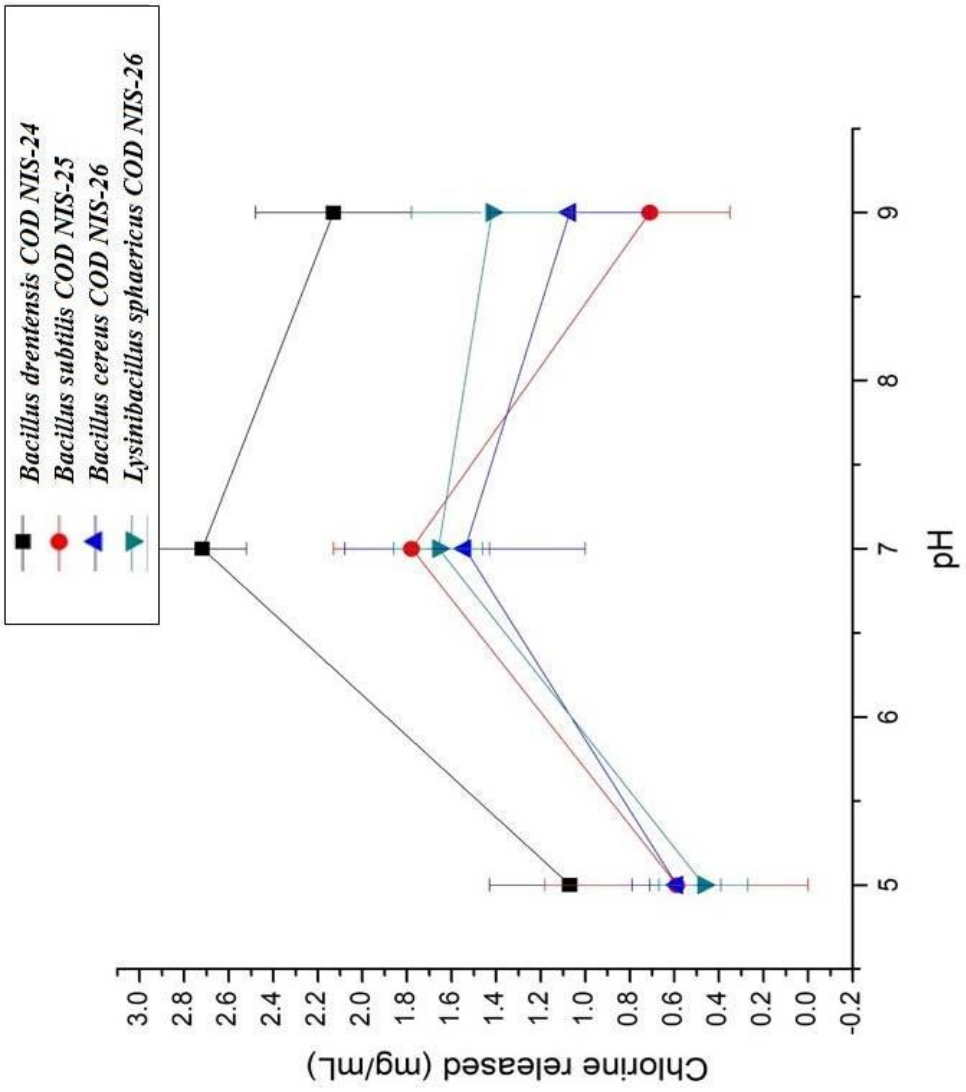

Fig. 6.2 Effect of pH on chlorine release

6.4 Conclusion

Biodegradation of lindane, recalcitrant and persistent organochlorine insecticides by utilizing the capacity of bacterial strains, COD NIS-24, COD NIS-25, COD NIS-26a and COD NIS-26b were isolated from the soils of

the study area by metabolizing them. These isolates were observed as potential strains for lindane degradation. Subsequently, these isolates were subjected to characterization and identification by whole-cell-based PCR amplification and sequence analysis of 16S rRNA genes. Accordingly, they were designated as *Bacillus drentensis COD NIS-24* (Accession No. MG581163), *Bacillus subtilis COD NIS-25* (Accession No. MG581164), *Bacillus cereus COD NIS-26* (Accession No. MG581165) and *Lysinibacillus sphaericus COD NIS-26* (Accession No. MG581166).

The lindane degrading efficiency of these microbes were determined by adopting argentometric methods by estimating chlorine ions and found as efficient lindane degrading potential. Among them, *Bacillus drentensis* COD NIS-24, exhibited maximum lindane degrading capacity nearly 81.1 % on an incubation period of 5 days. Lastly, the four organisms were optimized under varying physiological parameters and the maximum growth and degradation rate were denoted at 30 ºC and pH 7 for all the organisms. Consequently, these organisms can be effectively used for controlling lindane from the soil environment to a certain extent. Hence, the future aspects of the research endeavor relay on the ex situ biotreatment of the lindane contaminated soil environment by utilizing the capability of these four microbes through mass culturing them with the concurrent appropriate physiological conditions.

6.5 References

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Chapter

7

SUMMARY AND CONCLUSIONS

Contents

7.1 Salient features of the present study

This thesis is a first qualitative and quantitative report on heavy metal and OCI contaminants in the study area. Sites selected for this investigation covered agricultural zones, zones influencing urban activities and zones adjacent to industrial area. Heavy metal Cd, Zn, Co, Pb, and Fe, were found above the permissible limit and interestingly cannot deduce any seasonal pattern for their distribution. Similar trend of random behaviour was observed for the distribution of OCIs. Prominent OCI contaminants were α - HCH, β - HCH, γ - HCH, heptachlor, aldrin, heptachlor epoxide (B), 4,4'-DDE, dieldrin, endrin, 2,4'-DDD. Based on the information regarding the level of contaminants in the study region highlights the need for reclamation of these zones. In this region bioremediation is a feasible option considering its ecofriendly nature and cost effectiveness. As a starting step of the reclamation of these zones, the study focus on the isolating microbial strains holding prominent potential to degrade OCIs. Four strains of microbes namely, Bacillus drentensis COD NIS-24, Bacillus subtilis COD NIS-25,

Bacillus cereus COD NIS-26 and Lysinibacillus sphaericus COD NIS-26 having great potential to degrade lindane were isolated accordingly. Further, the growth conditions of these microbes were optimized to enhance the remediation potential. In future these strains can be used to reclaim these zones after field investigations.

Concerns regarding the lethal and chronic effects of intense practice of pesticides for enhancing the crop yield are raising the problem to the public over the past decades throughout the world. Nowadays, people or farmers use enormous variety of chemicals to meet their daily and agricultural deed, thereafter discharging these highly hazardous pollutants specifically, metals and toxic insecticides, through soil leaches frequently to the surrounding niche. These chemicals, originating from numerous sources, somehow persist in the soil environment for decades. Thus the assessment of environmental quality by estimating the concentration, quality, trends, etc. of quality indicating parameters are essential in the present world scenario. Contamination due to pollutants such as OCIs as well as toxic metals should be monitored through analysis of soil, and other systems of exposure such as water and marine species. Hence, conventional and innovated technologies should be adopted for the eradication of this 'hazard' globally, thereby maintaining a healthy, safe and green environment. However, the main pollutants addressed in the present research endeavour, include the distribution pattern of metals (Cu, Cd, Pb, Mn, Ni, Zn, Fe, Mg and Co) and OCIs (α - BHC, β - BHC, γ - BHC, heptachlor, aldrin, heptachlor epoxide (B), 4,4'- DDE, dieldrin, endrin, 2,4'-DDD, 4,4'- DDD, 2,4'- DDT, 4,4'- DDT, α - endosulphan and β - endosulphan). The thesis divided into 7 chapters and the important findings of the study are summarized as follows.

The first chapter, mainly focused on the general introduction about the metal and pesticide accumulation in the soil environment. Also details the adverse health impacts of the insecticides and metals on the ecosystem as well as to human beings. Now a days, the usage of synthetic chemicals mainly OCIs for the increased crop production, vector control, as well in other fields such as pharmaceutical and cosmetic products and their persistence are common outcome. In this context, the study of trace metals and pesticides, specifically OCIs have attained its significance. The research work is mainly concentrated on the lindane remediation, consequently because it was one of the major OCI detected in the selected study area. Furthermore, the microbial degradation techniques were discussed in detail and were successfully performed in this research work. Besides, the chapter discusses the main aim and objectives of the present research work.

The second chapter is the description of the selected study area (location map): Palakkad district, Kerala, India. The chapter also discusses the sampling procedure, sampling strategies and preservation strategies in detail.

Additionally, the third chapter provides the information on fundamental soil parameters (pH, texture, TOC), biochemical compositions (carbohydrates, lipids, proteins and tannin/lignin), and the concentration of metals (Cu, Cd, Pb, Mn, Ni, Zn, Fe, Mg and Co) present in the soil samples collected from the study area. The analytical procedures of the aforementioned parameters in the soil were discussed in detail in this chapter. Results indicated that the selected locations were rich in organic carbon content however, extensive variations in soil texture were observed

in all the stations throughout the sampling period. The pH of the scrutinized soil samples exhibited slight alkaline in nature. The metal distribution in the study region exhibited random behaviour throughout the sampling period. Nevertheless, based on the available threshold guidelines most of the metals such as Cd, Co, Zn, Pb and Fe were found above the permissible level. However, the pollution indices, EF, CF and Igeo indicated most of the stations exhibiting moderate to very high pollution throughout the sampling period. The PLI values implied deterioration of all the stations during MONSP14, PRMF15 and PRMMY15 especially the stations K II and K III and the major contribution derived from the metals Cd, Cu, Co, Zn and Pb. Furthermore, the enriched levels of these metals were sourced back to anthropogenic inputs from agricultural, pharmaceutical, domestic utilizes, random atmospheric deposition, geogenic inputs, constructive fields, etc. coupled with naturally occurring processes. Also, the soil structure and the organic matter defines the binding nature of the metals thereby accumulating them in the soil.

The fourth chapter portrays the complete picture of the organochlorine insecticide distribution in the study area throughout the sampling period. The chapter also provides information regarding the analytical methods for determining the concentration of these synthetic chemicals. The long term exposure of these xenobiotics in the environment has led to severe damage, consequently discovered chronic toxic effects on humans, animals, plants and aquatic organisms. Therefore, the control of their production, sale and application/usage in several countries were implemented, somehow trace level residues are detected frequently in all phases of the ecosystem owing to their persistent nature or frequent illegal usage. Therefore, monitoring of

OCIs were carried out in the agricultural soils of Palakkad were studied in detail and discussed in this chapter. Among the analyzed OCIs namely α - BHC, β - BHC, γ - BHC, heptachlor, aldrin, heptachlor epoxide (B), 4,4'-DDE, dieldrin, endrin, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, α – endosulphan and β - endosulphan, were observed in residual levels except 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, α - endosulphan and β - endosulphan. As discussed in chapter 2, similar trend i.e., random behavioural patterns were observed in all the stations, during the six sampling periods. The analyzed stations namely C I, C II, C III, K I, K II and K III were observed to be slightly contaminant within one or in another season. The highest concentrations were observed during PRMMH14 at the station K III. The accumulation of these synthetic compounds in these areas could be due to their recent application as well as their historic usage in various fields, mainly for agricultural purposes. The statistical analysis, Pearson correlation indicates, the nature of the soil in the Palakkad region influences the adsorption of these chemicals into the soil, specifically the presence of organic constituents. Further, the soil fractions such as clay, sand and silt indirectly contribute the sorption capacity. The health risk assessment of the study area with respect to the OCIs indicated a low risk of the health issues in humans. Hence, the study area is observed to be slightly contaminated, continuous monitoring assessments and usage of biofertilizeres were recommended for retaining the soil environmental quality of the region. Further, the study areas selected are ideal sites for discovering novel microbial strains to bio-eradicate OCIs specifically lindane from agricultural zones.

Fifth chapter discussed about the biodegradation technologies by utilizing the efficiency and capacity of microorganisms. It focused on the determination of microbial population and isolation of microorganisms from the soils of the study region capable of degrading lindane. The methods for isolating lindane degrading microbes were also discussed. Four bacterial isolates namely COD NIS-24, COD NIS-25, COD NIS-26a and COD NIS-26b were isolated, purified, and were identified by molecular level i.e., 16s rRNA gene sequencing analysis, as *Bacillus drentensis* COD NIS-24, *Bacillus subtilis* COD NIS-25, *Bacillus cereus* COD NIS-26 and *Lysinibacillus sphaericus* COD NIS-26. Further, these were deposited to NCBI Gen- Bank with accession numbers MG581163 - MG581166, respectively.

Finally, the sixth chapter described the potential of isolated lindane degrading soil microbes for the lindane degradation. The methods adopted were disclosed in the chapter. The efficiency of lindane degradation of the isolated microbes *Bacillus drentensis* COD NIS-24, *Bacillus subtilis* COD NIS-25, *Bacillus cereus* COD NIS-26 and *Lysinibacillus sphaericus* COD NIS-26, were determined. Argentometric method was utilized for estimating chlorine ions and an efficient lindane degrading potential was observed in the present study. *Bacillus drentensis* COD NIS-24, exhibited maximum lindane degrading capacity about 81.1 % on an incubation period of 5 days among the other bacterial isolates. The optimum temperature and pH for the maximum degradation were determined under different physiological parameters and maximum were observed at 30 ^{o}C and pH 7 for all the organisms. Hence, these organisms can be successfully utilized for removing lindane from the soil environment.

7.2 Scope of the study

The present study is first monitoring assessment data regarding the organic carbon content, metal as well as the OCI distribution in soils of selected locations in Palakkad district, Kerala, India. The four potential lindane degrading microbes were isolated from the study region which can be effectively used for the remediation processes considering their accessibility and comparatively cheap, low technological means and high public acceptance. However, the method has greatly relied on biological processes, no harmful chemical are involved in these remediation techniques, using these isolates can be undoubtedly, implemented in the soil environment.

7.3 Future scope of the study

Biodegradation processes discussed in the thesis, principally, focused on the insitu processes, the onsite performances of these isolates should be determined further. This study has considered for the extension of carrying out onsite performance of these microbes. Since the degradation studies were performed by a single compound, i.e., lindane, other OCIs, can also have accounted. Similarly, the structural elucidation of intermediate compounds, as well as the degradation pathways in the whole process, can also be focused on future research endeavours. Furthermore, these isolated organisms degrade toxic chemicals naturally, thus formulating adequate solution to develop genetically engineered microbes. Hence, these will provide a systematic and controlling tool for the maintenance of deteriorated/contaminated soil environment, thereby assuring the health of the environment.

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LIST OF PUBLICATIONS

1. **Gopalan, N.K.** and Chenicherry, S., 2018. Fate and distribution of organochlorine insecticides (OCIs) in Palakkad soil, India. *Sustainable Environment Research*, 28(4), pp.179-185.
2. **Nisa, K.G.** and Sujatha, C., 2019. Environmental Monitoring Assessment of Organochlorine Insecticides in Palakkad District, Kerala, India". *International Journal of Interdisciplinary Research and Innovations*, (7)1, pp.27-34.
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i. **Nisa, K.G**, Sujatha, C.H., Dibu, D., Prashob Peter, K.J, and Arsha, K., Bacillus drentensis COD NIS-24 (Accession No. MG581163), Isolation and Identification of Microbes from Palakkad Soil.

ii. **Nisa, K.G**, Sujatha, C.H., Dibu, D., Prashob Peter, K.J, and Arsha, K., *Bacillus subtilis* COD NIS-25 (Accession No. MG581164) Isolation and Identification of Microbes from Palakkad Soil.

iii. **Nisa, K.G**, Sujatha, C.H., Dibu, D., Prashob Peter, K.J, and Arsha, K.,*Bacillus cereus* COD NIS-26 (Accession No. MG581165), Isolation and Identification of Microbes from Palakkad Soil.

iv. **Nisa, K.G**, Sujatha, C.H., Dibu, D., Prashob Peter, K.J, and Arsha, K., *Lysinibacillus sphaericus* COD NIS-26 (Accession No. MG581166), Isolation and Identification of Microbes from Palakkad Soil.

Appendix I
Results of Analyzed Metals, Recovery Details and Analyzed Result of CRM Standards of OCIs

The following section contains the concentration of individual metals, EF, CF and Igeo values in each period with respect to the third chapter, Soil properties and Metal Distribution.

Table A1.1: Concentration (mg kg $^{-1}$) of Cu in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	4.48	5.63	66.5	25.07	16.91	4.24
KII	6.27	14.8	39.75	90.03	38.9	2.49
KIII	12.36	14.23	23.25	45.3	45.78	8.74
CI	10.55	9.72	9.39	14.3	15.68	3.71
CII	3.58	6.19	23.63	17.22	16.65	8.62
CIII	1.64	3.68	3.5	23.96	27.91	2.75

Table A1.2: Concentration (mg kg $^{-1}$) of Cd in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2.11	1.0	2.38	0.19	0.05	1.94
KII	1.23	0.64	2.75	1.06	0.33	1.58
KIII	2.68	1.46	3	0.49	ND	5.07
CI	1.85	0.25	0.73	0.03	ND	1.43
CII	2.62	1.7	1.63	0.01	ND	8.56
CIII	0.58	0.73	2.13	ND	0.11	1.82

Table A1.3: Concentration (mg kg $^{-1}$) of Mn in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	61.56	162.85	192.5	318.23	200.57	200.21
KII	10.52	ND	577.38	733.33	700.99	424.35
KIII	7.91	ND	413.38	722.9	462.35	480.44
CI	214.4	44.82	105	234.17	198.69	154.15
CII	118.31	24.28	148.63	164.42	332.77	180
CIII	58.38	106.48	42.88	249.07	400.71	58.74

Table A1.4: Concentration (mg kg $^{-1}$) of Mg in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	380.44	372.18	458.5	4100	400	462.42
KII	711.98	309.12	992.88	1600	5100	809.14
KIII	474.84	487.03	527.25	4500	600	542.26
CI	334.01	344.22	630.24	1700	800	549.45
CII	423.34	373.9	254.6	1700	2900	234.22
CIII	356.46	183.68	577.25	3000	700	480.12

Table A1.5: Concentration (mg kg $^{-1}$) of Co in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	ND	ND	15.13	13.52	14.35	14.16
KII	ND	1.37	12	28.86	29.2	16.21
KIII	1.99	3.6	10.38	20.49	28.25	11.87
CI	1.7	7.98	22.56	12.75	12.38	18.29
CII	ND	ND	16.13	12.39	11.68	15.7
CIII	2.01	ND	0.75	22.76	19.95	ND

Table A1.6: Concentration (mg kg $^{-1}$) of Zn in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	0.81	5.58	27.5	64.93	42.77	22.43
KII	16.12	23.53	26.13	455.63	236.87	24.16
KIII	13.19	21.73	54.13	200.3	203.43	32.1
CI	6.79	6.73	7.93	26.09	143.45	15.12
CII	5.04	6.08	22.25	28.84	44.6	19.01
CIII	0.42	3.74	6.13	42.91	68.54	11.64

Table A1.7: Concentration (mg kg $^{-1}$) of Ni in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	ND	1.11	ND	26.25	22.83	10.24
KII	1.4	1.28	ND	83.15	89.68	11.68
KIII	0.93	1.19	ND	35.34	65.89	18.14
CI	0.27	0.42	ND	14.92	36.17	23.53
CII	1.13	0.79	ND	18.85	32.22	29.39
CIII	ND	0.96	ND	38.7	20.16	16.47

Table A1.8: Concentration (mg kg $^{-1}$) of Pb in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2.99	ND	3.65	13.87	8.06	6.01
KII	1.24	5.94	2.43	45.37	16.96	4.25
KIII	5.7	4.67	4.56	112.84	27.09	7.58
CI	1.64	1.03	ND	10.57	20.86	5.56
CII	1.46	ND	ND	11.4	24.23	5.49
CIII	3.32	1.25	2.43	18.33	0.79	2.83

Table A1.9: Concentration (mg kg $^{-1}$) of Fe in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2277.23	14567.55	13850.75	27500	35700	35800
KII	6610.11	16558.87	13197.63	25500	52700	39600
KIII	22160.61	21304.92	11557	35200	100600	57500
CI	11981.09	38248.01	5619.63	16100	34700	67500
CII	13344.91	10634.65	12160.13	21100	22700	123500
CIII	1478.14	7516.3	8132	29100	23500	79800

Table A1.10: Values of Enrichment factor (EF) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	4.25	280.60	1.58	0.38	2.33	0.21	0.00	2.39
	KII	2.05	56.35	0.09	0.25	0.00	1.45	0.35	0.34
	KIII	1.20	36.62	0.02	0.05	0.24	0.35	0.07	0.47
	CI	1.90	46.76	1.05	0.06	0.00	0.34	0.04	0.25
	CII	0.58	59.46	0.52	0.07	0.00	0.22	0.14	0.20
	CIII	2.40	118.83	2.32	0.55	3.59	0.17	0.00	4.08
MONSP13	KI	0.83	20.79	0.66	0.06	0.00	0.23	0.13	0.00
	KII	1.93	11.70	0.00	0.04	0.22	0.84	0.13	0.65
	KIII	1.44	20.75	0.00	0.05	0.45	0.61	0.09	0.40
	CI	0.55	1.98	0.07	0.02	0.56	0.10	0.02	0.05
	CII	1.26	48.41	0.13	0.08	0.00	0.34	0.12	0.00
	CIII	1.06	29.41	0.83	0.06	0.00	0.30	0.21	0.30
PRMMH14	KI	10.37	52.04	0.81	0.08	2.91	1.18	0.00	0.48
	KII	6.51	63.10	2.56	0.17	2.42	1.18	0.00	0.33
	KIII	4.35	78.61	2.10	0.10	2.39	2.78	0.00	0.72
	CI	3.61	39.34	1.10	0.26	10.69	0.84	0.00	0.00
	CII	4.20	40.59	0.72	0.05	3.53	1.09	0.00	0.00

Table Cont.

	CIII	0.93	79.32	0.31	0.16	0.25	0.45	0.00	0.54
Seasons	**Stations**	**Cu**	**Cd**	**Mn**	**Mg**	**Co**	**Zn**	**Ni**	**Pb**
MONSP14	**KI**	1.97	2.09	0.68	0.34	1.31	1.40	1.59	0.92
	KII	7.63	12.59	1.69	0.14	3.01	10.61	5.42	3.23
	KIII	2.78	4.22	1.20	0.29	1.55	3.38	1.67	5.82
	CI	1.92	0.56	0.85	0.24	2.11	0.96	1.54	1.19
	CII	1.76	0.14	0.46	0.18	1.56	0.81	1.48	0.98
	CIII	1.78	0.00	0.50	0.24	2.08	0.88	2.21	1.14
PRMF15	**KI**	1.02	0.42	0.33	0.03	1.07	0.71	1.06	0.41
	KII	1.59	1.90	0.78	0.22	1.48	2.67	2.83	0.58
	KIII	0.98	0.00	0.27	0.01	0.75	1.20	1.09	0.49
	CI	0.98	0.00	0.34	0.05	0.95	2.46	1.73	1.09
	CII	1.58	0.00	0.86	0.29	1.37	1.17	2.36	1.94
	CIII	2.57	1.42	1.00	0.07	2.26	1.73	1.42	0.06
PRMMY15	**KI**	0.26	16.41	0.33	0.03	1.05	0.37	0.48	0.31
	KII	0.14	12.08	0.63	0.05	1.09	0.36	0.49	0.20
	KIII	0.33	26.70	0.49	0.02	0.55	0.33	0.52	0.24
	CI	0.12	6.42	0.13	0.02	0.72	0.13	0.58	0.15
	CII	0.15	20.99	0.09	0.00	0.34	0.09	0.40	0.08
	CIII	0.07	6.91	0.04	0.01	0.00	0.09	0.34	0.06

Table A1.11: Values of Contamination factor (CF) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	0.31	20.69	0.12	0.03	0.00	0.02	0.00	0.18
	KII	0.44	12.06	0.02	0.05	0.00	0.31	0.08	0.07
	KIII	0.86	26.27	0.02	0.04	0.17	0.25	0.05	0.34
	CI	0.74	18.14	0.41	0.02	0.15	0.13	0.01	0.10
	CII	0.25	25.69	0.22	0.03	0.00	0.10	0.06	0.09
	CIII	0.11	5.69	0.11	0.03	0.17	0.01	0.00	0.20
MONSP13	KI	0.39	9.80	0.31	0.03	0.00	0.11	0.06	0.00
	KII	1.03	6.27	0.00	0.02	0.12	0.45	0.07	0.35
	KIII	1.00	14.31	0.00	0.04	0.31	0.42	0.06	0.27
	CI	0.68	2.45	0.09	0.03	0.69	0.13	0.02	0.06
	CII	0.43	16.67	0.05	0.03	0.00	0.12	0.04	0.00
	CIII	0.26	7.16	0.20	0.01	0.00	0.07	0.05	0.07
PRMMH14	KI	4.65	23.33	0.37	0.03	1.30	0.53	0.00	0.21
	KII	2.78	26.96	1.10	0.07	1.03	0.50	0.00	0.14
	KIII	1.63	29.41	0.78	0.04	0.89	1.04	0.00	0.27
	CI	0.66	7.16	0.20	0.05	1.94	0.15	0.00	0.00
	CII	1.65	15.98	0.28	0.02	1.39	0.43	0.00	0.00
	CIII	0.24	20.88	0.08	0.04	0.06	0.12	0.00	0.14

Table Cont.

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
	KI	1.75	1.86	0.60	0.30	1.17	1.25	1.41	0.82
	KII	6.30	10.39	1.39	0.12	2.49	8.76	4.47	2.67
	KIII	3.17	4.80	1.37	0.33	1.77	3.85	1.90	6.64
MONSP14	**CI**	1.00	0.29	0.44	0.13	1.10	0.50	0.80	0.62
	CII	1.20	0.10	0.31	0.13	1.07	0.55	1.01	0.67
	CIII	1.68	0.00	0.47	0.22	1.96	0.83	2.08	1.08
	KI	1.18	0.49	0.38	0.03	1.24	0.82	1.23	0.47
	KII	2.72	3.24	1.33	0.38	2.52	4.56	4.82	1.00
	KIII	3.20	0.00	0.88	0.04	2.44	3.91	3.54	1.59
PRMF15	**CI**	1.10	0.00	0.38	0.06	1.07	2.76	1.94	1.23
	CII	1.16	0.00	0.63	0.21	1.01	0.86	1.73	1.43
	CIII	1.95	1.08	0.76	0.05	1.72	1.32	1.08	0.05
	KI	0.30	19.02	0.38	0.03	1.22	0.43	0.55	0.35
	KII	0.17	15.49	0.81	0.06	1.40	0.46	0.63	0.25
PRMMY15	**KIII**	0.61	49.71	0.91	0.04	1.02	0.62	0.98	0.45
	CI	0.26	14.02	0.29	0.04	1.58	0.29	1.27	0.33
	CII	0.60	83.92	0.34	0.02	1.35	0.37	1.58	0.32
	CIII	0.19	17.84	0.11	0.04	0.00	0.22	0.89	0.17

Table A1.12: Values of Geoaccumulation Index (Igeo) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	-2.26	3.79	-3.68	-5.74	0.00	-6.59	0.00	-3.09
	KII	-1.77	3.01	-6.23	-4.83	0.00	-2.27	-4.31	-4.36
	KIII	-0.80	4.13	-6.64	-5.42	-3.13	-2.56	-4.91	-2.16
	CI	-1.02	3.60	-1.88	-5.92	-3.36	-3.52	-6.69	-3.96
	CII	-2.58	4.10	-2.74	-5.58	0.00	-3.95	-4.63	-4.13
	CIII	-3.71	1.92	-3.76	-5.83	-3.11	-7.54	0.00	-2.94
MONSP13	KI	-1.93	2.71	-2.28	-5.77	0.00	-3.81	-4.65	0.00
	KII	-0.54	2.06	0.00	-6.03	-3.67	-1.73	-4.45	-2.10
	KIII	-0.59	3.25	0.00	-5.38	-2.27	-1.84	-4.55	-2.45
	CI	-1.14	0.71	-4.14	-5.88	-1.12	-3.53	-6.05	-4.63
	CII	-1.79	3.47	-5.02	-5.76	0.00	-3.68	-5.14	0.00
	CIII	-2.54	2.25	-2.89	-6.79	0.00	-4.38	-4.86	-4.35
PRMMH14	KI	1.63	3.96	-2.04	-5.47	-0.20	-1.50	0.00	-2.80
	KII	0.89	4.17	-0.45	-4.35	-0.54	-1.58	0.00	-3.39
	KIII	0.12	4.29	-0.94	-5.26	-0.75	-0.53	0.00	-2.48
	CI	-1.19	2.25	-2.91	-5.01	0.37	-3.30	0.00	0.00
	CII	0.14	3.41	-2.41	-6.31	-0.11	-1.81	0.00	0.00
	CIII	-2.62	3.80	-4.20	-5.13	-4.54	-3.67	0.00	-3.39
MONSP14	KI	0.22	0.31	-1.31	-2.31	-0.36	-0.26	-0.09	-0.88
	KII	2.07	2.79	-0.11	-3.66	0.73	2.55	1.58	0.83
	KIII	1.08	1.68	-0.13	-2.17	0.24	1.36	0.34	2.15

Table Cont.

	CI	-0.58	-2.35	-1.76	-3.58	-0.45	-1.58	-0.90	-1.27
	CII	-0.32	-3.94	-2.27	-3.58	-0.49	-1.44	-0.57	-1.16
	CIII	0.16	0.00	-1.67	-2.76	0.39	-0.86	0.47	-0.48
Seasons	**Stations**	**Cu**	**Cd**	**Mn**	**Mg**	**Co**	**Zn**	**Ni**	**Pb**
PRMF15	**KI**	-0.34	-1.61	-1.98	-5.66	-0.28	-0.87	-0.29	-1.66
	KII	0.86	1.11	-0.17	-1.99	0.75	1.60	1.68	-0.59
	KIII	1.09	0.00	-0.77	-5.08	0.70	1.38	1.24	0.09
	CI	-0.45	0.00	-1.99	-4.66	-0.49	0.88	0.37	-0.29
	CII	-0.37	0.00	-1.25	-2.80	-0.58	-0.81	0.21	-0.07
	CIII	0.38	-0.48	-0.98	-4.86	0.20	-0.19	-0.47	-5.01
PRMMY15	**KI**	-2.34	3.66	-1.98	-5.45	-0.30	-1.80	-1.45	-2.09
	KII	-3.11	3.37	-0.90	-4.65	-0.10	-1.69	-1.26	-2.58
	KIII	-1.30	5.05	-0.72	-5.22	-0.55	-1.28	-0.62	-1.75
	CI	-2.53	3.22	-2.36	-5.20	0.07	-2.37	-0.25	-2.20
	CII	-1.32	5.81	-2.13	-6.43	-0.15	-2.04	0.08	-2.22
	CIII	-2.96	3.57	-3.75	-5.40	0	-2.74	-0.76	-3.17

Table A1.13: The recovery details and analyzed result of CRM standards of OCIs (804-050) in soils.

OCIs	Recovery (%)	CRM STD (mg Kg $^{-1}$)	
		Certified value	Analyzed Value
α-HCH	90 ± 3	-	-
β-HCH	87 ± 4	-	-
γ-HCH	88 ± 3	491.6	526.1
Heptachlor	89 ± 5	-	-
Aldrin	90 ± 5	18.04	19.1
Heptachlor epoxide (B)	90 ± 3	-	-
4,4'-DDE	88 ± 2	1519.6	1246
dieldrin	88 ± 4	1862.5	1508.7
Endrin	103 ± 4	62.2	68.3
2,4'-DDD	92 ± 3	-	-
4,4'-DDD	91 ± 5	1530.6	1193.9
2,4'-DDT	90 ± 2	-	-
4,4'-DDT	87 ± 4	1060.1	795.1
α-endosulphan	89 ± 5	1464.3	1142.2
β- endosulphan	90 ± 5	1128.2	1028.8

Appendix II : Figures

Fig A2.1 Sampling stations: a) Station KI; b) Station KII; c) Station KII; d) Station CI; e) Station CII; f) Station CIII

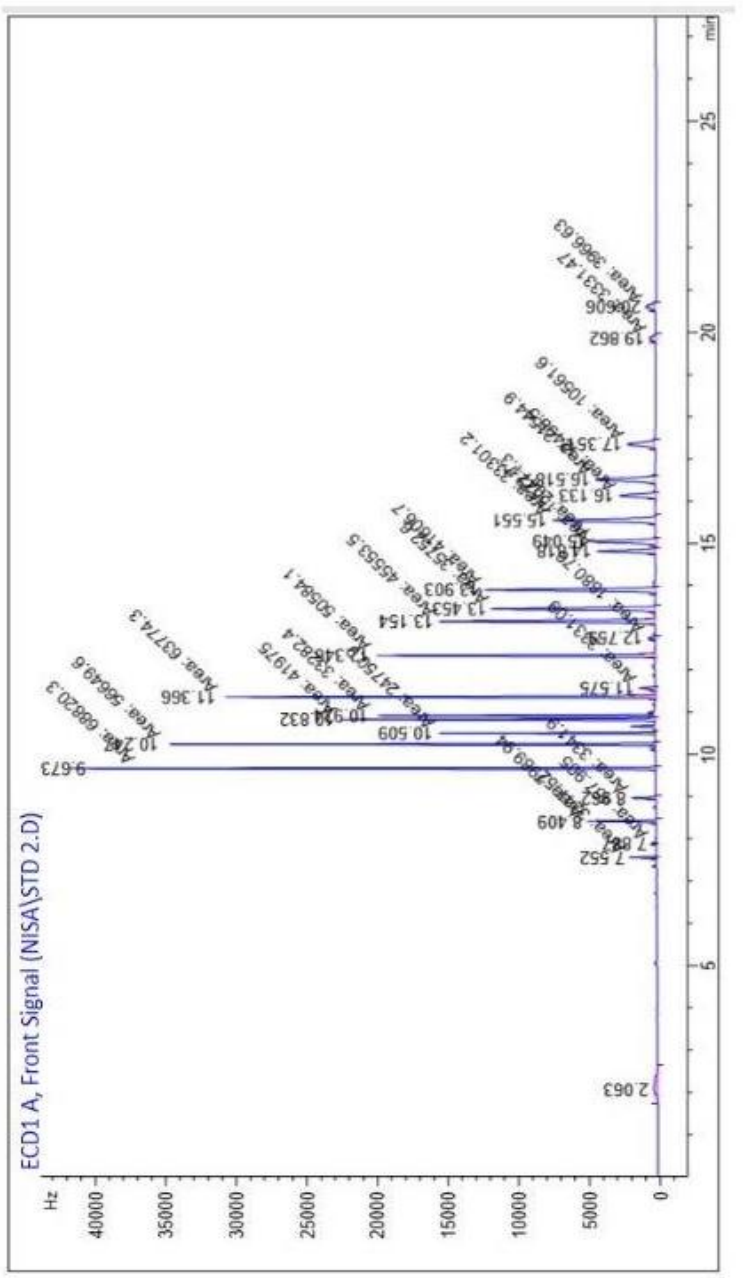

Fig A2.2 Representative Chromatogram (Standard solution) with respect to third chapter Spatio-Temporal Distribution Pattern of Organochlorine Insecticides (OCIs).

Fig A2.3 Biochemical test a) Indole; b) MR; c) VP; d) Citrate

Appendix I
Results of Analyzed Metals, Recovery Details and Analyzed Result of CRM Standards of OCIs

The following section contains the concentration of individual metals, EF, CF and Igeo values in each period with respect to the third chapter, Soil properties and Metal Distribution.

Table A1.1: Concentration (mg kg $^{-1}$) of Cu in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	4.48	5.63	66.5	25.07	16.91	4.24
KII	6.27	14.8	39.75	90.03	38.9	2.49
KIII	12.36	14.23	23.25	45.3	45.78	8.74
CI	10.55	9.72	9.39	14.3	15.68	3.71
CII	3.58	6.19	23.63	17.22	16.65	8.62
CIII	1.64	3.68	3.5	23.96	27.91	2.75

Table A1.2: Concentration (mg kg $^{-1}$) of Cd in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2.11	1.0	2.38	0.19	0.05	1.94
KII	1.23	0.64	2.75	1.06	0.33	1.58
KIII	2.68	1.46	3	0.49	ND	5.07
CI	1.85	0.25	0.73	0.03	ND	1.43
CII	2.62	1.7	1.63	0.01	ND	8.56
CIII	0.58	0.73	2.13	ND	0.11	1.82

Table A1.3: Concentration (mg kg $^{-1}$) of Mn in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	61.56	162.85	192.5	318.23	200.57	200.21
KII	10.52	ND	577.38	733.33	700.99	424.35
KIII	7.91	ND	413.38	722.9	462.35	480.44
CI	214.4	44.82	105	234.17	198.69	154.15
CII	118.31	24.28	148.63	164.42	332.77	180
CIII	58.38	106.48	42.88	249.07	400.71	58.74

Table A1.4: Concentration (mg kg $^{-1}$) of Mg in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	380.44	372.18	458.5	4100	400	462.42
KII	711.98	309.12	992.88	1600	5100	809.14
KIII	474.84	487.03	527.25	4500	600	542.26
CI	334.01	344.22	630.24	1700	800	549.45
CII	423.34	373.9	254.6	1700	2900	234.22
CIII	356.46	183.68	577.25	3000	700	480.12

Table A1.5: Concentration (mg kg $^{-1}$) of Co in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	ND	ND	15.13	13.52	14.35	14.16
KII	ND	1.37	12	28.86	29.2	16.21
KIII	1.99	3.6	10.38	20.49	28.25	11.87
CI	1.7	7.98	22.56	12.75	12.38	18.29
CII	ND	ND	16.13	12.39	11.68	15.7
CIII	2.01	ND	0.75	22.76	19.95	ND

Table A1.6: Concentration (mg kg $^{-1}$) of Zn in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	0.81	5.58	27.5	64.93	42.77	22.43
KII	16.12	23.53	26.13	455.63	236.87	24.16
KIII	13.19	21.73	54.13	200.3	203.43	32.1
CI	6.79	6.73	7.93	26.09	143.45	15.12
CII	5.04	6.08	22.25	28.84	44.6	19.01
CIII	0.42	3.74	6.13	42.91	68.54	11.64

Table A1.7: Concentration (mg kg $^{-1}$) of Ni in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	ND	1.11	ND	26.25	22.83	10.24
KII	1.4	1.28	ND	83.15	89.68	11.68
KIII	0.93	1.19	ND	35.34	65.89	18.14
CI	0.27	0.42	ND	14.92	36.17	23.53
CII	1.13	0.79	ND	18.85	32.22	29.39
CIII	ND	0.96	ND	38.7	20.16	16.47

Table A1.8: Concentration (mg kg $^{-1}$) of Pb in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2.99	ND	3.65	13.87	8.06	6.01
KII	1.24	5.94	2.43	45.37	16.96	4.25
KIII	5.7	4.67	4.56	112.84	27.09	7.58
CI	1.64	1.03	ND	10.57	20.86	5.56
CII	1.46	ND	ND	11.4	24.23	5.49
CIII	3.32	1.25	2.43	18.33	0.79	2.83

Table A1.9: Concentration (mg kg $^{-1}$) of Fe in soils from the study area

STATION	MONJN13	MONSP13	PRMMH14	MONSP14	PRMF15	PRMMY15
KI	2277.23	14567.55	13850.75	27500	35700	35800
KII	6610.11	16558.87	13197.63	25500	52700	39600
KIII	22160.61	21304.92	11557	35200	100600	57500
CI	11981.09	38248.01	5619.63	16100	34700	67500
CII	13344.91	10634.65	12160.13	21100	22700	123500
CIII	1478.14	7516.3	8132	29100	23500	79800

Table A1.10: Values of Enrichment factor (EF) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	4.25	280.60	1.58	0.38	2.33	0.21	0.00	2.39
	KII	2.05	56.35	0.09	0.25	0.00	1.45	0.35	0.34
	KIII	1.20	36.62	0.02	0.05	0.24	0.35	0.07	0.47
	CI	1.90	46.76	1.05	0.06	0.00	0.34	0.04	0.25
	CII	0.58	59.46	0.52	0.07	0.00	0.22	0.14	0.20
	CIII	2.40	118.83	2.32	0.55	3.59	0.17	0.00	4.08
MONSP13	KI	0.83	20.79	0.66	0.06	0.00	0.23	0.13	0.00
	KII	1.93	11.70	0.00	0.04	0.22	0.84	0.13	0.65
	KIII	1.44	20.75	0.00	0.05	0.45	0.61	0.09	0.40
	CI	0.55	1.98	0.07	0.02	0.56	0.10	0.02	0.05
	CII	1.26	48.41	0.13	0.08	0.00	0.34	0.12	0.00
	CIII	1.06	29.41	0.83	0.06	0.00	0.30	0.21	0.30
PRMMH14	KI	10.37	52.04	0.81	0.08	2.91	1.18	0.00	0.48
	KII	6.51	63.10	2.56	0.17	2.42	1.18	0.00	0.33
	KIII	4.35	78.61	2.10	0.10	2.39	2.78	0.00	0.72
	CI	3.61	39.34	1.10	0.26	10.69	0.84	0.00	0.00
	CII	4.20	40.59	0.72	0.05	3.53	1.09	0.00	0.00

Table Cont.

	CIII	0.93	79.32	0.31	0.16	0.25	0.45	0.00	0.54
Seasons	**Stations**	**Cu**	**Cd**	**Mn**	**Mg**	**Co**	**Zn**	**Ni**	**Pb**
MONSP14	**KI**	1.97	2.09	0.68	0.34	1.31	1.40	1.59	0.92
	KII	7.63	12.59	1.69	0.14	3.01	10.61	5.42	3.23
	KIII	2.78	4.22	1.20	0.29	1.55	3.38	1.67	5.82
	CI	1.92	0.56	0.85	0.24	2.11	0.96	1.54	1.19
	CII	1.76	0.14	0.46	0.18	1.56	0.81	1.48	0.98
	CIII	1.78	0.00	0.50	0.24	2.08	0.88	2.21	1.14
PRMF15	**KI**	1.02	0.42	0.33	0.03	1.07	0.71	1.06	0.41
	KII	1.59	1.90	0.78	0.22	1.48	2.67	2.83	0.58
	KIII	0.98	0.00	0.27	0.01	0.75	1.20	1.09	0.49
	CI	0.98	0.00	0.34	0.05	0.95	2.46	1.73	1.09
	CII	1.58	0.00	0.86	0.29	1.37	1.17	2.36	1.94
	CIII	2.57	1.42	1.00	0.07	2.26	1.73	1.42	0.06
PRMMY15	**KI**	0.26	16.41	0.33	0.03	1.05	0.37	0.48	0.31
	KII	0.14	12.08	0.63	0.05	1.09	0.36	0.49	0.20
	KIII	0.33	26.70	0.49	0.02	0.55	0.33	0.52	0.24
	CI	0.12	6.42	0.13	0.02	0.72	0.13	0.58	0.15
	CII	0.15	20.99	0.09	0.00	0.34	0.09	0.40	0.08
	CIII	0.07	6.91	0.04	0.01	0.00	0.09	0.34	0.06

Table A1.11: Values of Contamination factor (CF) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	0.31	20.69	0.12	0.03	0.00	0.02	0.00	0.18
	KII	0.44	12.06	0.02	0.05	0.00	0.31	0.08	0.07
	KIII	0.86	26.27	0.02	0.04	0.17	0.25	0.05	0.34
	CI	0.74	18.14	0.41	0.02	0.15	0.13	0.01	0.10
	CII	0.25	25.69	0.22	0.03	0.00	0.10	0.06	0.09
	CIII	0.11	5.69	0.11	0.03	0.17	0.01	0.00	0.20
MONSP13	KI	0.39	9.80	0.31	0.03	0.00	0.11	0.06	0.00
	KII	1.03	6.27	0.00	0.02	0.12	0.45	0.07	0.35
	KIII	1.00	14.31	0.00	0.04	0.31	0.42	0.06	0.27
	CI	0.68	2.45	0.09	0.03	0.69	0.13	0.02	0.06
	CII	0.43	16.67	0.05	0.03	0.00	0.12	0.04	0.00
	CIII	0.26	7.16	0.20	0.01	0.00	0.07	0.05	0.07
PRMMH14	KI	4.65	23.33	0.37	0.03	1.30	0.53	0.00	0.21
	KII	2.78	26.96	1.10	0.07	1.03	0.50	0.00	0.14
	KIII	1.63	29.41	0.78	0.04	0.89	1.04	0.00	0.27
	CI	0.66	7.16	0.20	0.05	1.94	0.15	0.00	0.00
	CII	1.65	15.98	0.28	0.02	1.39	0.43	0.00	0.00
	CIII	0.24	20.88	0.08	0.04	0.06	0.12	0.00	0.14

Table Cont.

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
	KI	1.75	1.86	0.60	0.30	1.17	1.25	1.41	0.82
	KII	6.30	10.39	1.39	0.12	2.49	8.76	4.47	2.67
	KIII	3.17	4.80	1.37	0.33	1.77	3.85	1.90	6.64
MONSP14	**CI**	1.00	0.29	0.44	0.13	1.10	0.50	0.80	0.62
	CII	1.20	0.10	0.31	0.13	1.07	0.55	1.01	0.67
	CIII	1.68	0.00	0.47	0.22	1.96	0.83	2.08	1.08
	KI	1.18	0.49	0.38	0.03	1.24	0.82	1.23	0.47
	KII	2.72	3.24	1.33	0.38	2.52	4.56	4.82	1.00
	KIII	3.20	0.00	0.88	0.04	2.44	3.91	3.54	1.59
PRMF15	**CI**	1.10	0.00	0.38	0.06	1.07	2.76	1.94	1.23
	CII	1.16	0.00	0.63	0.21	1.01	0.86	1.73	1.43
	CIII	1.95	1.08	0.76	0.05	1.72	1.32	1.08	0.05
	KI	0.30	19.02	0.38	0.03	1.22	0.43	0.55	0.35
	KII	0.17	15.49	0.81	0.06	1.40	0.46	0.63	0.25
PRMMY15	**KIII**	0.61	49.71	0.91	0.04	1.02	0.62	0.98	0.45
	CI	0.26	14.02	0.29	0.04	1.58	0.29	1.27	0.33
	CII	0.60	83.92	0.34	0.02	1.35	0.37	1.58	0.32
	CIII	0.19	17.84	0.11	0.04	0.00	0.22	0.89	0.17

Table A1.12: Values of Geoaccumulation Index (Igeo) for metals in the soils of the study area

Seasons	Stations	Cu	Cd	Mn	Mg	Co	Zn	Ni	Pb
MONJN13	KI	-2.26	3.79	-3.68	-5.74	0.00	-6.59	0.00	-3.09
	KII	-1.77	3.01	-6.23	-4.83	0.00	-2.27	-4.31	-4.36
	KIII	-0.80	4.13	-6.64	-5.42	-3.13	-2.56	-4.91	-2.16
	CI	-1.02	3.60	-1.88	-5.92	-3.36	-3.52	-6.69	-3.96
	CII	-2.58	4.10	-2.74	-5.58	0.00	-3.95	-4.63	-4.13
	CIII	-3.71	1.92	-3.76	-5.83	-3.11	-7.54	0.00	-2.94
MONSP13	KI	-1.93	2.71	-2.28	-5.77	0.00	-3.81	-4.65	0.00
	KII	-0.54	2.06	0.00	-6.03	-3.67	-1.73	-4.45	-2.10
	KIII	-0.59	3.25	0.00	-5.38	-2.27	-1.84	-4.55	-2.45
	CI	-1.14	0.71	-4.14	-5.88	-1.12	-3.53	-6.05	-4.63
	CII	-1.79	3.47	-5.02	-5.76	0.00	-3.68	-5.14	0.00
	CIII	-2.54	2.25	-2.89	-6.79	0.00	-4.38	-4.86	-4.35
PRMMH14	KI	1.63	3.96	-2.04	-5.47	-0.20	-1.50	0.00	-2.80
	KII	0.89	4.17	-0.45	-4.35	-0.54	-1.58	0.00	-3.39
	KIII	0.12	4.29	-0.94	-5.26	-0.75	-0.53	0.00	-2.48
	CI	-1.19	2.25	-2.91	-5.01	0.37	-3.30	0.00	0.00
	CII	0.14	3.41	-2.41	-6.31	-0.11	-1.81	0.00	0.00
	CIII	-2.62	3.80	-4.20	-5.13	-4.54	-3.67	0.00	-3.39
MONSP14	KI	0.22	0.31	-1.31	-2.31	-0.36	-0.26	-0.09	-0.88
	KII	2.07	2.79	-0.11	-3.66	0.73	2.55	1.58	0.83
	KIII	1.08	1.68	-0.13	-2.17	0.24	1.36	0.34	2.15

Table Cont.

	CI	-0.58	-2.35	-1.76	-3.58	-0.45	-1.58	-0.90	-1.27
	CII	-0.32	-3.94	-2.27	-3.58	-0.49	-1.44	-0.57	-1.16
	CIII	0.16	0.00	-1.67	-2.76	0.39	-0.86	0.47	-0.48
Seasons	**Stations**	**Cu**	**Cd**	**Mn**	**Mg**	**Co**	**Zn**	**Ni**	**Pb**
PRMF15	**KI**	-0.34	-1.61	-1.98	-5.66	-0.28	-0.87	-0.29	-1.66
	KII	0.86	1.11	-0.17	-1.99	0.75	1.60	1.68	-0.59
	KIII	1.09	0.00	-0.77	-5.08	0.70	1.38	1.24	0.09
	CI	-0.45	0.00	-1.99	-4.66	-0.49	0.88	0.37	-0.29
	CII	-0.37	0.00	-1.25	-2.80	-0.58	-0.81	0.21	-0.07
	CIII	0.38	-0.48	-0.98	-4.86	0.20	-0.19	-0.47	-5.01
PRMMY15	**KI**	-2.34	3.66	-1.98	-5.45	-0.30	-1.80	-1.45	-2.09
	KII	-3.11	3.37	-0.90	-4.65	-0.10	-1.69	-1.26	-2.58
	KIII	-1.30	5.05	-0.72	-5.22	-0.55	-1.28	-0.62	-1.75
	CI	-2.53	3.22	-2.36	-5.20	0.07	-2.37	-0.25	-2.20
	CII	-1.32	5.81	-2.13	-6.43	-0.15	-2.04	0.08	-2.22
	CIII	-2.96	3.57	-3.75	-5.40	0	-2.74	-0.76	-3.17

Table A1.13: The recovery details and analyzed result of CRM standards of OCIs (804-050) in soils.

OCIs	Recovery (%)	CRM STD (mg Kg $^{-1}$)	
		Certified value	Analyzed Value
α-HCH	90 ± 3	-	-
β-HCH	87 ± 4	-	-
γ-HCH	88 ± 3	491.6	526.1
Heptachlor	89 ± 5	-	-
Aldrin	90 ± 5	18.04	19.1
Heptachlor epoxide(B)	90 ± 3	-	-
4,4'-DDE	88 ± 2	1519.6	1246
dieldrin	88 ± 4	1862.5	1508.7
Endrin	103 ± 4	62.2	68.3
2,4'-DDD	92 ± 3	-	-
4,4'-DDD	91 ± 5	1530.6	1193.9
2,4'-DDT	90 ± 2	-	-
4,4'-DDT	87 ± 4	1060.1	795.1
α-endosulphan	89 ± 5	1464.3	1142.2
β- endosulphan	90 ± 5	1128.2	1028.8

Table A1.14: Analysis of standard reference materials BCSS-I for metals (mg kg^{-1} except Fe and Mg in %).

Metal	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Certified values	0.30	11.4	18.5	4.7	2.44	229	55.3	22.7	119
Measured values (n=3)	0.297	11.6	18.3	4.61	2.23	208.9	51.15	23.6	123.64

Appendix II: Figures

Fig A2.1 Sampling stations: a) Station KI; b) Station KII; c) Station KII; d) Station CI; e) Station CII; f) Station CIII

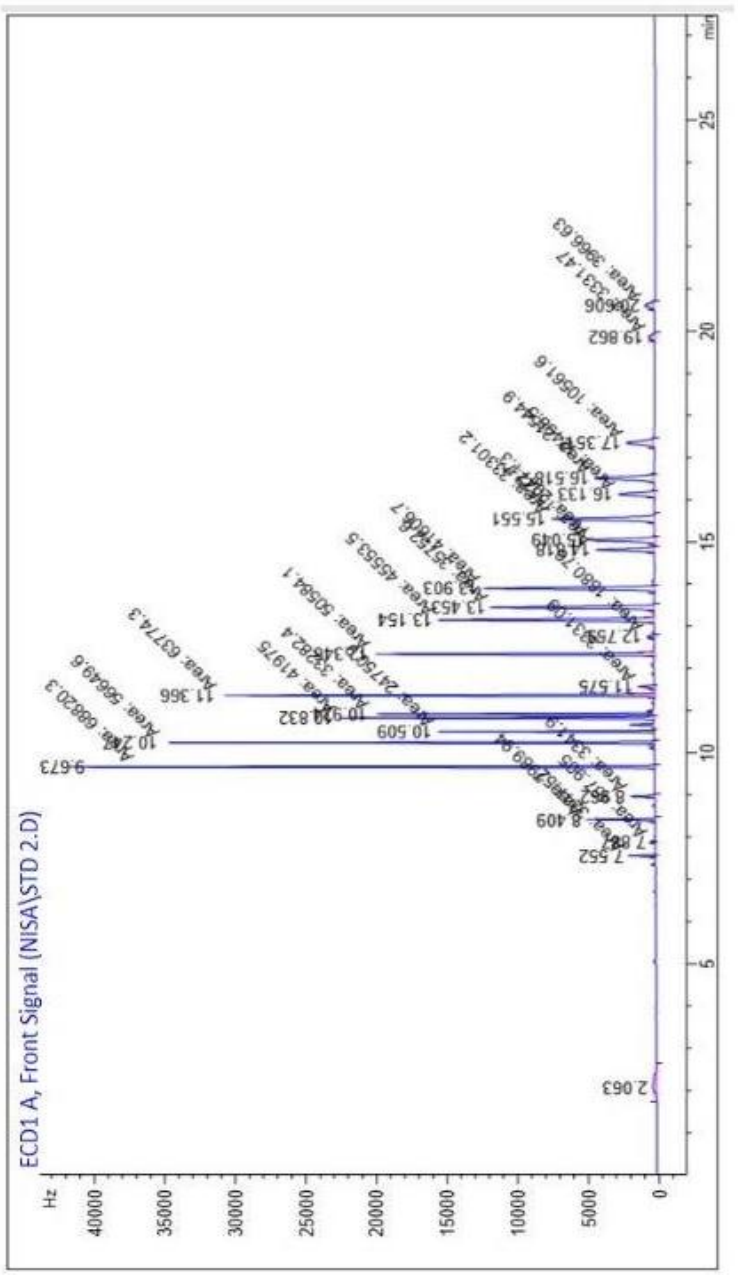

Fig A2.2 Representative Chromatogram (Standard solution) with respect to third chapter Spatio-Temporal Distribution Pattern of Organochlorine Insecticides (OCIs).