RHIZOREMEDIATION OF HEXACHLOROCYCLOHEXANE THROUGH PESTICIDE CONTAMINATED SOIL BY *Solanum nigrum*

Submitted By

Aneela Bibi

285- FBAS/MSES/F15

Department of Environmental Science

Faculty of Basic and Applied Sciences

INTERNATIONAL ISLAMIC UNIVERSITY ISLAMABAD

2017

Multipurpose plants
Medicinal plants-
Rhizospheric soil

RHIZOREMEDIATION OF HEXACHLOROCYCLOHEXANE THROUGH PESTICIDE CONTAMINATED SOIL BY *Solanum nigrum*

Submitted By

Aneela Bibi

285- FBAS/MSES/F15

Submitted to

Dr. Waqar-Un-Nisa

Assistant professor

INTERNATIONAL ISLAMIC UNIVERSITY ISLAMABAD

Faculty of Basic and Applied Sciences

Department of Environmental Science

Spring 2017

بسم الله الرحمن الرحيم

Department of Environmental Sciences International Islamic University Islamabad

Dated: 10-11-2017

FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. Aneela Bibi and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Environmental Sciences

COMMITTEE

External Examiner

Internal Examiner

Supervisor

Dr. Waqar-Un-Nisa

Assistant Professor

International Islamic University, Islamabad

Co-Supervisor

Dr. Tahir Hayat Malik

Assistant Professor

COMSAT University Abbottabad

Chairperson, DES
Dr. Rukhsana Tariq
Assistant Professor
International Islamic University, Islamabad

DR. RUKHSANA TARI[illegible]
A. Chairperson
Department of Environmental Scien[illegible]
International Islamic University Islama[illegible]

Dean, FBAS
Dr. Muhammad Arshad Zia
International Islamic University, Islamabad

Dissertation submitted to the Department of Environmental Science, as a part of the course of studies for Master`s Degree in Environmental Science of the International Islamic University, Islamabad

DEDICATION

This dissertation is dedicated to my beloved parents for their endless love, support and encouragement, whose hard work and prayers have enabled me to reach this stage and made me capable of facing troubles in life.

DECLARATION

I hereby declare that the work presented in this thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of this thesis report has been previously presented for any other degree.

Date _______________

____

Aneela Bibi

CONTENTS

ACKNOWLEDGEMENTS

In the Name of Allah the Most Beneficent and the Most Merciful.

I am grateful to Allah S.W.T our Lord and Cherisher, for guiding me to conceptualize, develop and complete my work. Indeed, without His help and will nothing be accomplished. I express my deep gratitude to Prophet Muhammad (SAW), who is ultimate torch bearer and guidance for entire humanity.

It is a matter of great privilege for me to present this dissertation to Dr. Waqar-Un-Nisa, Assistant Professor, who provided me all the guidance and support. She has taken pain to go through the project and make necessary correction as and when needed for her help and guidance. She has provided me with the quality of knowledge.

My deep sense of gratitude to Dr. Tahir Hayat Malik, Assistant Professor of Comsat institute of technology, Abbottabad his support and guidance. I would like to express my special gratitude and thanks to Dr. Nazneen Bangash, Assistant Professor at Comsat institute of technology, Islamabad, and Dr. Aansa Rukya Saleem, Assistant Professor at Bahria University, Islamabad for giving me such attention and time without their help and cooperation it was very difficult for me to complete my study. I would also thank my institution and my faculty members specially Dr. Rukhsana Tariq, Head of Department of Environmental Science, International Islamic University, Islamabad, and Dr. Rakhshanda Aziz, Assistant Professor without whom this project has been a distant reality.

My thanks and appreciations also go to my colleagues in developing the project and people who have willingly helped me out with their abilities. I also extend my heartfelt thanks to my families for their untiring and never-ending support.

Aneela Bibi

List of Abbreviations

Acronym	Abbreviation
ND	Not Determined
HCHs	HexaChlorocyclo Hexane
TOC	Total Organic Carbon
MBC	Microbial Biomass Carbon
OM	Organic Matter
POPs	Persistent Organic Pollutants
OCPs	Organo-Chlorine Pesticide
DDT	Dichlorodiphenoxytrichloro ethane
GDP	Gross Domestic Product
DDE	Dichlorodiphenoxydichloro ethylene
BHC	Benzene HexaChloride
HCB	HexaChloro Benzene
UNEP	United Nation Environmental Programme
ISQGs	Interim Sediment Quality Guidelines
PML	Probable Effect Limits
CSQGs	Canadian Sediment Quality Guidelines
FAO	Food Agriculture Organization
DHC	DehydroChlorination
DF	Dworkin and Foster
OD	Optical Density
UV	Ultra violet
CRD	Complete Randomized Design
EC	Electrical Conductivity
GC-MS	Gas Chromatography-Mass Spectrometry

List of Figures

List of Tables

ABSTRACT

Quality and magnitude of World's food have been improved using pesticides. However, these pesticides, such as Hexachlorocyclohexane, have unfavorably affected the quality of environment and health of human beings. These not only used in Agriculture but in public health activities. Their use has been banned in developed countries, but these are still being used in some developing countries including Pakistan. Present study was aimed at Assessment of HCHs concentration to be removed by rhizoremediation with *Solanum nigrum*; *Solanum nigrum* growth performance and identification of inoculate to be best for rhizo-microbial remediation. Pot experiments at 0, 5 and 10mg/kg HCHs spiked soil were conducted for 90 days. Soil physicochemical properties (pH, TOC, OM, EC, MBC) were considered. Residual HCHs concentration in spiked soil was 1.73,2.33, 3.9 and 6.1 mg/kg for 5% HCHs + Solanum nigrum + Inoculate; 5% HCHs + Inoculate; 10% HCHs Solanum nigrum + Inoculate; 10%HCHs + Inoculate respectively. While HCHs accumulation in *Solanum nigrum* in different treatments was 1.233, 2.133, 2.6667 mg/kg for 5% HCHs + *Solanum nigrum* + Inoculate; 10% HCHs + Solanum *nigrum*; 10% HCHs + *Solanum nigrum* + inoculate respectively. Strain which effectively improved the plant root and biomass was ST47 which improved root length almost 6.9cm. Results elucidated the use of *Solanum nigrum* along with ST47 strain as the effective and promising remediation technique for HCHs degradation.

INTRODUCTION

1 INTRODUCTION

As the population of world is growing day by day, sustenance of a country requires the use of techniques that not only benefit the country economically, industrially and in food industry but should support quality of environment.

To support the food requirement of large population pesticides have become the common source. These improve the quality and quantity of food to be feed by human beings. Pesticides control the disease-causing agents of crops i.e. insects, weeds, in this way it improves food production and feeds billions of people of world. Pesticides control the pests because these have comparatively lower cost, can be use used easily and have effective results in management of pests. If the use of pesticides exceeds the safe limit they pollute the environment, degrading its ecological aspects and hampering its natural processes to sustain life. However, some pesticides itself are dangerous in their low limit, that are persistent in nature and are called persistent organic pollutants. POPs sticks in environment for longer time and don't degrade easily, bio accumulates, and bio magnifies in food chains and food web and destroys the human being's health and environmental aspect. These compounds don't dissolve in water and sticks to soil strongly (Mertens, 2006). POPs exist in every section of environment, but these are transported mostly by soil, that delivers them through leaching to different parts of environment and contaminates the ground/ surface water, transports harmful substances to plants grown on contaminated soil thus contaminates the food chain, food web through plants and animals to humans (Tahir, 2012). Infectivity of different environmental portions like soil, sediment, ground waters and surface waters by pesticides is considered as the concern that need to be addressed on priority (Girish, 2013).

Some groups of pesticides including Organochlorine pesticides such as dichlorodiphenoxytrichloro ethane (DDT) and its degrading products dichlorodiphenoxydichloro ethylene (DDE) and hexachlorocyclohexane(HCH) have proven their self as more flourished mean of controlling pests since past decades and also being used in many industrial production but their practical use led many serious health and ecological evils so they were expelled to produce in many countries but the order is not being followed in different countries (Sandra *et al.*, 2015).

Pakistan is one of those developing countries which is having problem of management of pesticide residual. Agriculture has largest share (21%) in Pakistan's economy, 44% of labour force is employed in this sector (economic survey, 2009-2010). Pests and diseases are main cause of crops destruction in each year, so they should be managed in a proper manner to improve the food production. In this way growing population of world can be feed (Aftab *et al.*, 2007). To strengthen the economy, use of pesticides is mandatory but random use of pesticides in farming creates serious environmental problems and affects health of public and labours involved in this sector. Little research work has been done on sustainable practices for minimizing the risk effect of these pesticides (Sheikh, 2011). Several researchers have reported the presence of pesticides residues in different areas of Pakistan and the most common pesticides discern were those that retains in environment for longer period and don't decompose easily and effects every compartment of environment, such as organochlorine pesticides (Tahir *et al.*, 2012).

Hexachlorocyclohexane (HCH) isomers namely the alpha HCH, beta-HCH, and gamma-HCH (commercially known as lindane) are considered as new POPs and included in POPs list in 2009 (Vijgen *et al.*, 2010) and will therefore be addressed at global level because of their dumped waste remaining from the historic utilization and manufacture (Abhilash *et al.*, 2011).

Hexachlorocyclohexane is a type of organochlorine pesticide. Benzene molecule is chlorinated in presence of ultraviolet light to manufacture HCHs (Nagpal *et al.*, 2008). HCHs began to be produced in 1940s since then they have resulted in many serious environmental issues (Turnbull, 1996). Several biotic and a biotic factor such as soil physiochemical characteristics and availability of degraders decide the destiny and degradation of HCHs. Degradation of HCHs in soil is much slower as compared due to mass transfer problem (Rijnaarts *et al.*, 1990).

To protect the environment, and secure the public health different cleaning methods have been projected to remove HCHs from contaminated sites. One of those methods is remediation which returns contaminated environment to its natural condition.

Present study deals with Rhizo-microbial remediation of HCHs e.g. the dissipation of contaminants by microbes present in the rhizosphere of plants. This is a symbiosis relationship in which both species enhances each other's effect. Plant provides biological active sites to microbes and secretes nutrients which are used by microbes as their energy sources while microbes release enzymes that help plant against oxidative damage (Urgel and Ramos, 2001). In addition, plants may also inject microbes in soil to degrade contaminants (Gonzalez *et al.*, 2005).

Hexachlorocyclohexane has low aqueous solubility and it is highly hydrophobic so its degradation by microbes is difficult (Maritsa *et al.*, 2014). HCH not only destroys pests and insects but it also adversely affects the activities of other soil organisms (Shi *et al.*, 2007). Initially HCHs reduces the growth of plant grown on HCHs contaminated soil but tolerant plant can survive with it. It has been deregistered in many countries because of its non-target toxicity and longer persistence but residue problem, because of its past use, will continue for many decades and it also being manufactured in some countries (Norbert *et al.*, 2014).

The degradation of Hexachlorocyclohexane in soil by microorganisms depends on adsorption rate to the soil and its restricted availability for biological action (Thakur *et al.*, 2010). Effectiveness of microbes to degrade HCHs can be judged through its adaptive ability at which it starts to remove contaminant in an efficient manner (Elcey and Kunhi, 2010). So, the exploitation of native microbial strains and inoculation of these strains to *Solanum nigrum* is the best answer for onsite remediation purpose.

1.2 HYPOTHESIS

Rhizoremediation would effectively remove HCHs from contaminated soil utilizing best combination of *Solanum nigrum* and inoculate.

1.3 NEED OF STUDY

Usage of pesticide containing HCHs on Pakistan's crop to prevent the pest attack is the common strategy, but little steps have been taken for management of their adverse impacts on human health and environment. To improve the production from agricultural sector we can't restrict the use of pesticides, but their residual impacts can be reduced to level that is not harmful to humans and environment. For this purpose, major attention has to be given on different remedial technologies that are sustainable and feasible. So present study is conducted to identify the best rhizo-microbial inoculate that would help in effective remediation of HCHs.

1.4 SIGNIFICANCE OF STUDY

This study in its objective perspective will explore the rhizo-microbial inoculation *Solanum nigrum* for the removal of HCHs. This will provide the guidelines for best method of rhizo-microbial remediation. This would initiate further research on the best available methods for pesticide degradation and minimizing their expected risks to the environment.

1.5 OBJECTIVES OF STUDY

This study was aimed at:

- Isolation of inoculate to be best for combination with *Solanum nigrum* plant species for rhizo-remediation of HCHs.
- Assessment of HCHs concentration to be removed with rhizo-microbial remediation.
- Assessment of the growth performance of *Solanum nigrum* grown in HCHs contaminated soil.

LITERTATURE REVIEW

2 LITERATURE REVIEW

2.1 NEED OF MODERN SOCIETY AND ITS CONSEQUENCES

As the pesticide has increased the food productivity by reducing pests and combating different diseases because of this reason it has been used extensively from last decades. Application of agrochemicals is best for suppressing pests and different disease-causing agents. Use of pesticides work fast to control pests attack as compared to other common practices, such as biological and traditional methods that works in a longer period. But over use of these pesticides, lead to many issues that adversely affect the environmental aspects (Carson, 1962). This over usage resulted in higher pesticide contamination in our food chain, food web consequently destroying human health. Each year there are almost millions of cases about venomous contamination by pesticides over the globe. Detailed study on pesticides has cleared their sound adverse health impacts such as genetic disorder, diabetes, neurological problems and different respiratory effects. Concentration and contact method of pesticides decides about their respective impacts on health of humans and environment. people who are directly involved in pesticide contact, such as farmers, are at more risk of getting different diseases as compared to those whose exposure is less. After application it also contaminates the food grown on that farm which results in degrading health of common people who consumes that food. Discussion on management of pesticides through rules and regulation has always been a hot topic (Richter, 2002).

2.2 HEXACHLOROCYCLOHEXANES AS INSECTICIDE

HCHs are organochlorine pesticides that are being used as insecticides worldwide. These are toxic not only to humans and animals but also cause harm to the soil and water. Stockholm convention 2009 regarded these compounds as dangerous because these are persistent and carcinogenic in nature (Rochika and Dharmender, 2014).

2.3 RESPONSE OF ENVIRONMENT TO HCHs

Hexachlorocyclohexane such as gamma isomer of HCHs and Benzene hexachloride are compounds of organochlorine pesticides, which are poisonous in nature. These stay in environment for longer period and don't decompose easily e.g. gamma isomer of HCHs has half-life of 708 days in soil and 2, 292 days in water. When HCHs are sprayed on crops some of it (12 to 30%) volatilizes into air, transports over long distances and then through rain fall it come backs to earth surface and destroys the soil microflora (Shen *et al.*, 2004) and contaminates the soil by sticking to the organic matter of soil. Rodriguez and Toranzos, 2003 reported 50% drop in community of microbes that were grown in HCHs amended soil. The plants then grown on these contaminated soils will accumulate this contaminant in their tissues by their roots from soil or it will deposit on their leaves in volatilized form (Abhilash *et al.*, 2008). Bidlan *et al.*, 2004 studied that HCHs contamination affected the plant initial development and seeds germination. If HCHs contaminates the surface that has less organic matter and have high rainfall it will leach down to the ground water and will affect aquatic life and degrade water quality (Babu *et al.*, 2001).

Rachel Carson (United States) wrote a book "Silent Spring" in 1962. In which he daringly reported the imminence of pesticides on human and environmental health and emphasized for the first time that humans should pay special attention to issues that effects environment. Of those pesticides HCH are known to have adverse environmental and human effects. These retained in the environment for longer time affecting its quality (Yuxian *et al.*, 2012).

In the 1940s these HCHs were used for first time in United States, it was reported that only gamma isomer of HCHs has bio-cidal properties, so it was used as insecticide (commercially known as Lindane) (Vijgen, 2006). HCHs were used extensively (approximately 600, 000 tons) from their production period to 1990, for controlling different crop pests and insects causing public health issues, such as malaria etc. (Prakash *et al.*, 2004). Commercial manufacturing of HCHs were congested in 2008 and in 2009 Stockholm Convention reported these compounds as POPs (UNEP, 2009).

2.4 REPORTED IMPACTS OF HCHs

Different cases about recovery of insects, development of confrontation against HCHs, attacks on non-target species and progression of other insects have been mentioned. But despite that reality HCHs are still being used in all sectors, with greater extent in agriculture that has been adversely exaggerated the most (Sattler *et al.*, 2007). A study was conducted on assessment of HCHs in mango and pine needles by Calamari *et al.*, 1995 results revealed that noticeable amount of HCHs is present in leaves of mango and needles of pine.

Porta *et al.*, 2013 did a study in which blood samples of different people were analyzed and beta- HCH were assessed. Different parameters such as literacy, source of food, source of water and age factor were studied to better understand the presence of HCHs in people. People habitating near the river exhibited strong linkage with Beta HCHs. Outcome of study cleared it that people near to river possessed higher amount of Beta HCHs because of consumption of water and food grown by contaminated water.

Basanta and Subhas in 2000 did a study on Indian Major Carp (Labeo rohita) that was contaminated with toxic amount (1/10 and 1/15) of HCHs for 45 days. Tissues (kidney, liver, brain, skin, gills, muscle, heart) of test specie were thoroughly studied to understand the toxic effects on them. Results indicated that all the organs were damaged. Inflation in tubules of the kidney was observed, blood vessels of liver were swelled, obstruction of blood vessels in gills was obvious, and nervous system was deterioted.

The histopathological changes in the reproductive system and hormone levels of the fish were analyzed by Yuksel *et al* in 2016 after applying 0, methanol at 5, 10, 20, 40, 80 and 160μg/L/day lindane in a bath for 21 days to the fish separated for reproduction.

Eqani *et al.*, 2010 did a study in river Chenab in Pakistan to check concentration and allocation of some OCs pesticides and compared their amount with permissible limit provided by Interim Sediment Quality Guidelines (ISQGs), Canadian Sediment Quality Guidelines and Probable Effect Limit (PEL). Results revealed that Dieldrin, Heptachlor, Endoepoxide, DDTs and gamma isomer of HCHs in all sediment samples were above permissible limits.

A survey was conducted by Food and Agriculture Organization in 1998 to examine the amount of pesticides that have been banned, outdated results reported that large amount of HCHs (2785 tons technical HCHs, 304 tons gamma isomer and 45 tons indefinite) pesticides were present in the waste of Africa and East (http:///www.fao.org). A study done by Weber *et al.*, 2008 demonstrated that amount of HCHs (four to six million tons) present in the waste around the globe was approximately equal to total amount that waste of all other POPs that was stated by Stockholm Convention.

Production of commercial HCHs also generates large number of byproducts (alpha, beta and partial isomers of HCHs), considered as waste called "HCH Muck", e.g. 8 to 12 tons of other isomers of HCHs are generated along with 1 tons of commercial gamma isomer of HCHs manufacture (Weber *et al.*, 2008). These wastes are notorious in nature and are more problematic than commercial HCHs itself, and results in higher contamination at point source. Main problem is management of HCHs Muck (Vijgen *et al.*, 2006).

Increasing concern about adverse impacts of pesticides has resulted in lower number of residues of pesticides in waste since last two decades (Zhang *et al.*, 2003) but some countries such as Pakistan are still using expelled chemical (DDTs, HCHs etc.) because these are economically cheap (Malik *et al.*, 2011; Tariq *et al.*, 2007). Jan et al., 2008 examined chemical ware houses and confirmed presence of considerable amount of DDTs and its metabolites, although it has been banned in Pakistan since 1994.

Residues of HCH isomers have now been published for different countries in samples of atmosphere (Lammel *et al.*, 2007); Water (Kumari *et al.*, 2007); Soil (Raina *et al.*, 2008), Food products (Toteja *et al.*, 2003), milk (Zhao *et al.*, 2007), fish (Erkman *et al.*, 2006), and mammals (Gangwar *et al.*, 1970) and from blood samples of humans (Subramaniam *et al.*, 2006) and adipose tissue (Kutz *et al.*, 1991). Presence of HCHs residues in regions of Arctic, Antarctic and Pacific oceans have been mentioned because these are regions where it is produced and used in large amount (walker *et al.*, 1999). Global environmental problems are still resulting from their past usage although, now a day's these compounds are banned. Netherland, Brazil, Spain, Germany, United States, Spain, China Greece India and Canada have been declared as highly contaminated by HCHs (Lal *et al.*, 2010).

2.5 OVERVIEW OF HCHS IN PAKISTAN

Malik *et al.*, 2010 and Tariq *et al.*, 2010 conducted a study on pesticides in different areas of Pakistan and reported presence of larger reserves of obsolete pesticides. They declared approximately 2016 tons in Sindh; 3805 tons in Punjab; 128 tons in Baluchistan and 179 tons in KPK and 178 tons in Federal Department of Plant Protection. They further stated that from these reserves these pesticides enter environment degrading quality of all its compartments. Environmental laws are not properly implemented which results in illegitimate use of pesticides in different sectors. According to a report, written by Economic survey of Pakistan in 2005–2006, 74% of pesticides are insecticides, 14% are herbicide, 9% are fungicides, 2% are acaricides and 1 % is fumigants. 65% of theses pesticides are used in cotton crops, other main users of pesticides are crops of sugar cane, rice, fruits, maize and tobacco.

For 20 years Pakistan has increased its pesticide's application from 11 to 69%, extensively using insecticides on Cotton crops. In contrast to this stocks of hazardous, out dated and banned pesticides (3 thousand tons) are not according to environmental laws, which requires them to be under highly monitored stores. Illegitimate import of banned pesticides is common in Pakistan. So extensive system of environmental laws and innovation of new techniques to purify contaminated site is need of hour and should be done on immediate basis (Ghaffar *et al.*, 2014).

2.6 ISOLATION OF HCHs DEGRADERS FROM HCHs CONTAMINATED SITES

Till 1980s whole technical mixture of HCHs was used to destroy different insects (UNEP, 2005). Then due to best biocidal activities of lindane (gamma HCHs) it was separated from mixture of HCHs and was used for controlling insects till 1990s. But production of this commercial isomer of HCHs yields great amount of wastes that is hazardous to environment. HCHs are also manufactured to treat different insects causing public diseases such as used in shampoos to cure head lice and to cure scabies disease caused *Sarcoptes scabiei*. (Vijgen, 2006). Previously these compounds were used extensively, and their production's waste was not managed in an effective way that resulted pollution of every compartment of environment such as soil, water, atmosphere (Vijgen, 2006). By products which were produced from gamma isomer of HCHs had no biocial properties, so they were recklessly dumped into nearby sites that caused environmental contamination (Pereira *et al.*, 2006).

HCHs degrading microbes can screen from the areas that are polluted with HCHs (Lla et *al.*, 2010). It means the sites that are contaminated with HCHs provide the source from which different communities of microbes that are capable of degrading HCHs can be secluded and studied. Three Sphingobium strains were studied by Pal *et al.*, 2005 in France, Japan and India, this presence confirms that HCHs converting Bacteria can be found everywhere on the globe. HCHs degrading microbes possess special enzymes which help them to degrade HCHs and used them as energy source. Some HCHs degrading enzymes such as Dehydrochlorinase (Lin A) and Haloalkane (LinB) have been studies thoroughly (Bala *et al.*, 2013).

2.7 REMEDIATION TECHNOLOGIES

Several methods are reported for managing the pesticide residue. Dehydrochlorination (DHC) with thermal or base aided methods, use of metal catalyst to reduce the contaminant (Mertens *et al.*, 2007) and use of microorganisms to detoxify contaminants are some general practices to clean the environment (Phillip *et al.*, 2005). Pleurotusostreatus, Pleurotussajor-caju, Trameteshirsutus, Coriolus versicolor, Daldiniaconcentrica, Cyathusbulleri, Phanerochaetesordida and Phanerochaetechrysosporium are a small number of white rot fungi recognizedas degraders of pesticides (Nagpal, 2008). In comparison to chemical practices, remediation of environment by microbes, Phytoremediation and rhizoremediation of HCH has engrossed mounting notice because of their efficiencies in curing, economic feasibility and environmentally viable characteristics (Ngpal and Paknikar, 2006). HCH degradation occurs by removing Chlorine atoms i.e. Dechlorination and is dependent on oxygen content, pH, temperature and microbial biomass carbon (Rochika and Dharmender, 2014).

Study of degradation of HCHs by anaerobic microbes started in 1960s; later on aerobic microbes proficient in degradation of HCHs were developed. This study helped in understanding the genes of microbes (such as lin genes) involved in dissipation of HCHs then this information was used to make different gene products to improve degradation process. These genes were then recovered from different bacterial strains proving them as capable of degradaing HCHs. Lal *et al.*, 2006 proved Sphingomonads as the HCHs degraders because lin genes were discovered in them. Initially these genes were discerned from UT26 bacterial strains and then retrieved from B90A. Study of these genes helped in interpretation of spread and degrading capability of different HCHs degraders. Now there has been an increasing trend of taking Initiatives by different Environmental organizations and government to remedify the polluted soil by HCHs degrading microbes and species (Boltner *et al.*, 2007). Rup *et al.*, 2010 did a study in which he highlighted the efficiency and consequences of using bacterial strains to decontaminate the HCHs polluted site.

Phillips *et al* in 2006 purify 1100 tons of soil in United States that was spiked with 5000 mg /kg. He used Daramend product that was extracted from plant fibre to stimulate microorganisms for effective removal of HCHs from contaminated soil. Two Daramend products (D6390 and D6386) were developed by Cycled anaerobic and strictly aerobic methods described by Phillip *et al.*, 2004. Application of these products resulted in 50% drop in actual amount of HCHs over a time period of 1 year.

In a study carried out by Sagwan *et al.*, 2012 bioremediation of HCHs by different microbial community was observed by studying their composition and role in dissipating HCHs. This study was done by shotgun metagenomic sequencing and 16S rRNA technique. Samples were collected from three different soils spiked with different concentration of HCHs. contaminated soil was rich in Marinimicrobium, Sphingopyxis, Idiomarina, Chromohalobacter, Pseudomonas and Sphingomonas microbes. Evolutionary relationships between these organisms was studied which confirmed the presence of same genes which helps them to detoxify contaminant i.e. lin gene.

2.8 RHIZOREMEDIATION BEING THE MOST EFFECTIVE TECHNOLOGY TO REMEDIATE ENVIRONMENT

More work has been done on phytoremediation and bio remediation of HCHs, but little research work is available on rhizo-remediation strategy. Initially rhizodegradation of pesticides was studied (Jacobsen, 1997). Melling, 1993 reported the presence of approximately one hundred million bacteria and ten thousand fungi community in 1 gram of soil. Utilization of degrading abilities of different microbes to clean the environment is one of the sustainable methods that is 100 times better than other chemical methods to purify the environment (Vidali, 2001). Native microbes can work this function more effectively. Different researches on remediation of HCHs suggest that degrading bacteria protects plants from HCHs. Study on phytoremediation suggests that plants having deep roots and quick development are best to remove contaminants such as, Brassica species have good root system. Studies have proved Mulberry and Poplar trees as best for removing Trichloroethylene (TCE) and other chlorinated compounds, besides these various grass varieties and leguminous plants have proved their selves appropriate for phytoremediation (Kuiper *et al.*, 2001, 2004).

Association between the plant roots and their surrounding environment helps them to affect the activities of pollutant by ceasing their movement and changing their physical and chemical properties (Davis *et al.*, 2002). These pollutants enter into plants through various routes, volatile contaminants e.g. HCHs sublimates on the above ground parts of plants (Simonich and Hites 1994). More surface area revealed to environment results in more deposition of contaminants on plant leaves beside this environmental circumstance e.g wind speed, direction, temperature and rain fall also affects deposition of these compounds on plant parts (Barber *et al.*, 2005). To end when plants grown on contaminated soil decomposed into soil it results in higher concentration of HCHs in soil because of debris of contaminated plant (Horstman and McLachlan, 1996). With the passage of time availability of these contaminants declines due to mineralization of organic matter (Pereira *et al.*, 2009).

2.9 USE OF RHIZOREMEDIATION FOR REMEDIATION OF HCHs IN DIFFERENT AREAS

Kuiper and his colleagues in 2004 isolated bacteria from rhizosphere of plant grown on contaminated soil and prepared their cultures in laboratory and then inoculated those cultures into rhizosphere of different plants grown on polluted soil so that they can be seclude again and again to degrade contaminant (Dietmar *et al.*, 2008).

Fulekar, 2014 used Sorghum, Pennisetumpedicellatum and Cenchrussetigerus for rhizoremediation of different pesticides the potential micro-organism identified were stenotrophomonasmaltophila MHF ENV20, Stenotrophomonasmaltophila MHF ENV22 and Sphingo bacterium thalpophilum MHF ENV 23. The results have shown that complete bioremediation was found in rhizosphere.

In another study three poplar species along with HCHs degrading microorganisms were used to remediate contaminated soil in Fiume Sacco by Bianconi *et al.*, 2015. Results demonstrated that use of poplar along with HCHs degraders proved it to be effective and efficient in restoring contaminated soil back to its original form, thus that agricultural soil can again be used for crop's production.

Pannu *et al* in 2014 secluded bacteria from agricultural soil for degradation of lindane. 69.5 and 65% degradation were done by RP-1 and RP-3 after a rest period of 10 days while RP-9 showed 62% degradation after 15 days. Maximum performance was done by three isolates at incubation period 10-15 days, temperature 30°C, pH 7 and shaking speed 120rpm, initial substrate concentration 100mg/l. additional minerals were added to enhance degradation.

Becerra and his colleagues (2013) has proposed leguminous shrub *Cytisus striatus* (Hill) Rothm as applicant specie for cleaning soil contaminated with HCHs. Plant was inoculated with ETD4 and result revealed that this inoculation improved 120% root mass and 140% shoot mass and reduced the anti-oxidative enzymatic activity, hence increasing the rate of HCHs degradation.

A greenhouse experiment was designed by kidd *et al.*, 2008 in which *Cystisus striatus Rothm* and *Holcus lanatus L.* were used to stimulate rhizobacteria to improve their ability to degrade HCHs. Results suggested that use of tolerant plant species to improve microbial activity can effectively clean the site contaminated with HCHs.

Abhilash *et al.*, 2011 demonstrated a study to check the combine rhizospheric potential of *Withania somnifera* in the presence and absence of *staphylococcus cohnii* grown on lindane contaminated soil. The inoculation with microbes considerably improved the plant biomass and accumulation HCHs in plant. Results demonstrated that symbiosis relationship of plant (*Withania somnifera*) and bacterial strain (staphylococcus cohnii) proved it to be best combination for remediation of contaminated soil.

The literature reviewed helped in understanding the factors affecting the microbes that can degrade HCHs, favorable conditions for their growth and best combination of plant and inoculate to work effectively. So, this knowledge can be utilized for successful rhizoremediation of HCHs from pesticide contaminated soil. Based on this knowledge we initiated our research work using *Solanum nigrum* in association with microbe to degrade HCHs in a best way. *Solanum nigrum's* common names are Black Nightshade, Common Nightshade, Poisonberry, Black Nightshade and Mako. It is herb belonging to Solanaceae family. The specie has remediated the heavy metal and PCB's contaminated soil. Present research is based on improvement of its remediation potential by combining it with effective rhizomicrobe.

METHODOLOGY

3 METHODOLOGY

A set of experiments were carried out to assess the potential of rhizoremediation of *Solanum nigrum* to remediate HCHs and to assess plant growth aspects and subsequent uptake of HCHs from contaminated soil.

3.1 COLLECTION OF RIZOSPHERIC SOIL

Rhizospheric soil of plant grown on contaminated soil was collected. For rhizospheric soil, plants were uprooted. The uprooted plants were set aside at $4^{O}C$ in the laboratory. Plants were gently shaken to remove non rhizospheric soil from its roots. Same procedure was followed to remove rhizospheric soil.

3.2 ISOLATION OF HCHS DEGRADING BACTERIA

Enrichment technique was used to seclude numerous strains of rhizo bacteria, HCHs was used as source of carbon and energy in DF minimal medium (Dworkin and Foster, 1958). Bacterial strains were secluded from rhizospheric soil through dilution plate technique. Rhizospheric soil suspension was used to prepare series of dilution and from each dilution 200 µl suspension was plated on HCHs containing agar medium. Then it was incubated at 30 °C for 48 hours. Isolates were further tested on agar medium containing HCHs (50mg/l) and the most competent rhizobacteria were screened on the basis of their efficiency to consume HCH as the only source of carbon. The most efficient rhizobacteria were further evaluated for their ability to support the development of *Solanum nigrum* and potential to degrade HCHs under controlled conditions (Wollum II, 1982).

3.3 PREPARATION OF BACTERIAL CULTURE

Dworkin and Foster minimal Salt medium was used to grow selected rhizobacteria in 250 ml flasks. Flasks were then incubated ($30^{O}c$) at 100 rpm for 48 hours. Population of selected bacteria was then attained by calculating optical density (OD) of culture through UV visible spectrometer at 600nm. Each treatment was conducted with fresh inoculums. Procedure was carried out in clean cabinet to circumvent any contamination.

3.4 SAMPLE COLLECTION

Samples of uncontaminated soil were collected from the agricultural field of NARC. The samples were ground, sieved with 2 mm pore size sieve and air dried.

3.5 SOIL TEXTURE

Soil texture was measured by using Bouyoucos hydrometer method.

3.6 SOIL TREATMENTS

Soils samples were divided in eight treatments. Firstly, control soil with *Solanum nigrum* was used. Second treatment was comprised of Soil spiked with 5% HCHs and *Solanum nigrum*. Third was consist of *Solanum nigrum* and soil spiked with 10% HCHs. Fourth treatment was done by using inoculate in control soil. In fifth treatment inoculate was injected into 5% HCHs spiked soil. 10% HCHs spiked soil was treated with inoculate in sixth treatment. *Solanum nigrum* was used in presence of inoculate in soil spiked with 5% HCHs in seventh treatment. And last treatment was executed on 10% HCHs spiked soil along with *Solanum nigrum* and inoculates. The samples were stored in polythene bags for 15 days for aging process (Park *et al.*, 2011).

3.7 POT EXPERIMENT

Pot experiments were carried out to assess the remediation of HCHs from contaminated soil by plant. One plant species *Solanum nigrum* and inoculate isolated from plant grown on HCHs contaminated soil was selected. Pot trials were carried out in Environmental Sciences department of International Islamic University Islamabad. Small plants of *Solanum nigrum* were grown in pots. Each pot was filled with 1.5 kg sieved soil.

For pot trials the plants of *Solanum nigrum* was transplanted into pots after 15 days of spiking with HCH. In each pot, seedlings of *Solanum nigrum*were grown and inoculated with prepared inocula and irrigation was provided when needed. The pot trails were performed with three replications using completely randomized design (CRD). Ambient light and temperature was provided to pots in the green house. The plants were harvested after three (03) month and plant growth parameters e.g. fresh and dry biomass and root, shoot length were collected and analysed statistically.

3.8 PHYSICO-CHEMICAL ANALYSIS OF SOIL

Soil samples were examined for physico-chemical parameters before spiking the soil with HCH, and after harvesting of the plants. The analysis was performed by following the standard procedures as described below:

3.8.1 Soil moisture (SM)

Pre-weighed (10 g) soils were put in a Petri plate, weighed again. Soils were dried at 105°C for 24 hours in digital oven to remove moisture contents. Then SM was calculated by following formula:

$$Soil\ Moisture\ (\%) = \frac{loss\ of\ weight\ in\ soil\ samples}{weight\ of\ oven\ dried\ soil} \times 100$$

3.8.2 pH

pH meter was used to measure pH of soil (APHA, 2005). The model of the pH used was (BMS pH-200L).

3.8.3 Electrical Conductivity

Electrical conductivity was measured by EC meter in micro semen (µS) (Muhammad *et al.*, 2008).

3.8.4 Total Organic Carbon (TOC)

Total Organic Carbon (TOC) analyses were carried out by Walkley, 1997 titration method. One-gram air dry soil was added into a 500ml flask. 10 ml of normal solution of potassium dichromate and 20ml of conc. H_2SO_4 were added to flask dispenser and swirl were used to mix the solution then solution was allowed to stand for 30 minutes. Then for cooling 200 ml distilled H_2O and 10 ml conc. orthophosphoric acid was added. The contents of flask were titrated with 0.5 M $FeSO_4$ till the colour of the solution was tainted from violet blue to green.

Then Total organic carbon (TOC) was calculated by following formula:

$$\%\ Oxidizable\ Organic\ Carbon = \frac{(Vblank - Vsample)}{Wt} \times 0.3 \times M$$

$$\%Total\ Organic\ Carbon = 1.334 \times \%\ oxidizable\ Organic\ Carbon$$

Where

$$M = Molarityofferrousammoniumsulfatesolution\ (approx. 0.5\ M)$$

$$Vblank = Volumeofferrousammoniumsulfaterequiredtotitratetheblank$$

$$Vsample = Volumeofferrousammoniumsulfaterequiredtotitratethesample\ (ml)$$

$$Wt = weight\ of air\ dry\ soil\ (g)$$

$$1.334 = 3 \times 10 * -3 \times 100, where\ 3\ is\ the\ equivalentweight$$

3.8.5 Organic Matter

Organic matter was calculated through formula:

$$\%\ Organic\ Matter\ (w/w) = 1.724 \times \%\ Total\ Organic\ Carbon$$

3.8.6 Microbial Biomass Carbon (MBC)

Rapid microwave irradiation and extraction technique was used to calculate Microbial Biomass Carbon (Islam and Weil, 1988).

3.9 PLANT GROWTH PARAMETERS

The following growth parameters of *Solanum nigrum* were observed after harvesting.

3.9.1 Root Length (cm)

Meter rod was used to measure Root length.

3.9.2 Shoot length (cm)

Common meter rod was used to measure shoot length from the soil surface up to the tip of shoot.

3.9.3 Plant Biomass (Fresh and Dry weight)

Fresh and Dry weight was measured using an electrical balance.

3.10 HCHS ANALYSIS

3.10.1 HCHs Extraction from Soil

Soil samples were analyzed for hexachlorocyclohexane (HCHs) at the beginning and end of the pot trial.

1 g soil samples were transferred into 100 ml Teflon tubes then mixed with 5 ml of dichloromethane and each sample was extracted for 2 hr in water bath at temperature of 38°C. Then samples were centrifuged at 4000 r min^{-1} for 5 min to separate the supernatant from soil. The extract was eluted with 1 and 2 ml mix of n-hexane: dichloromethane (v/v 50:50) and the supernatant will the extract for HCH and then dried by sparging with N_2; Solid residues were re dissolved in 1 ml of acetonitrile (Li-hong *et al.*, 2006).

3.10.2 HCH Extraction from Plant Material

Pant material (root and shoot) were put through to water bath extractions for 20 min in ethyl acetate. Membrane filter was used to remove insoluble materials. The extract was conceded through silica gel packed column and the compounds on the silica gel column eluted with 250 ml of benzene. Elution was concentrated under reduced pressure, dried under nitrogen stream and then elute was re-dissolved in 1ml of acetonitrile for GC- MS analysis (Li-Hong *et al.*, 2006, Rhind et al., 2013).

3.11 GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

The Gas chromatography- Mass spectrometry (GC-MS) was used for the analysis of hexachlorocyclohexane. For HCH, the temperature will start at 70°C for 3 min; it will then ramped at 5°C/min to 250°C and held for1 min, will ramped to 300°C at 6C/min and was held for 6 min and finally was ramped to 325°C at 10C/min and was held for 5 min (Rhind *et al*., 2013). The standards of HCH mixture was run on GC- MS and retention times were optimized with individual HCH then samples were run on GC- MS.

3.12 STATISTICAL ANALYSIS

The data obtained were evaluated on excel sheets. The statistical analysis was carried out with SPSS. Other analysis including, comparison of means, was performed.

RESULTS AND DISCUSSION

4 RESULTS AND DISCUSSION

The results that have been obtained after conducting a thorough study, on evaluating the potential of *Solanum nigrum* to degrade HCHs in the presence and absence of Inoculate, through series of experiment are described in the following section.

4.1 SOIL PHYSICO-CHEMICAL ANALYSIS

Soil was collected from NARC to conduct experiment, after the experimental time soil was analysed for its physic-chemical properties. The result on soil properties as influenced by planting *Solanum nigrum* on spiked soil and inoculating it with bacterial strains in comparison to planting *Solanum nigrum* without bacterial strains on control soil for 90 days are presented and discussed as follows:

4.1.1 Soil Texture and Soil Moisture

The soil has a sandy clay loam texture with 16% Silt, 19.8% clay and 64.2% sand. The soil is brown in color with a moisture content of 35.89%. Soil physical properties are shown in table 4.1.

Table4.1: Soil Physical properties

Soil type	Sandy clay loam
Silt (%)	16
Clay (%)	19.8
Sand (%)	64.2
Color	Brown
Moisture (%)	35.89%

4.1.2 Soil pH

Plant type and organic matter of soil effects the pH of soil the most (kopittke *et al.*, 2004). pH of soil was measured before and at the end of experiment and it was cleared that it showed a decreasing trend as the treatment time swap. Figure 4.1 showing that pH of the soil before experiment was slightly alkaline having range of 7.9 to 8.2 while its range at the end of research was 6.5 to 7.8. Treatments having *Solanum nigrum* and inoculate showed greater variation while treatments with only inoculation showed minimum variation in pH ranges. Treatment contaminated with 10% HCHs and *Solanum nigrum* along with inoculate showed highest decreasing trend while control soil with inoculate represented lowest decreasing trend. As the experiment begins T8 had 7.9 pH and after 90 days it was 6.5 on the other hand T3 had 7.8 pH values at the end of treatment. pH values of all treatments have been shown in figure 4.1.

Decrease in pH value can be attributed to *Solanum nigrum* which releases specific organic acids thus decreasing the soil pH value. This study result coincides with another previous study in which pH of soil grown by *Solanum nigrum* was compared with S. lycopersicum and it was concluded that high organic acid secretion from *S. nigrum* lowers the pH value (Bao *et al.*, 2011). Fernandez *et al.*, 2008 resulted from a study based on HCHs effects on soil microbiological properties that the drop off in pH and the raise in amount of Cl_2 indicates that HCHs have been dissipated in spiked soil. Introduction of inoculate also resulted in lowering pH. The present study result is comparable to a study in which effect of legume inoculation was determined on soil pH. Inoculated plants can fix atmospheric nitrogen rather than relying on soil nitrogen this results in decrease of soil pH value (Kopittk *et al.*, 2004). Same result was reported in previous studies. This acidification is typically due to an increase in the net H+ excretion resulting from an increase in the cation/anion uptake ratio (Marschner and Römheld 1996).

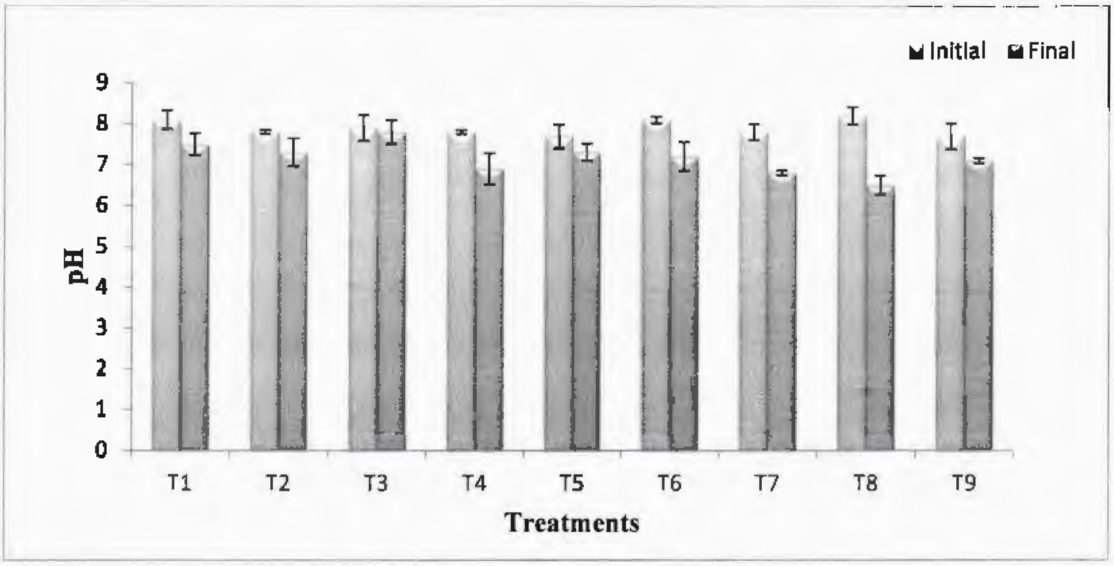


Figure 4.1 pH variations among different treatments in the soil samples after 90 days experiment. **T1**: control soil + *solanum nigrum*; **T2**: control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate. **T9**: 10% HCHs +inoculate

4.1.3 Organic Matter

Organic matter of different treatments has been represented in figure 4.2. It showed an increasing trend with the passage of experimental time. 10% HCHs spiked soil with *Solanum nigrum* in the presence of microbes (T8) represented higher organic matter 3.597%, at the start of experiment it was 1.39% while inoculated treatment on control soil (T3) showed decreased value from 0.93% to 0.79% in organic matter. All treatments in which plant was used showed relatively higher organic content while absence of contamination and inoculation resulted in lowering this organic matter. Some treatments showed reasonable organic matter contents such as T4, T5, T7 and T9 had 2.53%, 3.19%, 2.77% and 2.29% organic matter respectively (figure 4.2). Variation rate was different for all treatments. Treatment of control soil with inoculates showed an abrupt decrease in value. While all contaminated treatments with *solanum nigrum* represented slight change.

From the start of experiment to the end organic matter tend to increase but its percentage was different for different treatments. Higher concentration of organic matter may be endorsed to the presence of inoculated *Solanum nigrum* and contamination with HCHs. Decomposition of Plant roots and mineralization of HCHs by microbes increases the organic content of soil. Present work is in consistence with Leal et al., 2015 work, in which it was depicted that organic matter increased in crop cover soil as compared to bare soil. Treatments without contamination of HCHs depicted lower organic matter as compared to those having contamination and inoculated *Solanum nigrum*. This may be due to less mineralization and less microbial activity. Presence of inoculate also makes a part in increasing value of organic matter because it mineralizes the HCHs hence improving the carbon content of soil (spark *et al.*, 2002). Comparable results have been obtained by McMurry, 2000 and Clayden *et al.*, 2001.

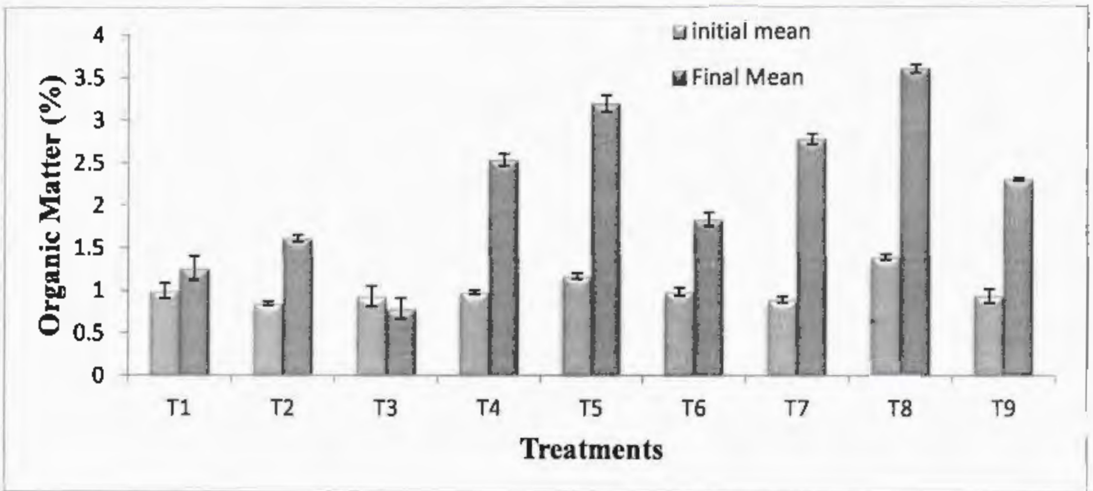


Figure 4.2 Organic matter of soil before and at end of experiment. **T1**: control soil + *solanum nigrum*; **T2**: control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate; **T9**: 10% HCHs +inoculate.

4.1.4 TOTAL ORGANIC CARBON

Results of the study showed that total organic carbon of treatments having HCHs and inoculated *Solanum nigrum* showed an increasing value from the start of experiment till the end. But these values didn't suffer great variation. Higher value 2.086% was observed at T8 in which 10% HCHs contamination was associated with *Solanum nigrum* and inoculate. It increased from 0.806% to 2.086%. While lowest 0.458% (initial concentration was 0.539%) was observed at T3 in which control soil amended with inoculate. All treatment's TOC values were with greater variation from the start to end of experiment. TOC of all treatments have been represented in figure 4.3. There was an abrupt change in treatments where inoculated *Solanum nigrum* was planted in contaminated soil.

Higher value of TOC is linked to higher organic matter and presence of HCHs which releases carbon into the soil by decomposition. Bacterial strains increase the rate of this decomposition resulting in higher concentration of TOC in the soil. Various previous studies confirmed the result of present study (Malik *et al.*, 2013, Akpan and Udoh, 2013). Mishra, K. and his colleagues found in a study that total organic carbon was positively associated with HCH of soil. Control soil amended with inoculate had lowest TOC because this had lowest organic matter and not contaminated with HCHs so little carbon is present in it that was used by microbes as their energy source.

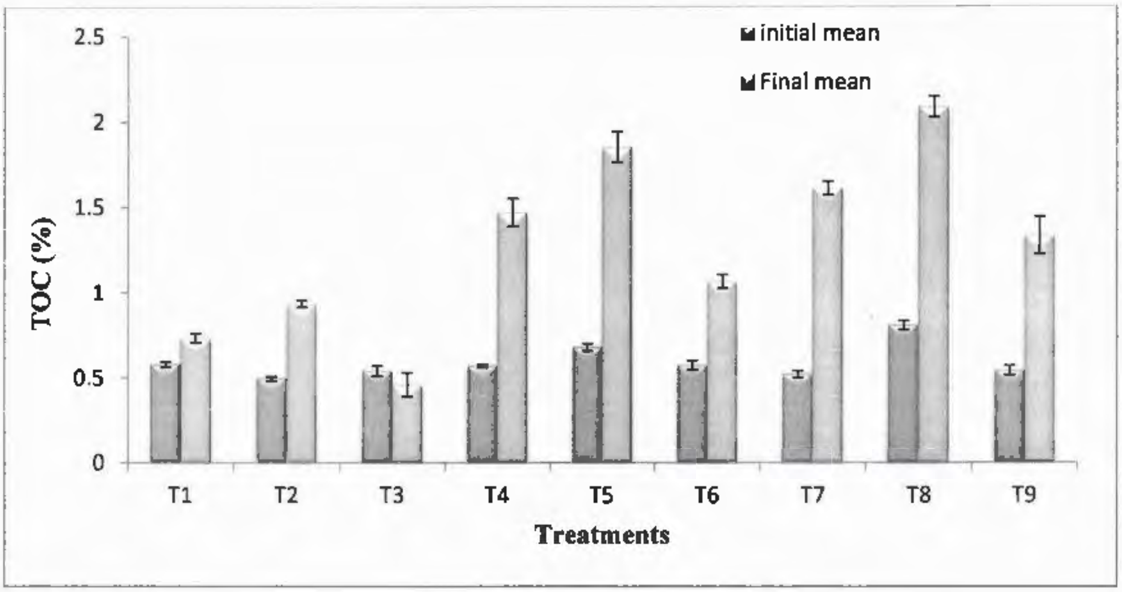


Figure 4.3 Change in TOC of soil after experimental time. T1: control soil + *solanum nigrum*; **T2:** control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4:** 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate; **T9**: 10% HCHs +inoculate.

4.1.5 Electrical Conductivity

Electrical conductivity decreased with increasing experimental time. At the beginning it was higher while with the passage of time it goes on decreasing. Treatments that were inoculated with microbes had higher electrical conductivity as compared to those that have only *Solanum nigrum*. Electrical conductivity values ranged from 81.33μs/cm to 93.53μs/cm represented in figure 4.4. Higher (93.53 μs/cm, initially it was 105.6 μs/cm) being observed at T8 (Inoculated *Solanum nigum* + 10% HCH) and lowest (81.33 μs/cm, initially it was 96.17 μs/cm) being observed at T1 (Control soil + *Solanum nigrum*). Other treatments T2, T3, T4, T5, T6, T7 and T9 showed EC values as 86.7μs/cm, 84.95μs/cm, 87.23μs/cm, 89.11μs/cm, 88.2μs/cm, 88.95μs/cm and 89.77μs/cm respectively.

Electrical conductivity may be credited to activities of microbes that decomposed and mineralized organic matter and HCHs and used it as a carbon source which increased the ions in soil thus enhancing the electrical conductivity of soil. But in the present study opposite of it has been observed. One explanation for this discrepancy might be due to taking up of ions, produced after mineralization, by plants. Similar trend was observed by Nisa *et al*., 2016 in which effect of diesel contamination was evaluated on physic chemical characteristics of soil and growth of vetiver grass. Inoculated treatments showed comparatively higher electrical conductivity as compared to uninoculated. Those treatments in which only *Solanum nigrum* was planted in control soil represented lowest electrical conductivity due to less microbial activity and low organic matter.

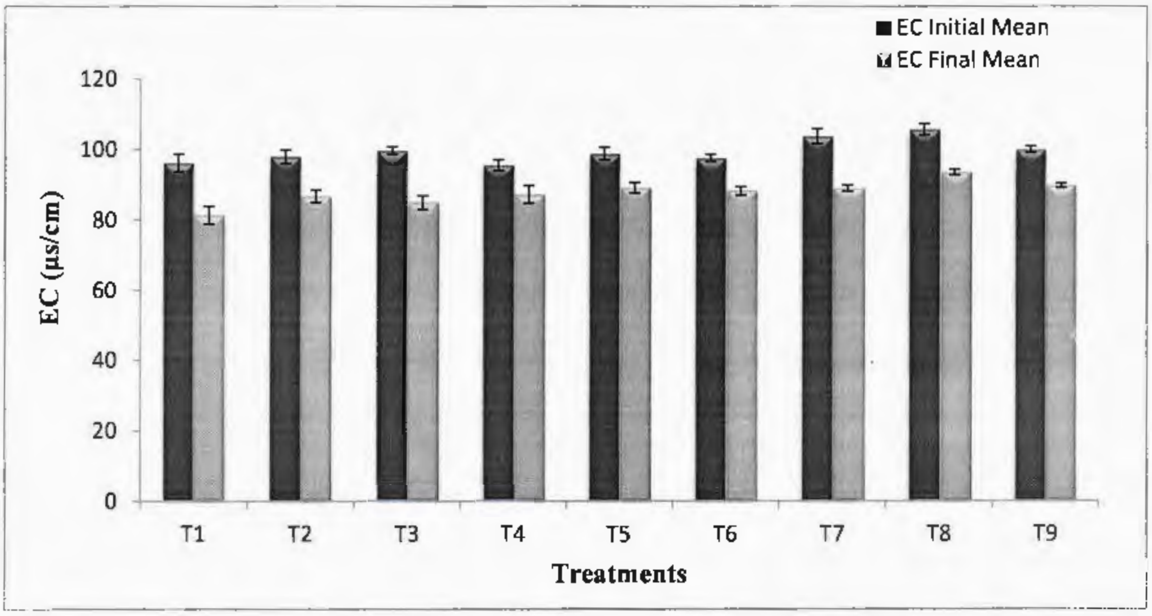


Figure 4.4 EC values of soil before and at the end of experiment. T1: control soil + *solanum nigrum*; **T2**: control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate; **T9**: 10% HCHs +inoculate

4.1.6 Microbial Biomass carbon

The overall parameters of experiment indicated the microbial development and metabolic activity in the soil, such as microbial biomass. Inoculation of microbes to *Solanum nigrum* in contaminated soil had higher concentration of microbial biomass as compared to those having only inoculation to *Solanum nigrum*. The treatment in which inoculated *Solanum nigrum* was planted in 10% HCHs contamination (T8) showed the peak value (1.78%) of microbial biomass while control soil with *Solanum nigrum* (T1) had lowest value (0.52%). T2, T5, T6 and T9 had 0.96%, 1.52%, 1.35%, and 1.32% significant MBC respectively (figure 4.5).

Promotion of microbial biomass in treatments with HCHs contamination and inoculated *Solanum nigrum* was due to availability of HCHs which serve as energy source for microbes and presence of plant which provide essential nutrients (photosynthesis based organic compounds) to microbes thus improved their number. Various studies represented the same result in which microbial growth was much higher in the presence of plant as compared to only microbes in soil (Kumar *et al.*, 1996). Some microbes are sensitive, cannot comply with contaminant and dies as exposed to it while other shows an increasing trend in their number as exposed to contaminant. The reason is they uses contaminant as their food source, secondly organic components released from dead microorganisms are also used as their power source and thirdly because of dead of some microbe's competition reduces that results in boost up of compatible MBC (Das and Mukherjee, 2000). In general microbial community flourished with introduction of Pesticides (Rodrigueez and Toranzos, 2003; Kid *et al.*, 2008; Wang *et al.*, 2008). Perucci *et al.*, 1994 reported that introduction of gemma hexachlorocyclohexane improved the microbial biomass carbon of soil. The microbes that combat contaminant can degrade that contaminant (Hernandez and Salinas, 2010). The present study resulted some microbial strains that can sustained with HCHs, similar results were obtained by Hussain *et al.*,2009 for different contaminants.

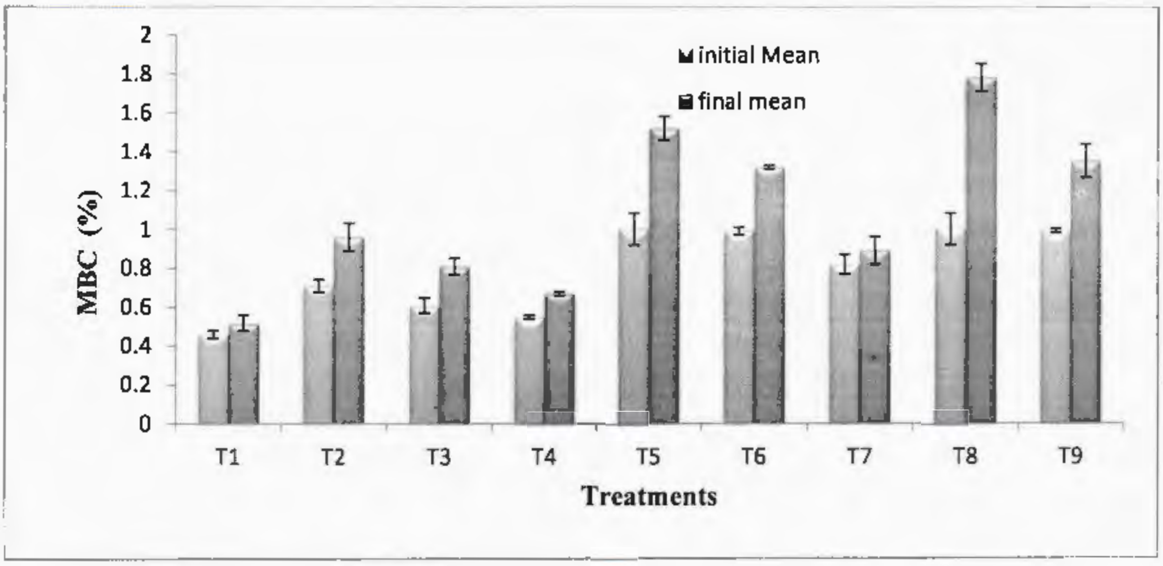


Figure 4.5 Fluctuations in MBC of soil at the start and end of experimental time. T1: control soil + *solanum nigrum*; **T2**: control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate; **T9**: 10% HCHs +inoculate.

4.2 ISOLATION OF RHIZOSPHERIC BACTERIA

Hexachlorocyclohexane (HCH) degrading bacteria were isolated from the rhizosphere of plants growing on HCH contaminated soil. The most competent rhizobacteria were screened based on their efficiency to consume HCH as the only source of carbon. The most efficient rhizobacteria were further evaluated for their ability to support the development of *Solanum nigrum* under greenhouse circumstances. The results of these experiments are discussed below.

4.2.1 Isolation of HCHs Degrading Bacteria

Various strains of bacteria were secluded from the rhizospheric soil based on their ability to use HCHs as the major source of carbon. 107 rhizobacterial isolates were chosen at the start, 82 isolates manifested productive growth on the agar medium containing 50 mg HCH. Of those 23% of the rhizobacteria were competent of mounting energetically on ACC medium.

4.2.2 Screening Rhizobacteria for Plant Growth Promoting Activity Under Controlled Conditions

Laboratory experiments were conducted in petri plates for selection of the bacterial isolates/ strains that efficiently improve the growth activities of *Solanum nigrum*.

4.2.3 Petri Plate Experiment

Petri plate experiment was conducted in controlled conditions to evaluate the effect of rhizobacteria on growth performance of *Solanum nigrum*. The outcome discloses that development of seedlings of inoculated *Solanum nigrum* appreciably improved as compared to uninoculated *Solanum nigrum*. Effect of different strains on seedling of *Solanum nigrum* has been shown in table 4.2. Observation of outcome shows that seedling of *Solanum nigrum* respond differently to different strains. No improvement was observed in some isolates while others improved the root length of *Solanum nigrum* in effective manner, highest (6.9 cm) being observed with SN47.

Table 4.2: Effect of Rhizobacterial Inoculation on Root Growth of Mako (*Solanum nigrum*) in Petri Plate Experiment under Axenic Conditions (Average of four replications)

Codes assigned to bacterial isolates	Root Length (cm)	Dry Root Weight (g)
	Mean±SE[a]	Mean±SE
SN4	6.1±0.93	0.038±0.018
SN9	6.8±0.41	0.017±0.001
SN13	6.4±0.51	0.014±0.001
SN27	4.81±0.34	0.037±0.014
SN33	5.84±0.67	0.026±0.001
SN39	5.58±0.91	0.021±0.008
SN47	6.9±0.88	0.048±0.019
SN54	5.85±0.64	0.016±0.001
SN62	5.52±0.98	0.017±0.002
SN66	5.06±0.97	0.020±0.002
SN71	5.69±0.22	0.026±0.001
SN80	6.18±1.03	0.017±0.004

[a]Standard error

4.2.4 Effect of Inoculation on *Solanum Nigrum* Under Soil Condition

Selected PGPR were tested for their efficiencies in improving *Solanum nigrum* biomass in HCHs contaminated soil, by Pot experiments under greenhouse conditions.

4.2.5 Biomass and Root Shoot Length

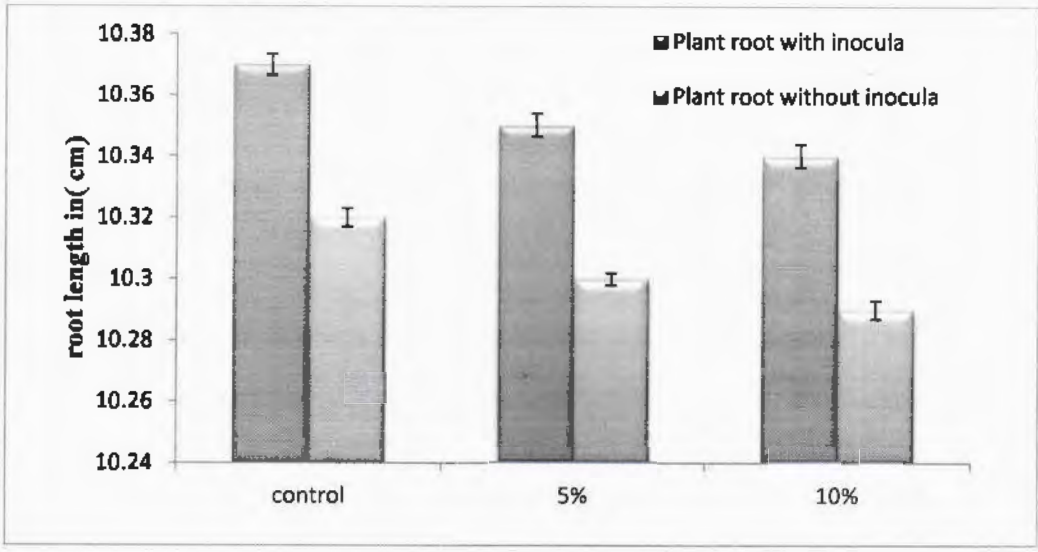
Our data indicated that plant biomass improved significantly with the passage of time, but its trend was different for different treatments. *Solanum nigrum* grown on contaminated soil improved biomass and root, shoot length but at a slow rate while that grown on control soil resulted at high biomass and root, shoot length. T7 (10% HCHs with *Solanum nigrum*) showed very slow growth rate while T2 (control soil with SN47 inoculated *Solanum nigrum*) had highest growth. Presence of inoculate increased biomass by increasing root length and improving plant growth. Initially average root length of all plants was 5cm while average shoot length was 11cm. After harvesting their root and shoot length and biomass increased at a very fast rate. Average root length of all treatments was 10cm, their shoot lengths were maximum for T2, T5 and T8 35.66cm, 30.66cm, and 28cm; their fresh weights were 7.9g, 7.0g, and 6.3g and their dry weights were 1.327g, 1.14g, and 0.99g respectively revealed in figure 4.6 a, b, c and d.

Results demonstrated that inoculation with rhizobacterial strains SN47 significantly improved the plant growth. Because symbiosis relationship between plant and rhizobacterial strains can amplify the antioxidant activities to relieve oxidative damage and enhance the growth of plant under stress. Result like present study was reported by Long *et al.*, 2014 who used rhizobacteria (*Pseudomonas brassicacearum)* to inoculate *Sonalum nigrum* and compared its biomass with *Solanum nigrum* grown in the absence of rhizobacteria and concluded that inoculation helps in plant growth. Tan *et al.*, 2015 also found that colonization of *Solanum photeinocarpum* with *Glomus versiforme* significantly improved the plant biomass. Abhilash *et al.*, 2011 assesed the growth of *W. somnifera* in control soil, and lindane spiked soil with and without microbial inoculation and concluded that *W. somnifera* enhanced the growth rate in the presence of microbial strain.

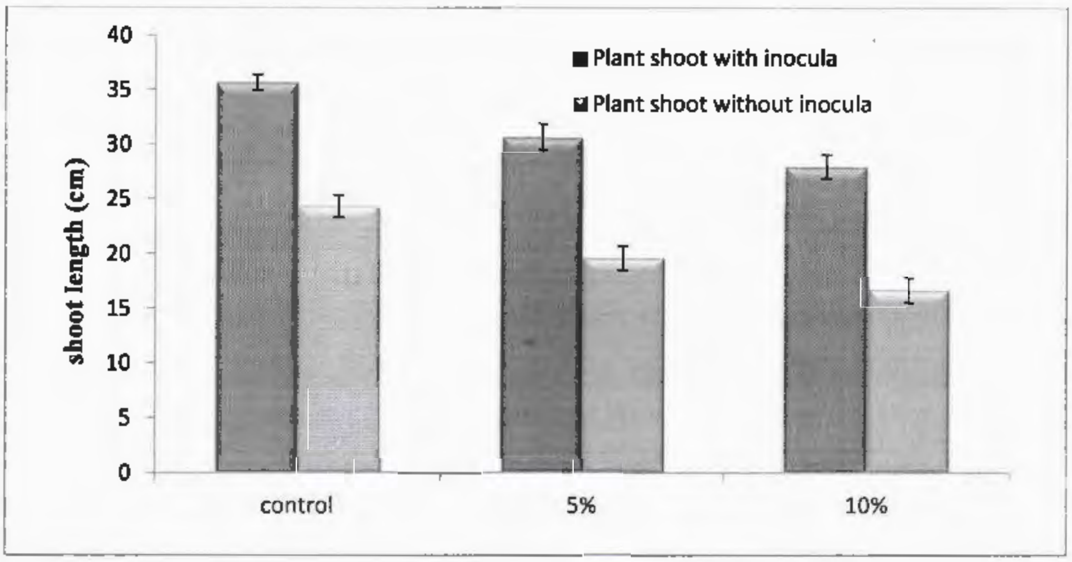
The above result had cleared that application of pesticide had a negative effect on plant growth because all plants that were grown on contaminated soil showed lower growth as compared to those on control soil, but the effect was not that much higher illustrating that

Solanum nigrum can tolerate HCHs. Biomass of *Solanum nigrum* decreased in all treatments contaminated with HCHs. The result agreed with Chuluun *et al*., 2009, in that reduction in plant biomass was observed after spiking the soil with organochlorine pesticide as compared to control soil. Kidd *et al.*, 2008 observed a small reduction in Cytisus biomass after planting it on HCH contaminated soil as compared to control soil. Previous study conducted by Abhilash *et al*., 2013 revealed that there was no considerable discrepancy in growth of Jatropha plant when exposed to low level of HCHs; however, the development was compacted to some extent as the HCHs application and contact time prolonged.

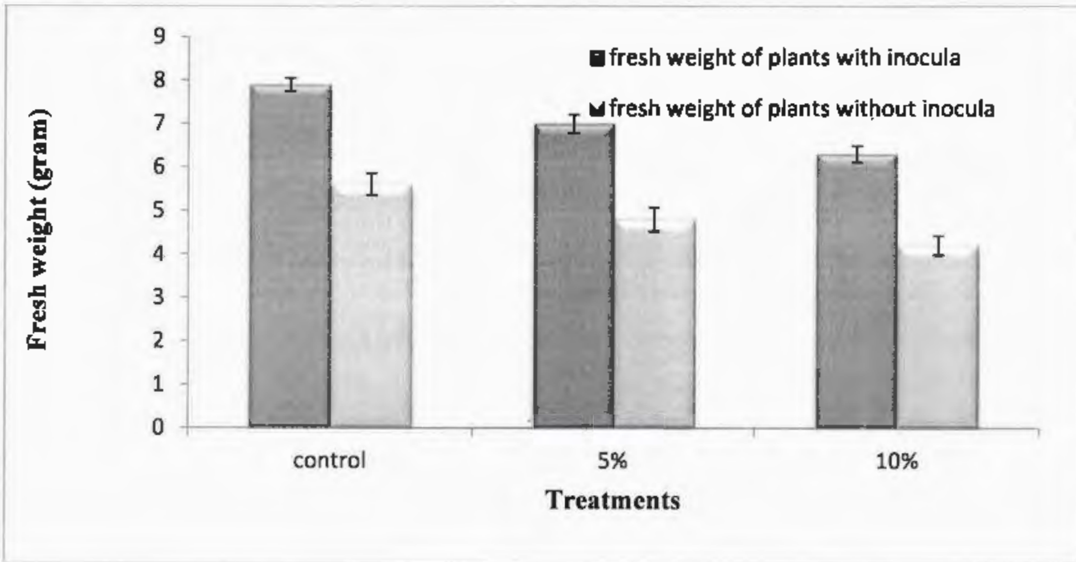
(a)


10.38
10.36
10.34
10.32
10.3
10.28
10.26
10.24
root length in(cm)
Plant root with inocula
Plant root without inocula
control
5%
10%

(b)


40
35
30
25
20
15
10
5
0
shoot length (cm)
Plant shoot with inocula
Plant shoot without inocula
control
5%
10%

(C)


9
8
7
6
5
4
3
2
1
0
Fresh weight (gram)
fresh weight of plants with inocula
fresh weight of plants without inocula
control
5%
10%
Treatments

(d)

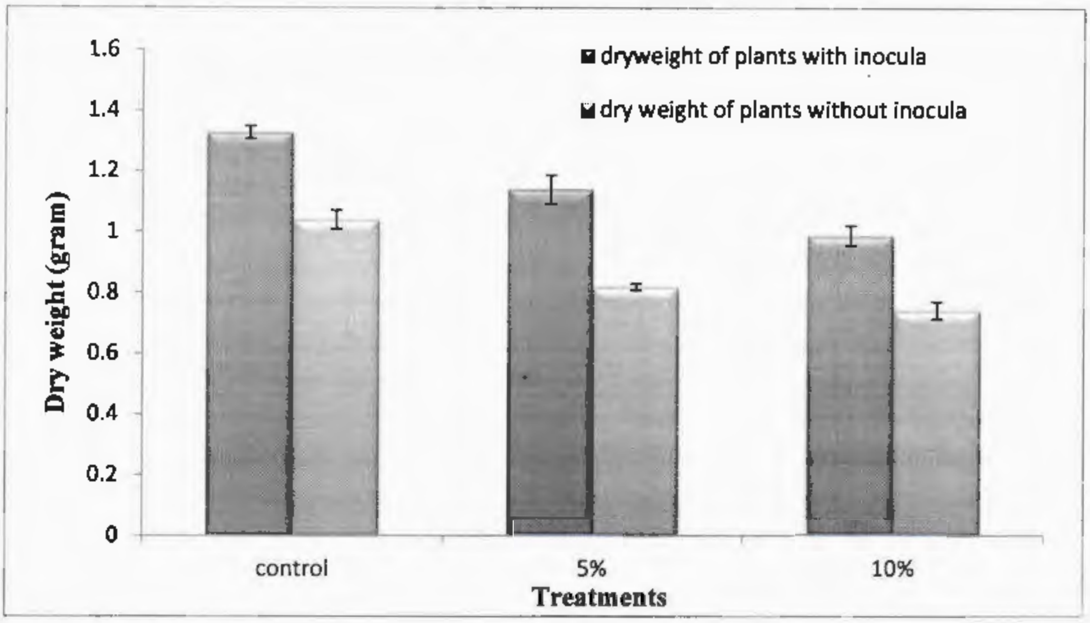


Figure 4.6Effect of HCHs contaminated soil on **(a) Root length** of *Solanum nigrum* with and without inoculate; **(b) Shoot length** of *Solanum nigrum* with and without inoculate; **(c) fresh weight** of *Solanum nigrum* with and without inoculate; **(d) Dry weigth** of *Solanum nigrum* with and without contaminated soil. **T1**: control soil + *solanum nigrum*; **T2:** control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate; **T6**: 5% HCHs + inoculate; **T7**: 10% HCHs + *Solanum nigrum*; **T8**: 10%HCHS + *Solanum nigrum* + inoculate;**T9**: 10% HCHs +inoculate.

4.3 HCHs ANALYSIS

HCHs concentration was deliberated in soil and *Solanum nigrum* before and after harvesting the plants. The samples were analysed through GC – MS. Results obtained are discussed below:

4.3.1 HCHs Extraction from Soil and *Solanum nigrum*

When the *Solanum nigrum* and bacterial isolates were grown on contaminated soil there was noticeable effect on HCHs degradation in all treatments. A drastic decrease in HCHs concentration in soil was observed in T8. In which *Solanum nigrum* was grown along with inoculate on 10% HCHs contaminated soil. T4 (5% HCHs + *Solanum nigrum*) represented lowest degradation. Detection limit of the HCHs in soil after harvesting plants ranged from 0.0013 to 7.733 for different concentration studied. T4, T5, T6, T7, T8, T9 had 4.2, 4, 3.9, 9, 9.3 and 9.1 mg/kg HCHs concentration respectively in soil before the experiment while at the end of 90 days this amount reduced to 3.3, 1.733, 2.533, 7.733, 3.9 and 6.1 mg/kg respectively. Figure 4.7 representing the residual HCHs for all treatments in spiked soil. HCHs concentration in *Solanum nigrum* at T5, T7 and T8 was 1.233, 2.1333 and 2.6667 respectively, shown in figure 4.8. Maximum was accumulated in *Solanum nigrum* grown in 10% HCHs contaminated soil along with SN47 strain.

HCHs accumulation in plants grown in control soil represented no significant difference even in presence of inoculate but on contaminated soil *Solanum nigrum* showed higher HCHs in its tissues in presence of inoculate.

The reduction in the HCHs concentration in soil after harvesting the *Solanum nigrum* could be attributed to secretions by *Solanum nigrum* and bacterial strain SN47 which improved the plant growth, helped in reducing oxidative stress in plant and used HCHs as food source. The physico–*chemical* properties of the soil have also been found to have a significant effect on the degradation of contaminant. pH of the soil was moderate for degradation of HCHs. Previous studies reported maximum degradation at approximately neutral pH. Gosh and Singh, 2005 reported that contaminant degradation in soils is principally controlled by pH, CEP, OC, TOC of soil.

HCHs degradation by *Solanum nigrum* showed the higher potential of this plant for toxic substances. Hyper accumulation of *S. nigrum* without showing toxicity symptoms possibly ascribed to sound extend detoxification system based on abstraction of contaminant in vacuole, by fastening them on suitable functional groups i.e. organic acids, proteins and peptides in the presence of enzymes that work efficiently even in presence of higher concentration of toxic substances (Cui *et al.*, 2007). *S. nigrum* secretes alkaloidal contents especially solanine, which is a neurotoxic glycoalkaloid (Abbas *et al.*, 1998). Solanine is toxic even in small quantity that's why it is the plant's major natural defense (Lin *et al.*, 2007). Recently it is reported by Ravi *et al.*,2009 that the berry of *S. nigrum* contains four steroidal alkaloid glycosides namely, solamargine, solasonine, α and β solanigrine. The accumulated alkaloids are involved in defensive mechanism against stress in the plant (Gogoi and Islam, 2012). Puhui *et al.*, 2016 represented *Solanum nigrum* as hyperaccumulator for Cd.

HCHs degradation by SN47 was comparatively higher to *Solanum nigrum*. Dissipation rate by microbes may be explained through growth profile. MBC content in different HCHs contaminated treatments tend to increase. This resulted reduction in HCHs concentration. Previous studies confirmed the present study's result. Abhilash *et al.*, 2011 who used isolated microbial strains from HCHs contaminated soil to degrade 5, 50 and 100mg/kg concentration of HCHs. The resulted residual HCHs amount in contaminated soil treatments were condensed to 0, 41 and 33 percent respectively. Similar result was obtained by Nagpal *et al.*, 2008 did a study on degradation of HCHs by white rot fungus with different nutrient conditions. He showed that stress conditions i.e. less availability of carbon, nitrogen and addition of veratryl alcohol (secondary metabolite of white rot fungus) results in production of oxidative enzymes which in turn speeds up the degradation process of HCHs by White Rot Fungus. A study was carried out by Benimeli *et al.*, 2008in which soil was spiked with HCHs in concentration of 100, 150, 200 and 300 g/kg, was treated with streptomyces M7 specie. The result showed residual concentration of HCHs in soil as 29.1%, 78.03%, 38.81% and 14.42% respectively. That ensures high potential of this specie for HCH's degradation. Another biodegrader, named autofluorescent Pseuomonas nitroreducens, have been developed by dehydrochlorinase activity which effectively degrades gemma hexachlorocyclohexane (Zhang *et al.*, 2010).

These results suggested that inoculation to *Solanum nigrum* resulted higher accumulation in plant tissues and lowest residual HCHs in spiked soil. Presence of microbes improves the plant growth and reduces the oxidative damage (due to presence of contaminant) of plant by increasing antioxidant acitivities. In addition to this, microbes utilize the HCHs as their food source, and secrete enzymes that mineralize the HCHs resulting further decrease in residual HCHs in spiked soil. In reaction plants provides biological active soil sites which improves microbial activity and enhances contaminant bioavailability (Gerhardt *et al.*, 2009). Previous study confirmed present result as a research addressed by Marques *et al.*, 2006 explained the higher detoxification level of *S. nigrum* in presence of *G. claroideum or G. intraradices*. The study result demonstrated*S. nigrum* as extractor and accumulator,with no apparent sign of toxicity, to higher amount of Zn that was concentrated in its parts as it was revealed to site polluted by Zinc.Addition of inoculate results in manufacturing of thiols (gulathione), by triggering the plant's natural ability to fight against any attack (Schutzendubel and Andrea, 2002). Theses thiols are produced by phytochelatin synthase enzyme. Which removes contaminant from the soil by sticking it and making it available for plant uptake (Cobbet and Goldsbrought, 2001).Khan *et al.*, 2015 did a study in which*Solanum nigrum*was used alone and inoculated with RSC-14 in Cd contaminated soil. RSC-14 secreted plant growth-promoting phytohormones such as indole-3-acetic acid, which improved the Plant roots, shoots and biomass significantly. The findings supported the beneficial uses of *Serratia* sp. RSC-14 in improving the phytoextraction abilities of *S. nigrum* plants in Cd contamination. Another study in comparison to present study conducted by Abhilash *et al.*, 2011 indicated that supplementation of plant *(Withania somnifera)* with bacterial strains effectively condensed the destructive impacts of HCHs (lindane) to plant and supported plant biomass. The plants that were inoculated with bacteria showed increment in buildup of HCHs (29.91 µg/g dry matter) in their respective tissues as compared to non-inoculated plants (23.22 µg/g dry matter). Result of present study also represented significant accumulation of HCHs in *Solanum nigrum* that were inoculated with bacterial strain SN47 proving this combination to be effective for remediating HCHs from environment.

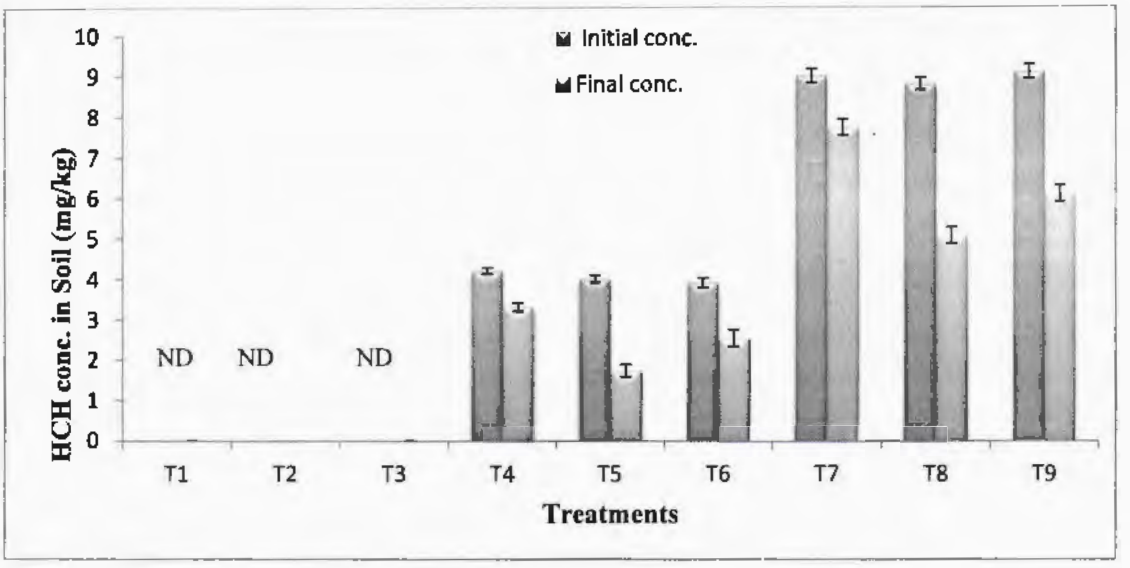


Figure 4.7 Concentration of HCHs in soil before and after harvesting the *Solanum nigrum*. **T1**: control soil + *solanum nigrum*; **T2:** control soil + *Solanum nigrum* + inoculate; **T3**: control soil + inoculate; **T4**: 5% HCHs + *Solanum nigrum;***T5**: 5% HCHs + *Solanum nigrum* + inoculate;**T6**: 5% HCHs + inoculate;T7: 10% HCHs + *Solanum nigrum*;**T8**: 10%HCHS + *Solanum nigrum* + inoculate; **T9**: 10% HCHs + inoculate.

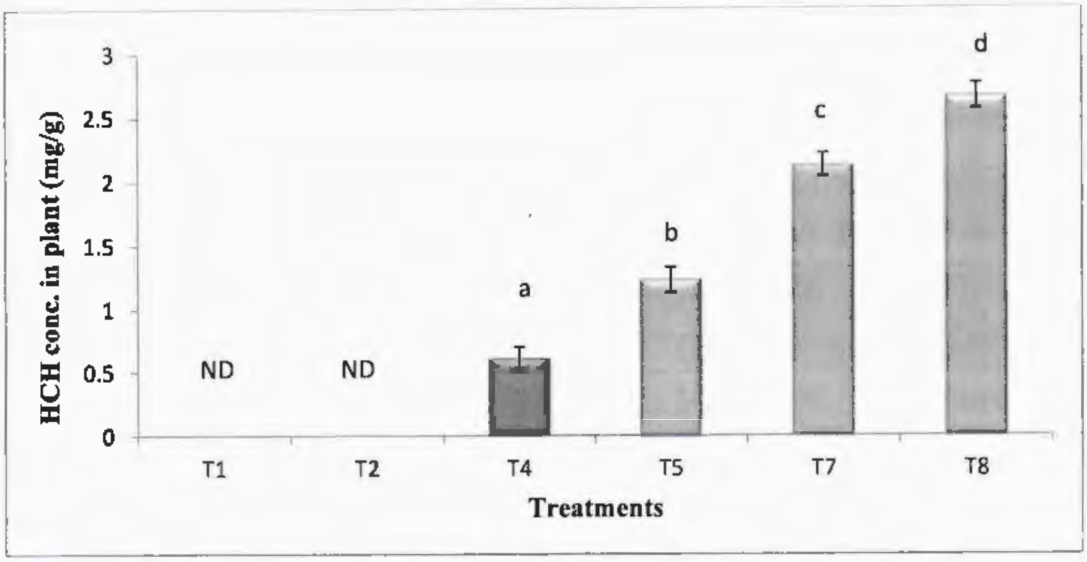


Figure 4.8 Concentration of HCHs in *Solanum nigrum* harvesting after 90 days.

T1: control soil + *solanum nigrum*; **T2:** control soil + *Solanum nigrum* + inoculate; **T4**: 5% HCHs + *Solanum nigrum;* **T5**: 5% HCHs + *Solanum nigrum* + inoculate;**T7**: 10% HCHs + *Solanum nigrum*;**T8**: 10%HCHS + *Solanum nigrum* + inoculate.

CONCLUSION AND RECOMMENDATIONS

5 CONCLUSION AND RECOMMENDATIONS

The food requirements of present generation need improved and advanced technologies in agriculture. But these technologies should not be at the cost of environment. Human beings are so smart, for their own advantage, they have developed some agrochemicals that have effectiveness on some insects. Of those agrochemicals pesticides are foremost mean of controlling disease-causing agents because these are economically affordable, convenient to use and are fruitful. But some pesticides are POPs, they bioaccumulate in environment posing risk to human health and quality of environment. Humans should adapt to earth environment, which he depends to survive, and abide by the native laws, in this regard he can lead nourishing and viable life. One of the pesticides is Hexachlorocyclohexane, being used as insecticide. It accumulates in environment and affects its quality. Appreciable attempts have been made to developed technologies to purify contaminated environment back to its original form, one sustainable solution (that includes social, environmental and economic aspects of life) to remove this contaminant from environment is rhizoremediation. In which effected sites are refurbish to their pure variety, by using a symbiosis relationship between plant and their rhizospheric microbes, to enable them to persue their services. So, present study was conducted to identify HCHs degrading Plant and microbes.

. On a concluding note outcome of study include that *Solanum nigrum* is best for phytoremediation of HCHs but its inoculation with SN47 microbes presents it to be promising and effective remover of HCHs from contaminated soil releasing no adverse impact on environment. HCHs accumulation in *Solanum nigrum* didn't show any toxic symptoms showing higher tolerance of this plant for HCHs contamination. Inoculation to *Solanum nigrum*with SN47 showed highest dissipation rate of HCHs as compared to only *Solanum nigrum*. Degradation rate was in the order of *Solanum nigrum* + SN47 strains > SN47 Strain >*Solanum nigrum*. Physicochemical properties of soil also contributed in degradation potential of HCHs by *Solanum nigrum* and SN47 strains.

The rhizoremediation technology developed for the bioremediation of HCHs in *Solanum nigrum* rhizosphere by developed microbial consortium would be applicable for bioremediation of pesticide contaminated sites, for the treatment of pesticide waste or effluents generated through pesticide manufacturing and formulation units and from their agricultural and domestic usages. This is socially acceptable, environmentally viable and economically feasible remediation technique.

Study has proved that *Solanum nigrum* can flourish in contaminated soil but suitable agro practices can improve its production. Nutrients should be added to boost Microbial Biomass and enhance plant growth further improving the degradation rate. This study enriches the diversity of HCH degraders.

Although present study focused on single species of plant and microbe, it can also be used to identify other species of plant and microbes that can prove their selves as best for removing pollutants from the environment. So, in this way study of other species of microbes, which can flourish and colonize in the roots of plants, and plants which can be excellent tolerant of toxic substances, can defer competent approaches for management of environment. Those enthralling approaches can develop as well as extend remediation by microorganisms as a universally acknowledged approach.

Study of tolerance and adaptation of respective non-cultivated crop can help in betterment of cultivated crops by giving information about their sustaining requirements. Present study proved *Solanum nigrum* as high tolerant plant so; its genes can be transplanted to other species of same genera or different to make them best for phytoremediation.

REFERENCES

REFERENCES

Abhilash, P.C., Vijgen, Y. F. L., Lal, M., Nandita, S., Mohammad, Y., Chongguo, T., Andreas, S., Roland, W. 2011. *Environmental Science and Pollution Research.* **18**(2):152–162.

Abhilash, P. C., Jamil, S., Singh, V., Singh, A., Singh, N. and Srivastava, S. C. 2008. Occurrence and distribution of hexachlorocyclohexane isomers in vegetation samples from a contaminated area. *Chemosphere,***72**:79-86.

Abhilash, P. C., Singh, B., Srivastava, P., Schaeffer, A. and Singh, N. 2013. Remediation of lindane by Jatropha curcas L: Utilization ofmultipurpose species for rhizoremediation. *Biomass and bioenergy,***51**: 18 9—1 9 3.

Aftab, T., Ahmed, D., Saud, H., Yusaf, S., Ahmad, M. A. and Afzal. 2007. Evaluation suspected chronic pesticide poisoning among residents near agriculture fields. *Biomedica,***23**: 1553-1560.

Akpan, G. U. and Udoh, B. T. 2013. Evaluation of Some Properties of Soils Affected by Diesel Oil Pollution in Uyo, Niger Delta Area, Nigeria. *Journal of Biology, Agriculture and Healthcare.***3** (**8**): 2224-3208.

Anderson, J. M. and Ingram, J.S. I. 1993. Tropical soil biology & fertility – *a handbook of methods, CAB International.*

Babu, G. S., Hans, R. K., Singh, J., Viswanathan, P. N. and Joshi, P. C. 2001. Effect of lindane on the growth and metabolic activities of cyanobacteria. *Ecotoxicol. Environ. Saf,* **48**:219-221.

Bala, A., Roy, A., Behura, N., Hess, D. and Das, S. 2013. Insight to the mode of action of Allium sativum leaf agglutinin (ASAL) expressing in T3 rice lines on brown planthopper. *Am J of Plant Sci.* 4: 400-407.

Barber, J. L., Sweetman, A.J., van, W. D., Jones, K.C., 2005. Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *Science of the Total Environment,* **349**: 1-44.

Basanta, K. D. and Mukherjee, S. C. 2000. A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *Veterinarski arhiv,70*(4): 169-180.

Becerra, C., Prieto, A., Kidd, P.S. 2013. Phytoremediation of hexachlorocyclohexane (HCH)-contaminated soils using Cytisusstriatus and bacterial inoculants in soils with distinct organic matter content Plant Soil. **362**: 247.

Biancon, D., Paolis, M. R., Agnello, M. C. D., Pietrini,F. Zacchini, M. C. Polcaroa, E. D., Paris, P., Spinab, S. and Massacci, A. 2015. Field-scale rhyzoremediation of a contaminated soil with hexachlorocyclohexane (HCH) isomers: the potential of poplars for environmental restoration and economical sustainability. *Handbook of Phytoremediation*. 4(1): 783-794.

Bidlan, R., Afsa,r M., Manonmani, H. K. 2004. Bioremediation of HCH-contaminated soil: elimination of inhibitory effects of the insecticide on radish and green gram seed germination. *Chemosphere*,**56**:803-811.

Boltner, D., Godoy, P., Muoz-Rojas, J., Duque, E., Moreno-Morillas, S., Sanchez, L. and Ramos, J. L. 2007. Rhizoremediation of lindane by rootcolonizing*Sphingomonas*. *Microb. Biotechnol*, **1**:87–93.

Calamari, D., Bacci, E., Focardi, S., Gagg,i C., Morosini, M. and Vighi, M. 1995. Role of plant biomass in the global environmental partitioning of chlorinated hydrocarbons. *Environmental Science & Technology*, **25**(8): 1489–1495.

Chen, S. K., Edwards, C. A., Subler, S. 2001. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biol Biochem*,**33**:1971–1980.

Chuluun, B., Iamchaturapatr, J. and Rhee, J. S. 2009. Phytoremediation of Organophosphorus and Organochlorine Pesticides by *Acorus gramineus*. *Environ. Eng. Res*,**14**(4): 226-236.

Clayden, J. and Greeves, N. 2001. Organic Chemistry.*Oxford University Press*, 524–525.

Cobbett, C., Goldsbrough, P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology*, **54**: 159–182.

Das, A. C., Mukherjee, D. 2000. Soil application of insecticides influences microorganisms and plant nutrients. *Appl Soil Ecol,* **14**:55–62.

Davis, L. C., CastroDiaz, S., Zhang, Q., and Erickson, L. E. 2002. Benefits of vegetation for soils with organic contaminants. Crit. Rev. Plant Sci. **21**(5): 457–491.

Dietmar, B., Patricia, G., Jesús, M. R., Estrella, D., Silvia, M. M., Lourdes, S. and Ramos, J. L.2008. Rhizoremediation of lindane by root-colonizing Sphingomonas. *MicrobBiotechnol.* **1**(1): 87–93.

Donald, D. B., Block, H., Wood, J. 1997. Role of ground water on hexachlorocyclohexane (lindane) detections in surface water in western Canada. *Environ. Toxicol. Chem.,* **16**:1867-1872.

Elcy, C. D. and Kunhi, A. M. 2010. Substantially enhanced degradation of hexachlorocyclohexane isomers by amicrobial consortium on acclimation. *J Agric Food Chem,* **58**:1046–1054.

Eqani, S. M. A. S., Riffat, N. M. and Ashiq, M. 2010. The level and distribution of selected organochlorine pesticides in sediments from River Chenab, Pakistan. *Environ Geochem Health.*

Erkmen, B. and Kolankaya, D.2006. Determination of organochlorine pesticide residues in water, sediments and fish samples from the Meric Delta Turkey. *Int. J. Environ. Anal. Chem.,* **86:**161–169.

Fernandez, P., Poisa-Beiro, A., Acea, M. J. 2008. Effect of hexachlorocyclohexane isomers on some soil microbiological properties. *EDAFOLO gía.,* **15**: 155-168.

Fulekar, M. H. 2014. Rhizosphere Bioremediation of Pesticides by microbial consortium and potential microorganism. *Int.J.Curr.Microbiol.App.Sci.* **3**(7): 235-248.

Gangwar, M. P., Lakhchaura, B. D. and Sud, S. C.1979. In vitrostudies on the effect of certain pesticides on rumen microorganisms of the Buffalo. *Indian Vet. J.,* **56**:1001–1006.

Gerhardt, K. E., Huang, X. D., Glick, B.R., Greenberg, B.M., 2009. Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Sci.*, **176**: 20–30.

Ghaffar, A., Masaaki, T. and Mashiatullah, A. 2014. Two-Phase Dechlorination/Detoxification of Lindane (Hexachlorocyclohexane). *Advances in Environmental Chemistry*, **3**: 222–245.

Girish, K. and Kunhi, M. A. 2013. Microbial degradation of gamma-hexachlorocyclohexane (lindane). *African Journal of Microbiology Research.* 7(17):1635-1643.

Gogoi, P. and Islam, M. 2012. Phytochemical screening of *Solanum nigrum* L. and *S. myriacanthus* Dunal from districts of upper Assam, India. J. Pharm., **2**: 455-459.

Gonzalez, M. I., Campos, M. J. and Ramos, J. L. 2005. Analysis of Pseudomonas putida KT2440 gene expression in the maize rhizosphere: in vivo expression technology capture and identification of root-activated promoters. *J Bacterial.* **187**: 4033–4041.

Horstmann, M. and McLachlan, M. S. 1996. Evidence of a novel mechanism of semivolatile organic compound deposition in coniferous forests. *Environmental Science & Technology*. **30**(5): 1794–1796.

Hernandez, M. L. and Sanchez-Salinas, E. 2010. Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. *Rev. Int. Contam. Ambient*, **26**: 27-38.

http://www.finance.gov.pk/survey/chapter 10/02 Agriculture.pdf economic survey 2009-2010.

Hussain, S., Siddique, T., Saleem, M., Arshad, M. and Khalid, A. 2009. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. *Advances in Agronomy*, **102**: 159-200.

Islam, K.R. and Weil, R. W. 1988. Microwave irradiation of soil for routine measurement of microbial biomass carbon. Biology and Fertility of Soils **27**: 408-416.

Jacobsen, B. J. 1997. Role of plant pathology in integrated pest management. *Annu Rev Phytopathol.* **35**:373–391.

Jana, T. K., Debnath, N. C., Basak, R. K. 1998. Effect of insecticides on the composition of organic matter, ammonification and nitrification in a Fluventic ustochrept. *J. Int. Soil Sci.*, **46**:133–134.

Karthikeyan, R., Davis, L. C., Erickson, L. E., Al-Khatib, K., Kulakow, P. A., Barnes, P. L., Hutchinson, S. L., Nurzhanova, A. A. 2004. Potential of plant-based remediation of pesticide-contaminated soil and water using non-target plants such as trees, shrubs, and grasses. *Crit Rev Plant Sci.***23**:1–11.

Khan, A. R., Ihsan, U., Abdul, L. Khan., Gun-Seok, P., Muhammad, W., Sung-Jun, H.. Byung, K. J. and Yunyoung, K. 2015.Improvement in phytoremediation potential of *Solanumnigrum* under cadmium contamination through endophytic-assisted *Serratia* sp. RSC-14 inoculation. *Environmental Science and Pollution Research*, **22**(18): 14032–**14042.**

Kidd, P. S., Prieto-Fernandez, A., Monterroso, C., Acea, M. J. 2008. Rhizosphere microbial community and hexachlorocyclohexane degradative potential in contrasting plant species. *Plant Soil*,**302**: 233-247.

Kopittke, P. M., Menzies, N. W., H.B. and Fulton, I. 2004. Effect of Mn deficiency and legume inoculation on rhizosphere pH in highly alkaline bauxite residue. *International Soil Conservation Organisation Conference*, **13**: 122–134.

Kuiper, E. L., Lagendijk, G. V., Lugtenberg, B. 2004. Rhizoremediation: A beneficial plant microbe interaction. *Mol Plant Microbe Int.***17**: 6–15.

Kumar, K., Mukerji, G. and Lal, R. 1996. Molecular aspects of pesticide degradation by microorganisms. *Critic Rev. Microbiol.*, **22:** 1-26.

Kumar, R., Pandey, S., Pandey, A. 2006. Plant roots and carbon sequestration. *CurrSci,* **91**: 885-90.

Kumari, B., Madan, V. K. and Kathpal, T. S. 2007. Status of insecticide contamination of soil and water in Haryana, India. *Environ. Monit. Assess.*,**136:**239–244.

Kutz, F. W., Wood, P. H. and Bottimore, D. P.1991. Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev. Environ. Contam. Toxicol.,* **120:**1–82.

Lal, R., Dogra, C., Malhotra, S., Sharma, P. and Pal, R. 2006. Diversity, distribution and divergence of *lin* genes in hexachlorocyclohexane-degrading sphingomonads. *Trends Biotechnol,*. **24:**121–130.

Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H. P., Holliger, C., Jackson, C., Oakeshott, J. G. 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *MicrobiolMolBiol Rev.* **74**:58–80.

Lammel, G., Ghimb, Y. S., Gradosa, A., Gaod, H., Huhnerfussc, H. and Lohmann, R.2007. Levels of persistent organic pollutants in air in China andover the Yellow Sea. Atmos. Environ. **41:**452–464.

Leal, O. D., Rosa, M. V. C., Pauletto, A. E.,Fernando, L., Pinto, S. and Pillo, N. 2015. Organic Matter Fractions and Quality of the Surface Layer of a Constructed and Vegetated Soil After Coal Mining. *Rev. Bras. Ciênc,*39(3): 25−**37.**

Long, H. H., Schmidt, D. D., Baldwin, I. T. 2008. Native Bacterial Endophytes Promote Host Growth in a Species-Specific Manner; Phytohormone Manipulations Do Not Result in Common Growth Responses. *PLoS ONE*, **3**(7): 270−279.

Malik, M.A., Khan, K. S., Marschner, P. and Hassan, F. 2013. Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *Journal of Soil Scence and Plant Nutrition,* **13**(4): 955-966.

Malik, M. T., Akem,C. N., Kazmi,M. R. and Fateh,F. S. 2011. Towards chemical management of mango sudden death disease in Pakistan. International conference on "New Frontiers in Plant Pathology for Asia and Oceania, Darwin- Australia" *VirologicaSinica.* **18**: 571–575.

Marques, A. P. G. C., Rui, S., Kalina, A.. Samardjievab , Jose, P., Castro, P. M. L. 2006. Solanum nigrum grown in contaminated soil: Effect of arbuscular mycorrhizal fungi on zinc accumulation and histolocalisation. *Environ Pollut,* **145**(3): 691−699.

Martin, B. C., George, S. J., Price, C. A., Ryan, M. H., and Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. *Sci Total Environ.* **472**: 642-53.

McMurry, J. 2000. Organic Chemistry Cole: *Thomson Learning*, **5**: 7581.

Melling, F. B. 1993. Soil microbial ecology: Applications in agricultural and environmental management. New York: *Marcel Dekker*.

Mertens, B., Boon, N. and Verstraete, W. 2006. Slow-release inoculation allows sustained biodegradation of gamma-hexachlorocyclohexane. *Appl Environ Microb,* **72**: 622–627.

Metcalf, R. L. 1955. "Physiological basis for insect resistance to insecticides,"*Physiol Revs.***35**: 192–232.

Matsumoto, E., Kawanaka, Y., Yun, S. J. and Oyaizu, H. 2009. Bioremediation of the organochlorine pesticides, dieldrin and endrin, and their occurrence in the environment. *Applied Microbiology and Biotechnology*, **84**(2): 205–216.

Muhammad, S., Muller, T. and Joergensen, R. G. 2008. Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. *J. Arid Environ.***72**: 448-457.

Nagpal, V., and Paknikar, K. M. 2006. Integrated biological approach for the enhanced degradation of lindane. *Indian J. Biotechnol.* **5**: 400- 406.

Nagpal, V., Srinivasan M. C. and K. Paknikar, M. 2008. Biodegradation of γ-hexachlorocyclohexane (Lindane) by a non-white rot fungus conidiobolus 03-1-56 isolated from litter. *Indian Journal of Microbiology.* **48**:134.

Nelson, D.W. and Sommers, L. E. 1982. Total carbon, organic carbon and organic matter. In: Methods of Soil Analysis, (Eds.): A. L. Page, R. H. Miller and D. R. Keeney. Part II, *American Soc. Agro., Madison.* 555-579.

Newman, L. A. and Reynolds, C. M. 2004. Phytodegradation of organic compounds. *Curr. Opin. Biotechnol.* **15**: 225–230.

Nisa, W. U., Bangash, N., Saleem, A. R., Rashid, A. and Dawson, L. 2016. Effect of diesel contamination on the physico-chemical characteristics of soil and growth of vetiver grass.*Soil Environ.* **35**(1): 91-98.

Park, S., Kim, S. K., Kim, J. T., Kang, D. and Sung, K. 2011. Effects of humic acid on phytodegradation of petroleum hydrocarbons in soil simultaneously contaminated with heavy metals. *J. Env. Sci.* **23**(12): 2034-2041.

Pereira, R. C., Arbestain, M. C. and Monterroso, C. 2006. Behaviour of a-, b-, g-, and d hexachlorocyclohexane in the soil plant system of a contaminated site. *Environmental Pollution,* **144**: 210-217.

Pereira, R., Antunes, S. C., Castro, B. B., Marques, C. R., Gonçalves, A. M. M., Gonçalves, F. and Pereira, R. 2009. Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: Commercial formulation versus active ingredient. *Ecotoxicology.***18**:455–463.

Perucci, P. and Scarponi, L. 1994. Effects of the herbicide imazethapyr on soil microbial biomass and various soil enzyme activities. *Biol Fertil Soils*,**17**:237–240.

Phillips, M. R., Yang, G. and Zhang, Y. 2002. Risk factors for suicide in China: a national case control psychological autopsy study. *Lancet*,**360**:1728–1736.

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

Phillips, T. M., H. Lee, J. T. Trevors, and A. G. Seech. 2004. Mineralization of hexachlorocyclohexane in soil during solid-phase bioremediation. *J. Ind. Microbiol. Biotechnol.***31**:216–222.

Porta, D., Fiorella, F., Francesco, B., Annalisa, A., Fano1, V.,Silvia, N. and Forastiere, F. 2013. A biomonitoring study on blood levels of beta-hexachlorocyclohexane among people livingclose to an industrial area. *Environmental Health*,**12**: 57-67.

Prakash, O., Suar, M., Raina, V., Dogra, C., Pal, R., and Lal, R. 2004. Residues of hexachlorocyclohexane isomers in soil and water samples from Delhi and adjoining areas. *Current Science.***87**(1): 73–77.

Puhui, J., Yufang, S., Yongji, J., Xiwang, T., Yanan, T., Pengcheng, G. and Wenshe, H. 2016. A two-year field study of phytoremediation using *Solanum nigrum* L. in China. International *Journal of Phytoremediation,***18** (9): 924 –928.

Raina, V., M. Suar, A. Singh, O. Prakash, M. Dadhwal, S. K. Gupta, C. Dogra, K. Lawlor, S. Lal, J. R. van der Meer, C. Holliger, and R. Lal. 2008. Enhanced biodegradation of hexachlorocyclohexane (HCH) in contaminatedsoils via inoculation with *Sphingobium indicum* B90A. Biodegradation **19:**27–40.

Rhind, S. M., Kyle, C. E., Kerr, C., Osprey, M., Zhang, Z. L., Duff, E. I. 2013. Concentrations and geographic distribution of selected organic pollutants in Scottish surface soils. *Environ Pollut***182**:15–27.

Richter, E. D. 2002. Acute human poisonings. In: Encyclopedia of Pest Management (Ed D Pimentel). *Dekker, New York,* 3–6.

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

Rochika. P and Dharmender. K. 2014. Biodegradation study of gemma HCH using selected bacteria isolated from agricultural soil. African Journal of Microbiology Research, 8(36): 3335-3346.

Rodriguez, R. A. and Toranzos, G. A. 2003. Stability of bacterial populations in tropical soilupon exposure to Lindane. *Int Microbiol.,*6:253–258.

Rup L., Gunjan P., Pooja S., Kirti K., Shweta M., Rinku P., Vishakha R., Christof H., Colin J., and John G. 2010. Biochemistry of Microbial Degradation of Hexachlorocyclohexane and Prospects for Bioremediation. *microbiology and molecular biology reviews*, **74**(1): 58–80.

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

Sangwan, N., Lata, P., Dwivedi, V., Singh, A. and Niharika N. 2012. Comparative Metagenomic Analysis of Soil Microbial Communities across Three Hexachlorocyclohexane Contamination Levels. *PLoS ONE*, 7(9): 46−99.

Sattler, C., Kächele, H. and Verch, G. 2007. Assessing the intensity of pesticide use in agriculture. *Agr. Ecosyst. Environ.* **119**: 299–304.

Schutzendubel, A., Andrea, P. 2002. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, **53**: 1351−1365.

Sheikh, S.A., Nizamani, S. M., Jamali,A. A. and Kumbhar, M.I.2011. Pesticides and Associated Impact on Human Health: A Case of Small Farmers in Southern Sindh. *Pakistan. Journal of Pharmacy and Nutrition Sciences.* **1**: 82-86.

Shen, L., Wania, F., Lei, Y. D., Teixeira, C., Muir, D. C. and Bidleman, T. 2004. Hexachlorocyclohexanes in the North American atmosphere. *Environ. Sci. Technol.* **38**:965-975.

Shi, S. J. and Bending, G. D. 2007. Changes to the structure of Sphingomonas sp. Communities associated with biodegradation of the herbicide isoproturon in soil. *FEMS Microbiol. Lett.* **269**: 110–116.

Singh J, Singh D K. 2005c. Dehydrogenase and phosphomonoesterase activities in groundnut (Arachis hypogeae L.) field after diazinon, imidachlorprid and lindane treatments. *Chemosphere*,**60**: 32–42.

Spark, K. M. and Swift, R. S. 2002. Effect of soil composition and dissolved organic matter on pesticide sorption. *Sci Total Environ*., **21**(1-3):147-161.

Subramaniam, K., and Solomon, R. D. J. 2006. Organochlorine pesticides BHC and DDE in human blood in and around Madurai, India. *Indian J. Clin. Biochem.*,**21**:169–172.

Tahir, A., Imtiaz, A. and Seema, T. 2012. Determination of Pesticide Residue in Nawabshah District Pakistan. *Pakistan. J. Zool.,* 44(1): 87-93.

Tan, S. Y., Jiang, Q. Y., Zhuo, F., Liu, H., Wang, Y. T. and Li, S. S. 2015. Effect of Inoculation with *Glomus versiforme* on Cadmium Accumulation, Antioxidant Activities and Phytochelatins of *Solanum photeinocarpum*. *PLoS ONE,* **10**(7): 132−347.

Tariq, M. I., Afzal, S., Hussain, I. and Sultana, N. 2007. Pesticide Exposure in Pakistan: A review. *Environment International.***33**: 1107-1122.

Thakur, H. M., M. S., Manonmani, H. K. and Rajashekara, M. 2010. Degradation of Technical Grade Hexachlorocyclohexane in Soil Slurry by a Defined Microbial Consortium. *Int. J. Environ. Res.* **4**(3):471-478.

Toteja, G. S., A. Mukherjee, S. Diwakar, P. Singh, and B. N. Saxena. 2003. Residues of DDT and HCH pesticides in rice samples from different geographicalregions of India: a multicentre study. *Food Addit. Contam.,***20:** 933–939.

Turnbull, A., 1996. Chlorinated pesticides. In: Hester, R.E., Harrison, R.M. (Eds.), Chlorinated Organic Micropollutants. Environmental Science and Technology.The Royal Society of Chemistry, Cambridge.113-135.

United Nations Environment Programme: Annual Report 2009.

Urgel, M. and Ramos, J.L. 2001. Expression of a Pseudomonas putida aminotransferase involved in lysine catabolism is induced in the rhizosphere. *Appl Environ Microbial.*67: 5219–5224.

Vidali, M. 2001. Bioremediation: An overview.*Pure and Applied Chemistry,* **73**:1163–1172.

Vijgen, J., Li, Y., Martin, F., Lal, R. and Weber, R. 2006. The legacy of lindane and technical HCH production. Dioxin Conference proceedings. *Organohalogen Compd.***68**:899–904.

Walkley, A. 1997. A critical examination of rapid method for determining organic carbon in soil: Effect of variations in digestion conditions and of organic soil constituents. *Soil Science***63**: 251-263.

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

Wang, M. C., Liu, Y. H., Wang, Q., Gong, M., Hua, X. M., Pang, Y. J., Hu, S. and Yang, Y. H. 2008. Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. *Soil Biol Biochem*,**40**:778–788.

Weber, R., C. Gaus, M. Tysklind, P. Johnston, M. Forter, H. Hollert, H. Heinisch, I. Holoubek, M. Lloyd-Smith, S. Masunaga, P. Moccarelli, D. Santillo, N. Seike, R. Symons, J. P. M. Torres, M. Verta, G. Varbelow, J. Vijgen, A. Watson, P. Costner, J. Woelz, P. Wycisk, and M. Zennegg. 2008.Dioxin- and POP-contaminated sites—contemporary and future relevanceand challenges. *Environ. Sci. Pollut. Res.*,**15**:363–393.

Willett, K. L., Ulrich, E. M., Hites, R. A. 1998. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. *Environ Sci Technol.* **32**: 2197–2207.

Wollum II, A.G. 1982. Cultural methods for soil microorganisms.*ASA and SSSA Pub. Madison Wisconsin*,**2**: 719—802.

Yuksel, H., Ispir, U., Ulucan, A., Turk, C. and TaysI, R. 2016. Effects of Hexachlorocyclohexane (HCH-γ-Isomer, Lindane) on the Reproductive System of Zebrafish (*Danio rerio*). *Turkish Journal of Fisheries and Aquatic Sciences,* **16**: 917-921.

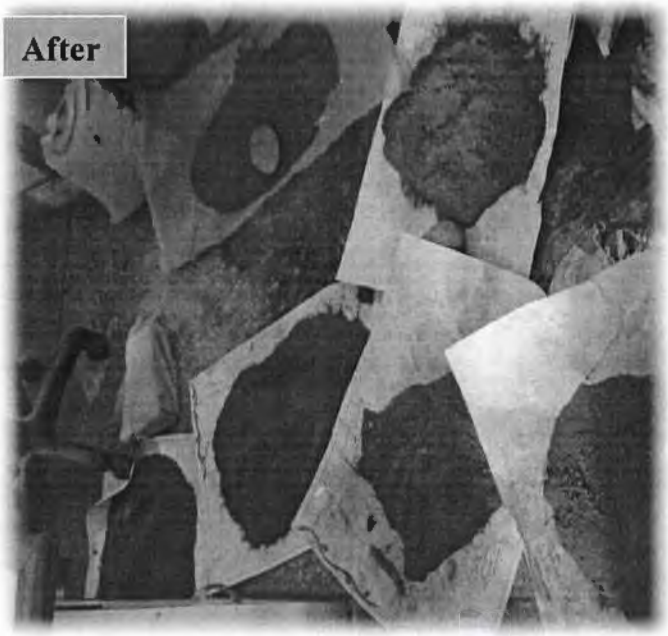
Yuxian, H., Jianwei, Z., Yu, Z., Nicolas, D. and Kongming, W. 2012. Lethal effect of imidacloprid on the coccinellid predator Serangiumjaponicum and sublethal effects on predator voracity and on functional response to the whitefly Bemisiatabaci. *Ecotoxicology.* **21**:1291–1300.

Zhao, G., Y. Xu, W. Li, G. Han, and B. Ling.2007. PCBs and OCPs in human milk and selected foods from Luqiao and Pingqiao in Zhejiang, China. *Sci. Total Environ.***378**:281–292.

ANNEXURES

ANNEXURE

Collection of soil from NARC to conduct experiment

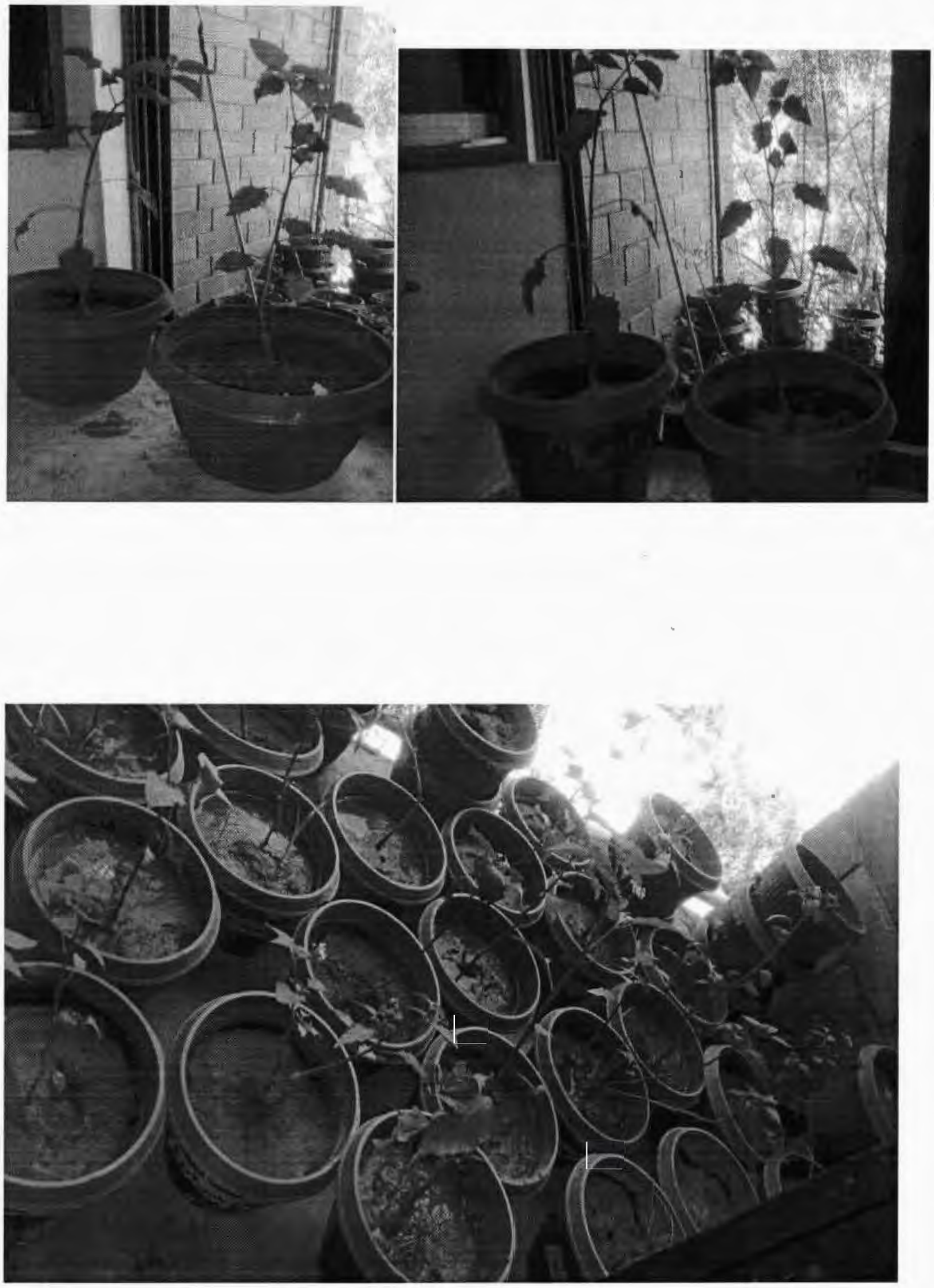


Preparing soil for conducting experiment

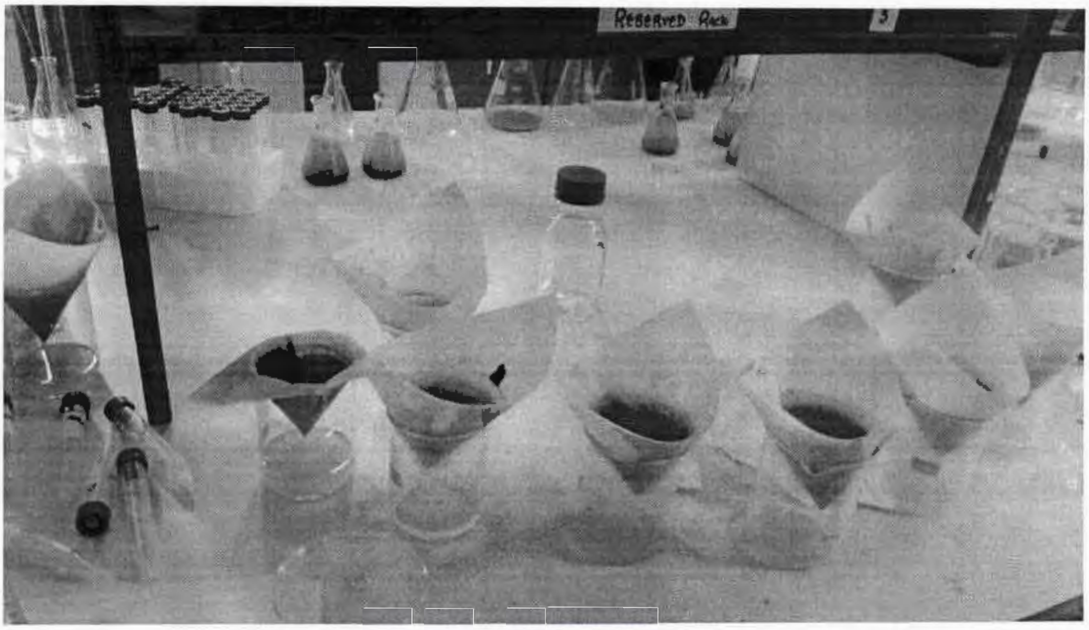
Tagging of pots and aging of spiked soil

Plants wilt at the start of experiment

Later, as the time swap they showed good growth



Growth of *Solanum nigrum* during experimental period of 90 days



Preparing soil samples for physico–chemical analysis of soil