International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-5839, Volume 9, Issue 2; April -June, 2022, pp. 6-13

https://www.krishisanskriti.org

Isolation and Screening of Bacterial Strains from Sewage Water Enriched on Non-organochlorine Pesticide Mixture for Potential Degraders of Lindane

Dr. Satish Kumar*[1&2], Dr. Rajkumar Bidlan[2], Dr. Shukla Saluja[1] and Prof. Jai Gopal Sharma[2#]

[1]Department of Botany, Sri Venkateswara College, University of Delhi, New Delhi- 110021, India
[2]Department of Biotechnology, Delhi Technological University, Delhi- 110042, India
E-mail: skbiodelhi@gmail.com

Abstract—*Lindane (γ-hexachlorocyclohexane) belongs to organochlorine pesticides. Organochlorine pesticides including DDT and lindane have been used for many years in agriculture, public health programmers and cosmetic products. Its persistence in nature is an important environment and health concern. New formulated pesticides from organophosphates and pyrethroids are gaining acceptance in the global market, it becomes imperative that strategies be developed to clear the environment off the residues of organochlorine pesticides without getting affected by the other pesticide classes. We enriched a consortium on non-organochlorine pesticide mixture i.e. commercial formulations from local market and utilised this consortium for studying the degradation of lindane in a synthetic effluent generated in the lab by spiking minimal medium with lindane. We found that the consortium could be resolved into 18 bacterial strains when induced with lindane and was able to degrade 85% and 75% of 5 ppm and 10 ppm lindane by 5 days of incubation respectively. Higher concentrations such as 15 ppm to 30 ppm were degraded up to 60% by the end of 5 days. We hypothesise that the growth leads to the secretory enzyme production that usually act on the residual lindane. This would reduce the chances of our consortium becoming a cause of concern as the inoculum might gradually die with the reduction in the residual pesticide levels in the agricultural land and environment.*

Introduction

Pesticides are natural or synthetic substances used to eradicate and/or controller of pest, insects and unwanted species. These could be either a single substance or mixture of various substances. Apart from killing the pests and insects they are used as preservative in packed food items and beverages. Lindane {Gamma (γ)-hexachlorocyclohexane} belongs to the organochlorine pesticide class of the synthetic pesticides. Organochlorine is one of the oldest classes of synthetic pesticides. Organochlorine pesticides including DDT and lindane have been used over many years in agriculture, public health programmers and cosmetic products, and few OC's are still used for health program and cosmetic products. Gamma isomer of Hexachlorocyclohexane (Lindane; CAS No. 58-89-9) is one of the major OCPs used by several countries. Lindane (γ-HCH) was first synthesized in 1825 by Michael Faraday and first time isolate and describe by Teunis van der Linden in 1912. According to Safe (1993), hexachlorocyclohexane (HCH) consists of eight isomers, whereas according to Vijgen (2006) HCH consists of five isomers namely α, β, γ, δ and ϵ, while only γ isomer shows insecticidal property. The formulation of HCH contains 60-70% α isomer and 10-12% γ isomer. Alpha isomer do not have any insecticidal property, that wise it was dumped into soil approximate 6 million tons in many part of World even in India (Weber *et al*., 2008).

Lindane and other polychlorinated pesticides were acquired into the environment (Simonich and Hites, 1995) and have been suspected causative agents for numerous health problems including respiratory symptoms (ATSDR, 2005; Ye *et al.*, 2013). In a research survey in the national capital city of India it was observed that at least half of the collected samples of buffalo milk harboured lindane above the WHO/FAO tolerance limits (Aslam *et al*., 2013). According to Vankar and Sahu (2011) estimated 10.03 ppm lindane residues have been detected near the lindane dumping unit from a HCH manufacturing unit in the Lucknow city of India. According to Agrawal and Shahi (2015) lindane is a causative agent for many health problems like respiratory disorders and influence on neurotransmitter by affecting the nervous system and many organs like liver, kidney etc. due to increased pesticides from the environment. With this in view, we tried to enrich the bacterial strains from microbial consortium that is tolerate to the combination of organophosphate and pyrethoids pesticides and studied its effectiveness towards lindane degradation in the laboratory.

Materials and methodology

Chemicals: Lindane (98% purity) was procured from Sigma - Aldrich, USA. Commercial formulations of mixed pesticides (pyrethroids and organophosphates) were procured from Azad Market, Delhi and Saharanpur, Utter Pradesh, India. Nutrient broth and agar, were purchased from Hi-Media Lab Private Limited, India. Other chemicals like *o*-tolidine was used in this study were of standard manufacturers and purchased from authorized vendors.

Solvents: Acetone, cyclohexane, n-hexane, dichloromethane, N, N'- dimethylformamide, ethanol, hydrochloric acid, amyl alcohol, glacial acetic acid, immersion oil, Glycerol. All other analytical reagents were HPLC and GC grade. These solvents were procured from authorized vendors as per Institution.

Glassware and plasticwares: Petridishs, conical flask, volumetric flask, separatory funnel, measuring cylinders, beakers, pipettes, test tubes, and others were manufacturers Borosil and Tarson brand and procured from authorized vendors.

Culture medium: LB and NA medium were used for bacterial growth and minimal medium (M_4) was used for lindane degradation study. pH (7±0.2) of cultures medium were adjusted with help of 1N HCL and 1N NaOH.

Sterilization: Culture and minimal medium, distilled water, petri-plates and other glasswares were disinfected by autoclaving at 121ºC (115 lb psi) for 20 to 30 minutes, depending on volume and size of materials. Plastic wares were cleaned with 70% ethanol before use. After autoclave prior to their use all culture tools except bacterial culture and distilled water, tissue paper, micro-tips spite lamp, etc., were exposed to ultraviolet light in a laminar flow cabinet for 30 minutes. The exterior of laminar air flow and fingers of hands were sanitized with 70% ethanol before the starting the experiment.

Collection and preparation of sample: Following steps were involved for sample preparation.

- Water sample collected from contaminated sites and sludge.
- Samples thoroughly mixed and filtered through Whatmann No.1 Filter paper
- Added incremental amounts of pesticide mix (commercial formulations) periodically.

Enrichment of the microbial population: The enrichment technique was developed according to Kumar *et al* (2016) with minor modifications. The sewage sludge filtrate was incubated at room temperature (R.T. = 25-30 ^{O}C) for 9 months with addition of gradual increasing pesticide concentrations to it at regular intervals. This was done by initially adding 1/10 NB media and 2 ppm each of commercial formulations of various pesticides (organophosphates and pyrethroids but not organochlorine) and then increasing the pesticide concentrations gradually to 100 ppm by the end of 9th month. As per table No. 1 mixture of commercial formulations further diluted with autoclaved distilled water.

Table 1: Pesticides used for enrichment of bacterial consortium from commercial formulations.

Sl. No.	Pesticides	% (EC)	Dilution	Volume used
1	Chloropyriphos	20%	100ml >1000ml	10 ml
2	Profenofos & Cypermethrin	40% 4%	50ml > 1000ml	40 ml
3	Deltamethrin & Triazophos	1% 35%	50ml > 1000ml	30 ml
4	Lambda-Cyhalothrin	5%	100ml > 1000ml	10 ml
5	Dichlorvos	76%	10ml > 1000ml	8 ml
6	Cypermethrin	25%	100ml > 1000ml	12 ml

Screening for lindane degraders: The cultures thus enriched were then isolated at regular intervals for viability of bacterial strains and screened for their potential to degrade lindane. Screening process was performed by two methods, first various concentration (5ppm to 30ppm) of lindane added into M_4 agar medium and observed the growth of bacterial strains after 24 hours. In second method bacterial strains from enriched consortium were cultured in nutrient agar plates by streaking without added any concentration of lindane. Dense growth of bacterial strains were observed into nutrient agar plates within 24 hours. Lindane (0.5% in acetone) were sprayed all over the culture plates under aseptic conditions. After evaporation of acetone a thin film of lindane was found in culture plates. Few bacterial strains were tolerate and consumed this lindane film and the zone of partial clearance were observed after 48 hours and completely clearance after 144 hours of incubation. These positive bacterial strains were isolated and purified for further investigation of lindane degradation.

Inoculum: The consortium was inoculated to nutrient broth and nutrient agar, and incubated at 25-30 ^{o}C for 24 hours. 5 ppm of lindane was supplementary for acclimatization of culture and was incubated further for 72 hour at 25-30 ^{O}C. Incubated culture was harvested by centrifugation, washed two times in minimal medium and further induced with 5 ppm lindane in minimal medium for 72 h at 25-30 ^{O}C. This induced culture was harvested by centrifugation, washed again two times in minimal medium and was used as inoculum for degradation studies. The concentration of inoculum was optimized by UV-Vis Spectrophotometer at 600 nm.

Lindane degradation by the consortium: The mixed population obtained as enriched culture from the 9 month enrichment was screened for lindane degradation in broth. 5

ml minimal medium was placed in test tubes and autoclaved for 20 minutes at 121 ^{o}C. After cooling to 25-30 ^{o}C, lindane (as dimethylformamide solution) was added to the medium to a final concentration of 5 ppm through 30 ppm separately in triplicate sets. The induced consortium was inoculated at 0.01 OD_{600}, mixed well and incubated at 25-30 ^{o}C for various time periods. Whole tube samples (in triplicates) were collected at the pre- determined periods for estimating growth and residual lindane. Growth was estimated by reading OD_{600} against the minimal medium and residual lindane was estimated after extraction and performing thin layer chromatography. Tubes with minimal medium and lindane were taken as controls for each concentration and each incubation period.

Extraction of residual lindane: Residual lindane was extracted twice using various organic solvent in glass separating funnel. Experimental samples were terminated by adding few drops of concentrated HCl at various interval (0 hour to 144 hour) of every 24 hours. Acidification process was helpful to stop the growth and metabolic reactions in sample. Acidified samples were used for extraction of lindane with equal volume of various organic solvent in glass separating funnel. Maximum concentration of lindane was extracted by dichloromethane solvent. The solution of dichloromethane and lindane were separated with funnel having anhydrous sodium sulphate in filter paper. The organic solutions were filtered into glass test tube and after evaporating the organic solvent, the dry content of lindane were dissolved in acetone twice. Acetone solutions were transferred to 1.5 ml micro-centrifuge tube. Dry sample having residues of lindane was dissolved in 0.1 ml of acetone just before analysis of degradation by TLC.

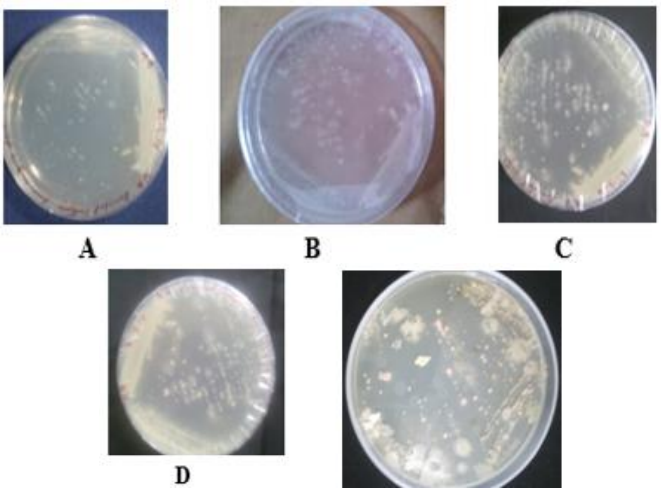
Silica thin layer chromatography: The residues of lindane was detected by using thin layer chromatography on G silica gel coated plates. The 10 x 10 cm^2 and 20 x 20 cm^2 plates were arranged on TLC platform by a spreader using silica gel. The thickness of coated plates were 0.25 mm and uniform layer. The coated plates were activated at 120 ^{o}C for 1 hour. A fixed volume (0.1 ml) of the extracted lindane was loaded on the activated plates. Various combination of organic solvents were used as mobile phase. Cyclohexane: *n*-hexane (4:1) solvent system was significant solvent as mobile phase. When solvent front reached around 2/3 height of the plate the loaded plates were removed from chromatography chamber and allowed to dry. The plates were sprayed with a solution of 2% *o*-tolidine (in acetone) was sprayed to detect the lindane spots, these spots intensity were observed in bright or sunlight. The intensity of spots were observed and calculated the spot area. The range of known amounts of lindane were spotted on TLC plate for preparing the standards curve. This standards curve was used as a reference curve.

Conditions of gas chromatography:
The residues lindane was determined and quantified by gas chromatography (Shimadzu AQC - 2010). Conditions for gas chromatography were following.

- Column Name: Rxi-SilMS with internal diameter of 0.25 mm
- Temperature 260 ˚C, pressure 15.6 kPa
- Total Flow: 16.3 mL/min
- Column Flow: 1.21 mL/min
- Detector: Flame Ionization Detector
- Gas: He
- Solvent for sample: Acetone
- Injection volume: 2µL
- The retention time of lindane was 13.097 min.

Results and Discussion

Enriched population by the mixed commercial formulation of pesticides: The mixed population that got enrichment till 9 months with regularly increase the concentrations (2ppm to 100 ppm) of commercial formulated pesticides (without organochlorine). In bacterial consortium consisted of various colour colonies on NA medium in petri plates, which were morphologically different. These were further isolated and purified by frequently streaking and preserved on nutrient agar medium with 2ppm to 10ppm mixture of non organochlorine pesticides. Morphologically 18 bacterial strain were reconstitute in equal sizes with help of spectrophotometer. This consortium having 18 bacterial strain was used for the present study of lindane degradation.



Figs 1 Plates during enrichment by mixed pesticides (After: A = 24H; B = 1M; C = 3M; D = 6M, and 9 Months).

Purification and screening of the lindane degrading bacterial strains: The screening process was performed on agar plate. Bacterial isolates in the plates are those with 48 h incubation with the lindane layer sprayed over them (Fig. 2). 18 cultures were degraded the lindane film and made a zone of clearance the lindane film. Thus these bacterial isolates have

the potency for lindane degradation. The reconstituted consortium of bacterial isolates was induced in the laboratory with 2ppm, 5ppm and 10ppm lindane for 3 cycles in broth and then considered for further studies on lindane degradation. Similar study also done by Bidlan and Manonmani (2002) via three *Pseudomonas* strains and *Serratia marcescens* DT- 1P for DDT degradation. According to Pannu and Kumar, 2014 nine bacterial strains were capable to degradating lindane film within 7 days of inoculation, out of 9 bacterial strains only 3 bacterial strains were survived in the presence of 100 ppm lindane. Effect of cadmium and other toxic supplement were study by Kumar *et al*., 2010 and other workers respectively.

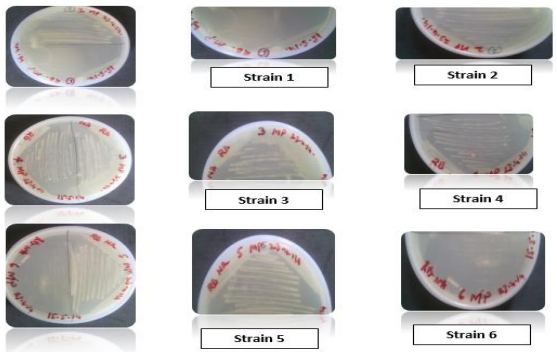


Fig. 2 Bacteria capable of lindane degradation from the enriched consortium after purification.

Lindane degradation by the reconstituted consortium

Reconstituted consortium obtained in the laboratory after nine months of enrichment of the native population from the sewage studied for the degradation of different concentrations of lindane (5 ppm through 30 ppm) in 144 h incubation under stationary conditions at 25-30 ^{o}C showed degradation between 60% - 85% (Fig. 3).

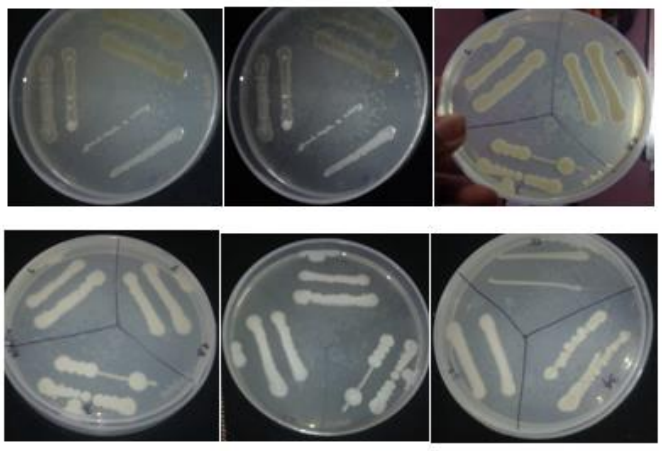


Fig. 3 Potential bacterial strains for degrading lindane

We found that the consortium could be resolved into 18 bacterial strains when induced with lindane and was able to degrade 85% and 75% of 5 ppm and 10 ppm lindane by 5 days of incubation respectively. Higher concentrations such as 15 ppm to 30 ppm were degraded up to 60% by the end of 6 days. The zig-zag pattern of growth for each concentration is depicted in Fig. 15 to 20. One common trend observed was the initial increase in biomass in all concentrations of lindane considered for the study up to 36 h except for 30 ppm, where the growth increased till first 48 h. Hence, the other sets of experiments were conducted with inoculum size of culture OD_{600} = 0.020 to 0.035. The lower concentration of 5 ppm was readily taken up by the consortium and around 60% of the available lindane disappeared in the first 3 h while the next immediate concentration of 10 ppm took 18 h to degrade the same percentage of the supplied lindane. Jilani (2013) also observed same type of research work. Some organic compounds and pesticides were used for enrichment of microbial consortium and various bacterial strains is done by Manonmani *et al*., 2000; Bidlan, 2003; Bidlan *et al.*, 2004 and Nagpal & Paknikar, 2006 and Murthy & Manonmani, 2007. *Eschericia coli* was obtained from rat faeces for lindane degradation studies by Francis *et al.* (1975) that 10% degradation of the supplementary 0.04% lindane, when enrichment of sewage and soil could not yield any promising strain. Our methodology was to acclimatize that could be helpful in the present and future situation, because most of the countries of world were used and in present time applying the commercial formulations of pyrethroids and organophosphates as a substitute of organochlorine pesticides like DDT and lindane. Similar type of study were performed by various groups of researcher like Slotkin and Seidler (2009), Kamel *et al.*, 2012; while David *et al.* (2014) demonstrated various harmful effect of organophosphates. We required to develop microbial population that is tolerant and degradation to the present or future higher concentrations of various organochlorine and non- organochlorine pesticides in the environmental samples.

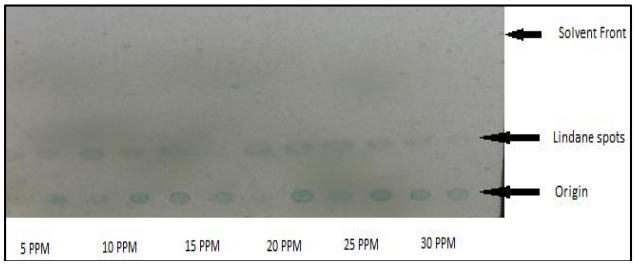


Fig. 4 TLC plate for estimating of different concentrations of lindane (5 ppm to 30 ppm) at 0 hour.

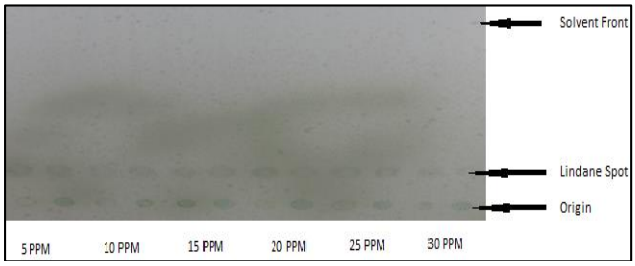


Fig. 5 TLC plate for estimating of different concentrations of lindane (5 ppm to 30 ppm) at 24 hour.

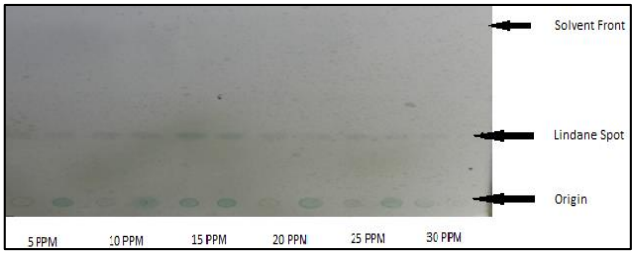


Fig. 6 TLC plate for estimating of different concentrations of lindane (5 ppm to 30 ppm) at 96 hour.

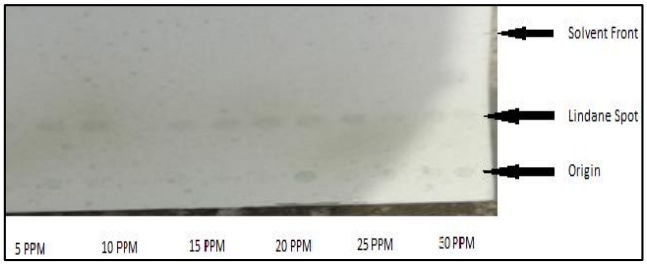
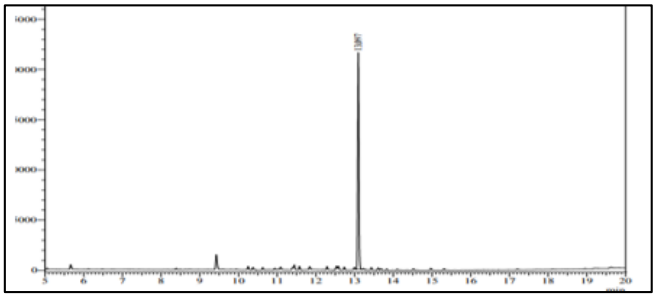
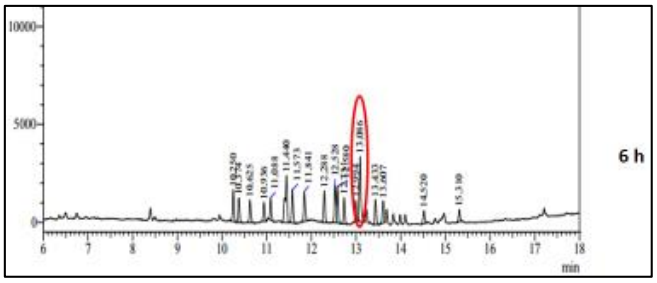


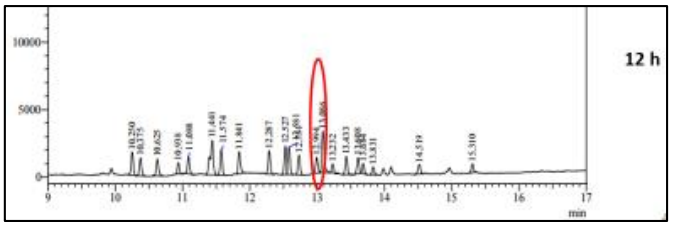
Fig. 7 TLC plate for estimating of different concentrations of lindane (5 ppm to 30 ppm) at 144 hours.



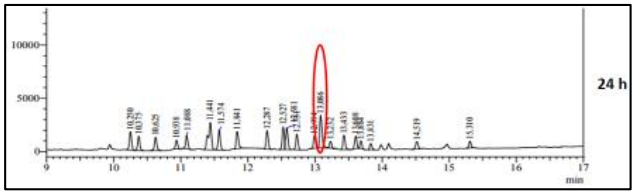
Figs: 8 GC standard lindane chromatogram.



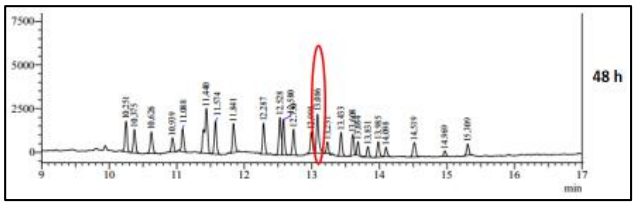
Figs: 9 GC chromatogram for degradation of 30 ppm lindane at 6 hour.



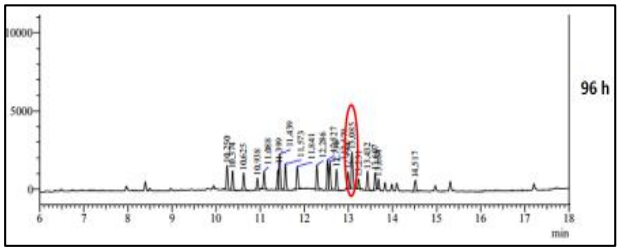
Figs: 10 GC chromatogram for degradation of 30 ppm lindane at 12 hour.



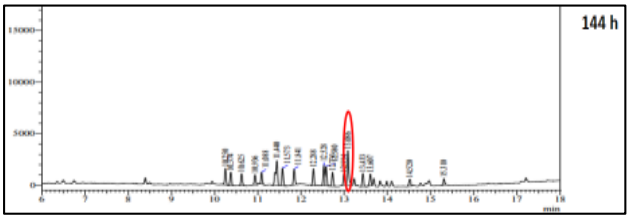
Figs: 11 GC chromatogram for degradation of 30 ppm lindane at 24 hour.



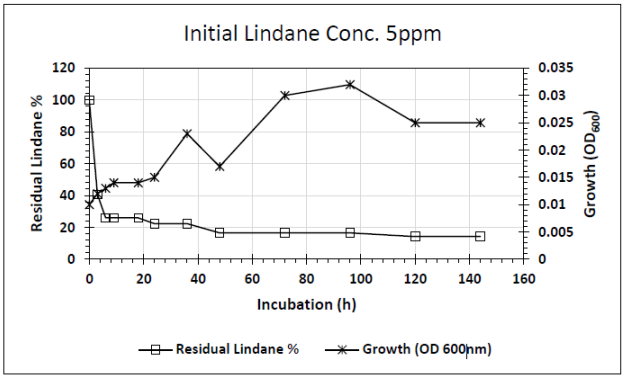
Figs: 12 GC chromatogram for degradation of 30 ppm lindane at 48 hour



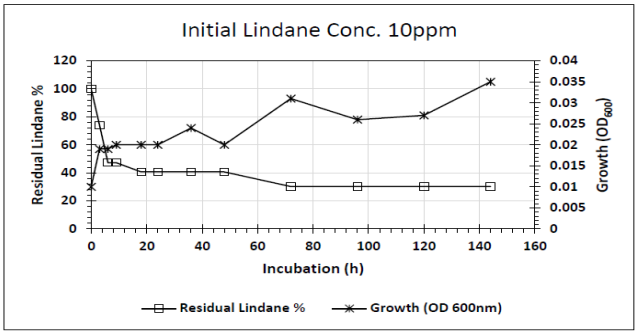
Figs: 13 GC chromatogram for degradation of 30 ppm lindane at 96 hour



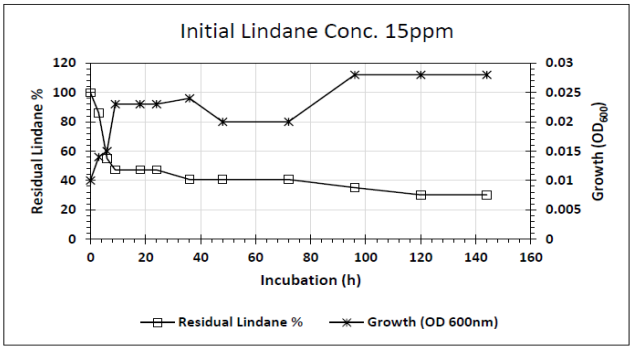
Figs: 14 GC chromatogram for degradation of 30 ppm lindane at 144 hour.



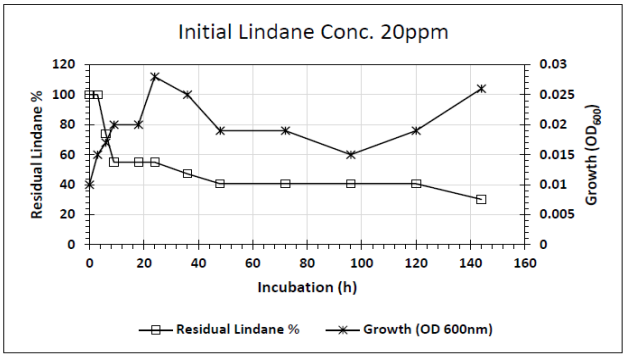
Figs 15: Bacterial growth versus degradation, 5ppm initial concentration of lindane



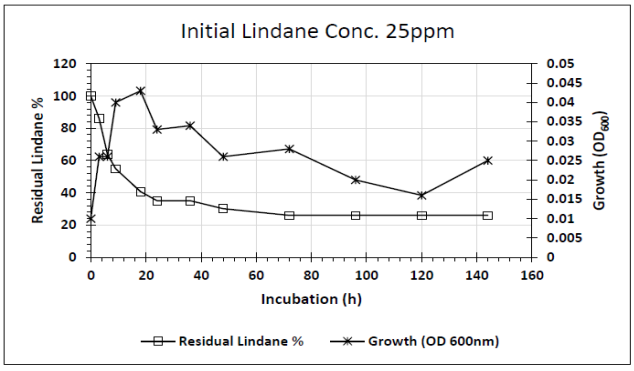
Figs 16: Bacterial growth versus degradation, 10ppm initial concentration of lindane



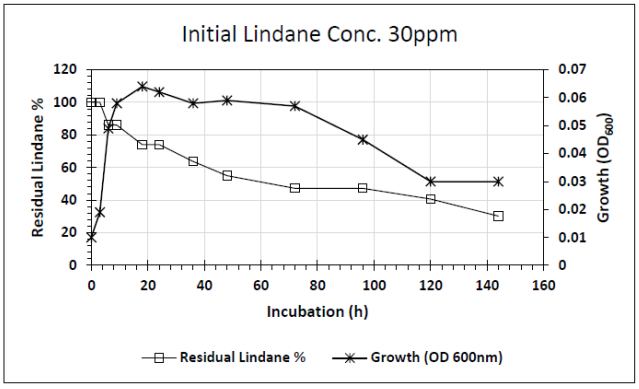
Figs 17: Bacterial growth versus degradation, 15ppm initial concentration of lindane



Figs 18: Bacterial growth versus degradation, 20ppm initial concentration of lindane



Figs 19: Bacterial growth versus degradation, 25ppm initial concentration of lindane



Figs 20: Bacterial growth versus degradation, 5ppm to 30ppm initial concentration of lindane

Conclusion

Microbial degradation is the safe approach and cost effective and that can be established and adapted for improving the environmental condition by remediating lindane contamination. We enriched a microbial consortium (bacterial population) consisting of morphologically 18 distinct bacteria using commercial formulations (pyrethroids and organophosphates) and mixture but without organochlorine. Our bacterial consortium facilitated 85% to 60 % degradation of 5ppm to 30ppm lindane within 5 days. In our study first time reported that bacterial population gradually completely enriched on the mixtures of commercial formulations pesticides. In this study bacterial population from the enriched consortium had the potency to degrade these new pesticides, because we were enriched by the mixture of pesticides from two group and degradation of pesticides from third group. The present research work and strategies are helpful in saving the environmental from toxic substances for the future generations

Acknowledgements

This research work was financial supported by University Grants Commission (UGC) for providing the post- doctoral fellowship and junior/senior research fellowship to Bidlan and Kumar respectively.

References

[1] Agrawal N. and Shahi S. K. 2015. An environmental cleanup strategy- Microbial transformation of xenobiotic compounds. Int. J. Curr. Microbiol. App. Sci. 4(4): 429-461.

[2] Aslam M., Rais S. and Alam A. 2013. Quantification of organochlorine pesticide residues in the buffalo milk samples of Delhi city, India. J. Environ. Protec. 4: 964-974.

[3] ATSDR Agency for Toxic Substances and Disease Registry: ToxFAQs for hexachlorocyclohexane. 2005. Hexachlorocyclohexane (HCH). Atlanta, USA.

[4] Baczynski T., Pleissner D. and Grotenhuis T. 2010. Aerobic biodegradation of organochlorine pesticides in contaminated soil- Significance of temperature and availability. Chemosphere. 78(1): 22-28.

[5] Bartha R. 1990. Isolation of microorganisms that metabolize xenobiotic compounds. In: Labeda D. P., editor. Isolation of

Biotechnological Organisms from Nature. New York: Mc Graw Hill. 283-305.

[6] Bhuyan S., Sahu S. K., Adhya T. K. and Sethunathan N. 1992. Accelerated aerobic degradation of gamma-HCH in suspensions of flooded and non-flooded soils pretreated with HCH. Biol. Fert. Soils. 12(4): 279-284.

[7] Bidlan R. 2003. Studies on DDT degradation by Bacterial strains. In: Isolation, purification and identification of microbes capable of DDT- degradation. PhD thesis, University of Mysore, India. pp 90-142.

[8] Bidlan R. and Manonmani H. K. 2002. Aerobic degradation of dichlorodiphenyl- trichloroethane (DDT) by *Serratia marcescens* DT-1P. Process Biochem. 38: 49-56.

[9] Bidlan R., Afsar M. and 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.

[10] Cerkvenik V., Doganoc D. Z. and Jan J. 2000. Evidence of some trace elements, organochlorine pesticides and PCBs in Slovenian cow's milk. Food Technol. Biotechnol. 38(2): 155-160.

[11] Chen Y. 2012. Organophosphate- induced brain damage: mechanisms, neuropsychiatric and neurological consequences and potential therapeutic strategies. Neurotoxicology. 33: 391-400.

[12] David P., Sogorb M. A., Fabbri M., Gribaldo L., Collotta A., Scelfo B., Vilanova E., Harris G. and Price A. B.. 2014. Genomic and phenotypic alterations of the neuronal- like cells derived from human embryonal carcinoma stem cells (NT2) caused by exposure to organophosphorus compounds paraoxon and mipafox. Int. J. Mol. Sci. 15: 905-926.

[13] Deepthi N., Rastogi N. K. and Manonmani H. K. 2007. Degradation of DDT by a defined microbial consortium: optimization of degradation conditions by response surface methodology. Res. J. Microbiol. 2: 315-326.

[14] Focht D. D., Alexander M. 1970. DDT metabolites and analogs: ring fission by *Hydrogenomonas.* Science. 170(3953): 91-92.

[15] Francis A. J., Spanggord R. J. and Ouchi G. I. 1975. Degradation of lindane by *Eschericia coli*. App. Microbiol. 29(4): 567-568.

[16] Grung M., Lin Y., Zhang H., Steen A. O., Huang J., Zhang G., Larssen T. 2015.

[17] Jilani S. 2013. Comparative assessment of growth and biodegradation potential of soil isolate in the presence of pesticides. Saudi J. Biol. Sci. 20: 257-264.

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

[19] Kamel F., Umbach D. M., Bedlack R. S., Richards M., Watson M., Alavanja M. C. R., Blair A., Hoppin J. A., Schimdt S. and Sandler D. P. 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology. 33(3): 457-462.

[20] Kumar A. Cameotra S. S. and Gupta S. 2010. Screening and characterization of potential cadmium biosorbent *Alcaligenes* strain from industrial effluent. J. Basic Microbiol. 51:1-7.

[21] Latha R. 2012. Biochemical and molecular characterization of DDT- degrading dehalogenase from *Pseudomonas* Spp. In: Review of literature. PhD thesis. University of Mysore, Mysore, India. pp 9- 105.

[22] Liu Z., Yang C, Jiang H., Mulchandani A., Chen W. and Qiao C. 2009. Simultaneous degradation of organophosphates and 4-substituted phenols by *Stenotrophomonas* Species LZ-1 with surface- displayed organophosphorus hydrolase. J. Agric. Food Chem.57: 6171-6177.

[23] **23.** Manonmani, H. K., Chandrashekaraiah D. H., Reddy S. N., Elcey C. D. and Kunhi A. A. M. 2000. Isolation and acclimation of a microbial consortium for improved aerobic degradation of gamma- hexachlorocyclohexane. J. Agric. Food Chem. 48: 4341– 4351.

[24] **24.** Mathur H. B., Agarwal H. C., Sapna Johnson and Nirmali Sarikia. 2005. CSE India Report: Analysis of Pesticide Residues in Blood Samples from Villages of Punjab. CSE/PML/PR-21/2005.

[25] **25.** Murthy H. M. R. and Manonmani H. K. 2007. Aerobic degradation of technical hexachlorocyclohexane by a defined microbial consortium. J. Hazard. Mater. 149(1): 18-25.

[26] **26.** Nagpal V. and Paknikar K. M. 2006. Integrated approach for the enhanced degradation of lindane. Indian J. Biotechnol. 5(suppl): 400-405.

[27] **27.** Nigam U. and Siddiqui K. J. 2001. Organochlorine insecticide residues in dairy milk samples collected in Lucknow, India. Bull. Environ. Contam. Toxicol. 66: 678-682.

[28] **28.** Noren K. and Meironyte D. 2000. Organochlorine and organobromine contaminanting Swedish human milk in perspective of past 20- 30 years. Chemosphere. 44: 1111-1123.

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

[30] **30.** Pannu R. and Kumar D. 2014. Biodegradation study of gamma- hexachlorocyclohexane using selected bacteria from agricultural soil. Afr. J. Microbiol. Res. 8(36): 3335-3346.

[31] **31.** Pino N. and Penuela G. 2011. Simultaneous degradation of the pesticides methyl parathion and chloropyrifos by an isolated bacterial consortium from a contaminated site. Int. Biodeter. Biodegr. 65: 827-831.

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

[33] **33.** Sander P., Wittich R. M., Fortnagel P., Wilkes H. and Francke W. 1991. Degradation of 1,2,4- Trichloro- and 1,2,4,5- Tetrachlorobenzene by *Pseudomonas* strains. Appl. Environ. Microbiol. 57(5): 1430-1440.

[34] **34.** Saxena M. C. and Siddiqui M. K. J. 1982. Pesticide pollution in India: Organochlorine pesticides in milk of women, buffalo and goat. J. Dairy Sci. 65: 430-434.

[35] **35.** Selvi A. A., Rastogi N. K. and Manonmani H. K. 2013. Degradation of isoprothiolane by a defined microbial consortium using response surface methodology. J. environ. Biol. 34: 545-554.

[36] **36.** Simonich L.S., Hites R.A. 1995. Global distribution of persistent organochlorine compounds. Science. 269:1851-1854.

[37] **37.** Slotkin T. and Seidler F. 2009. Transcriptional profiles reveal similarities and differences in the effects of developmental neurotoxicants on differentiationbinto neurotransmitter phenotypes in PC12 cells. Brain Res. Bull. 78(4-5): 211-225.

[38] **38.** Sonkong K., Prasertsan P. and Sobhon V. 2008. Screening and identification of *p, p'*-DDT degrading soil isolates. J. Sci. Technol. 30 (Suppl.1): 103- 110.

[39] **39.** Sreedharan B, Singh N, Sethunathan N. 1999. Aerobic degradation of soil-sorbed isomer of hexachlorocyclohexane by *Pseudamonas paucimobilis*. In: Abstract book, Abstract No. TRC 2. 2nd Int. Conf. On Contam. Soil Environ. In Australasia-pacific regions. 12-17 Dec. 1999, New Delhi, pp. 109-110.

[40] **40.** Stanciu G., Dobrinas S., Birghila S. and Popescu M. 2008. Determination of organic compounds from different typs of coffee by HPLC and GC-ECD analysis. Environ. Engg. Mgmt J. **7**(6): 661-666.

[41] **41.** Toledo M. V. M., Salmeron V., Rodelas B., Pozo C. and Lopez J. G. 1993. Studies on the effects of a chlorinated hydrocarbon insecticide, lindane, on soil microorganisms. Chemosphere. 27(11): 2261-2270.

[42] **42.** Vankar, P. S. and Sahu R. 2011. Lindane levels in the dumping grounds in Chinhat area, Lucknow, India. Electron. J. Environ. Agri. Food Chem. 10(4): 2081-2089.

[43] **43.** Viel J. F., Warembourg C., Idrissi G. L. M., Lacroix A., Limon G., Rouget F., Monfort C., Durand G., Cordier S., Chevrier C. 2015. Pyrethroid insecticide exposure and cognitive development disabilities in children: The PELAGIE mother-child cohort. Environ. Int. 82:69-75

[44] **44.** Wada H, Senoo K, Takai Y. 1989. Rapid degradation of gamma-HCH in upland soil after multiple applications. Soil Sci. Plant Nutr. 35(1): 71-77.

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

[46] **46.** Yang J., Liu R., Song W., Yang Y, Cui F. and Qiao C. 2012. Appl. Biochem. Biotechnol. 166: 590-598.