UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

Remediation of PPO-Tengeru and NHC-Morogoro contaminated sites in Tanzania relating to the project "Capacity Strengthening and Technical assistance for the Implementation of the Stockholm Convention NIPs/AFDCs of the COMESA/SADC sub-regions"

Final Report

BACAS

Bureau of Agricultural Consultancy and Advisory Service
Sokoine University of Agriculture, Morogoro, Tanzania
bacas@suante.ac.tz
November, 2017

Provision of remediation of PPO-Tengeru and NHC-Morogoro contaminated sites in Tanzania relating to the project "Capacity strengthening and Technical assistance for the Implementation of the Stockholm Convention NIPs/AFLDCs of the COMESA/SADC sub-regions"

Final Project Report

Prepared by

Prof. Ernest E. Semu- PhD

Prof. John J. Msaky- PhD

Miss Monica Nakei

Department of Soil and Geological Sciences,

Sokoine University of Agriculture, Morogoro, Tanzania

January, 2019

Table of Content

Contents

1 General Introduction

Persistent organic pollutants are posing a threat to human health. As a result countries have invested in identifying and remediating or at least protecting contaminated sites to control the risk of exposure, by humans and animals alike, to such toxins. A total of 19 regions of Tanzania were surveyed for contamination by persistent organic pollutants. In the process, over 40 stores have been found to have severe environmental contamination with possible risks. Out of these, 4 sites located in Kibaha and Korogwe are contaminated with DDT, Aldrin and Toxaphene (NIP, 2005). A site at PPO Tengeru was found to be heavily contaminated by Lindane.

Several strategies are followed for remediation or control and management of soils contaminated by pesticides. Contaminated soils may be excavated and shipped to a certified landfill or incinerated (Paulson, 1998). The cost of these methods is often prohibitive and may pose many environmental concerns because excavating the soil and storing it in a landfill literally means transferring the danger from original place to a more regulated enclosure. Therefore, alternative low-cost yet safer methods are more preferred (and especially so to low-income contries) to accelerate the degradation and natural attenuation of pesticides from multiple chemical classes. Among them, bioremediation, use of zerovalent iron and/or soil biosolarization have been proposed in the last years as alternatives for the reduction or elimination of pesticide residues in soils (Navarro et al., 2007).

Bioremediation, which is a general concept that includes all those processes and actions that take place in order to biotransform the contaminants, to less or non-toxic products, has been observed to be an efficient, ecofriendly and economically feasible method of detoxification (Kavita and Geeta, 2014). It refers to the use of microorganisms or plants to detoxify or degrade the xenobiotics. The utility of bioremediation in the degradation of pollutants in the environment has been successfully demonstrated for several xenobiotics, including pesticides (Singh et al., 2006).

In this study we demonstrate two bioremediation approaches as initial steps towards a consolidated assortment of efforts to remediate two POPs contaminated sites of in Tanzania. We show that common plant species with proven record of bioaccumulation of POPs from the soil can be used into a program of remediation of a Lindane and other biopesticide-contaminated site at PPO Tengeru. We also demonstrate that certain plant and microbial species are capable of withstanding and biotransforming DDT and its metabolites thereby reducing toxicity levels of the same from a DDT-contaminated site at NHC-Morogoro.

For purposes of convenience, this report is, therefore, subdivided into two major parts. Part A dealing with the demonstration of the phyto- and micro-mediation of chiefly DDT and metabolites at NHC-Morogoro site and Part B which explains the demonstration of phytoremediation of both Lindane and DDT at PPO-Tengeru site.

Part A: Bioremediation of Lindane and DDT (and their metabolites) at NHC Morogoro site.

2. Background and methodological approach

2.1 Historical background of the site

The NHC Morogoro contaminated is well known and famous for being among the central key sites for formulation of DDT and endosulfan, among other pesticides since pre-independence times. The history about the study site is scanty. However, due to perceived side effects to humans and the environment (Stockholm Convention, 2005), production of such chemicals was banned and the site was eventually abandoned in 1997 (NIP, 2005). To-date, in this site, human settlements and commercial premises are found within 200 meters from the store and also the railway station is just near the site. Vegetable cultivation takes place along Mjimwema stream. The closest garden downstream is situated at about 300 meters from the site. Due to proximity to human activities and river systems, this site has a very high potential for environmental and social impacts. The environmental impact may include the pollution of Mjimwema steam, exposure of local communities to the pesticide residues including railway station workers, students of the secondary school, workers of Quick Security Company currently renting the main building and workers of Quality Progress Limited also renting the newly refurbished godown through emitted gases from the pesticides or by direct contacts with residual pesticide contaminants.

As indicated above, in resource poor countries like Tanzania, the most feasible options include the deployment of cheap but effective methods of remediation such as pyto-remediating plants and effective micro-remediation microbial communities. We chose to demonstrate at the NHC site how, if carefully chosen certain microbial species can effectively bio-degrade the DDT and other pesticides to less non-toxic degradation products. We also opted to demonstrate that certain plant species (both domesticated and wild) can be used as bio accumulator bio-agents to slowly but effectively get rid of the toxic chemicals from the soils.

2.2 Profile, fertility and microbiological soil sampling and analysis for the NHC site

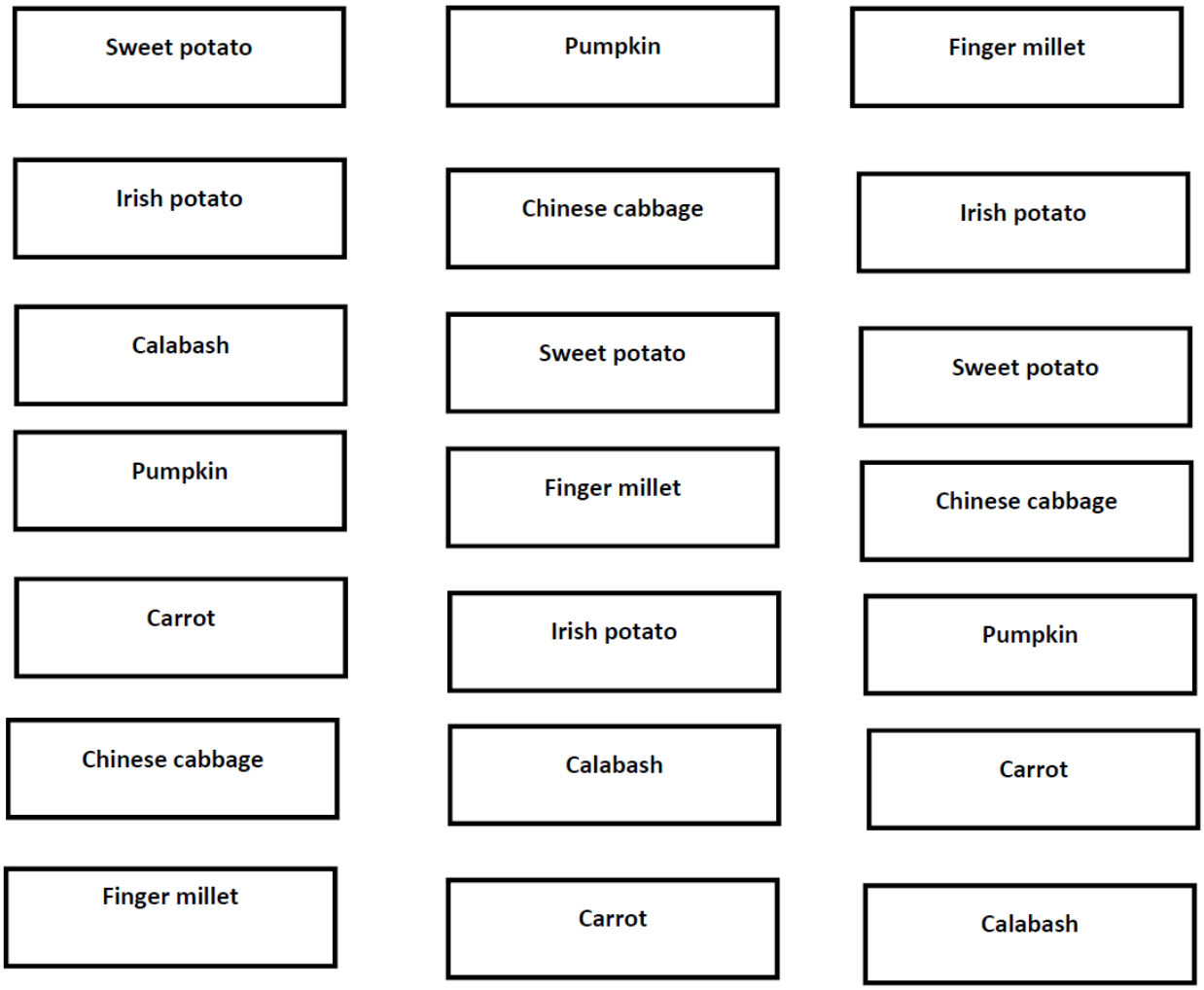
Activities at the site included surface and profile sampling to obtain soil samples that would shade light on the lateral and downward movement of DDT, lindane and other pesticides at the site. Using hand-held auger, as shown on the sketch diagram below, the site, was sampled to 100 cm down the profile from the soil surface at an interval of 20 cm. Soils were packed in plastic bags, clearly labelled and shipped to the lab for further processing. In the Soil Science laboratory at Sokoine University of Agriculture, the samples were air-dried re-parked into translucent brown bottles for transfer to capable laboratory for analysis of the levels and types of POPs and metabolites.

Microbiological samples were also taken for to the lab for isolation of DDT tolerant microorganisms. For this purpose, the soil from spots within the site previously confirmed to have relatively high DDT (or its metabolites) levels (between 950- 1400 ppm) were sampled as source soils for both the resistant microbes and DDT-contaminated microbiological medium. The soil samples were carefully taken to the soil microbiology lab at SUA for processing and isolation of microorganisms including fungi and bacteria. A portion of the same soil was used to prepare DDT-contaminated soil extract broth and agar using standard procedure.

2.3 Phytoremediation plant establishment, management and harvesting

This activity was preceded by selection of phytoremediatory plant species to be used at the demonstration site. Phytoremediation plants selection for the NHC Morogoro site was guided and informed by both previous season studies under screenhouse conditions and past research experience as well as literature search on similar studies. Briefly, following a successful screehhouse-based trial of candidate crops that could bio-accumulate DDT and its metabolite using soils collected from the site, it was decided that calabash, pumpkin, carrots, sweet potato, Irish potato, finger millet and Chinese cabbage to be used.

Field experimental layout used to establish phytoremediation plants at NHC Morogoro



Based on this layout, the experimental site was cleared, tilled, harrowed and then seedbeds prepared for plant establishment.

Plant performance was monitored agronomically observing and recording plant's vegetative performance from germination to flowering or to stage of plant harvesting for eventual laboratory analysis.

After all plants had achieved sufficient vegetative growth (around 50 days after planting), they were harvested, air-dried, ground to powder form and transport to a Tropical Pesticide Research Institute (TPRI) in Arusha-Tanzania and later to Tshwane University of Technology (TUT) in Pretoria- South

Africa for chemical analysis. For analytical purposes, each test plant harvested was processed to separate the above-ground from below ground biomass.

Team from UNIDO was joined by BACAS consultants and Division of Environment to inspect the experimental field at NHC Morogoro when plants had reached sufficient vegetative growth

Part of Plant samples from NHC Morogoro packed in brown translucent glass bottles for international transfer to TUT laboratory for analysis

2.4 Laboratory extraction and quantification of lindane and DDT from soil and plant samples

2.4.1 Extraction of soil samples

Soil samples (ca. 2.5 g) were weighed into pre-cleaned cellulose thimbles and were thereafter transferred into cleaned Soxhlet apparatus for extraction. The samples were extracted for 16 h using a mixture of n-hexane:acetone (2:1, v/v). Upon completion, the extracts were allowed to cool down to room temperature. The extracts were then carefully transferred into pre-clean round bottom flasks and were rotary evaporated to approximately 2 mL. Two laboratory reference soil samples were spiked with known amounts of the targeted compounds, and were similarly prepared following the aforementioned procedures. The spiked reference samples serve as a quality assurance measure to assess the efficiency of the extraction method by estimating the analytical recoveries of the targeted compounds.

2.4.2 Extraction of plant samples

The root and shoot parts of test plants namely: pumpkin, calabash, Irish potato, sweet potato, simsim, and finger millet were prepared for the determination of residual levels of the targeted POPs namely lindane and DDT (plus its metabolites). Approximately 2.5 g of the plant samples were weighed into pre-cleaned amber bottles. The samples were soaked overnight with 50 mL of n-hexane:acetone (1:1, v/v) followed by ultrasonic-assisted extraction for 30 min. The set-up was allowed to cool down to room temperature and the extract was carefully transferred into a clean round bottom flask. The extraction was repeated using the same volume of the extraction solvent and time. The extracts were

combined and subjected to rotary evaporation as was previously indicated for soil samples. Similarly, two laboratory reference plant samples (lettuce) were spiked with known amounts of the targeted compounds, and were similarly prepared following the aforementioned procedures. The spiked reference samples were employed for the estimation of the analytical recoveries of the targeted compounds.

2.4.3 Soil and plant extract clean-up prior to instrumental analysis

The extracts from soil and plant samples were purified separately using deactivated silica gel packed into glass columns. Prior to the clean-up procedure, the packed columns were conditioned using 25 mL of n-hexane to remove trapped air and possible interfering contaminants. The concentrated extracts (either from soil or plant samples) were quantitatively transferred into the glass columns and were eluted under gravity with 40 mL of n-hexane:acetone (2:1, v/v). The eluate were rotary evaporated to approximately 2 mL, and transferred, thereafter, into amber vials where the eluates were further concentrated with high purity nitrogen gas until they reached incipient dryness. The extracts were, then re-constituted with 1 mL of Toluene followed by the addition of 50 µL of 500 pg µL-1 of DDT-d8 that was employed as an internal standard.

2.4.4 Instrumental analysis and quantification of lindane, DDT and metabolites

The quantitative estimation of all the targeted compounds from the extracts (soil or plant) was performed using an Ultra-trace 2010 Shimadzu GC equipped with QP 2010 Ultra mass spectrometer operated in EI mode. The chromatographic separation of these compounds was achieved using DB-5 MS (15 m, 0.25 mm i.d., 0.10 µm film thickness) capillary column. The optimal conditions employed for the GC-EI-MS instrument are shown in Table 1-1. To enhance the sensitivity of the instrument and to overcome the inherent problems of interfering co-extractants, the MS acquisition was carried out in selected ion monitoring (SIM) mode. In this case, a target ion and two reference ions were selected for each targeted compound as well as internal standard (DDT-d8) for their identification and quantification.

Table 1-1: Optimised GC-EI-MS conditions employed for OCPs analysis and quantification

Parameters	Optimum conditions
GC parameters	
Injection volume	1 µL
Carrier gas (% purity)	Helium (99.999%)
Injection mode	Splitless
Flow control mode	Linear velocity
Injector temperature	270^{o}C
Linear velocity	63.5 cm/sec
Column flow	1.5 ml/min.
Purge flow	3.0 ml/min.
Equilibrium time	3.0 min.
Sampling time	2.00 min.
Oven temperature programming	70^{o}C held for 1.0 min., ramped @ 25^{o}C/min. to 180^{o}C, ramped @8^{o}C/min to 300^{o}C held for 5 min.
MS parameters	
Ion source temperature	270^{o}C
Interface temperature	280^{o}C
Solvent cut time	2.0 min.
Acquisition mode	SIM
Ionisation method	EI

2.5 Isolation and characterization of Dichloro- Diphenyl Trichloroethane (DDT)-resistant microorganisms for their potential role in biodegradation of DDT

1.5.1 Preamble

There is a basic assumption that microorganisms exposed to extreme environmental conditions have a general tendency to adapt to ways that allow their survival in such surroundings. As such, it is generally accepted that microbial communities living in soils with high levels of DDT must have evolved ways to detoxify the chemical, - rendering the surrounding habitable by such microbial species. A variety of microorganisms can, over time, detoxify hazardous chemicals like DDT through metabolizing them to less toxic variants either via degradative pathways or ligand formation approaches. Because DDT biodegradation can happen in soil, albeit at a slow rate, scientists have explored various ways to expedite the process. Some of the approaches that are used to enhance in-situ biodegradation rate of DDT include manipulation of soil conditions by treating it with surfactants to improve access of microbes to DDT and addition of DDT-degrading microbial species to DDT-contaminated soils (Aislabie et al., 1997; Chauhan and Singh, 2015).

2.5.2 Methodological approach

In this demonstration project we chose to isolate microbes from soils contaminated with DDT, multiply them in the lab and re-introduce them back to site to boost their numbers and enhance DDT biodegradation. Samples of soils were, therefore, collected from points previously identified as having elevated levels of DDT (and or metabolites) and brought to a Soil microbiology lab at Sokoine University of Agriculture for isolation of microbes.

The isolation procedure followed was the use of soil extract solution. The soil samples from each sampled point was divided into two halves, one half was used to prepare a soil extract agar. Four hundred grams of air-dried soil was dissolved in 1 liter of pure distilled water, autoclaved for 15 minutes at 121˚C, allowed to cool and sediment for overnight at room temperature, Then the mixture was centrifuged to obtain the clear supernatant. After adjusting the pH of the supernatant to 7.0, 15g of agar were added, re-autoclaved prior to pouring into the sterile petri dishes to get soil extract agar plates.

The second half of the soil samples was used to isolate potential DDT (and or lindane) resistant microorganisms using a serial dilution technique and plating of previously prepared soil extract agar. Isolates successfully developing colonies in this POPs contaminated soil were presumed to be at DDT-metabolizing or at least DDT-resistant microorganisms. After repeated purification cultures on DDT-rich media, five isolates showing unimpeded growth rates on DDT-contaminated soil extract agar were

selected for further identification. rRNA of pure cultures was extracted from fresh colonies and purified, amplified and then sent for molecular sequencing using the 16S rRNA PCR products at Inqaba Biotech, Nairobi.

3. Major Findings at the NHC Morogoro site

3.1 Status of DDT (and Lindane) contamination of the soil at NHC Morogoro site

To draw a clear picture of the extent of contamination of the site by the two POPs, lindane and DDT, both surface and below surface soils were taken for laboratory quantification. Results of the laboratory analysis quantifying the two chemicals in soil (Table 2-1) show that unlike lindane, DDT contamination is widespread at the site. Surface (0 -30 cm deep) soil levels of DDT ranged from as low as 4, 210 to as high as 74, 944 ng/g soil. There is also sufficient evidence to believe that the contamination has also percolated down the soil profile. This is because a soil sample taken 200 cm below ground was also found to have total DDT of 16733 ng/ g soil. It is noted also that all the common metabolites of DDT were also detected at every point sampled, implying that indeed microbially assisted biodegradation of DDT has been happening in the soils at the site. Lindane was also detected in some spots at the site with values ranging from 9 – 203 ng /g soil.

Table 2-1. Concentration of lindane and DDT plus its metabolites from soils of a contaminated site at NHC Morogoro

	Concentration (ng/g)							
Soil sample	**Lindane**	**Mitotane (o,p-DDD)**	**p,p'-DDD**	**o,p-DDE**	**p,p'-DDE**	**o,p-DDT**	**p,p-DDT**	**Total DDT**
FERT_X1	13	1003	10059	357	3529	1917	10351	**27216**
FERT_X2	ND	1108	12987	761	9351	2475	13364	**40046**
FERT_X3	9	323	3632	114	887	692	3737	**9385**
FERT_X4	ND	1152	10425	740	7435	1993	10722	**32467**
FERT_X5	30	2510	28751	642	7970	5485	29586	**74944**
FERT_X6	181	550	2519	443	3688	517	2706	**10423**
FERT_X7	203	121	902	123	1928	195	941	**4210**
FERT_X8	157	1379	5329	791	10961	1030	5473	**24963**
P2_O_200m	ND	752	6299	190	1807	1201	6484	**16733**

3.2 Uptake of DDT, its metabolites and lindane by phytoremediation plants at NHC Morogoro site

Seven different plants namely carrots, finger millet, sweet potato, irish potato, pumpkin, calabash and Chinese cabbage were deployed for the phytoremediation demonstration trial at NHC Morogoro site. The plants exhibited varying abilities to bio-accumulate both lindane and/or DDT into their below-ground and above ground biomass. While carrots failed to even germinate in the field presumably due to toxic effects of DDT (and other pesticides in the soil, Irish potato plants exhibited retarded growth.

The rest of the plants exhibited know vegetative growth but varying degrees of accumulation of DDT and lindane into their biomass as shown in Table 2-2 below. Results show that uptake of lindane by any of the test plants at the NHC site was insignificantly small. Small amounts of lindane were detected in sweet potato, Irish potato and finger millet biomass (Table 2-2). Majority of the test plants, however, showed substantial abilities to extract DDT and or its metabolites and bio-accumulate it in the biomass either below ground or above ground. Results show further that uptake of DDT (Total DDT) by plants ranged from a minimum of 45 to a maximum of 181.55 ng/g soil for root biomass of calabash and Chinese cabbage, respectively. Similarly, the highest concentration in shoot biomass was recorded at 536.94 ng/g in Tembele- a variant of sweet potato commonly grown in Tanzania for its leaves closely followed by pumpkin shoot with 306.03 ng/g. The lowest shoot concentration of total DDT was recorded in Irish potato at 70.4 ng/g of biomass.

It is worth noting here that even after repeated sowing and close monitoring carrots failed to germinate completely at NHC Morogoro site, Irish potato emergence was also erratic leading to comparatively weaker seedlings.

Table 2-1: Concentration of DDT and lindane in biomass of selected phyto-remediation plants grown at NHC Morogoro site

		Concentration (ng/g)							
Sample code	**Sample name**	**Lindane**	**Mitotane (o,p-DDD)**	**p,p'-DDD**	**o,p-DDE**	**p,p'-DDE**	**o,p-DDT**	**p,p-DDT**	**Total DDT**
1b	NHC CHINESE ROOT R2	ND	7.24	69.91	18.11	1.77	0.45	71.94	169.42
13b	NHC CHINESE ROOT R2	ND	17.09	70.36	18.63	1.80	13.41	72.38	193.67
		0.00	24.33	140.27	36.74	3.57	13.86	144.32	363.09
Average	**NHC CHINESE ROOT R2**	**0.00**	**12.17**	**70.14**	**18.37**	**1.79**	**6.93**	**72.16**	**181.55**
17b	NHC CHINESE SHOOT R1	ND	10.71	55.76	10.66	1.33	0.27	57.38	136.11
29b	NHC CHINESE SHOOT R2	ND	13.69	46.17	11.51	1.42	8.80	47.50	129.09
		0.00	24.40	101.93	22.17	2.75	9.07	104.88	265.20

Average	**NHC CHINESE SHOOT R2**	**0.00**	**12.20**	**50.97**	**11.09**	**1.38**	**4.54**	**52.44**	**132.60**
2b	NHC PUMPKIN ROOT R3	ND	2.41	26.44	4.29	0.82	0.18	27.20	61.34
4b	NHC PUMPKIN ROOT R1	ND	3.85	19.89	1.94	0.35	0.07	20.46	46.56
5b	NHC PUMPKIN ROOT R2	ND	6.95	27.73	5.47	0.75	0.14	28.53	69.57
		0.00	13.21	74.06	11.70	1.92	0.39	76.19	177.47
Average	**NHC PUMPKIN ROOT**	**0.00**	**4.40**	**24.69**	**3.90**	**0.64**	**0.13**	**25.40**	**59.16**
3b	NHC PUMPKIN SHOOT R2	ND	27.05	261.27	51.96	6.19	48.34	259.47	654.28
9b	NHC PUMPKIN SHOOT R1	ND	10.39	91.64	6.60	2.21	17.46	94.29	222.59
31b	NHC PUMPKIN SHOOT R3	ND	3.80	15.21	3.42	0.25	2.90	15.65	41.23
		0.00	**41.24**	**368.12**	**61.98**	**8.65**	**68.70**	**369.41**	918.10
Average	**NHC PUMPKIN SHOOT**	**0.00**	**13.75**	**122.71**	**20.66**	**2.88**	**22.90**	**123.14**	**306.03**
	Total (Plant)								
6b	NHC F.MILLET ROOT R3	ND	25.09	64.46	12.97	0.04	2.26	66.54	171.36
30b	NHC F.MILLET ROOT R1	0.03	25.76	37.68	12.29	0.70	7.18	38.77	122.38
34b	NHC F.MILLET ROOT R2	ND	14.41	32.10	9.29	0.42	6.12	33.03	95.37
		0.03	**65.26**	**134.24**	**34.55**	**1.16**	**15.56**	**138.34**	389.11
Average	**NHC F.MILLET ROOT**	**0.01**	**21.75**	**44.75**	**11.52**	**0.39**	**5.19**	**46.11**	**129.70**
8b	NHC CALABASH ROOT R2	ND	8.82	19.28	5.92	0.60	0.36	19.84	54.82
33b	NHC CALABASH ROOT R1	ND	5.18	12.26	2.57	0.20	2.34	12.62	35.17
		0.00	14.00	31.54	8.49	0.80	2.70	32.46	89.99
Average	**NHC CALABASH ROOT**	**0.00**	**7.00**	**15.77**	**4.25**	**0.40**	**1.35**	**16.23**	**45.00**
32b	NHC CALABASH SHOOT R2	ND	8.46	40.50	6.36	0.54	7.72	41.67	105.25
37b	NHC CALABASH SHOOT R3	ND	32.80	83.96	39.07	2.74	16.00	86.38	260.95
		0.00	41.26	124.46	45.43	3.28	23.72	128.05	366.20
Average	**NHC CALABASH SHOOT**	**0.00**	**20.63**	**62.23**	**22.72**	**1.64**	**11.86**	**64.03**	**183.10**
14b	NHC TEMBELE ROOT R1	0.36	3.69	51.73	3.17	0.01	9.86	53.22	121.68
22b	NHC TEMBELE ROOT R2	ND	6.35	41.59	7.38	1.50	7.92	42.79	107.53
		0.36	10.04	93.32	10.55	1.51	17.78	96.01	229.21
Average	**NHC TEMBELE ROOT**	**0.18**	**5.02**	**46.66**	**5.275**	**0.755**	**8.89**	**48.005**	**114.61**

36b	NHC TEMBELE SHOOT R3	0.08	12.62	72.03	6.76	1.23	13.84	74.06	180.54
12b	NHC TEMBELE SHOOT R2	ND	13.07	391.56	9.21	1.94	74.62	402.92	893.32
		0.08	25.69	463.59	15.97	3.17	88.46	476.98	1073.86
Average	**NHC TEMBELE SHOOT**	**0.04**	**12.845**	**231.795**	**7.985**	**1.585**	**44.23**	**238.49**	**536.93**
15b	NHC IRISH P.SHOOT R2	ND	9.22	42.61	2.78	0.94	8.12	43.84	107.51
35b	NHC IRISH P.SHOOT R3	ND	3.28	13.37	1.21	0.20	2.55	13.76	34.37
		0.00	12.50	55.98	3.99	1.14	10.67	57.60	141.88
Average	**NHC IRISH P.SHOOT**	**0.00**	**6.25**	**27.99**	**2.00**	**0.57**	**5.34**	**28.80**	**70.94**
23b	NHC IRISH P.ROOT R1	ND	4.42	22.27	1.82	0.42	4.24	22.91	56.08
26b	NHC IRISH P.ROOT R1	ND	7.97	41.57	3.36	1.28	8.06	42.74	104.98
		0.00	12.39	63.84	5.18	1.70	12.30	65.65	161.06
Average	**NHC IRISH P.ROOT**	**0.00**	**6.20**	**31.92**	**2.59**	**0.85**	**6.15**	**32.83**	**80.53**
18b	NHC S.POTATO SHOOT R3	0.13	9.02	56.48	4.60	1.24	10.76	58.12	140.22
19b	NHC S. POTATO SHOOT R2	0.33	10.22	66.33	8.85	1.63	12.64	68.24	167.91
22b	NHC S. POTATO SHOOT R1	ND	6.35	41.59	7.38	1.50	7.92	42.79	107.53
		0.46	25.59	164.40	20.83	4.37	31.32	169.15	415.66
Average	**NHC S. POTATO SHOOT**	**0.15**	**8.53**	**54.80**	**6.94**	**1.46**	**10.44**	**56.38**	**138.55**

3.3 Characterization of potential DDT-degrading microorganisms

Five microbial isolates exhibiting persistence and unaffected growth in soils rich in DDT were selected, further purified in the laboratory and processed for further identification to molecular level. Molecular identification was preceded by the polymerase chain reactions to extract total DNA as shown in Figure 1 below. After purification of the PCR products the 16S rRNA sequencing was done at the Inqaba Biotech, Nairobi. The sequence results were blasted in the NCBI BLASTn tool to find the closest identity of each of the sequenced organism. BLASTn search yield the identity of the five organisms sequenced as shown in Table 3 below.

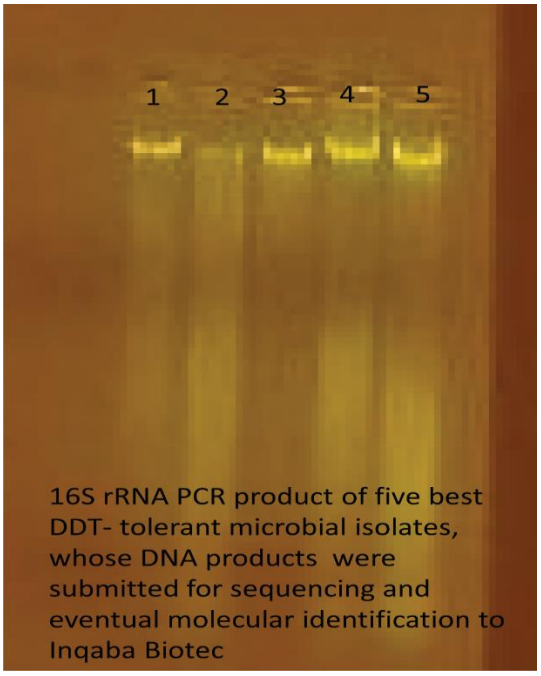


Figure 1: 16S rRNA **PCR products of best DDT-tolerant/degrading isolates from NHC Morogoro site.**

Table 3-1: Identity of Potential DDT-degrading microorganisms isolated from high DDT-contaminated spots at NHC Morogoro site

Sample ID	Identical to	(%) identity
4	*Streptomyces sp.* RM535	99
9	*Streptomyces sp.* RM365	97
17	*Streptomyces muensis* strain	85
20	*Streptomyces sp.* strain EAJJ-R19	99
23	*Streptomyces iakyrus* strain JF35	99

Strikingly all the five isolates were identified as being Streptomyces species. The role of Streptomyces in metabolizing DDT and other persistent organic pesticides has been previously reported in literature

(Kantachote, 2001; Javaid, et al., 2016). These strains could then be multiplied in the lab and re-introduced back to the site in huge numbers to stimulate an expedited the co-metabolism of DDT and its metabolites

Part B: Bioremediation of Lindane and DDT at the Plant Protection Office-Tengeru site.

4.0 Historical background and Methodological approach for PPO Tengeru Site

4.1 Location and history of the site

The PPO site is located at approximately S 03° 39.092 - E 036° 79.9200 in Arumeru District, one of six districts in the Arusha Region of Tanzania. This site was instituted by the government of Tanzania to manage migratory pest prevalence in Arusha and surrounding areas, Northern Tanzania. History shows that approximately 5 tonnes of hexachlorocyclohexane (HCH) were stored in drums and bags adjacent to the PPO between 1970 and the mid-1990. In 1995, a new storage facility was built 10 m from the open storage area. A few years later, a noticeable leakage from the containers was reported and, in the course of containing the danger, lindane (almost pure γ-HCH) was buried near the offices in 2000. Several sampling and analyses carried out over the site did reveal that in addition to lindane, which was rated the largest contaminant Persistent Organic Pesticide (POP) at the site, other pesticides found in the soils at the site include DDT(and metabolites), Diazinon, Fenthion and permethrin (Li, 1999; Kishimba, 2009, McDowall, 2009).

4.2 Site preparation, phytoremediation plant establishment and management

This demonstration project was designed to show that certain plant species (domesticated or otherwise) can be used to remove some of these pesticides from the soil as alternative to expensive remediation options. With knowledge that the site has gone through both preliminary and detailed site investigation to confirm POPs contamination, we started by identifying plant species with known abilities to bio-accumulate POPs such as lindane and DDT from soils out of both our own preliminary research and literature reports. Identified plants had proven abilities to extract either lindane or DDT from soil and they included, *Sesamum indicum* L, (lindane), *Ricinus communis* (DDT, Aldrin,

Heptachlor, Methoxychlor and lindane), *Hordeum vulgare* L (DDT), *Tricum sp*.), *Capsicum annuum* and *Brassica oleracae* var. *acephala*), (Lindane and DDT). (Abhilash and Singh (2010) Rissato et al., (2015; Mitra and Raghu, 1989),

Identification of phytoremediation plants was followed by actual clearing and tilling of the site which involved removal of a polyethylene sheet lining the ground used as a temporary measure to contain the strong smell leaking from the site to the office building. After tillage, the site was organized into 1.5 x 4 m seedbeds onto which seeds of the selected phytoremediation plants were directly planted/sowed at respective recommended agronomic spacing. Each of these plant species were directly sowed into their randomly selected plots based on recommended spacing specific to each crop. All other agronomic practices were observed during planting, growth and development of the plants. Three replications were used for each involved plant species as shown in the field experimental layout below.

PPO-TENGERU EXPERIMENTAL LAYOUT

5 SIMSIM		4 COLLARD		2 WHEAT	
1 CHILLI	P	6 CATOR PLANT	P	3 LETTUCE	
3 LETTUCE	A	2 WHEAT	A	1 CHILLI	
4 COLLARD	T	5 SIMSIM	T	6 CATOR PLANT	
2 WHEAT	H	3 LETTUCE	H	5 SIMSIM	
6 CATOR PLANT		1 CHILLI		4 COLLARD	

Key:

- Treatment plots: (4 * 1.5) m; Path between blocks: 1 m
- Path between treatments: 0.5 m

- Length of the experimental plot: 14 m
- Width of the experimental plot: 11.5 m ; Total Area: 161.00 m^2

Plants were allowed to continue with the vegetative growth until just before flowering, where it is expected that they would have reached their peak of bioaccumulation of target compounds in the biomass. Representative samples were taken from each plot by separating above-ground from below-ground biomass, packaged in clean containers and shipped to the lab for analysis of POPs including lindane and DDT (plus their respective metabolites) content in the biomass of the plants sampled. In each plot, three representative plants randomly chosen were carefully cut at the base just above the soil level and separately packaged and labelled. The below ground biomass (whole root system was also carefully recovered packaged and labelled prior to shipping to TPRI for analysis. Eventually, the samples were shipped to Tshwane University of Technology in Pretoria, South Africa for analysis.

Site preparation management and vegetative plant growth at various stages at PPO-Tengeru

4.3 Laboratory extraction and quantification of lindane and DDT from soil and plant samples

4.3.1 Extraction of plant samples from PPO Tengeru

The procedure followed here is similar to one explained under section 2.4.2 above. Briefly, the root and shoot parts of test plants namely: Castor oil, wheat, oat, collard, simsism, and hot pepper were prepared for the determination of residual levels of the targeted POPs namely lindane and DDT (plus its metabolites). Approximately 2.5 g of the plant samples were weighed into pre-cleaned amber bottles. The samples were soaked overnight with 50 mL of n-hexane:acetone (1:1, v/v) followed by ultrasonic-assisted extraction for 30 min. The set-up was allowed to cool down to room temperature and the extract was carefully transferred into a clean round bottom flask. The extraction was repeated using the same volume of the extraction solvent and time. The extracts were combined then carefully transferred into pre-clean round bottom flask before subjecting them to rotary evaporation to approximately 2 mL. Two laboratory reference plant samples (lettuce) were spiked with known amounts of the targeted compounds, and were similarly prepared following the aforementioned procedures. The spiked reference samples were employed for the estimation of the analytical recoveries of the targeted compounds.

4.3.2 clean-up of PPO plant sample extracts prior to instrumental analysis

The extracts from plant samples of PPO-Tengeru were purified using deactivated silica gel packed into glass columns. Prior to the clean-up procedure, the packed columns were conditioned using 25 mL of n-hexane to remove trapped air and possible interfering contaminants. The concentrated extract was quantitatively transferred into the glass columns eluted under gravity with 40 mL of n-hexane:acetone (2:1, v/v). The eluate was rotary evaporated to approximately 2 mL, and transferred, thereafter, into amber vials where the eluate was further concentrated with high purity nitrogen gas until it reached incipient dryness. The extracts were, then re-constituted with 1 mL of Toluene followed by the addition of 50 µL of 500 pg µL-1 of DDT-d8 that was employed as an internal standard.

5. Results and general interpretation of observations

5.1 Agronomic performance of phytoremediation plants used

In addition to differential performance during seed emergence and seedling growth, agronomic performance of the test plants was quantified through whole plant weight per plot, number and weight of seeds per plant or plot. Seed germination and seedling growth were specifically weaker in Collard, lettuce and chilli pepper. However, the plants from these species tolerated the toxic effects during the vegetative growth stages with obvious physical signs of stress as one moves from the most contaminated spots of the test site. Overall, most of the phytoremediation test plants established at the PPO Tengeru did manage to complete their life cycles when grown in the contaminated site as supported by the data presented in Tables 4-1 4-2 and 4-3 below.

Table 4-1 Vegetative performance of test plants at PPO-Tengeru- Weight of seeds per plant

	WEIGHT OF SEED/ PLANT (grams)		
Treatment plant	R1	R2	R3
WHEAT	11.96	11.73	14.06
OAT	9.22	15.71	9.95
*COLLARD	0	0	0
SIMSIM	7.08	44.93	9.62
HOT PEPPER	65.43	73.84	34.69
CASTOL OIL PLANT	254.17	104.58	396.98

CV (%) = 3.6

*= No seeds at the time of measurement

Table 4.2 Vegetative performance of test plants at PPO-Tengeru- Weight of whole plants per plot

Treatment plant	WEIGHT OF WHOLE PLANT/PLOT (grams)		
	R1	R2	R3
WHEAT	1129.14	951.2	736.3
OAT	979.6	1440.75	647.2
COLLARD	802.71	6609.1	2649.01
SIMSIM	1480.95	1399.2	462.88
HOT PEPPER	5846.82	1591.5	5139.12

CV (%) = 7.1

Table 4.1 Vegetative performance of test plants at PPO-Tengeru- Number of seeds per plant

Treatment plant	NUMBER OF SEEDS PER PLANTS		
	R1	R2	R3
WHEAT	300.4	284.8	353.4
OAT	164.4	280.6	179.4
COLLARD*	0	0	0
SIMSIM	35.4	67	13.4
HOT PEPPER	16.4	18.4	8.4
CASTOL OIL PLANT	146	53.33	213.33

CV (%) = 14.8

** = No seeds at the time of measurement*

5.2 Uptake of two major POPs-Lindane and DDT by tested phytoremediation plants at PPO-Tengeru site.

Uptake of lindane and DDT at the PPO-Tengeru site by the test plants established there was tested by six different plant types previously widely reported of being capable of bio-accumulating either lindane or DDT into their below- and/or above ground biomass. Results presented in Table 5-1 indicate that uptake of lindane was undetected in all but two of the test plants namely oat and castor oil. Castor oil plant root had the lowest average lindane concentration of 0.05 ng/g while oat shoot recorded an average of 0.19 ng/g.

Unlike lindane, the concentration of DDT in the biomass of plant samples was significantly higher in most of the test plant samples. Simsim shoot biomass recorded the highest concentration of total DDT of 362.22 ng/g closely followed the amount in wheat shoot at 202.56 ng/g. Similarly, the lowest concentration of DDT was recorded in castor oil root at 0.745 ng/g.

Table 5.1: Concentration of DDT and lindane in biomass of selected phyto-remediation plants grown at PPO Tengeru site in Arusha

		Concentration (ng/g)							
Sample code	Sample name	Lindane	Mitotane (o,p-DDD)	p,p'-DDD	o,p-DDE	p,p'-DDE	o,p-DDT	p,p-DDT	Total DDT
15	TNR OAT ROOT R2	ND	2.11	6.93	2.19	0.14	1.3	6.93	19.6
21	TNR OAT ROOT R3	ND	7.25	15.84	2.58	0.05	3.46	16.09	45.27
Total		0	9.36	22.77	4.77	0.19	4.76	23.02	64.87
Average	**TNR OAT ROOT**	**0.00**	**4.68**	**11.39**	**2.39**	**0.10**	**2.38**	**11.51**	**32.44**
26	TNR OAT SHOOT R3	ND	0.26	0.73	0.13	<0.01	ND	ND	1.12
10b	TNR OAT SHOOT R2	0.38	0.36	1.51	0.26	0.05	0.29	1.54	4.01
Total		0.38	0.62	2.24	0.39	0.05	0.29	1.54	5.13
Average	**TNR OAT SHOOT**	**0.19**	**0.31**	**1.12**	**0.20**	**0.03**	**0.15**	**0.77**	**2.565**
2	TNR C.OIL ROOT R1	0.1	0.1	0.3	0.24	0.04	9.63	51.38	61.69
6	TNR C.OIL ROOT R3	ND	0.55	34.6	1.06	0.09	6.54	35.42	78.26
Total		0.1	0.65	34.9	1.3	0.13	16.17	86.8	139.95
Average	**TNR C.OIL ROOT**	**0.05**	**0.33**	**17.45**	**0.65**	**0.07**	**8.09**	**43.40**	**69.975**
19	TNR C.OIL SHOOT R1	ND	1	ND	0.38	0.02	ND	ND	1.4
25	TNR C.OIL SHOOT R2	ND	0.04	ND	0.04	0.01	ND	ND	0.09
Total		0	1.04	0	0.42	0.03	0	0	1.49
Average	**TNR C.OIL SHOOT**	**0**	**0.52**	**0**	**0.21**	**0.015**	**0**	**0**	**0.745**
3	TNR COLLARD SHOOT R2	ND	1.01	ND	0.18	<0.01	ND	0.8	1.99
4	TNR COLLARD SHOOT R3	ND	0.85	0.33	0.13	0.01	ND	0.23	1.55
Total		0	1.86	0.33	0.31	0.01	0	1.03	3.54
Average	**TNR COLLARD SHOOT**	**0**	**0.93**	**0.165**	**0.155**	**0.005**	**0**	**0.515**	**1.77**
10	TNR COLLARD ROOT R1	ND	2.39	9.81	0.83	0.01	1.54	8.13	22.71
28	TNR COLLARD ROOT R3	ND	2.85	12.4	2.56	0.88	ND	12.58	31.27
Total		0	5.24	22.21	3.39	0.89	1.54	20.71	53.98
Average	**TNR COLLARD ROOT**	**0.00**	**1.75**	**11.11**	**1.70**	**0.45**	**0.77**	**10.36**	**26.11667**
5	TNR HOT PEPPER SHOOT R3	ND	0.14	8.82	0.46	0.01	2.54	8.99	20.96
18	TNR HOT PEPPER SHOOT R2	ND	1.33	11.03	0.45	0.01	2.16	11.28	26.26
		0	1.47	19.85	0.91	0.02	4.7	20.27	47.22
Average	**TNR HOT PEPPER**	**0.00**	**0.74**	**9.93**	**0.46**	**0.01**	**2.35**	**10.14**	**23.61**

	SHOOT								
22	TNR HOT PEPPER ROOT R2	ND	0.66	99.03	0.67	0.65	ND	ND	101.01
	TNR H. PEPPER R1	ND	0.33	9.25	1.13	0.3	9.65	9.4	30.06
		0	0.99	108.28	1.8	0.95	9.65	9.4	131.07
Average	TNR HOT PEPPER SHOOT	0	0.495	54.14	0.9	0.475	4.825	4.7	**65.535**
8	TNR SIM SIM SHOOT R3	ND	21.59	279.61	67.44	7.29	53.32	287.71	716.96
30	TNR SIM SIM SHOOT R1	ND	0.05	7.32	0.10	0.01	ND	ND	7.48
		0	21.64	286.93	67.54	7.30	53.32	287.71	724.44
Average	TNR SIM SIM SHOOT	0	10.82	143.47	33.77	3.65	26.66	143.86	**362.22**
9	TNR SIM SIM ROOT R1	ND	1.64	ND	4.21	0.61	ND	1.92	8.38
13	TNR SIM SIM ROOT R2	ND	0.04	ND	0.01	<0.01	0.00	ND	0.05
		0.00	1.68	0.00	4.22	0.61	0.00	1.92	8.43
Average	TNR SIM SIM ROOT	0.00	0.84	0.00	2.11	0.31	0.00	0.96	**4.215**
12.00	TNR WHEAT SHOOT R2	ND	7.56	37.09	0.60	0.11	8.14	37.82	91.32
20.00	TNR WHEAT SHOOT R1	ND	0.07	26.73	1.40	0.01	5.81	27.37	61.39
31.00	TNR WHEAT SHOOT R3	ND	0.40	429.60	2.33	0.19	7.70	14.76	454.98
		0.00	8.03	493.42	4.33	0.31	21.65	79.95	607.69
Average	TNR WHEAT SHOOT	0.00	2.68	164.47	1.44	0.10	7.22	26.65	**202.56**
14.00	TNR WHEAT ROOT R2	ND	1.26	ND	1.14	1.57	ND	2.85	6.82
16.00	TNR WHEAT ROOT R1	ND	0.79	ND	1.40	0.10	5.22	ND	7.51
		0.00	2.05	0.00	2.54	1.67	5.22	2.85	14.33
Average	TNR WHEAT ROOT	0.00	1.03	0.00	1.27	0.84	2.61	1.43	**7.165**

General Discussion

This demonstration project emerged out of several confirmatory studies that the two sites at PPO Tengeru and NHC Morogoro were indeed contaminated with various POPs. Phyto- and micro-remediation options were then recommended for a demonstration pilot project at NHC Morogoro and only phytoremediation was earmarked for the PPO-Tengeru site. We have managed, in this study, to show that the dominant contaminating POP (i.e. DDT) has indeed been percolating down the profile and significant amounts of the chemical were found in soil samples taken 2 m down the soil column. The concentrations of DDT and its metabolites detected in profile sample exceeded maximum permissible limits according to the Tanzanian Environmental Management (Air, Water and Soil) regulations, 2007. Government Notice Number 237, 238 and 239 (URT, 2007). The discovery that DDT has indeed migrated down the profile consolidates our initial assumption that both underground and on surface lateral movements of the contaminant can put both water streams and vegetable production activities downstream towards the railway station at risk of contamination.

At both NHC Morogoro and PPO Tengeru sites, all phytoremediation plants deployed were able to extract significant amounts of DDT although amounts extracted were far greater at NHC Morogoro than PPO Tengeru. This observation can be attributed to the fact that DDT levels were clearly higher at NHC Morogoro.

Observations that the test plants successfully extracted DDT from the contaminated soil are in line with a handful of previous scientific reports on this subject matter. Whitefield et al. (2010), for example, reported significant extraction and bioaccumulation of DDT into the biomass of pumpkin, *Cucurbita pepo* ssp. pepo. In a separate study *Cucurbita pepo* (species pumpkin and zucchini) were reported to achieve the highest phytoremediation indices (measured through their respective amounts of total DDT bioaccumulated from contaminated medium) compared to other vascular plants (Lunney et al., 2004). Uptake and developmental effects of DDT on other test plants used both at PPO Tengeru and NHC Morogoro sites observed in this report, are generally in line with a handful of previous scientific reports. For example, the uptake of DDT by Chilli pepper *Capsicum annuum* has been reported previously (Mitra and Raghu, 1998). Castor oil plant, *Ricinus communis* L has been shown to be an excellent remover of both o,p′-DDT and p,p′-DDT from contaminated soils (Rissato et al., 2015).

Observations that lindane uptake by the tested plants was either undetected or very insignificant are not surprising because phytoremediation of Lindane from soils is not widely reported in literature, apparently because lindane is generally hardly taken up by most plant species. A report by the International programme on Chemical Safety (IPCS) of the World Health Organization (1991) indicated that uptake and translocation of lindane and -y-PCCH in plants is limited, especially in soils with a high content of organic matter. Previous studies have shown that both lindane taken up from soil and its metabolites were not evenly

distributed within the plants: Comparatively high residue levels were always detected in the roots, whereas only small amounts were translocated into stems, leaves, and fruits (WHO, 1991)

Bioremediation potential of the five microbial isolates characterized in this study, has shown that these isolates indeed tolerate relatively high levels of DDT in their growth medium and that they could potentially be either further trained or directly released into a contaminated field to aid with bioremediation of DDT and other related metabolites.

Conclusion:

In this general study, we have shown that certain plant species, when carefully managed, can be deployed to sites contaminated by DDT and other POPs to act as phytoremediation agents, extracting sizable amounts of the pesticides and locking them into their biomass. The resultant biomass will then need to be consigned for a designated degradation facility. Clearly, most of the plants tested in this study were good DDT absorbers but all plants experienced reasonable difficulties at uptake of lindane from the soil. Similarly, We have exhibited in this report that certain microbial species have the capacity to biotransform POPs such as DDT to lesser toxic metabolites

References

Commission for Environmental Cooperation. (2006). *North American Regional Action Plan (NARAP) on lindane and other hexachlorocyclohexane (HCH) isomers.*

World Health Organization (WHO), International Programme on Chemical Safety. Health and Safety guide no. 54. Lindane (Gamma HCH) health and safety guide. Geneva. Pp 44.

Dutta, M. and Schafer, K. S. (2003). *Lindane: answers to common questions.*

Hayes, W. J. (1982). *Pesticides studied in man*. Williams and Wilkins.

IARC. (1979). IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some halogenated hydrocarbons. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 20: 195-241.

Lunney, A. I., Zeeb, B. A. and Kenneth J. Reimer, K.J. (2004). Uptake of Weathered DDT in Vascular Plants: Potential for Phytoremediation. *Environmental Sciences and Technology* 38 (22): 6147–6154. DOI: 10.1021/es030705b

Meister, R., Berg, G., Sine, C., Meister, J. Poplyk, J. (Eds) (1998). *Farm Chemicals Handbook.* Willoughby: Meister Publishing Co. pp. C48-C234.

SRI. (1987). *Directory of Chemical Producers* (pp. 173). Menlo Park: SRI International.

Whitfield Aslund ML1, Lunney AI, Rutter A, Zeeb BA. (2010). Effects of amendments on the uptake and distribution of DDT in Cucurbita pepo ssp pepo plants. *Environ Pollution*. 158(2):508-13. doi: 10.1016/j.envpol.2009.08.030.

Weinberg, J. (2008). *An NGO Guide to Persistent Organic Pollutants, A Framework for Action To Protect Human Health and the Environment From Persistent Organic Pollutants (POPs).*

Dubey RK1, Tripathi V1, Singh N2, Abhilash PC3 (2014) . Phytoextraction and dissipation of lindane by Spinacia oleracea L. Ecotoxicol Environ Saf. 109: 22–26.

Abhilash and Singh , 2010. Effect of growing Sesamum indicum L. on enhanced dissipation of lindane (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) from soil. Int J Phytoremediation. 12(5):440-53.

Rissato, S.R., Galhiane, M.S., Fernandes, J.R. Gerenutti, M, Gomes, H.M, Ribeiro, R and Almeida, M.V. (2015). Evaluation of *Ricinus communis* L. for the Phytoremediation of Polluted Soil with Organochlorine Pesticides. BioMed Research International. http://dx.doi.org/10.1155/2015/549863.

J. M. Aislabie , N. K. Richards & H. L. Boul (1997) Microbial degradation of DDT and its residues—A review, New Zealand Journal of Agricultural Research, 40:2, 269-282, DOI: 10.1080/00288233.1997.9513247

Chauhan A. and Singh, J (2015). Biodegradation of DDT. Journal of Textile Science and Engineering. 5:1 DOI: 10.4172/2165-8064.1000183

Javaid, M.K., Ashiq, M. and Tahir, M. (2016). Potential of Biological Agents in Decontamination of Agricultural Soil. Scientifica. http://dx.doi.org/10.1155/2016/1598325

Abhilash PC and Singh N (2010). Effect of growing *Sesamum indicum* L. on enhanced dissipation of lindane (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) from soil.

Mitra, J. and Raghu, K. 1989. Effects of DDT on the growth of crop plants. Environ. Pollut. 61: 157–170.

Mitra, J. and Raghu, (1998). Detrimental effects of soil residues of DDT on chillies (Capsicum annuum). Fresenius Environmental Bulletin 7(1):008-013

Karthikeyan, R., Davis, L.C., Erickson, L.E., Al-Khatib, K., Kulakow, P.A, Barnes, P.L., Hutchinson, S.L. and Nurzhanova, A. (2004). Potential for Plant-Based Remediation of Pesticide-Contaminated Soil and Water Using Nontarget Plants such as Trees, Shrubs, and Grasses. Critical Reviews in Plant Sciences, 23(1):91–101.

URT (2007): The Environmental Management (Air, Water and Soil) regulations, 2007. Government Notice Number 237, 238 and 239