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On

Bioremediation of Soils Contaminated with Chlorinated Pesticides

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Bioelimination of pesticides and other chemical residues from soils and waste waters

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ABSTRACT

That the adipose tissue of Indian population has got the highest load of pesticide residues is well documented. It is also a very disturbing fact that mother's milk in our country has alarmingly high levels of pesticide residues. BHC and DDT are the major pesticide residues that were detected in human body and milk. Other organochlorine insecticides such as heptachlor, endosulphan, aldrin, dieldrin, etc also have been detected though not at very high concentrations. Residues of 2,4,5-T and products of partial degradation of several other herbicides and insecticides also pose serious environmental problems. The deleterious effects of organochlorine compounds on human health has now been well recognised. Research data are rolling out day-by-day from different laboratories around the world on the effects of pesticides on nervous, immune, and reproductive systems. Endocrine disrupting activity of some of these compounds is well established. All these chemical residues reach human body mainly through food. The residue that accumulate in soil and water due to their recalictrance enter the food chain and get bioconcentrated. Almost every food commodity in India carries traces to heavy loads of pesticide residues. This, no doubt, is a threat to our own health, but also has affected the export market of various crop produces. Work on development of treatment and bioremediation technologies based on microbial degradation of various xenobiotic and hazardous chemicals has been going on in our Department for the last 8-9 years. Potent microbial consortia that can degrade, α-, β-, γ- and δ-isomers of

hexachlorocyclohexane (HCH) and technical-HCH (upto 400 ppm) in shake flasks were developed. Four bacterial strains capable of degrading upto 25 ppm of DDT in shake flasks and upto 15 ppm in soil, within 48-72 hr, have been isolated and degradation kinetics were studied. 2,4,5-T, an extensively used and highly recalcitrant herbicide, was found to inhibit tomato and brinjal seed germination completely at 20 ppm level. Inoculation of soil with *Burkholderia cepacia* AC1100, 7 days before sowing the seeds, effectively protected the seeds resulting in normal germination. 4-Chlorobenzoate (4-CBA), an intermediary metabolite of biodegradation of DDT and PCBs generally accumulate in soil. 3- Chlorobenzoate (3-CBA) is another important pollutant chemical. Both these inhibit germination of seeds particularly of the numbers of Solanaceae family (e.g., tomato, tobacco, brinjal, etc.). Bioremediation of soil by inoculation with *Pseudomonas aeruginosa* 3mT, a potent degrader of 3-CBA and 4-CBA (upto 8 and 12 g/L, respectively) efficiently eliminated the inhibitory effect. The kinetics of degradation of chlorobenzoates in shake flasks and their biochemistry were worked out. Phenolic compounds originating from petroleum, coal gasification and other industries are major pollutants of underground water. Five potent bacterial strains that can mineralise phenol were isolated. *Pseudomonas* sp. CP4 could degrade 1.5 g/L phenol by free cells and upto 4.0 g/L by agar-agar encapsulated cells. Strains CP4, *Pseudomonas* sp. strains CPC-1, CoPC-4 and SoPC-5 could degrade all the three isomers of cresol and other aromatic compounds through a *meta*-cleavage pathway. *P.stutzeri* SPC-2 degraded phenol and hydroxybenzoates through an *ortho*-pathway. A mixed culture containing eight bacterial strains could efficiently degrade all the three isomers of mononitrophenol individually and in mixtures. Nitrophenols are intermediary metabolites of certain herbicides and insecticides.

PATENTS AND PUBLICATIONS ARISING FROM THE WORK

PATENTS

1. A.A.M.Kunhi and P.V.Ajith Kumar (1995). A process for the isolation of a potent bacterial strain that can degrade high concentrations of 3-chlorobenzoate, 4-chlorobenzoate and other aromatic compounds (filed vide No.1243/DEL/95 dated 4th July 1995).

2. H.K.Manonmani, D.H.Chandrashekaraiah, N.Sreedhar Reddy, P.V.Ajith Kumar, P.Y.Aneez Ahamad and A.A.M.Kunhi. (1995). An improved process for the mineralisation of alpha-hexachlorocyclohexane useful for treatment of industry effluent and bioremediation of contaminated soil (filed vide No.2448/DEL/95 dated 29-12-1995).

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LIST OF PUBLICATIONS

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6. Ahamad,P.Y.A. and **Kunhi,A.A.M.** 1995. Degradation of phenol through ortho-cleavage pathway by *Pseudomonas stutzeri* strain SPC-2. Lett. Appl. Microbiol. 22: 26-29.

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16. Karuna Rao, Shabana Basheer, Manonmani H.K., Elcey C.D. and **Kunhi,A.A.M.** (1999). Degradation of mononitrophenols by a microbial consortium. Biodegradation. (communicated).

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1. **Kunhi,A.A.M.** Development of genetically engineered microbial strains for fast degradation of recalcitrant pesticide residues. National Symp. on "New Trends in Biotechnology", 3-4 June 1988, Trivandrum, India

2. **Kunhi,A.A.M.** *Pseudomonas* - an efficient system for gene cloning and manipulations. National Symposium on "Recent trends in microbial gene technology", 9-11 Dec 1988. Osmania University, Hyderabad, India

3. Ajith Kumar,P.V. and **Kunhi,A.A.M.** Isolation and characterisation of microorganisms degrading chlorinated aromatic compounds **Intl. Symposium on Industrial Biotechnology,** Nov 18-20, 1990, Osmania University, Hyderabad, India

4. Ajith Kumar,P.V. and **Kunhi,A.A.M.** (1991) Microbial degradation of chlorinated pesticides - the realities. 31st Ann. Conf. AMI, Jan 23-25, Tamil Nadu Agric. Univ., Coimbatore, India

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6. Ajith Kumar,P.V. and **Kunhi,A.A.M.** Isolation and characterisation of chlorobenzoic acid degrading strain of Pseudomonas. IX ICFOST National Symposium of AFST(I), June 10-12,1991, CFTRI, Mysore, India **(Won First Prize in Poster Presentation)**

7. Ajith Kumar,P.V. and **Kunhi,A.A.M.** Degradation of 3-chlorobenzoic acid and phenol by pure and mixed culture of Pseudomonas spp. Diamond Jubilee Ann. Gen. Body Meeting of Soc. Biol. Chem. (India), Dec. 26-30, 1991. Indian Institute of Chemical Biology, Calcutta, India

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9. Ibid. Degradation of phenols by *Pseudomonas* strain CP4. Abstract No.BCP 18, ibid, p. 81

10. **Kunhi,A.A.M.** and Valli,K. Biodegradation of pesticide residues and other hazardous organic wastes. Dept. of Biotechnology (New Delhi) sponsored Brain Storming Session in Food Biotechnology, May 7-8, 1993. CFTRI, Mysore, India

11. Ajith Kumar,P.V. and **Kunhi,A.A.M.** Mineralization of chlorobenzoic acids by *Pseudomonas aeruginosa* 3mT - the metabolic studies. Abstract No.BTM-32, 3rd Intl. Food Convention, Sep 7-12, 1993, Mysore, India, p.48

12. Chandrasekhariah,D.H., Ajith Kumar,P.V. and **Kunhi,A.A.M.** Degradation of hexachlorocyclohexane isomers by microbial consortia. Abstract No.BTM-33, 3rd Intl. Food Convention, Sep 7-12, 1993, Mysore, India, p.49

13. Ahamad,P.Y.A. and **Kunhi,A.A.M.** Degradation of cresols by a strain of *Pseudomonas* sp. CP4. Abstract No.34, **ibid**, pp.49-50

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15. **Kunhi,A.A.M.** Microbial degradation of xenobiotic compounds - A review of the work done in our Laboratory. Abstract No. IL-8, IICT Golden Jubilee Seminar on Emerging Trends in Applied Biology, 21-22 Oct 1994, Hyderabad, India

16. **Kunhi,A.A.M.** Aerobic biodegradation of hexachlorocyclohexane (HCH) isomers - its Biochemistry and Genetics, Abst. No.SAM-24, Microbiol. Intl. Conference (MICON 94) and 35th Annual Conf. of the Association of Microbiologists of India, Nov 1994, Mysore, India, p. 122

17. Sreedhar Reddy,N. Gowthaman,M.K. and **Kunhi,A.A.M.** Estimation of basic Kinetic parameters in the microbial degradation of alpha-hexachlorocyclohexane. Abst. No.EMB-70, ibid, p. 50

18. Ajith Kumar,P.V. and **Kunhi,A.A.M.** Degradation of monochlorobenzoates by *Pseudomonas aeruginosa*. Abst. No.EMB-72, ibid, p. 50

19. Aneez Ahamad.P.Y. and **Kunhi,A.A.M.** A *Pseudomonas stutzeri* strain that degrades phenol through ortho-pathway. Abstr No.EMB-80, ibid, p.80,

20. Sreedhar Reddy,N. and **Kunhi,A.A.M.** Biomineralization of alpha, beta, gamma and delta-isomers of hexachlorocyclohexane by microbial consortia. Abstr No.EMB-88, ibid, p.53

21. **Kunhi, A.A.M.** (1995). Biochemical incompatibility in simultaneous degradation of chloro- and non-chloroaromatic compounds. Abst. No. OE 1 Louis Pasteur's Centenary Memorial Symposium. 20th Nov. 1995. Regional Research Laboratory (CSIR), Trivandrum, India. p.9.

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24. **Kunhi,A.A.M.** (1997). Biodegradation of xenobiotics and hazardous chemicals. Invited lecture. Refresher course in chemistry for College and University Teachers, 25th Nov 1997, Academic Staff College and Dept of Chemistry, University of Mysore, Mysore, India

25. **Kunhi, A.A.M.** (1997). Biodegradation of pesticide residues - the national scenario. DBT meeting on Pesticides residues in food - detection and degradation. 23rd December 1997. Industrial Toxicology Research Centre, Lucknow, India.

26. **Kunhi, A.A.M.** (1998) Environmental pollution - solution through biotechnology. Inaugral address to the Society of Applied Botany, Kuvempu University, Shankaraghatta, Karnataka. 12th Feb 1998.

27. **Kunhi, A.A.M**. (1998) Biotechnological management of pesticides residue problem in food and biobeneficiation of coffee processing wastes. Workshop on Formulation of programmes in Biotechnology for sustainable coffee production. Coffee Board and Dept of Biotechnology, Govt. of India. 9-10 March 1998, CFTRI, Mysore.

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29. Shabana Basheer, Jayachandran V.P., Manonmani, H.K. and **Kunhi,A.A.M.** (1998). Co-degradation of 3-chlorobenzoate and phenol by defined mixed bacterial cultures. Abstract No.F-01. Abst. Book p.59. **IFCON-98 (4th Intl. Food Convention)** 23-27 Nov.1998. Mysore, India.

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RADIO TALKS

Kunhi,A.A.M. How safe is the food we eat? Talk in English, broadcasted at 9-16 PM on 1st October 1993 by All India Radio (Govt. of India) Mysore Station

Kunhi,A.A.M. Biotechnological Approaches to Pollution Control. Broadcast at 9-16 PM on 4th April 1997 by All India Radio (Govt. of India) Mysore Station

POPULAR ARTICLE

Kunhi,A.A.M. and Ajith Kumar,P.V. (1990). "Pesticides - the boon and the bane". ANVESHAKA. A Souvenir of Mysore Amateur Naturalists (MAN), Mysore

THESES PREPARED

Ph.D.

1. Dr.P.V.Ajith Kumar, 1996. "**Studies on degradation of 3-chloro and 4- chlorobenzoic acids by *P.aeruginosa* 3mT**, Mangalore University, Mangalore

2. Dr.P.Y.Aneez Ahamad, 1997. "**Studies on microbial degradation of phenolic compounds**", University of Mysore

3. Mr.Rajkumar Bidlan. **Studies on biochemistry and genetics of degradation of DDT by a strain of *Pseudomonas*** sp. (work in progress).

4. Ms. Shabana Basheer. Biodegradation of nitrophenols by bacterial mixed cultures. (Work in progress).

M.Tech (Environ. Engg.)

1. Mr.K.Sudhakar Babu, "Degradation of phenols and chlorobenzoates by Pseudomonas spp. 1992, University of Mysore

2. Mr.D.H.Chandrashekaraih, "Studies on biomeneralisation of HCH isomers by microbial consortia, 1993, University of Mysore

3. Mr.N.Sreedhar Reddy, "Biodegradation of hexachlorocyclohexane isomers by mixed bacterial cultures. 1994, University of Mysore

4. Ms.Karuna Rao, 1997. "Studies on biodegradation of nitrophenols in synthetic effluents. University of Mysore

M.Phil

Mr K.P.Gangadhara (1991). Effect of aromatic hydrocarbons on seed germination and their degradation by microbial strains. M.Phil (Seed Technol.), University of Mysore, Mysore

M.Sc

1. Mr S.Vijay Kumar (1997). Some studies on the plasmid profiles of hexachlorocyclohexane (HCH)-degrading bacterial strains. Resarch Project Report, M.Sc. (Bioscience), University of Mysore, Hemagangothri, Hassan campus

2. Mr V.P.Jayachandran (1998). Simultaneous degradation of phenol and chlorobenzoate by axenic and mixed bacterial cultures. Research Project, M.Sc. (Apppl. Microbiol.)., M.G.R. College, Hosur, University of Madras

3. Jitendra Ozarkar (1998). Study on plasmid profiles of phenol and chlorobenzoate degrading *Pseudomonas* strains. Project. M.Sc. (Microbiology). University of Pune, Pune, Maharashtra State.

MICROORGANISMS ISOLATED/USED IN THE WORK

α-HCH degrading microbial consortium	
Ps.fluorescens CFR 1002 *Ps.stutzeri* CFR 1003 *Ps.pseudoflova* CFR 1004 *Ps.palleronii* CFR 1005 *Ps.diminuta* CFR 1006 *Ps.mendocina* CFR 1007 *Ps.caryophilli* CFR 1008 *Ps.solanacearum* CFR 1009 *Fusarium* sp. CFR 217	1. Consortium isolated by a long term enrichment technique 2. Degrades: upto 100 ppm of α-HCH within 72 hr in shake flasks 3. Also degrades β-, γ, and δ-HCH 4. Individual strains degrade only upto 10 ppm of α-HCH
γ-HCH-degrading microbial consortium	
Pseudomonas sp. CFR-1010 *Pseudomonas* sp. CFR-1011 *Pseudomonas* sp. CFR-1012 *Pseudomonas* sp. CFR-1013 *Pseudomonas* sp. CFR-1014 *Pseudomonas* sp. CFR-1015 *Pseudomonas* sp. CFR-1016 *Pseudomonas* sp. CFR-1017 *Pseudomonas* sp. CFR-1018 *Fusariums* sp. CFR-217	1. Consortium isolated by enrichment of sugarcane field soil 2. Degrades upto 300 ppm of lindane in shake flasks 3. Degrades other isomers also 4. Individual isolates can degrade only 10 ppm of γ-HCH
DDT degrading bacteria	
1. Strain DT-Ct-1 2. Strain DT-1P 3. Strain DT-2 4. Strain DT-Ct-2	All the strains degrade upto 25 ppm ppm of p-p-DDT All the strains degrade upto 25 ppm DDT within 48-96 hr in shake flasks In soil DT-Ct-2 degrades upto 25 ppm within 120 hr

Phenol degrading strains 1. *Pseudomonas* sp. CP4 2. *Pseudomonas* sp. CPC-1 3. *Ps. aeruginosa* CoPC-4 4. *Pseudomonas* sp. SoPC-5	1. All degrade 1-1.5 g/L phenol in shake flasks 2. CP4 tolerates upto 4 g/L phenol when encapsulated in agar-agar	1. All degrade cresols 2. Follow a *meta*-cleavage pathway
5. *Ps.stutzeri* SPC-2	Degrades upto 1.2g/L phenol and hydroxy-benzoates	Follows an *ortho*-cleavage pathway
Nitrophenol-degrading consortium 1. *Klebsiella ozaenae* SNP-1a 2. *Ps.stutzeri* SNP-1b 3. *Xanthomonas maltophila* SNP-2 4. *Ps.aeruginosa* SNP-3 5. *Flavobacterium odoratum* SNP-4a 6. *Ps.aeruginosa* SNP-4c 7. *X.maltophila* SNP-5 8. *Pseudomonas* sp. SNP-6	1. The consortium was developed by enrichment of soil collected from the vicinity of a pharmaceutical industry 2. Can degrade *o*-Nitrophenol upto 210 ppm *m*-Nitrophenol upto 210 ppm *p*-Nitrophenol upto 280 ppm 3. Can also degrade mixture of all the isomers (56 ppm each) simultaneously	

Chlorobenzoate degrading strain *Ps.aeruginosa* CFR 1001	1. Degrades upto 8 g/L of 3-chloro-benzoate and 12 g/L of 4-chloro-benzoate in shake flasks 2. Bioremediates 3-CBA/4-CBA-contaminated soil and protects tomato seeds from germination inhibition
2,4,5-T degrading *Burkholderia cepacia* AC1100	Obtained from Prof Anand Chakrabarty, U.S.A. Used for bioremediation of 2,4,5-T-contaminated soil for protecting seeds from the inhibition of germination

Future plan of work

1. To study the biochemistry of degradation of HCH, particularly that of b-d, and a-HCH.

2. Deciphering the parthway(s) of degradation of DDT by bacterial strains

3. To study the genetics of degradation of HCH isomers and DDT with a view to improve the strains

4. Field trials of bioremediation of HCH and/or DDT-contaminated soils

5. Development of microbial strains/consortia for the degradation of heptachlor, aldrin, dieldrin, endosulfan, synthetic halogenated pyrethroids, etc

Personnel involved in the work

Dr A.A.M.Kunhi	Scientists
Dr H.K.Manonmani	
Dr P.V.Ajith Kumar	Students
Dr P.Y.Aneez Ahamad	
Mr K.P.Gangadhara	
Mr K.Sudhakar Babu	
Mr D.H.Chandrashekariah	
Mr N.Sreedhar Reddy	
Dr C.D.Elcey	
Ms Karuna Rao	
Ms Shabana Basheer	
Mr V.P.Jayachandran	
Mr Rajkumar Bidlan	