REFFERENCES

Aiumsupasit, N. Survey of pesticides in the Thailand environmental. [online]. (2005). Available from: http://san.anamai.moph.go.th/Heli/TextHELI/env.doc.

Alexander, M. 1994. Biodegradation and Bioremediation. San Diego, CA: Academic Press,

Alvey, S., Crowley, D.E. 1995. Influence of organic amendments on biodegradation of atrazine as a nitrogen source. Journal of Environmental Quality 24: 1156-1162.

Ambrosoli, R., Negre, M., Divapra, M.G.1996. Indications of the occurrence of enhanced biodegradation of carbofuran in some Italian soils. Soil Biology and Biochemistry 28: 1749-1752.

AOAC. 2000. Official method of analysis of AOAC international/William Herwitz, editor. 17^{th} ed. Gaithersburg, Maryland: AOAC International,

Atagana, H.J. 2004. Biodegradation of phenol, *o*-cresol, *m*-cresol and *p*cresol by indigenous soil fungi in soil contaminated with cresolate. World Journal of Microbiology and Biotechnology 20: 851–858.

Atagana, H.J. 2006. Biodegradation of polyacyclic aromatic hydrocarbons in contaminated soil by biostimulation and bioaugmentation in the presence of copper(II) ions. World Journal Microbiology Biotechnology 22: 1145-1153.

Bailey, H.C., Digiorgio, C., Kroll, K., Miller, J., Hinton, D.E., Starrett, G. 1996. Development of Procedures for Identifying Pesticide Toxicity in Ambient Waters: Carbofuran, Diazinon, Chlorpyrifos. Environmental Toxicology Chemistry 15(6): 837-845.

Barahona, L.M., Vazquez, R.R., Velasco, M.H., Jarquin, C.V., Perez, O.Z., Cantu, A.M., Albores, A. 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Applied Soil Ecology 27: 165-175.

Baron, R.L. 1991. Carbamate insecticides. Handbook of pesticide toxicology. 3–8. New York: Academic Press,

Benitez, F.J., Acero, J.L., Real, F.J. 2002. Degradation of carbofuran by using ozone UV radiation and advanced oxidation processes. Journal of Hazardous Material 89: 51-66.

Bento, F.M., Camargo, F.A.O., Okeke, B.C., Frankenberger, W.T. 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation biostimulation and bioaugmentation. Bioresource Technology 96: 1049-1055.

Bernard, A., Ferda, M. 1991. Biochemical Engineering and Biotechnology Handbook. 2nd. Newyork: Stockton,

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I. 1997. Enhanced accumulation of Pb in Indian Mustard by soil applied chelating agents. Environmental Science and Technology 31: 860-865.

Brown, S.L., Chaney, R.L., Angle, J.S., Baker, A.J.M. 1994. Phytoextraction potential of *Thlaspi caerulescens* and Bladder Campion for zinc and cadmium-contaminated soil. Journal of Environmental Quality 23: 1151-1157.

Burns, R.G. 1978. Soil Enzymes. London: Academic Press,

Burken, J.G., Schnoor, J.L. 1997. Uptake and metabolism of atrazine by poplar trees. Environmental Science and Technology 31: 1399-1406.

Cain, R.B., Head, I.M. 1991. Enhanced degradation of pesticides: its biochemical and molecular biological basis pesticide in soil and water. BCPC monography 47: 23-40.

Chapalamadugu, S., Chaudry, G.R. 1992. Microbiological and biotechnological aspects of metabolism of carbamates and organophosphates. CRC Critical Review in Biotechnology 12: 357-389.

Chaudhry, G.R., Ali, A.N. 1988. Bacterial Metabolism of Carbofuran. Applied and Environmental Microbiology 54: 1414-1419.

Chemichael, M., Pfaender, F.K. 1997. The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. Journal Biodegradation 8(1): 1-13.

Chen, H.J., Guo, G.L., Theng, D.H., Cheng, C.L., Huang, D.L. 2006. Growth factor kinetics and biodegradation mechanism associated with *Pseudomonas nitroreducens* TX1 grown on octylphenol polyethoxylates. Journal of Environmental Management 80: 279-286.

Chiron, S., Torres, J.A., Fernandez-Alba, A., Alpendurada, M.F., Barcelo, D. 1996. Identification of carbofuran and methiocarb and their transformation products in estuarine waters by on-line solid phase extraction liquid chromatography-mass spectrometry. Journal of Environmental Analysis Chemistry 65: 37-52.

Chu, K.H., Alvarez-Cohen, L. 1998. Effect of nitrogen source on growth and trichloroethylene degradation by methane-oxidizing bacteria. Applied and Environmental Microbiology. 64: 3451-3457.

Chulalongkorn University. Hazardous Chemical Imported Quantity Statistic. [online]. (2004). Available from: http://www.chemtrack.org/Stat Import/25 38JAN_DEC02_KGM_ID.pdf.

Cohen, S.Z. 1996. Pesticides in ground water in the United States: monitoring, modeling, and risks form the U.S. perspective. Environmental Science and Health. 31: 345-352.

Crecchio, C., Curci, M., Pizzigallo, D.R., Ricciuti, P., Ruggiero, P. 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology and Biochemistry 36: 1595-1605.

Crossno, S.K., Kalbus, L.H., Kalbus, G.E. 1996. Determinations of Carbon Dioxide by Titration: New Experiments for General, Physical and Quantitative Analysis Courses. Journal of Chemical Education 73:175.

DC Chemical Co., Ltd. Carbofuran. [online]. (nd). Available from: http://www.dcchem.co.kr/english/product/p_detail/p_detail10.html.

Dejonghe, W., Boon, N., Seghers, D., Top, E.M., Verstraete, W. 2001. Bioaugmentation of soils by increasing microbial richness: missing links. Environmental Microbiology 3(10): 649-657.

Devries, S., Evans, O. Carbofuran. [online]. (1999). Available from: http://wvlc.uwaterloo.ca/biology447/Assignments/Assignment1Submissions /On-Campus/carbofuran/ Carbofuran.html.

Dibyendu, S., Michael, F., Rupali, D., Stuart, B. 2005. Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition carbon supplementation, and monitored natural attenuation. Environmental Pollution 136: 187-195.

Deuel, L.E.Jr., Price, J.D., Turner, F.T., Brown, K.W. 1979. Persistence of carbofuran and its metabolites, 3-keto and 3-hydroxy carbofuran under flooded rice culture. Journal of Environmental Quality 8: 23-26.

Dungan, S.R., Kukier, U., Lee, B., 2006. Blending foundry sands with soil effect on dehydrogenase activity. Journal of Science of the Total Environment 357: 221-230.

Duquenne, P., Parekh, N.R., Catroux, G., Fournier, J.C. 1996. Effect of inoculant density, formulation, dispersion and soil nutrient amendment on the removal of carbofuran residues from contaminated soil. Soil Biology and Biochemistry 28: 1805-1811.

Dushenkov, S., Vasudev, D., Kapulnik, Y., Gleba, D., Fleisher, D., Ting, K.C., Ensley, B. 1997. Removal of uranium from water using terrestrial plants. Environmental Science and Technology 31(12): 3468-3474.

EPA. Groundwater and drinking water. [online]. (2003). Available from: http://www.epa.gov/safewater/contaminants/dw_contamfs/carbofur.html.

Evert, S. Environmental Fate of Carbofuran. [online]. (2002). Available from: http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/carbofuran.pdf.

Extension Toxicology Network. Pesticide Information Profiles; Carbofurn [online]. (1996). Available from: http://extoxnet.orst.edu/pips/carbofur.htm.

Felsot, A.S., Maddox, J.V., Willis, B. 1981. Enhanced microbial degradation of carbofuran in soils with histories of Furadan® use. Bulletin of Environmental Contamination and Toxicology 26: 781-788.

Ferguson, H.S., Franzmann, D.P., Revill, T.A., Snape, I., Rayner, L.J. 2003. The effects of nitrogen and water on mineralisation of hydrocarbon in diesel-contaminate terrestrial Antractic soils. Cold Regions Science and Technolog 37: 197-212.

Ferrell, M.A., and Aagard, S.D. Pesticide storage facility, design, and management plan; pesticide education program fact sheet. [online]. (2003). Available from:http://www.uwyo.edu/CES/PUBS/MP93_Series/mp-93.21_facility.pdf patein.

Gallego, J.L.R., Loredo, J., Llamas, J.F., Vázquez, F., Sánchez, J. 2001. Bioremediation of diesel-contaminated soils: Evaluation of potential in-situ techniques by study of bacterial degradation. Biodegradation 12: 325–335.

Gao, J., Garrison, A.W., Hoehamer, C., Mazur, C.S., Wolfe, N.L. 2000. Uptake and phytotransformation of o,p′-DDT and p,p′-DDT by axenically cultivated aquatic plants. Journal of Agricultural and Food Chemistry 48(12): 6121–6127.

Gentry, T.J., Josephson, K.L., Pepper, I.L. 2004. Functional establishment of introduced chlorobenzoate degraders following bioaugmentation with newly activated soil: Enhanced contaminant remediation via activated soil bioaugmentation. Biodegradation 15(1): 67-75.

Getzin, L.W. 1973. Persistence and degradation of carbofuran in soil. Environmental Entomology 2: 461-467.

Gibert, O., Pablo, J., Cortina, J.L., Ayora, C. 2004. Chemical characterization of natural organic substrates for biological mitigation of acid mine drainage. Water Research 38: 4186-4196.

Glinski, J., Stepniewski, W. 1985. Soil Aeration and its Role for Plants. Boca Raton, Florida: CRC Press,

Hance, R.J. 1973. Effect of pH on the degradation of atrazine, dichlorprop, linuron and propyzamide in soil. Pesticide Science 10(1): 83-86.

Hollender, J., Dreyer, U., Kornberger, L., Kampfer, P., Dott, W. 2002. Selective enrichment and characterization of a phosphorus removing bacteria consortium from activated sludge. Applied Microbiology and Biotechnology 58: 106-111.

Howard, P.H. 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals Pesticides. Chelsea, MI: Lewis Publishers,

Huston, L.P., Pignatello, J.J. 1999. Degradation of selected pesticide active ingredients and commercial formulation in water by the photo-assisted fenton reaction. Water Research 33: 1238-1246.

ICPS INCHEM. Carbofuran. [online]. (nd). Available from: http://www.inchem.org/documents/jmpr/jmpmono/v96pr03.htm.

Information Division of the Plant Industry Directorate (IDPID). Special Review of Carbofuran Insecticide: Effects on Avian Fauna and Value to Agriculture. [online]. (1993). Available from: http://www.hc-sc.gc.ca/pmra-arla/English/pdf/prdd/prdd_d9302-e.pdf.

Jasper, D.A. 1994. Bioremediation of agricultural and forestry soils with symbiotic microorganisms. Australian Journal of Soil Research 32(6): 1301-1319.

Johnson, W.G., Lavy, T.L. 1995. Persistence of carbofuran and molinate in flooded rice culture. Journal of Environmental Quality 24: 487-493.

Kale, S.P., Murthy, N.B.K., Raghu, K. 2001. Degradation of C-carbofuran in soil using a continuous flow system. Chemosphere 44: 893-895.

Kara, E.E. 2000. Effects of some plant residues on nitrogen mineralization and biological activity in soils. Turkish Journal of Agriculture and. Forestry 24: 457-460.

Karpouzas, D.G., Walker, A., Froud-Williams, R.J., Drennan, D.S.H. 1999. Evidence for the enhanced biodegradation of ethoprophos and carbofuran in soils from Greece and the UK. Pesticide Science 55: 301-311.

Kaufman, D.D., 1978. Degradation of pentachlorophenol in soil and by soil microorganisms in Pentachlorophenol, Chemistry. Rao, K.R. Phamacology and Environmental Toxicology. New York: Plenum Press,

Kidd, H., James, D.R. 1991. The Agrochemicals Handbook. 3rd. Cambridge, UK: Royal Society of Chemistry Information Services,

Kowalchuk, G.A., Gerards, S., Woldendrop, J.W. 1997. Detection and characterization of fungal infections of Ammophila arenaria (marram grass) roots by denaturing gradient gel electrophoresis of specifically amplified 18S rDNA. Applied and Environmental Microbiology 63: 3858-3865.

Kulkarin, M., Chaudhari, A. 2005. Biodegradation of *p*-nitrophenol by *P. putida*. Bioresource Technology 97(8): 982-988.

Lalah, J.O., Wandiga, S.O., Dauterman, W.C. 1996. Mineralization, Volatilization, and Degradation of Carbofuran in Soil Samples from Kenya. Bulletin of Environmental Contamination Toxicology 56: 37-41.

Lee, W., Wood, T K., Chen, W. 2006. Engineering TCE-Degrading Rhizobacteria for Heavy Metal Accumulation and Enhanced TCE Degradation. Applied and Environmental Microbiology 72(12): 7503-7509.

Levanon, D. 1993. Roles of fungi and bacteria in the mineralization of the pesticides atrazine, alachlor, malathion and carbofuran in soil. Soil Biology and Soil Biochemistry 25(8): 1097-1105.

Li, Z., Xu, J., Tang, C., Wu, J., Muhammad, A., Wang, H. 2006. Application of 16S rDNA-PCR amplification and DGGE fingerprinting for detection of shift in microbial community diversity in Cu-, Zn-, and Cd-contaminated paddy soils. Chemosphere 62: 1374-1380.

Lin, C.H., Lerch, R.N., Garrett, H.E., George, M.F. 2005. Incorporating forage grasses in riparian buffers for bioremediation of atrazine, idoxaflutole and nitrate in Middouri. Agroforestry Systems 63: 91-99.

Lucas, A.M., Rubertol, S., Vazquenz, A., Lobalbol, W.P., Cormack, M. 2005. Psychrotolerant hydrocarbon-degrading *Rhodococcus* strains isolated from polluted Antarctic soils. Antarctic Science 17(1): 47-56.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D. 2001. A fern that hyperaccumulates arsenic. Nature 409: 579.

Maria, S., Holtzea, S.R., S, J.S., Hans, C.B., Hansenc, J.A. 2006. Biostimulation and enrichment of 2,6-dichlorobenzamide-mineralising soil bacterial communities from dichlobenil-exposed soil. Soil Biology and Biochemistry. 15: 258-165.

Metcalf and Eddy Inc. 2003. Wastewater engineering treatment and reuse, 4th Ed. rev.G.Tchobanoglous. Mc Graw-Hill, New York: Burton, G.F., Stensel, L. H.D.

Miles, J.R.W., Tu, C.M., Harris, C.R. 1981. A Laboratory Study of the Persistence of Carbofuran and its 3-Hydroxy- and 3-Keto-Metabolites in Sterile and Natural Mineral and Organic Soils. Journal of Environmental Science and Health 16(4): 409-417.

Mocek, A., Drzymala, S., Maszner, P. 1997. Genesis analysis and classification of soils. AR Poznen 416.

Montiel, E.M., Ordaz, N.R., Granados, C.R., Ramirez, C.J., Mayer C.J.G. 2006. 2,4-D-degradation bacterial consortium isolation, kinetic characterization in batch and continuous culture and application for bioaugmenting an activated sludge microbial community. Process Biochemistry 41: 1521-1528.

Monteiro, A.A.M.G., Boaventura, R.A.R., Rodrigues, A.E. 2000. Phenol biodegradation by *Pseudomonas putida* DSM 548 in a batch reactor. Journal Biochemistry and Engineering 6: 45-49.

Moorman, T.B., Cowan, J.K., Arthur, E.L, Coats, J.R. 2001. Organic amendments to enhance herbicide biodegradation in contaminated soils. Biology and Fertility Soils 33: 541-545.

Mora, A., Revilla, C.E., Hermosin, M.C. 1996. Persistence and degradation of carbofuran in Spanish soil suspensions. Chemosphere 32: 1585-1598.

Moscinski, K.J. 1995. Bioaugmentation and Biostimulation techniques to bioremediate soils contaminated with herbicide mixtures. Master Thesis in Science. The Graduate School, Iowa State University.

Nakatsu, H.C., Torsvik, V., Ovreas, L. 2000. Soil Community Analysis Using DGGE of 16S rDNA Polymerase Chain Reaction Products. Soil Science Society of America Journal 64: 1382-1388.

Nelson, D.W., Sommers, L.E. 1996. Total carbon, organic carbon, and organic matter In: Methods of Soil Analysis, Part 2, 2nd. Madison, WI: American Society of Agronomy Inc.

Ngampongsai, A. 1990. Degradation and residues of carbofuran in soil and sweet corn. Master Thesis in Agricultural. The Graduate School, Kasetsart University.

Okpokwasili, G.C., Nweke, C.O. 2005. Microbial growth and substrate utilization kinetics. African Journal of Biotechnology 5: 305-317.

Olaniran, O.A., Pillay, D., Pillay, B. 2005. Biostimulation and bioaugmentation enhances aerobic biodegradation of dichloroethanes. Chemosphere 63: 1-9.

Ovreas, L., Forney, L., Dae, F.L., Torsvik, V. 1997. Distribution of bacterioplankton in meromictic lake saelenvannet, as detemined by denatureing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16s rRNA. Applied and Environmetal Microbiology 63: 3367-3373.

Perez, B.A., Corral, O.L., Linares, L.F., Garcia, F.E., Vazquez, R.R. 2004. Biostimulation of micro-organisms from sugarcane bagasse pith for the removal of weathered hydrocarbon from soil. Applied Microbiology Letters 38: 373-377.

Plangklang, P. 2004. Degradation of carbofuran by rhizosphere soil microorganisms. Master Thesis in Environmental Management (Inter-Department). The graduate School, Chulalongkorn University.

Qu, L.T., Gancarz, D.H., Wheeler, W.B., Rao, P.S.C., Davidson, J.M. 1982. Influence of soil temperature and soil moisture on degradation and metabolism of carbofuran in soil. Journal of Environmental Quality 11: 293-298.

Raha, P., and Asit, D.K. 1990. Photodegradation of carbofuran. Chemosphere 21: 99-106.

Rahman, K.S.M., Rahman, T.J., Kourkoutas Y., Petsas, I., Marchant, R., Banat, I.M. 2003. Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresource Technology 90: 159–168.

Read, D.C. 1983. Enhanced microbial degradation of carbofuran and fensulfothion after repeated applications to acid mineral soil. Agricultural Ecosystem and Environmetal 10: 37-46.

Riffaldi, R., Levi-Minzi R., Cardelli, R., Palumbo, S. Saviozzi, A. 2006. Soil Biological Activities in Monitoring the Bioremediation of Diesel Oil-Contaminated Soil. Journal of Water and Air and Soil Pollution 170: 3-15.

Rittman, B.E., Whiteman, R. 1994. Bioaugmentation: a coming of age. Biotechnology 1: 12-16.

Ruberto, L., Vazquez, S.C., Cormack., W.P.M. 2003. Effectiveness of the natural bacterial flora, biostimulation and bioaugmentation on the bioremediation of a hydrocarbon contaminated Antarctic soil. International Biodeterioration and Biodegradation 52(2): 115-125.

Sahoo, A., Adhya, T. K., Bhuyan, S., Sethunathan, N. 1993. Effect of moisture Regime, temperature, and organic matter on soil persistence of carbosulfan. Environmental Contamination and Toxicology. 50: 29-34.

Scelza, R., Rao, M.A., Gianfreda, L. 2007. Effects of compost and bacterial cell on the decontamination and the chemical and biology properties of an agriculteral soil artificially contaminated with phenanthrene. Soil Biology and Biochemistry 39: 1303-1317.

Schmidt, S.K., Alexander, M. 1995. Effect of dissolved organic carbon and second substrates on the biodegradation of organic compounds at low concentration. Applied and Environmetal Microbiology 49: 822-827.

Seiber, J.N., Catahan, M.P., Barril, C.R. 1978. Loss of carbofuran from rice paddy water: chemical and physical factors. Journal of Environmental Science and Health 13(2): 131-148.

Shibamoto, T., Mourer, C., Hall, G. 1993. Pilot Monitoring Study of Two Pesticides in Air. Air Resources Board 95: 581-595.

Shuler, M.L., Kargi, F. 1992. Bioprocess Engineering Basic Concepts. New Jersey: Prentice-Hall Inc.

Shelton, D.R., and Parkin, T.B. 1991. Effect of moisture on sorption and biodegradation of carbofuran in soil. Journal of Agricultural and Food Chemistry 39: 2063-2068.

Siddaramappa, R. 1978. The degradation of carbofuran in paddy water and flooded soil of untreated and retreated rice fields. Journal of Environmental Science and Health 13: 369-380.

Sims, J.R., Solum, D.J., Wichman, D.M., Kushnak, G.D., Welty, L.E., Jackson, G.D., Stallknecht, G.F., Westcott, M.P., Carlson, G.R. 1993. Canola variety yield trials. Agricultural Research 10: 15-20.

Skipper, H.D. 1999. Bioremediation of contaminated soils In: Sylvia. D.M., Principles and Applications of Soil Microbiology Upper Saddle River. NJ: Prentice Hall.

Smith, G.J. 1992. Toxicology and Pesticide Use in Relation to Wildlife: organophosphorus and Carbamate Compounds. Boca Raton FL: C. K. Smoley.

Sopade, P.A., Lee, S.B., White, E.T., Halley, P.J. 2007. Glass transition phenomena in molasses. Food and Science Technology 40: 117-1122.

Spectrum Laboratory Inc. Chemical Fact Sheet. [online]. (2003). Available from: http://www.speclab.com/compound/c1563662.htm

Stefenson, W.A., and Alexander, M. 1995. Role of competition for inorganic nutrients in the biodegradation of mixtures of substrates. Applied and Environmental Microbiology 61: 2859–2862.

Struthers, J.K., Jayachandran, K., Moorman, T.B. 1998. Biodegradation of Atrazine by Agrobacterium radiabacter J14a and use of this strain in bioremediation of contaminated soil. Applied and environmental microbiology 64(9): 3368-3375.

Teerakun, M., Reungsang, A., Virojanakud, W. 2004. Phytoremediation of carbofuran in soil. Songklanakarin Journal of Science and Technology. 26: 171-176.

Tomasek, P.H., Karns, J.S. 1989. Cloning of a carbofuran hydrolase gene from *Achromobacter* sp. WM111 and its expression in gram-negative bacteria. Journal of Bacteriology 171: 4038-4044.

Trabue, S.L., Feng, X., Ogram, A.V., Ou, L.T. 1997. Carbofuran degradation in soil profiles. Journal of Environmental Science and Health 32: 861-878.

Trindade, P.V.O., Sobral, L.G., Rizzo, A.C.L., Leite, S.G.F., Soriano, A.U. Evaluation valuation of the biostimulation and bioaugmentation techniques in the bioremediation process of petroleum hydrocarbons contaminated soil. [online]. (2002). Available from: http://ipec.ululsa.edu/Conf2002/trindade_soriano_21.pdf

Trindade, P.V.O., Sobral, L.G., Rizzo, A.C.L., Leite, S.G.F., Soriano, A.U. 2005. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparison study. Chemosphere 58: 515-522.

Trotter, D.R., Kent, R., Wong, M. 1991. Aquatic fate and effect of carbofuran. Critical Review in Environmental Contaminant 21: 137-176.

U.S. Environmental Protection Agency. Handbook of remedial action at waste disposal sites. [online]. (1985). Available from: http://books.nap.edu/openbook.php record_id=1412&page=221

Venkateswarlu, K., Siddarame, G.T.K., Sethunathan, N. 1977. Persistence and biodegradation of carbofuran in flooded soils. Journal of Agricultural Food Chemistry 25: 533-536.

Venkateswarlu, K., Sethunathan, N. 1978. Degradation of carbofuran in rice soils as influenced by repeated applications and exposure to aerobic conditions following anaerobiosis. Journal of Agricultural Food Chemistry 26: 1148-1151.

Venkateswaru, K., Sethunathan, N. 1979. Metabolism of carbofuran in rice straw-amended and Unamended rice straw. Journal of Environmetal Quality 8: 365-368.

Venkateswarlu, K., Chendrayan, K., Sethunathan, N. 1980. Persistence and biodegradation of carbaryl in soils. Journal of Environmental Science and Health 4: 421-429.

Venkateswarlu, K., Sethunathan, N. 1984. Degradation of carbofuran by *Azospirillum lipoferum* and *Streptomyces* spp, isolated from flooded alluvial soil. Bulletin of Environmental Contamination Toxicology 33: 556-560.

Vidali, M. 2001. Boremediation and overview. Pure and Applied Chemistry 73: 1163-1172.

Vroumsia, T., Steiman, R., Seigle-Murandi, F., Benoit-Guyod, L. 1999. Effects of culture parameters on the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-dichlorophenol (2,4-DCP) by selected fungi. Chemosphere 39: 1397-1405.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Science 29-38.

Walworth, J.M., Woodard, C.R., Braddock, J.F., Reynolds, C.M. 1997. Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: an approach to determining optimum levels. Journal of Soil Contamination 6: 465-480.

Wikipedia. Bioremediation. [online]. (n.d.). Available from: http://en.wikipedia.org/wiki/Bioremediation.

Wilson, P.C., Whitwell, T., Klaine, S.J. 2000. Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*. Bulletin of Environmetal Contamination Toxicology 39(3): 282-288.

Wong, K.W., Toh, B.A., Ting-Obbard, J.P. 2005. Biodegradation of penanthrene by the indigenous microbial biomass in a zinc amended soil. Applied Microbiology Letters 40: 50-55.

Yen, J.H., Hsiao, F.L., Wang, Y.S. 1997. Assessment of the insecticide carbofuran's potential to contaminate groundwater through soils in the subtropics Ecotoxicity and Environmentaol Safety 38: 260-265.

Yu, C.B., Gary M., Hansen, D.J., Larsen, J.R. 1974. Fate of Carbofuran in a Model Ecosystem. Journal of Agriculture Food and Chemistry 22: 431-434.

Zhang, Y., Frankenberger, T.W. 2002. Factors affecting removal of selenate in agricultural drainage water utilizing rice straw. The Science of the Total Environment 305: 207-216.

APPENDICES

APPENDIX A

Degradation of carbofuran, metabolite of carbofuran, dehydrogenase activity and growth curve of carbofuran degraders in soil

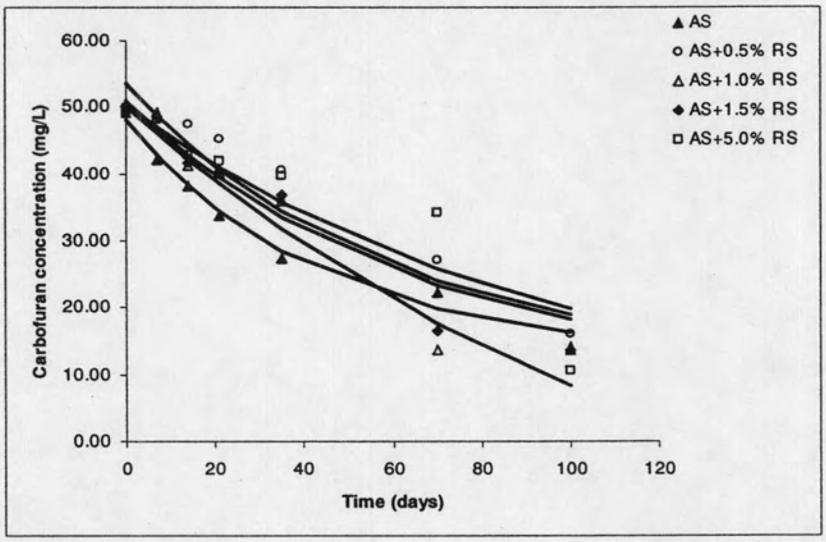


Figure A-1 Degradation of carbofuran in soil amended with autoclaved RS (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

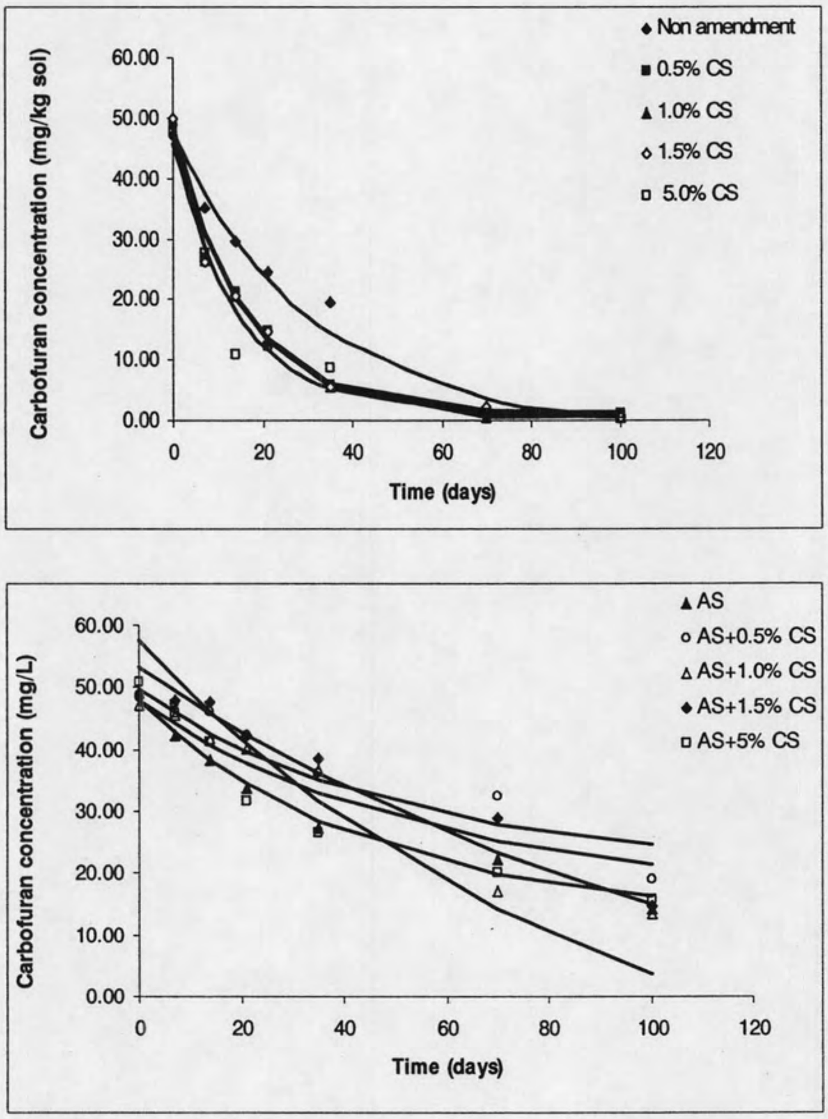
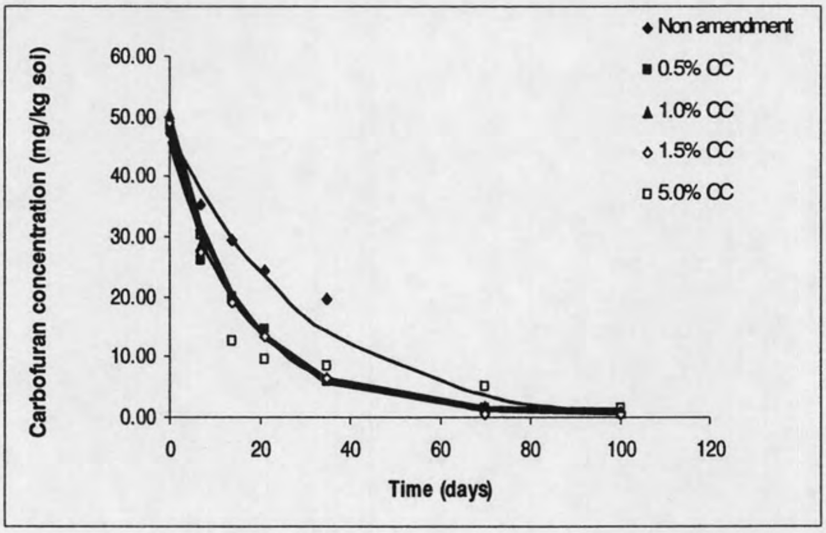


Figure A-2 Degradation of carbofuran in soil amended with CS and autoclaved CS (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)



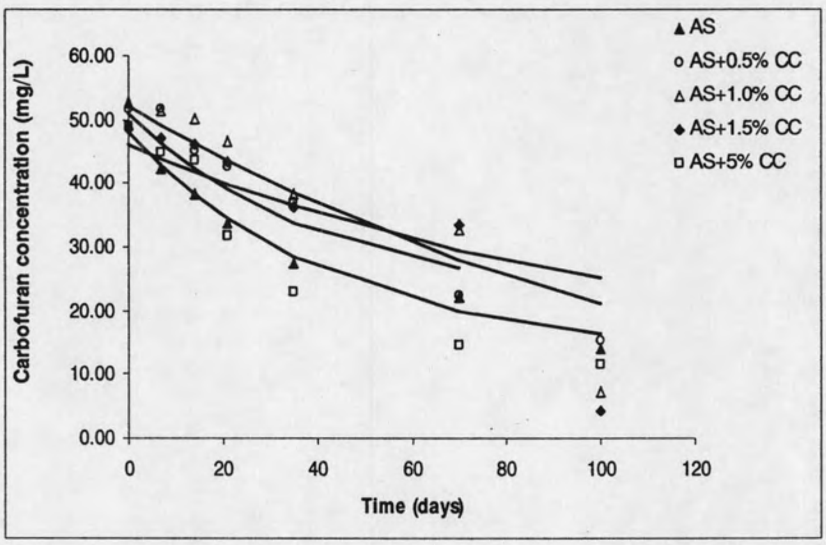
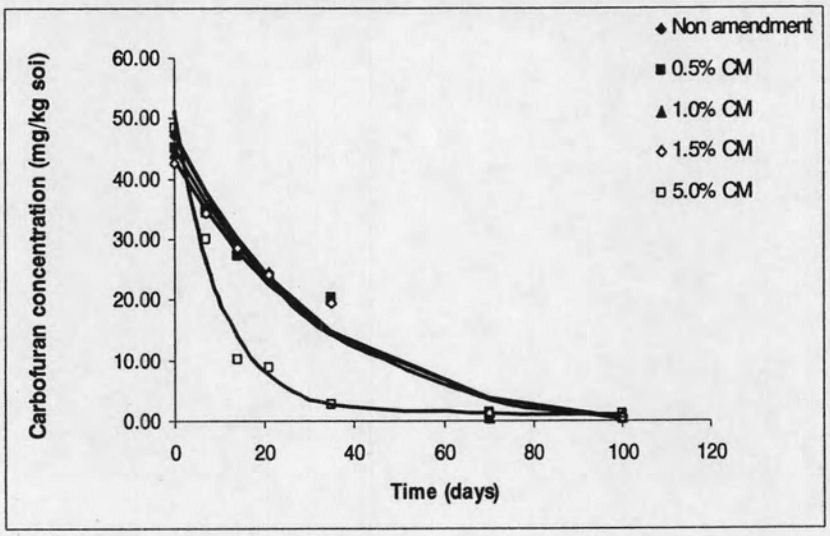


Figure A-3 Degradation of carbofuran in soil amended with CC and autoclaved CC (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)



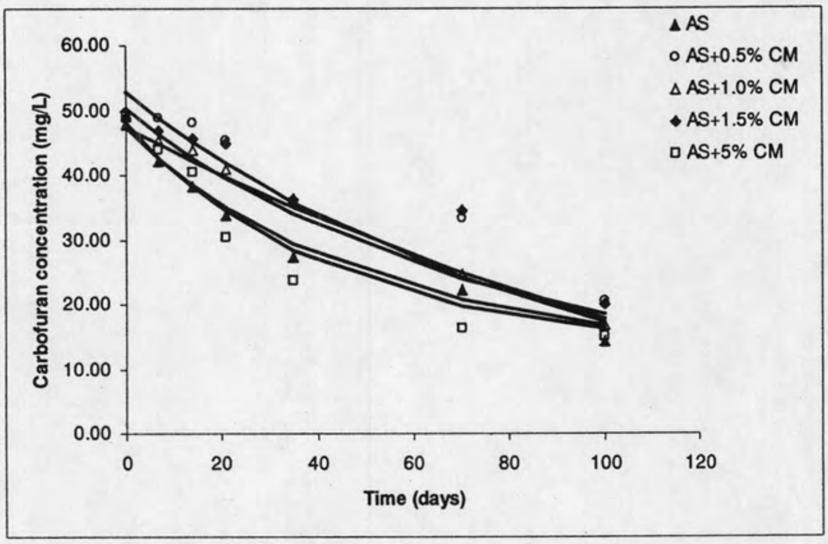


Figure A-4 Degradation of carbofuran in soil amended with CM and autoclaved CM (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

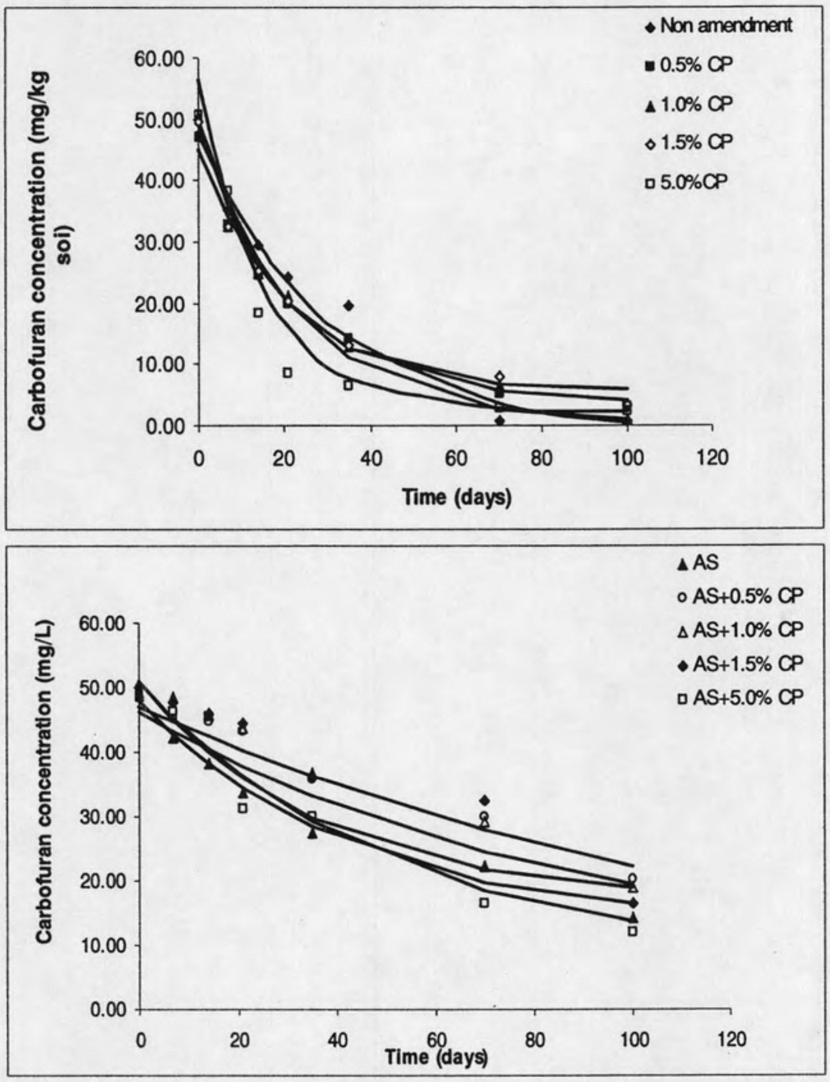
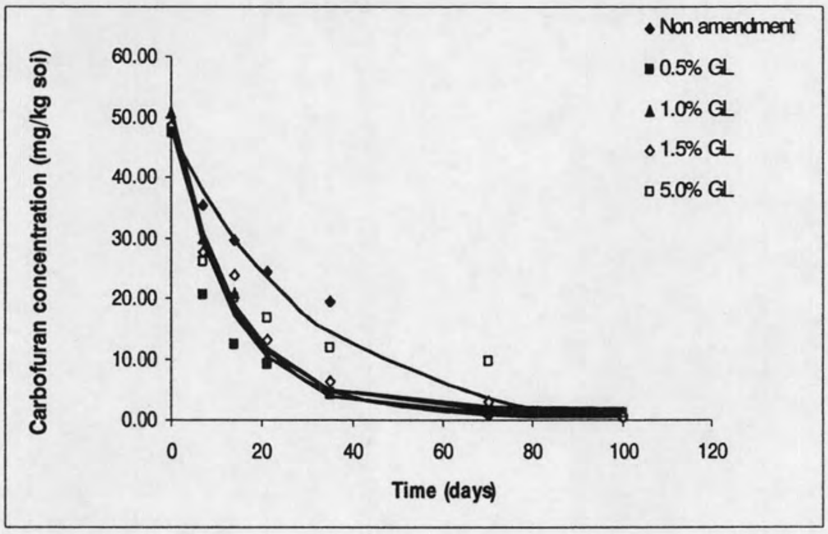


Figure A-5 Degradation of carbofuran in soil amended with CP and autoclaved CP (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)



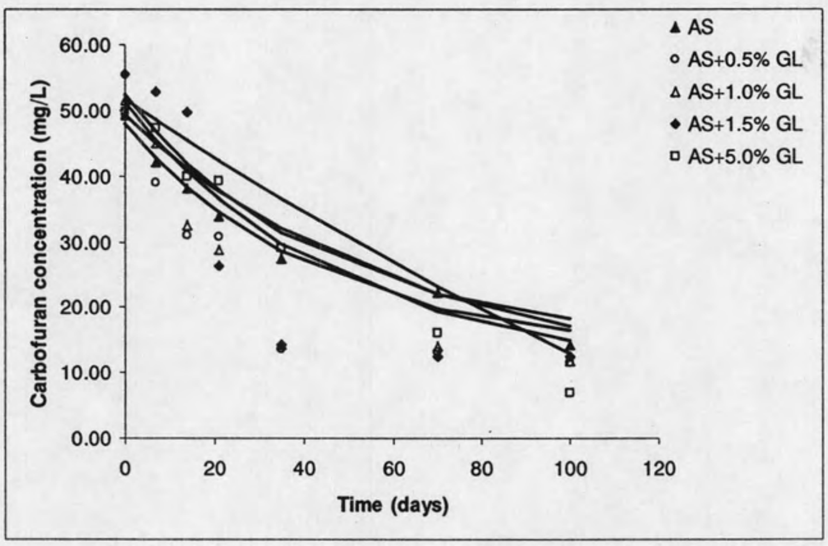
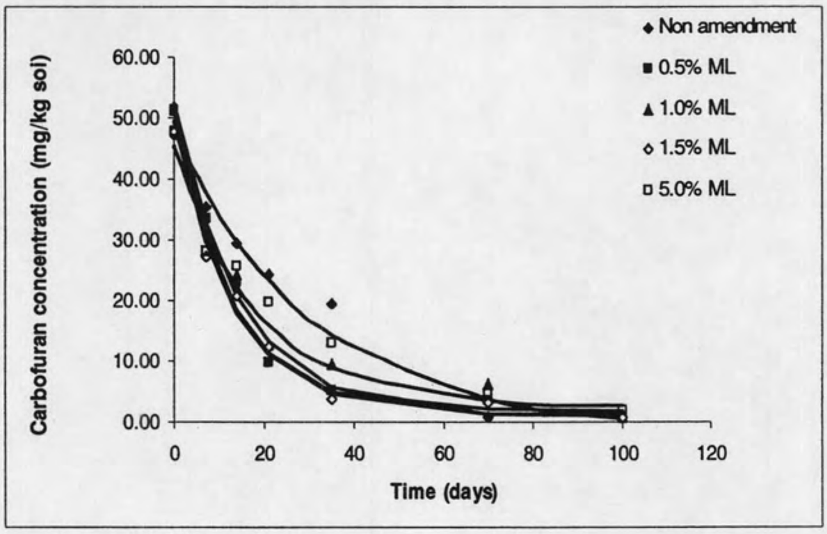


Figure A-6 Degradation of carbofuran in soil amended with GL and autoclaved GL (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)



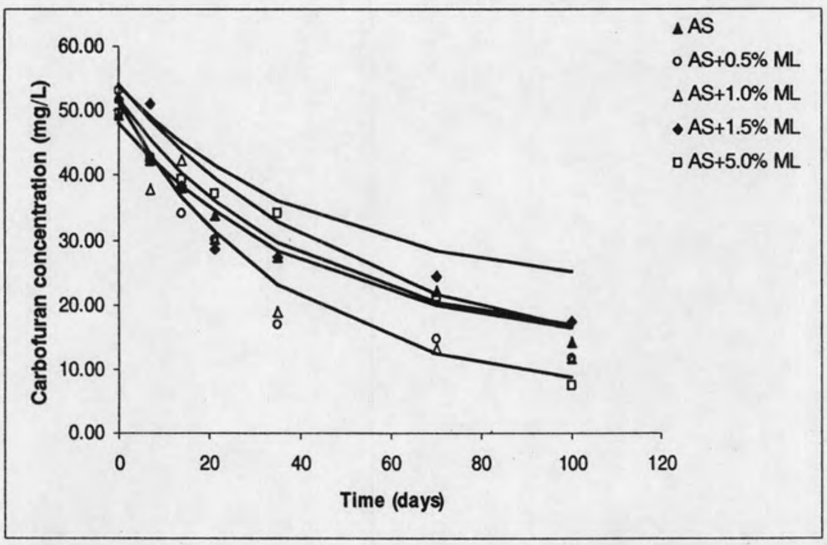


Figure A-7 Degradation of carbofuran in soil amended with ML and autoclaved ML (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

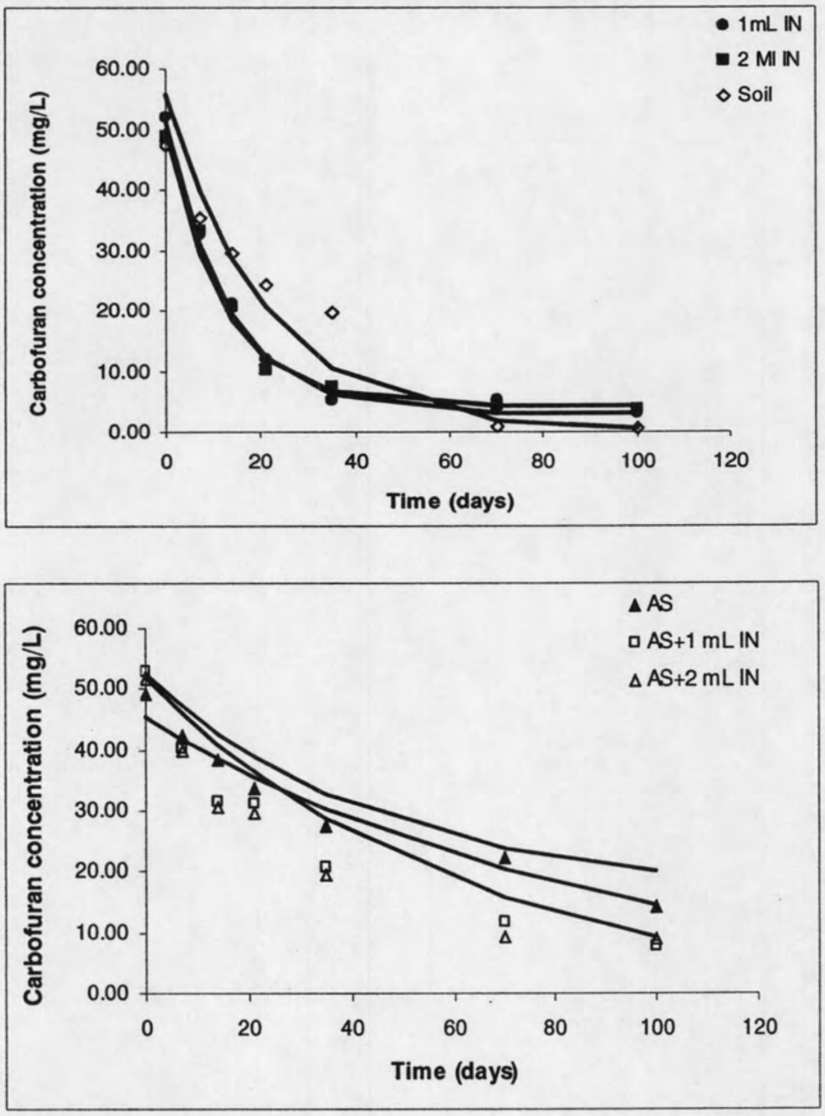
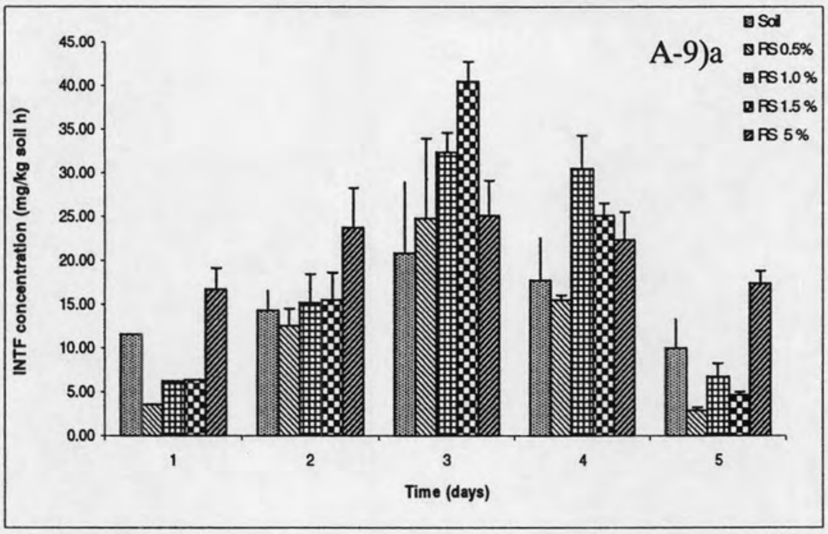


Figure A-8 Degradation of carbofuran in soil and autoclaved soil amended with inorganic amendment (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)



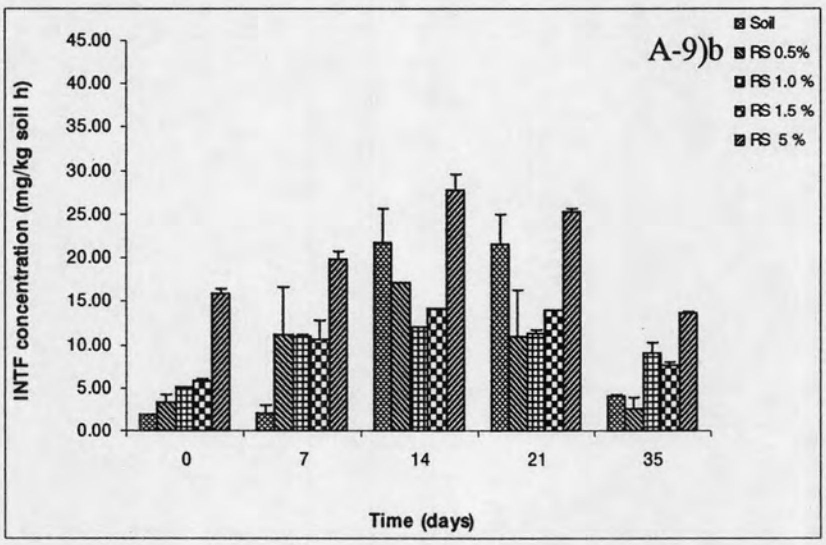
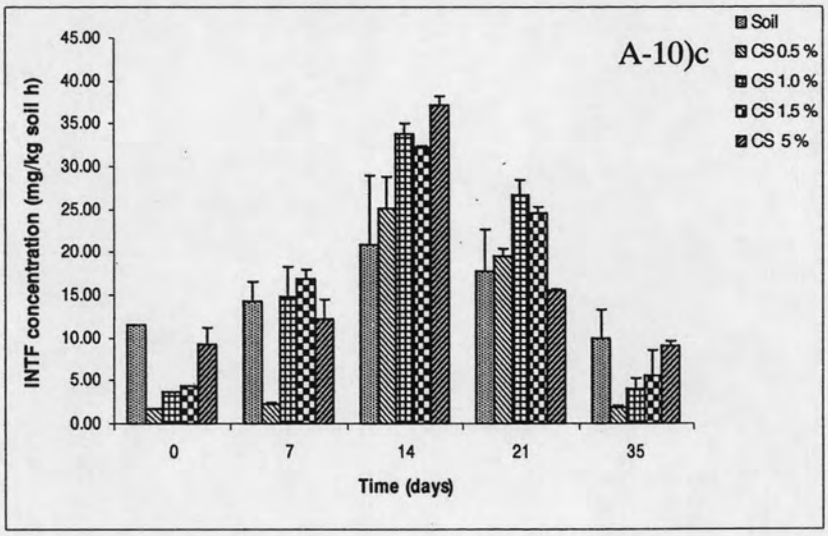


Figure A-9 Dehydrogenase Activity in soils: A-9a) soil with CF and A-9b) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



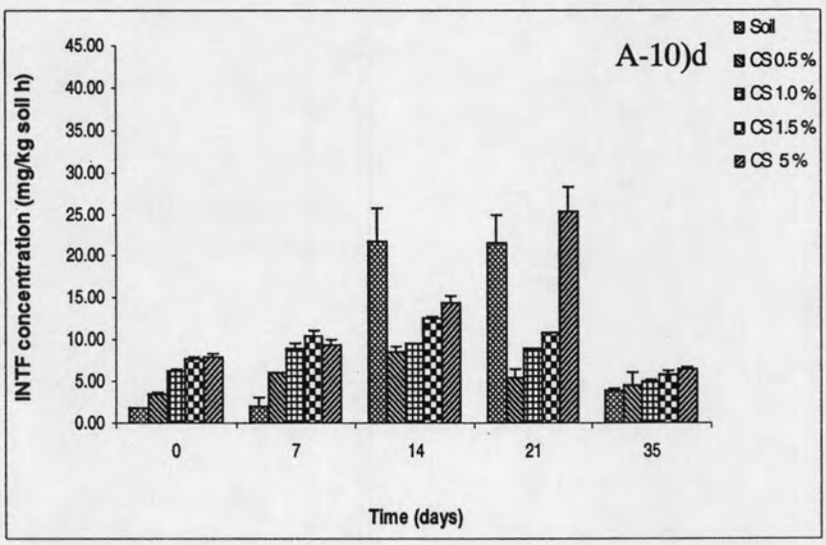
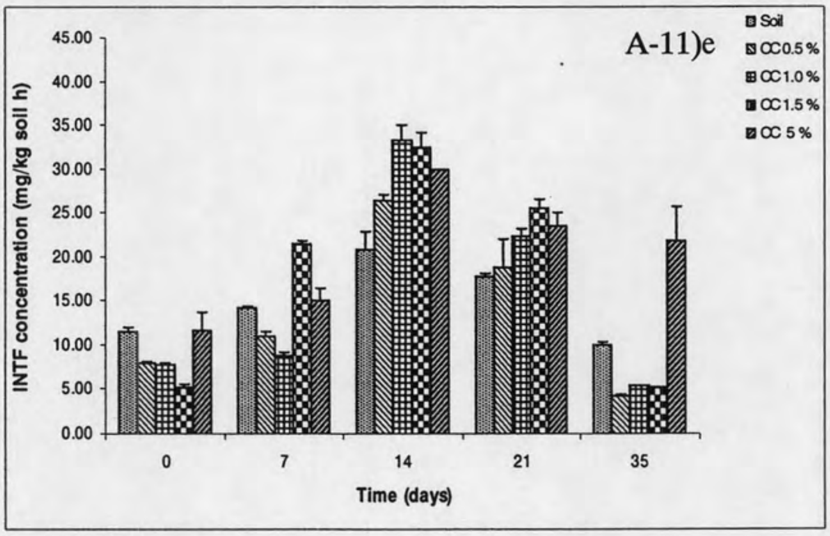


Figure A-10 Dehydrogenase Activity in soils: A-10c) soil with CF and A-10d) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



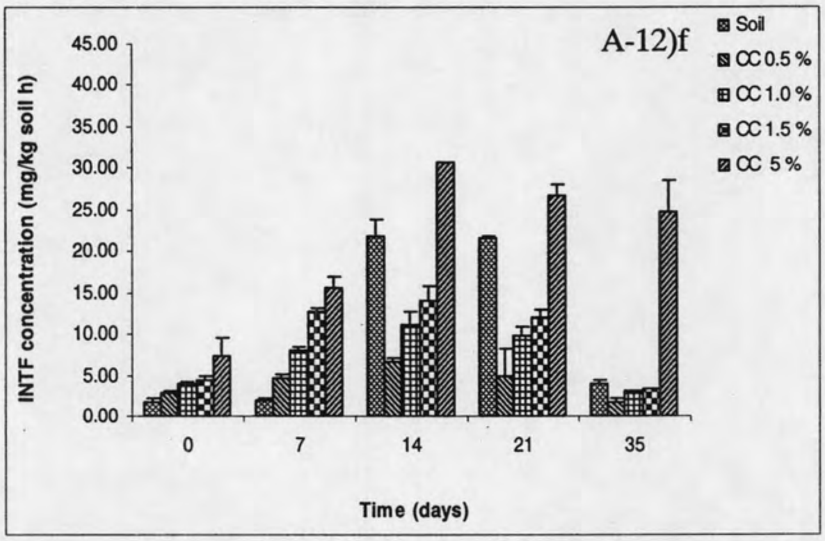
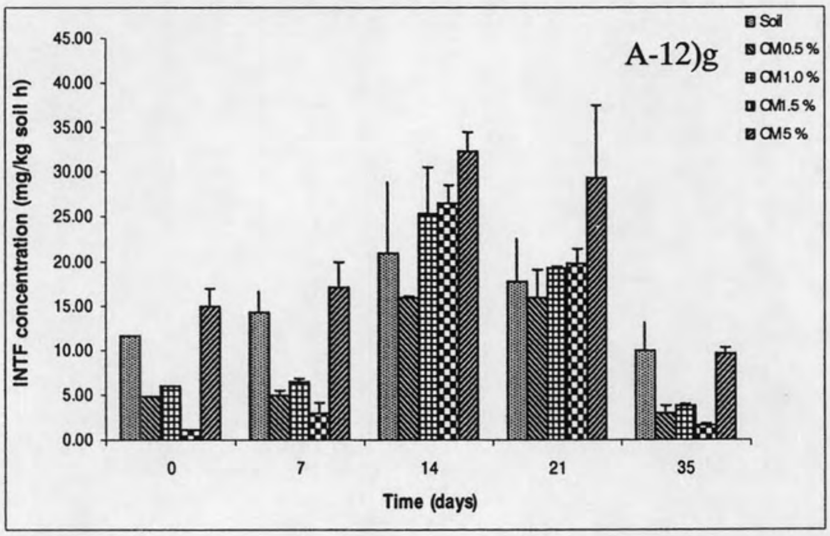


Figure A-11 Dehydrogenase Activity in soils: A-11e) soil with CF and A-11f) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



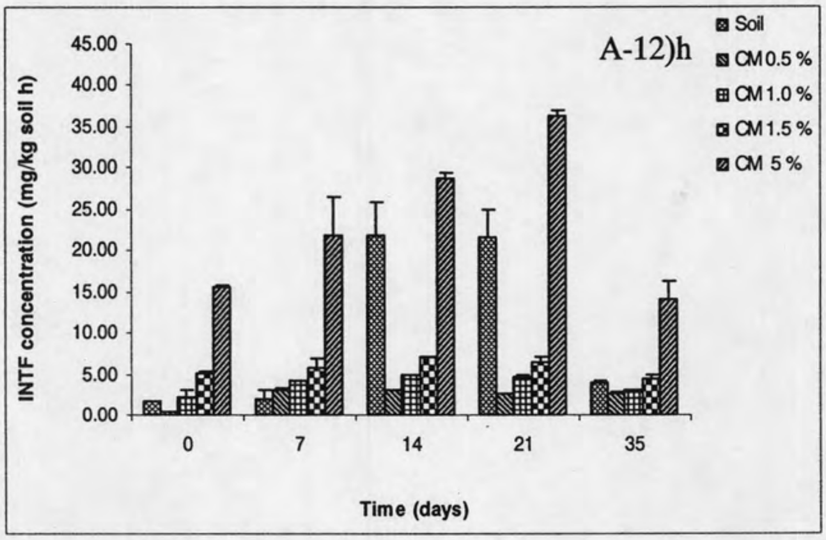
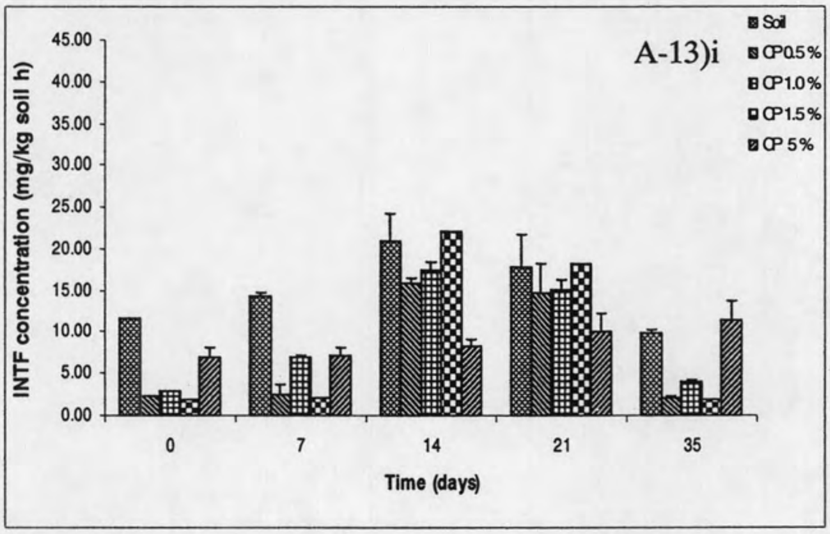


Figure A-12 Dehydrogenase Activity in soils: A-12g) soil with CF and A-12h) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



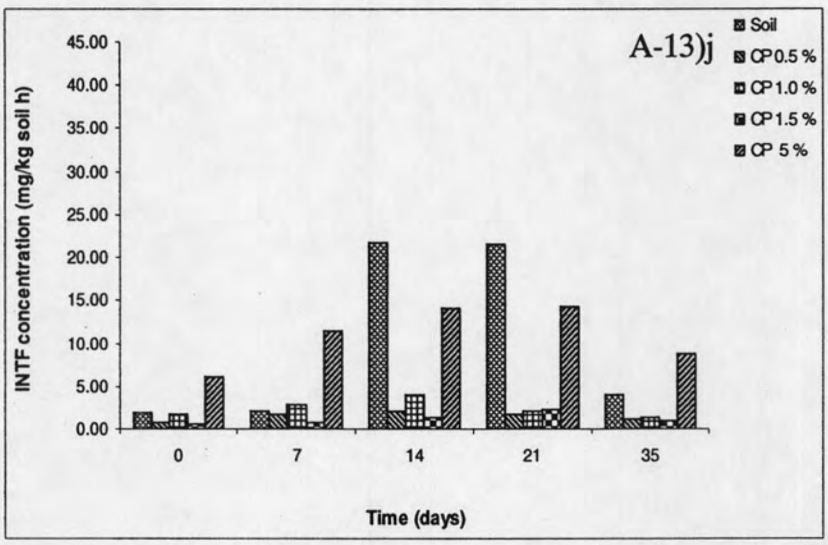
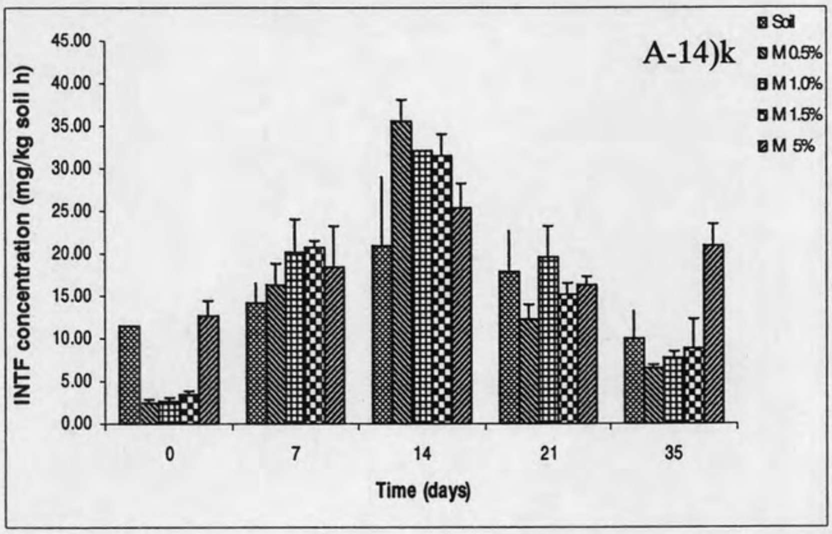


Figure A-13 Dehydrogenase Activity in soils: A-13i) soil with CF and A-13j) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



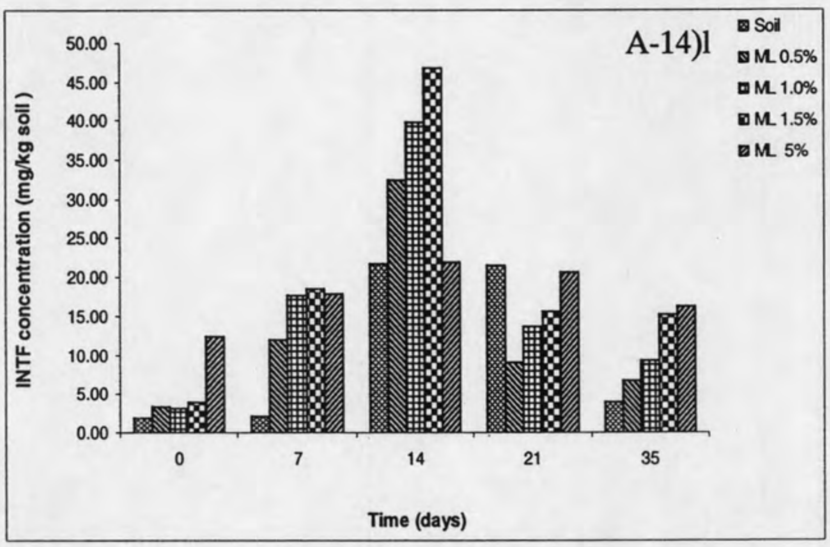
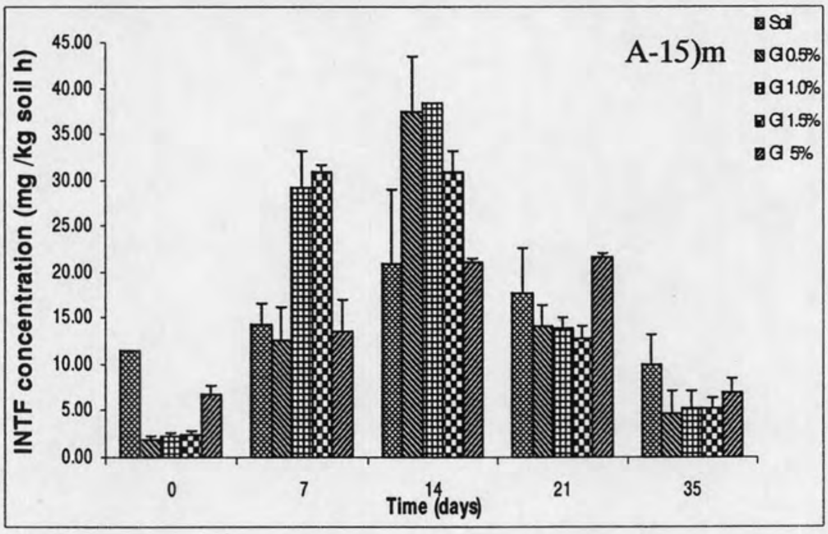


Figure A-14 Dehydrogenase Activity in soils: A-14k) soil with CF and A-14l) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.



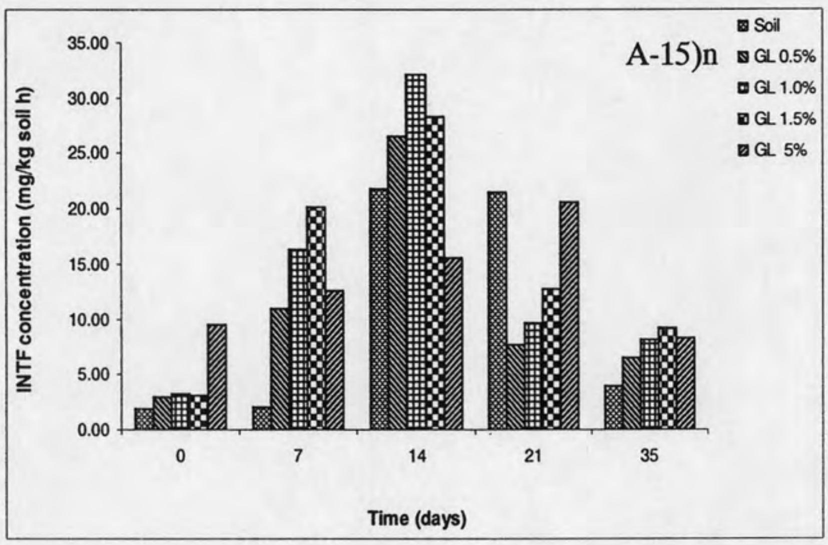


Figure A-15 Dehydrogenase Activity in soils: A-15m) soil with CF and A-15n) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.

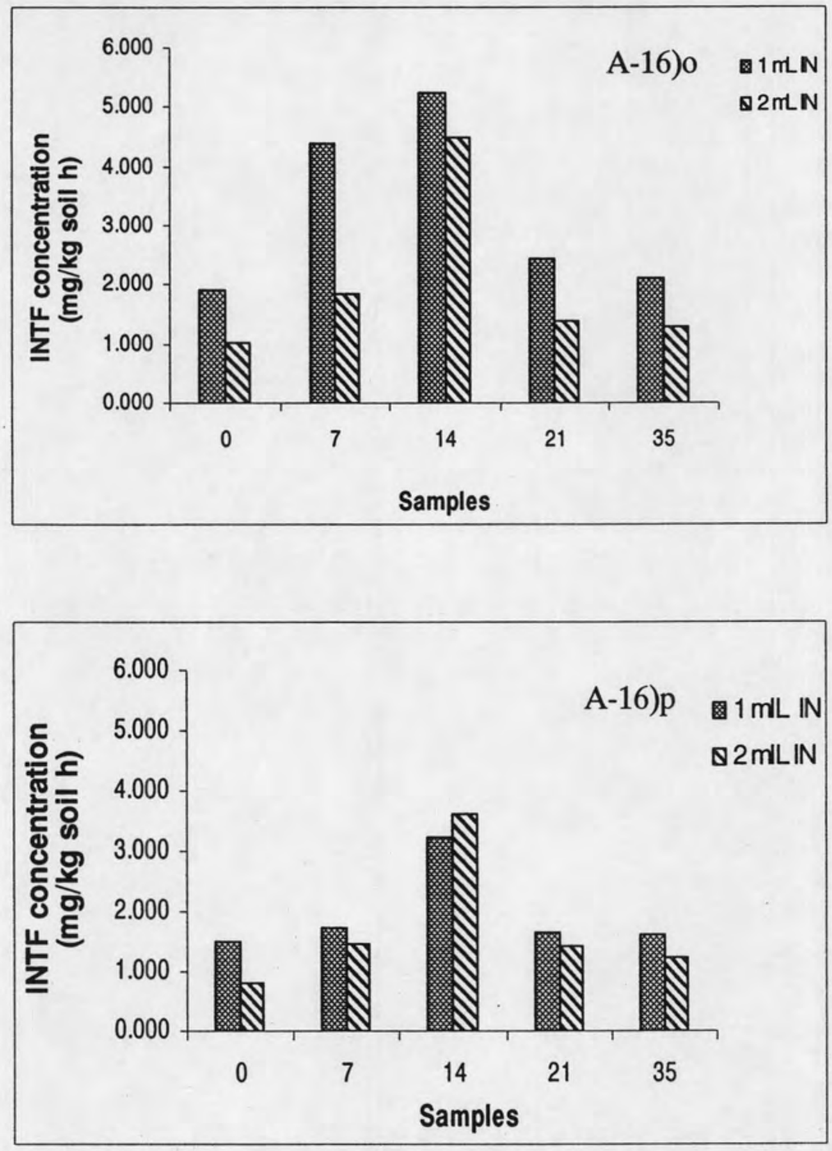


Figure A-16 Dehydrogenase Activity in soils: A-16o) soil with CF and A-16p) soil without CF (control) at the concentration of 50 mg kg^{-1} dry soil.

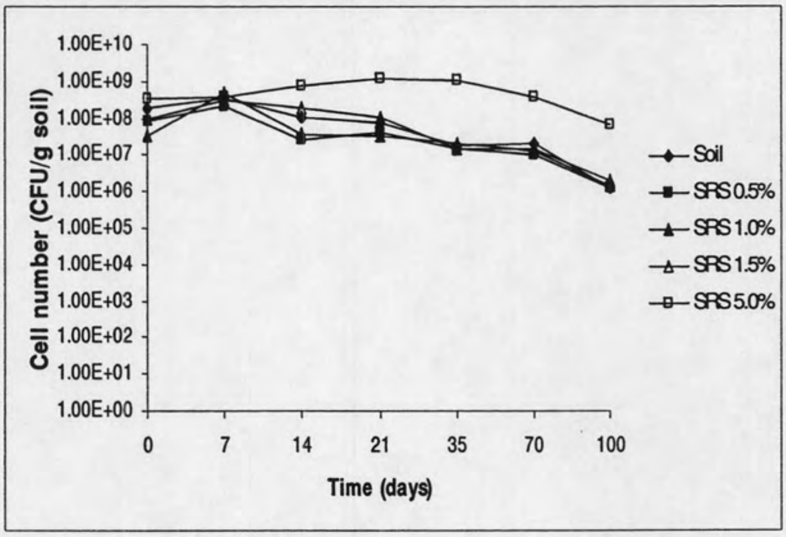


Figure A-17 Growth of carbofuran degraders from soil amended with RS

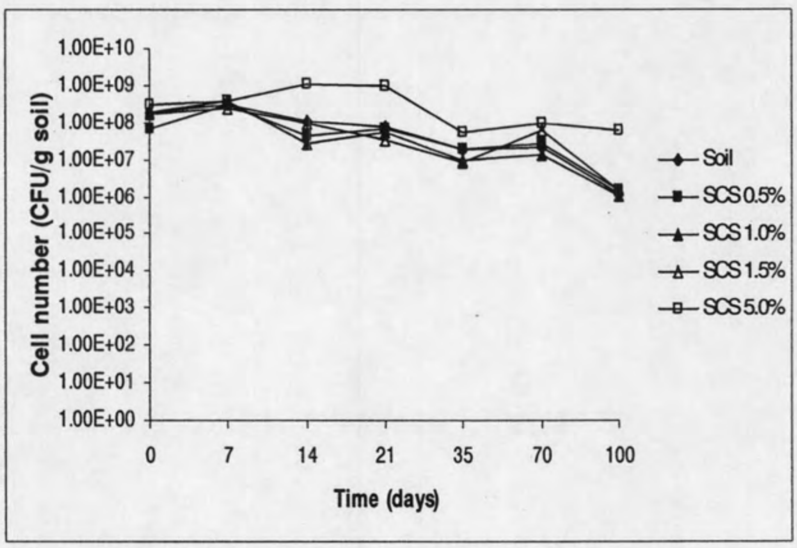


Figure A-18 Growth of carbofuran degraders from soil amended with CS

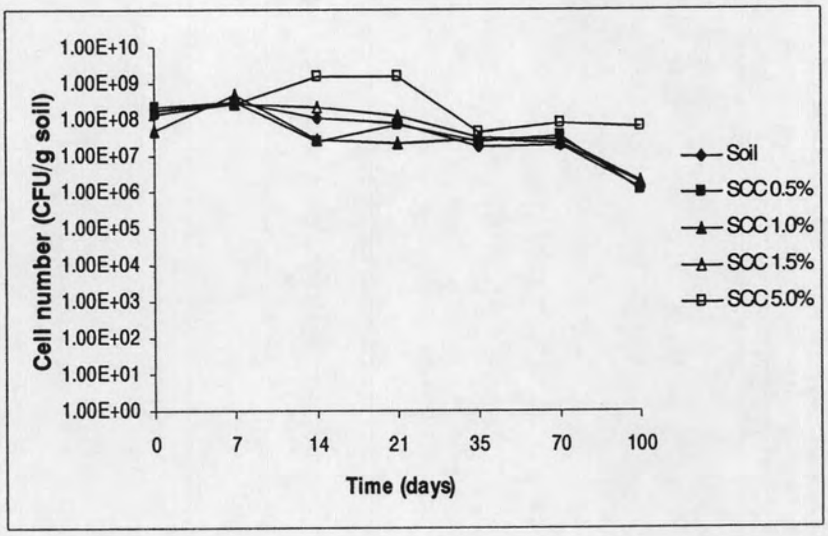


Figure A-19 Growth of carbofuran degraders from soil amended with CC

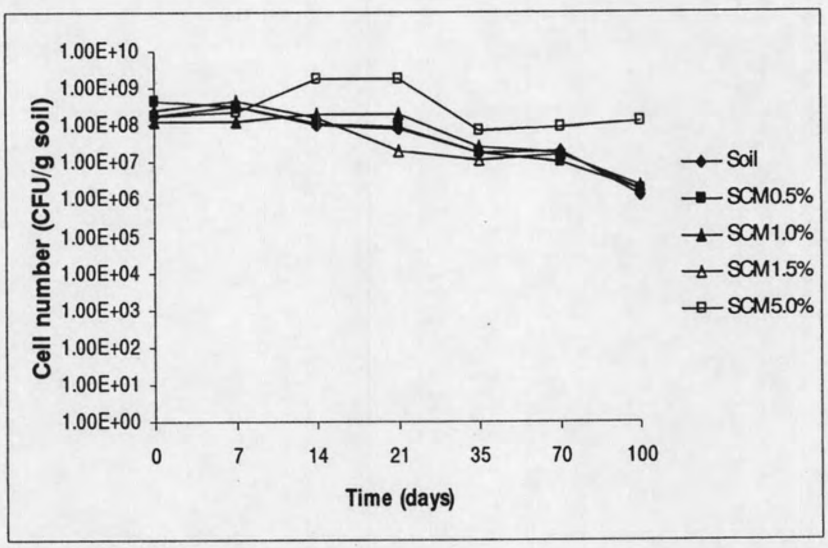


Figure A-20 Growth of carbofuran degraders from soil amended with CM

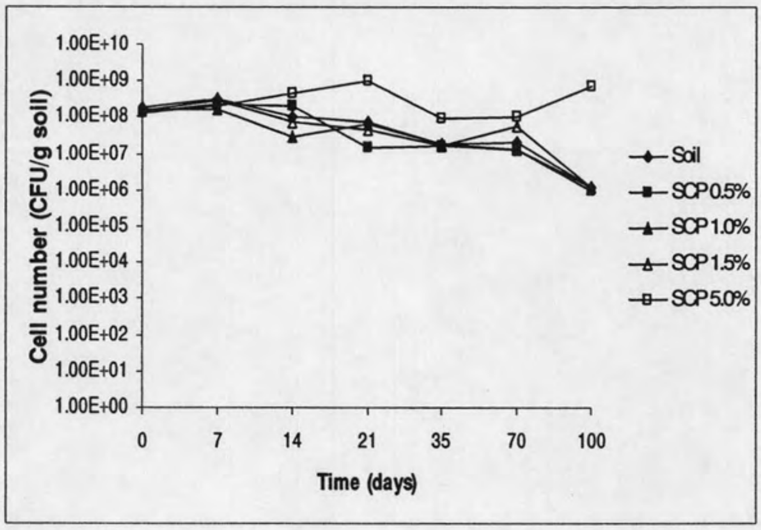


Figure A-21 Growth of carbofuran degraders from soil amended with CP

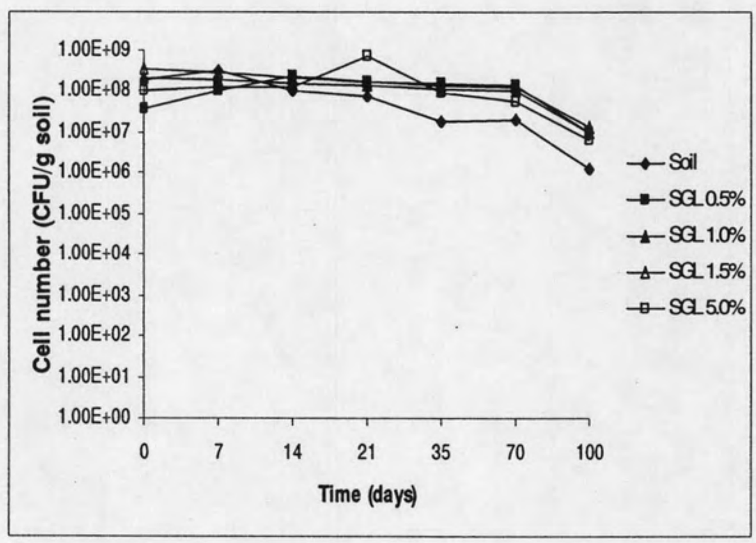


Figure A-22 Growth of carbofuran degraders from soil amended with GL

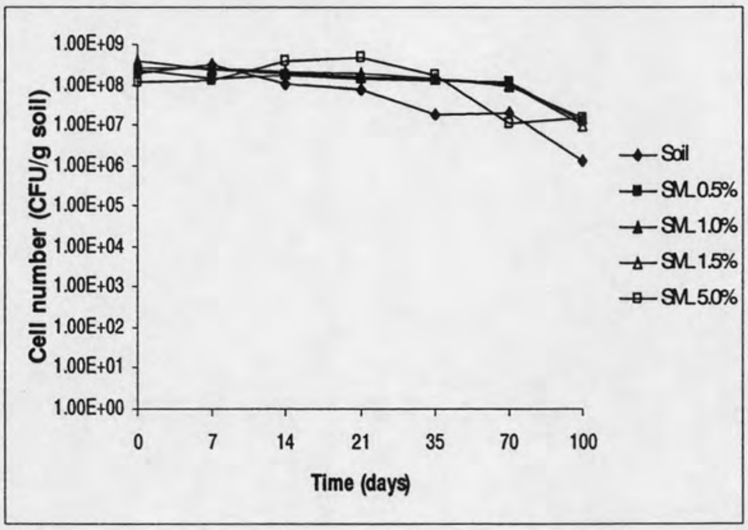


Figure A-23 Growth of carbofuran degraders from soil amended with ML

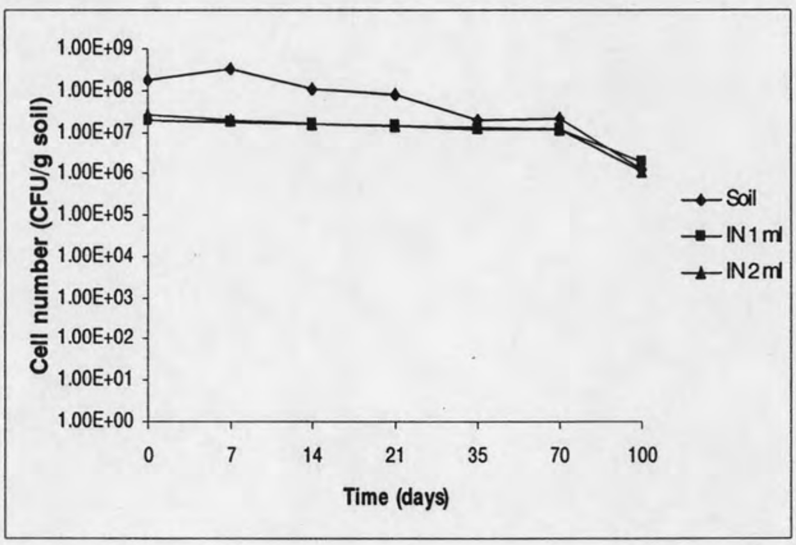


Figure A-24 Growth of carbofuran degraders from soil amended with inorganic amendment

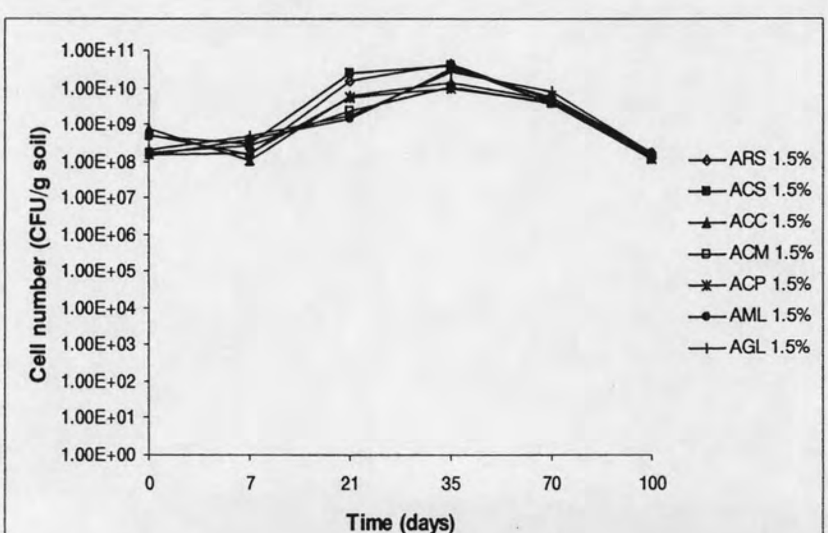
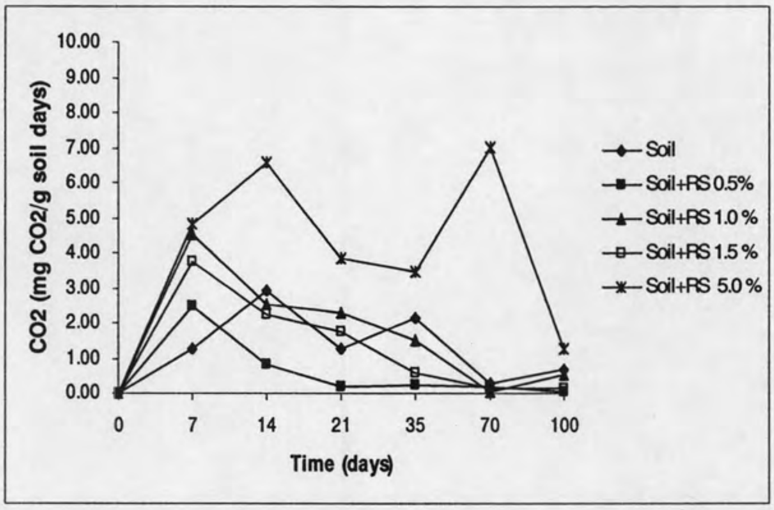
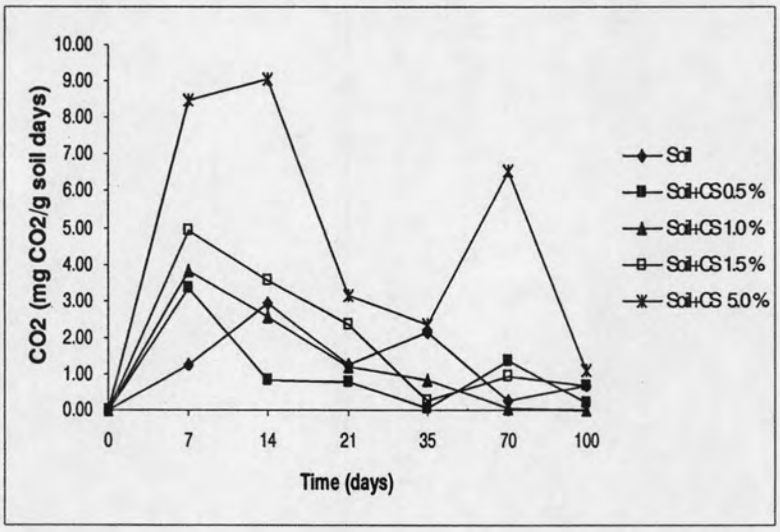


Figure A-25 Growth of carbofuran degraders from soil amended with autoclaved amended





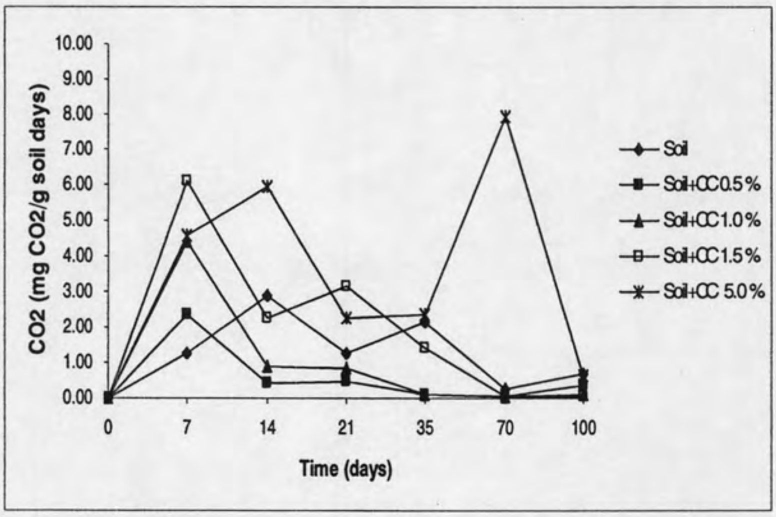


Figure A-26 CO_2 generated from soil amended with RS, CS, CC without carbofuran

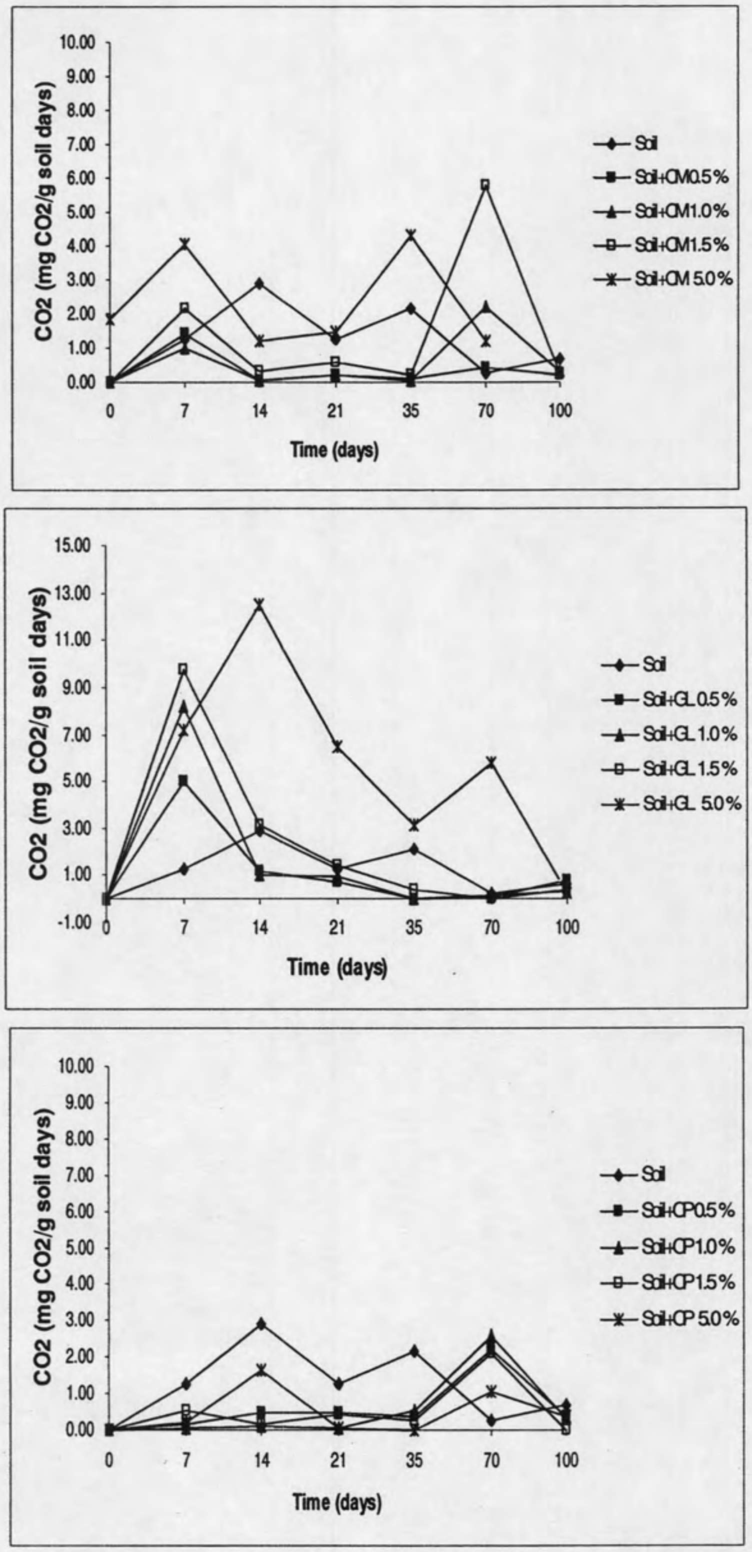


Figure A-27 CO_2 generated from soil amended with CM, CP, ML without carbofuran

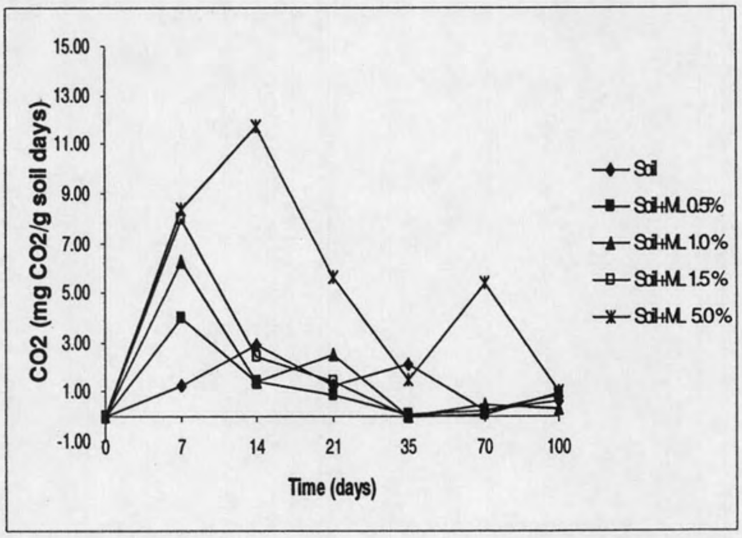


Figure A-28 CO_2 generated from soil amended with GL without carbofuran

Table A-1 Concentraion of carbofuran phenol in soil amended with organic amendments

Day	Carbofuran phenol concentration (mg/kg soil)						
	0	7	14	21	35	70	100
Non-amendment	0.14	0.04	0.14	0.02	0.02	0.02	0.14
0.5% RS	0.39	1.04	0.04	0.37	0.01	0.03	0.11
1.0% RS	0.89	0.27	0.05	0.02	0.01	0.06	0.14
1.5% RS	1.30	0.27	0.01	0.08	0.02	0.04	0.10
5.0% RS	0.00	0.04	0.31	0.35	0.00	0.00	0.00
0.5% CS	0.14	0.01	0.02	0.63	0.03	0.10	0.14
1.0% CS	0.11	0.27	0.08	0.57	0.00	0.08	0.15
1.5% CS	0.11	0.26	0.02	0.39	0.04	0.04	0.04
5.0% CS	0.00	0.07	0.27	0.00	0.03	0.00	0.00
0.5% CC	0.12	0.06	0.13	1.43	0.00	0.09	0.11
1.0% CC	2.41	0.05	0.11	0.05	0.02	0.16	0.14
1.5% CC	3.43	0.02	1.90	0.75	0.00	0.18	0.10
5.0% CC	0.00	0.00	0.81	0.16	0.00	0.00	0.00
0.5% CP	0.11	0.04	0.02	0.01	0.01	0.16	0.07
1.0% CP	0.05	0.14	0.06	0.01	0.01	0.16	0.13
1.5% CP	1.02	0.06	0.03	0.01	0.01	0.23	0.15
5.0%CP	0.00	0.12	0.04	0.03	0.03	0.00	0.00
0.5% CM	0.07	0.10	0.05	0.01	0.02	0.20	0.16
1.0% CM	0.02	0.10	0.04	0.05	0.00	0.23	0.09
1.5% CM	0.03	0.03	0.06	0.00	0.00	0.13	0.11
5.0% CM	0.00	0.00	0.01	0.07	0.00	0.00	0.00
0.5% ML	0.00	0.12	0.26	0.08	0.00	0.03	0.07
1.0% ML	0.00	0.08	0.17	0.07	0.04	0.08	0.03
1.5% ML	0.00	0.19	0.16	0.41	0.00	0.01	0.00
5.0% ML	0.00	0.01	0.00	0.03	0.00	0.00	0.00
0.5% GL	0.00	0.13	0.01	0.07	0.02	0.02	0.01
1.0% GL	0.00	0.10	0.01	0.15	0.02	0.06	0.01
1.5% GL	0.00	0.19	0.24	0.37	0.00	0.17	0.01
5.0% GL	0.00	0.36	0.05	0.05	0.00	0.00	0.00

Table A-2 Concentraion of 3-keto carbofuran in soil amended with organic amendments

	3-keto carbofuran concentration (mg/kg soil)						
Day	0	7	14	21	35	70	100
Non-amendment	0.09	0.21	0.08	0.01	0.01	0.01	0.03
0.5% RS	0.54	0.16	0.11	0.65	0.22	0.01	0.01
1.0% RS	0.78	0.13	0.10	0.15	0.29	0.01	0.02
1.5% RS	0.00	0.29	0.08	0.00	0.33	0.02	0.02
5.0% RS	0.00	0.08	0.36	0.07	0.00	0.00	0.00
0.5% CS	0.00	0.05	0.03	0.02	0.13	0.02	0.03
1.0% CS	0.00	0.52	0.62	0.01	0.10	0.01	0.06
1.5% CS	0.00	0.51	0.12	0.33	0.08	0.03	0.07
5.0% CS	0.00	0.06	0.75	0.15	0.00	0.00	0.00
0.5% CC	0.07	0.27	0.14	0.36	0.10	0.01	0.04
1.0% CC	0.41	0.33	0.12	0.00	0.10	0.02	0.04
1.5% CC	0.40	0.61	0.23	0.59	0.13	0.01	0.04
5.0% CC	0.00	0.61	1.08	0.18	0.00	0.00	0.00
0.5% CP	0.10	0.24	0.04	0.42	0.13	0.01	0.02
1.0% CP	0.04	0.10	0.07	0.14	0.22	0.01	0.03
1.5% CP	0.04	0.12	0.09	0.20	0.05	0.01	0.02
5.0%CP	0.00	0.03	0.06	0.15	0.00	0.00	0.00
0.5% CM	0.05	0.07	0.07	0.11	0.09	0.01	0.03
1.0% CM	0.00	0.13	0.07	0.05	0.04	0.01	0.05
1.5% CM	0.07	0.07	0.05	0.04	0.09	0.01	0.09
5.0% CM	0.00	0.03	0.07	0.01	0.00	0.00	0.00
0.5% ML	0.00	0.02	0.00	0.03	0.00	0.02	0.03
1.0% ML	0.00	0.01	0.02	0.04	0.00	0.01	0.01
1.5% ML	0.00	0.00	0.01	0.07	0.01	0.03	0.02
5.0% ML	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5% GL	0.00	0.02	0.03	0.05	0.04	0.02	0.01
1.0% GL	0.00	0.07	0.02	0.04	0.00	0.01	0.02
1.5% GL	0.00	0.03	0.00	0.11	0.07	0.00	0.01
5.0% GL	0.00	0.01	0.01	0.05	0.00	0.00	0.00

APPENDIX B

Dehydrogenase activity and growth curve of carbofuran degraders in aged soil

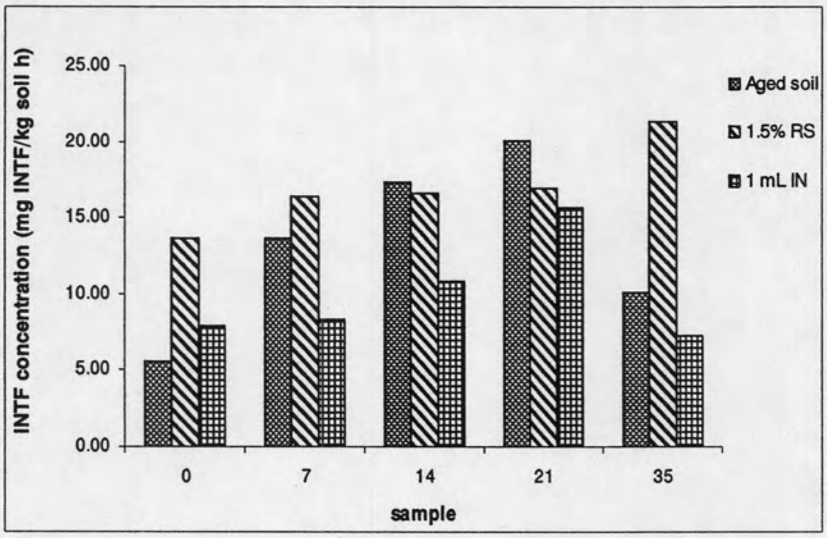


Figure B-1 Dehydrogenase Activity in aged soils amended with 1.5% RS and 1 mL of inorganic amendment

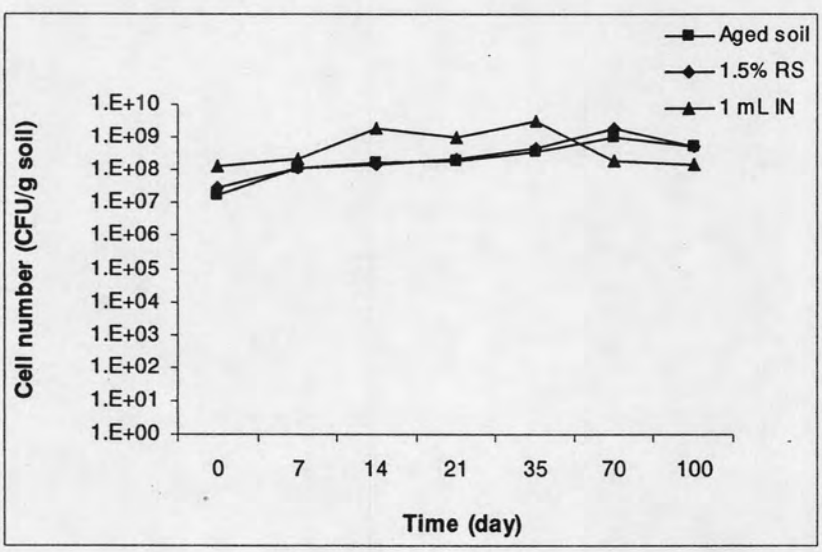


Figure B-2 Growth of carbofuran degraders from soil amended with 1.5% RS and 1 mL of inorganic amendment

APPENDIX C

Standard curves of carbofuran, carbofuran phenol and 3-keto carbofuran in methanol

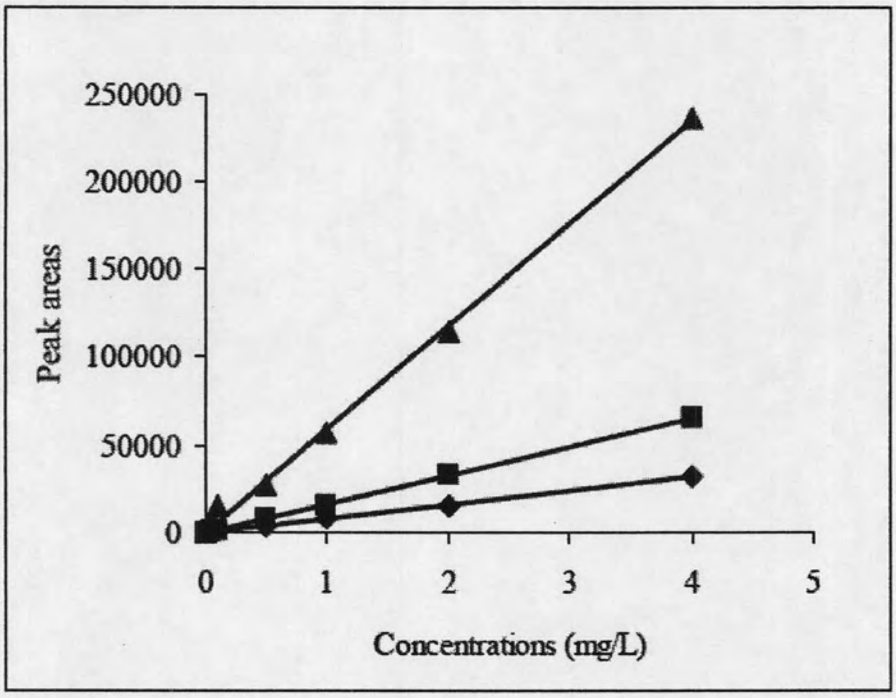


Figure C-1 Standard curve of carbofuran (◆), carbofuran phenol (■) and 3-ketocarbofuran (▲) in methanol

Linear equation of carbofuran standard

$y = 7921.1x$ (1)

$R^2 = 0.9994$

Linear equation of carbofuran phenol standard

$y = 16315x$ (2)

$R^2 = 0.9997$

Linear equation of 3-keto carbofuran standard

$y = 58846x$ (3)

$R^2 = 0.9969$

APPENDIX D

Degradation of carbofuran in BSM and growth curve of stimulated and non-stimulated carbofuran degraders

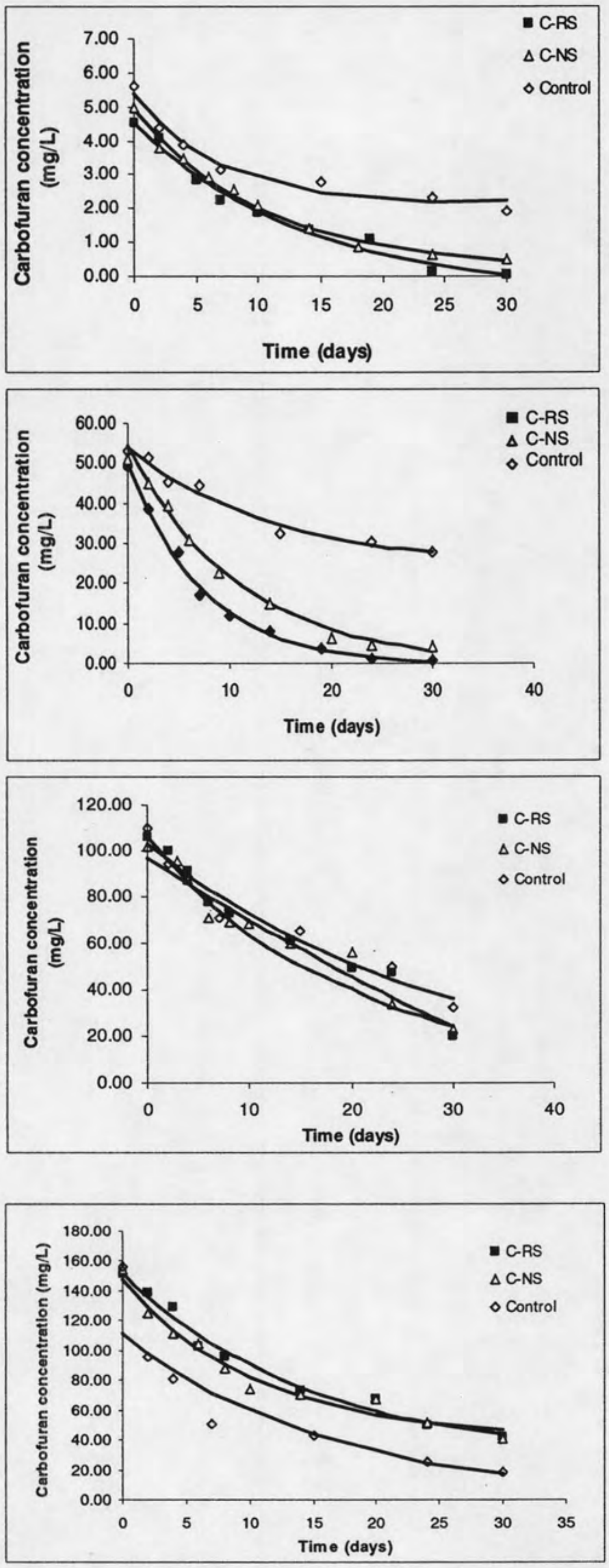


Figure D-1 Degradation of carbofuran by carbofuran degraders in C-limited BSM containing carbofuran at the initial concentration of 5, 50, 100, 150 mg/L (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

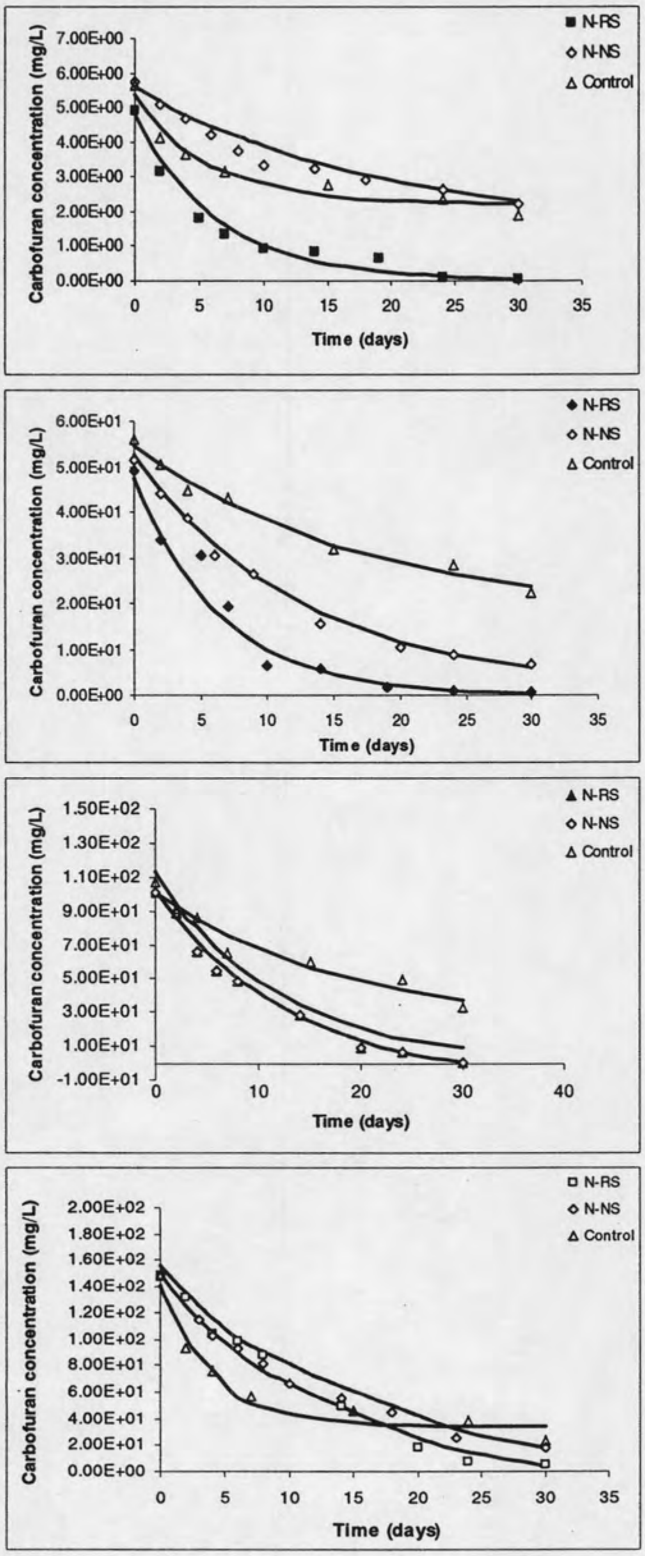


Figure D-2 Degradation of carbofuran by carbofuran degraders in N-limited BSM containing carbofuran at the initial concentration of 5, 50, 100, 150 mg/L (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

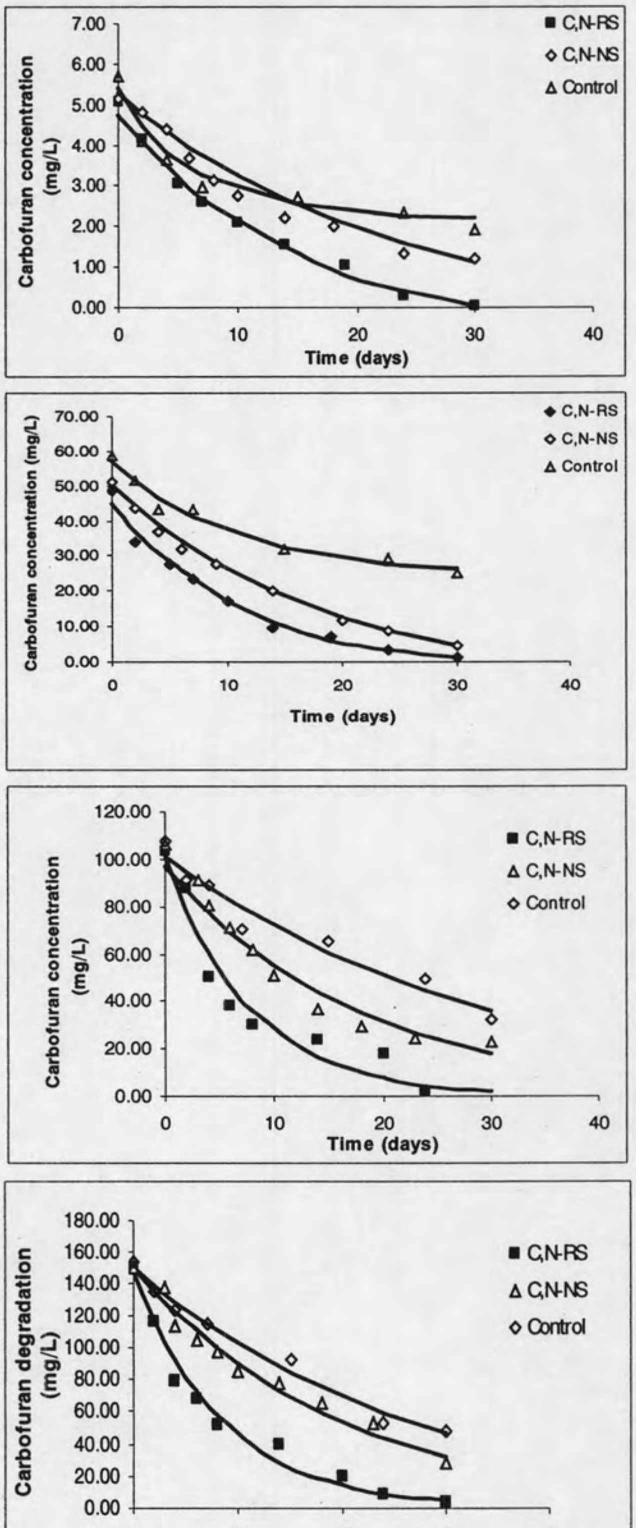


Figure D-3 Degradation of carbofuran by carbofuran degraders in C,N-limited BSM containing carbofuran at the initial concentration of 5, 50, 100, 150 mg/L (Lines = Carbofuran concentration fitted to the Modified First order Kinetic Model)

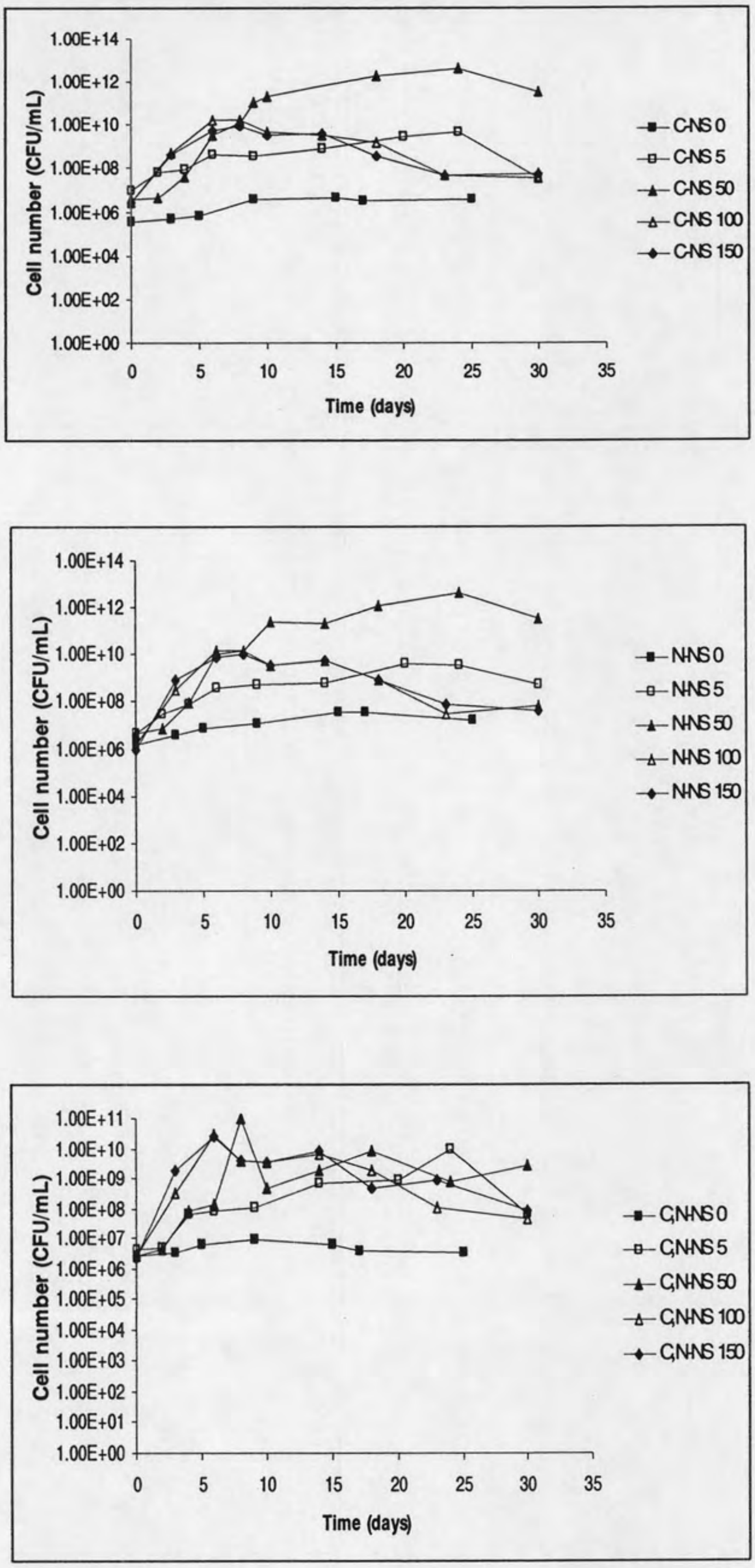


Figure D-4 Growth curves of C-NS, N-NS, C,N-NS in C-limited BSM, N-limted BSM, C,N-limited BSM at each initial concentrations during incubation

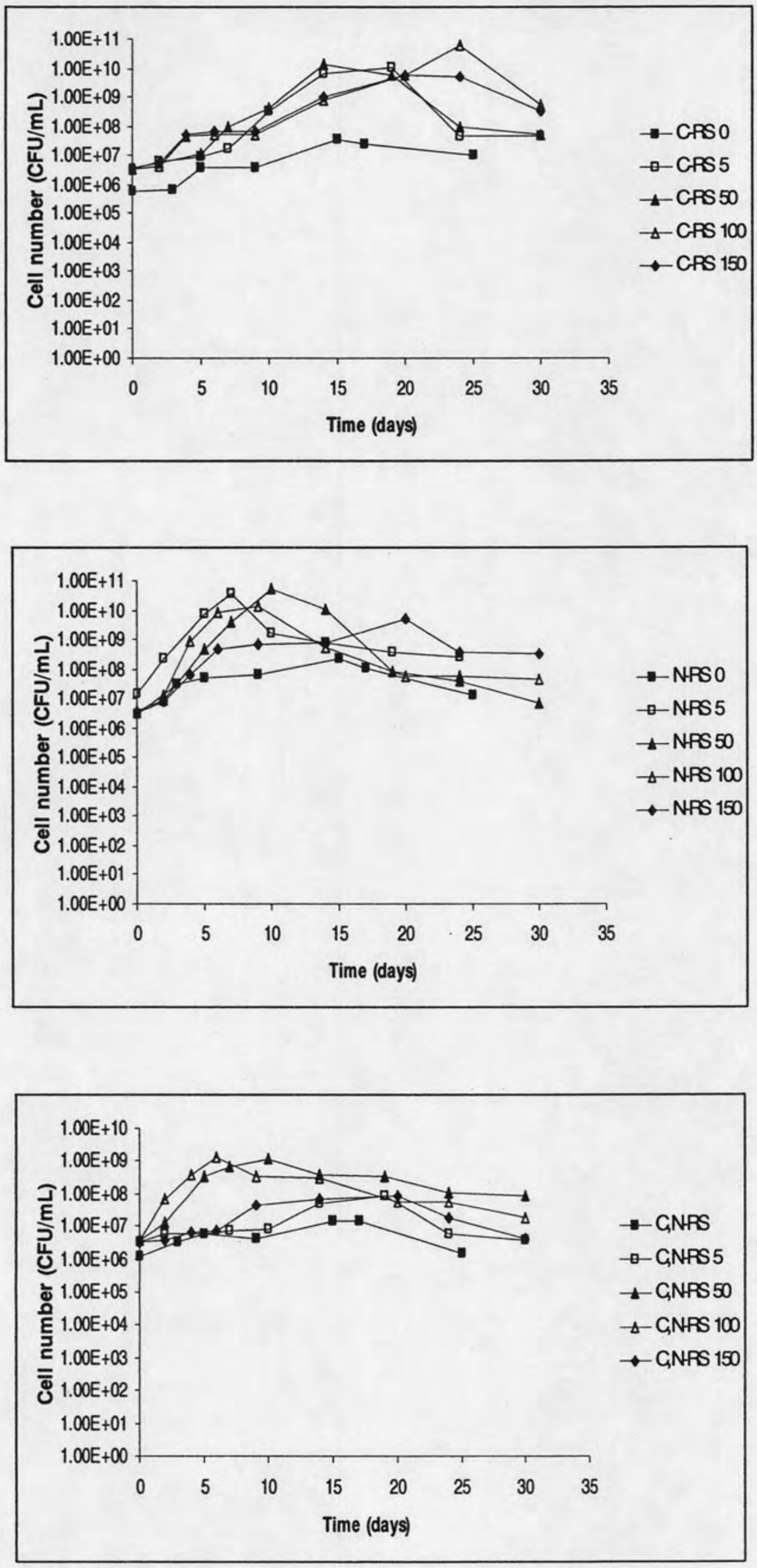


Figure D-5 Growth curves of C-RS, N-RS, C,N-RS in C-limited BSM, N-limted BSM, C,N-limited BSM at each initial concentrations during incubation

APPENDIX E

Biostimulation of carbofuran degradation in soil by agricultural residues

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BIOSTIMULATION OF CARBOFURAN DEGRADATION IN SOIL BY AGRICULTURAL RESIDUES

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ABSTRACT

This research was conducted to investigate the biostimulation technique using agricultural residues i.e., rice straw, cassava pulp, cattle manure, compost and corn cob as a bioremediation tool for improving the degradation of carbofuran residues in rice field soil. Soil microbial activity was evaluated by measuring dehydrogenase activity and carbon dioxide (CO_2) evolved from the soil as soil respiration. Results indicated that carbofuran was more rapidly degraded in soils amended with 1.5% rice straw ($t_{1/2}$ of 6 days) than in control which was soil without amendment ($t_{1/2}$ of 11 days). Rice straw, cassava pulp and corn cob used in this study increased number of microorganisms and microbial activity in the soil comparing to control. Compost (CP) was found to inhibit microbial activity suggesting by a decrease of dehydrogenase activity and resulting in a decrease of carbofuran degradation efficiency in the soil. From these results, it could be concluded that rice straw was observed to be the most suitable organic amendment for biostimulation of carbofuran indicating by the shortest half-life of carbofuran and highest microbial activity presenting in the soil amended with this material.

Key words: agricultural residue; biostimulation; bioremediation; carbofuran

INTRODUCTION

Carbofuran (2,3-dihydro-2,2 dimethylbenzofuran-7-yl methylcarbamate) is a broad-spectrum carbamate insecticide widely used in crops and plants such as corn, rice, sorghum, potato, and sugarcane, etc. [1]. Carbofuran is known to be toxic to mammals, fish and birds through cholinesterase inhibition with an acute oral LD50 in rats 8-14 mg kg^{-1}, and dogs 19 mg kg^{-1}. The US EPA reported that the maximum contaminant level (MCL) for carbofuran is 40 $\mu g\ l^{-1}$[2]. Carbofuran is soluble in water and mobile in soil resulting in a high potential for groundwater contamination [3].

Biostimulation refers to the addition of nutrients, air or oxygen into the contaminated systems with an attempt to stimulate the indigenous microbial population to degrade the contaminants of concern. Advantages of this method are simple to maintain, applicable over large areas, cost-effective, and leads to the complete destruction of the contaminant [4]. Biostimulation has been used to remediate various types of contaminant such as petroleum hydrocarbon [5, 6] and pesticides [7].

Thailand is an agricultural country that produces several hundred million tons of agricultural products such as vegetables waste, fruits waste, rice straw, cassava pulp, cattle manure, etc. and some millions of tons are wasted each year [8]. These large amounts of wastes become environmentally concerns. Therefore, there is a need to added value into these agricultural wastes and biostimulation is one of the solutions to make use of these agricultural wastes. Agricutural wastes are well recognized as the sources of carbon and nitrogen that can be used by microorganisms as their food or energy source. Several researchers investigated the effectiveness of agricultural residues on stimulation of the degradation of contaminants. For example, Zhang and Frankenberger [9] successfully used rice straw to accelerate the removal of selenate in agricultural drainage water. Baranona et al. [6] added 3% corn and crop residues at the C/N ratio 100:10, and 30% moisture content into the diesel contaminated soil. Results showed that the diesel removal was increased to 67% compared with non-stimulated diesel-contaminated soils.

Previous research indicated a promise of adding agricultural residues to stimulate the degradation of contaminants. As of our knowledge, however, there is a very limited data on using agricultural residues to biostimulate carbofuran degradation in soil. Therefore, in this study we investigated the effect of agricultural residues on stimulation of the indiigenous microorganisms to degrade carbofuran in contaminated rice field soils.

MATERIALS AND METHODS

Agricultural Amendments

Agricultural amendments used for biostimulation study were cassava pulp (CS) which provided by Asia Ctric Co. Ltd (Kalasin Province, Thailand), cattle manure (CM), compost (CP), rice straw (RS) and corn cob (CC) which obtained from the Faculty of Agriculture, Khon Kaen University, Thailand. These materials were blended and passed through 2 mm sieves and then kept in plastic bag prior the usage. Carbon and nitrogen contents in these materials (Table 1) were analyzed by Wakley black method [10], and Kjeldahl method [11], respectively.

Table: 1 Carbon and Nitrogen Contents in Agricultural Amendments

Content	RS	CS	CC	CM	CP
Organic carbon (%)	41.48	51.70	43.66	29.78	8.88
Total nitrogen (%)	0.43	0.25	0.82	1.58	0.28

Chemicals

Carbofuran (98% purity) and carbofuran phenol (99% purity) were purchased from Sigma-Aldrich, USA and 3-keto carbofuran (98.5% purity) was purchased from Ehrenstorfer Quality, Germany. Methanol (HPLC and analytical grade) was purchased from Merck, Germany.

Soil Sample

Soil sample was collected from the rice field of Ban Non-Muang, Khon Kaen, Thailand, with a history of carbofuran application, at a depth of 0-15 cm. Soil was air dried, passed through a 2-mm sieve, kept in plastic bag and stored at 4 °C prior the usage. The analysis of soil properties indicated that the soil sample was classified as sandy loam with total nitrogen, organic carbon and pH of 0.89%, 0.1% and 6.9, respectively. Background concentration of carbofuran was approximately 1.28 mg kg^{-1}.

Biostimulation Experiment

Fifty g of soil was mixed with carbofuran at an average concentration of 50 mg kg^{-1} dry soil in a 280 ml glass bottle capped with a plastic lid. Each agricultural residue was added to the soil at the varying concentrations of 0.5% and 1%, 1.5% (w/w). The initial moisture content was adjusted to 15-18% before incubating at room temperature. Controls were autoclaved soils with amendment and soil without amendment which were conducted by a similar manner. At days 0, 7, 14, 21 and 35, soil samples were sacrificed to determine soil microbial activity and analyzed for carbofuran and its metabolites concentrations.

Carbofuran and its metabolites in soil samples were extracted using ASE 100 Accelerate Solvent Extractor under the following: 100 ^{o}C extraction temperature, 5 min static extraction time. Methanol was used as the extraction solvent and two extraction cycles.

The extracts were determined for carbofuran and its metabolites concentrations by HPLC equipped with 4.6x150 mm-Lunar 0.5 μm C-18 column (Phenomenex, USA), a UV detector operating at 220 nm and a 20 μL injector loop. The operating parameters were: mobile phase, methanol-water (60:40); flow rate, 1 ml min^{-1} at the ambient temperature.

Numbers of carbofuran degraders were counted on the BSM agar coated with 50 mg l^{-1} of carbofuran. The half-lives of carbofuran in these soils were calculated by fitting with a modified first-order kinetic model [12].

Soil Microbial Respiration

Soil microbial respiration was measured by determining an accumulation of CO_2 emitted from the soil by titration method. This was conducted to investigate microbial activity in soil. Forty grams of dry soil was added into 280 ml glass bottle and the carbofuran was then added at the initial concentration of approximately 50 mg kg^{-1} dry soil. The organic amendments i.e. rice straw, cassava pulp, corn cob, compost, and cattle manure were added to the soil at the various concentration of 0.5%, 1.0%, 1.5% (w/w). Control was soil mixed with carbofuran at a concentration of 50 mg kg^{-1} soil without agricultural amendment. After soil was mixed with carbofuran and amendments, plastic box contained 15 ml of 1N NaOH solution was inserted above the soil sample in bottle and then the bottle was capped with plastic lid. Evolved CO_2 trapped in NaOH solution was determined at days 0, 7, 14, 21 and 35 by titration excess of NaOH with 1 N HCL using phenolphthalein as indicator. At each sampling date, 0.1 N NaOH solution in plastic box was replaced. CO_2 evolved from soil microbial respiration was calculated by using an equation of CO_2 = (B-V) NE, where B, V, N and E were amount of HCL that use to titrate with NaOH of the control, amount of HCL that used to titrate with NaOH of each samples, concentration of HCL (1 N) and equivalent weight (CO_2 = 22), respectively [13].

Dehydrogenase Activity Assay

Dehydrogenase is an intracellular enzyme involved in microbial respiratory metabolism indicating microbial activity [14]. The DHA assay was conducted by determining a iodonitrotetrazolium formazan (INTF) which was a product from a reduction of iodonitrotetrazolium violet (INT) using dehydrogenase enzyme as a catalyst. A 2.5 g dry soil sample was mixed with 1.25 ml of DI water and 0.5 ml of INT solution (5 g l^{-1}) in vial bottle sealed with septum lid before incubating at 27° C for 22 h in dark. After incubation, the metabolic product i.e., INTF was extracted by adding 12.5 ml methanol and then filtrated through a Whatman paper No.42. The absorbance of the metabolic product in the filtrates were measured at λ_{max}= 428 nm using a spectrophotometer. The INTF standard was prepared in methanol. DHA was expressed as mg INTF kg^{-1} dry soil h^{-1} [15].

RESULTS AND DICUSSIONS

Dissipation of Carbofuran in Soil as a Result of Biostimulation Technique

This experiment investigated the effect of agricultural amendments on stimulation of the indigenous microorganisms to degrade carbofuran in contaminated rice field soils. Degradation of carbofuran over time in soil amended and non-amended (controls) with an agricultural residues were shown in Fig.1. Results indicated that the shortest half life of carbofuran (6 days) was found in soil amended with 1.5% RS compared to 11days in controls (Table 2), indicating that RS was the most suitable material for biostimulation of carbofuran among the amendedments used in this study. This might due to the fact that microorganisms in the soil might be able to use RS as their energy sources i.e., C and N sources and/or electron donors thus enhancing their carbofuran degradation ability [9]. In addition, when compared RS to the other agricultural residues used in this study, rice straw was more bulky and light in weight leading to an increase in the soil porosity which in turn improving air ventilation in soil resulting in a better carbofuran degradation than other amendments. However, levels of concentration of amendments did not affect carbofuran degradation in soil (Table 2). Our results were similar to the findings of Venkateswarlu and Sethunathan [16] who reported that the addition of RS 0.5% w/w to the flooded undisturbed soil effected more rapid degradation of carbofuran than in unamended soil.

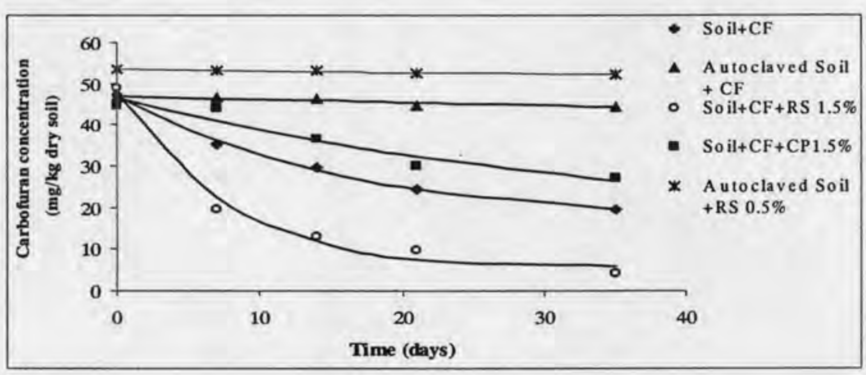

Figure 1: Degradation of Carbofuran in Soils (Lines = Carbofuran Concentration Fitted to the Modified First-order Kinetic Model)

The addition of CS, CM and CC did not affect carbofuran degradation in soil in which carbofuran half lives in soil amended with these material were in the range of 9-11days which were similar to control (half-life of 11days) (Table 2). This might due to the fact that soil used in this experiment was collected from rice field with a history of carbofuran application. Thus, microorganisms present in this soil might adapt themselves to have a capability to use carbofuran as their energy sources i.e., C- and/or N-sources and could degrade carbofuran without the stimulation. Repeated application of carbofuran in soil was reported to enhance the degradation of carbofuran [17, 18, 19] resulted from an increase number of carbofuran degraders in soil. Morel-Chevillet [20] reported that soil previously treated with 15 N-methylcarbamate enhanced the degradation rate of carbofuran and increased in the number of carbofuran degraders. An approximately 12 times shorter half-life of carbofuran in soil than in autoclaved soil (Table 2) confirmed that microorganism in the soil played an important role in carbofuran degradation.

Numbers of carbofuran degraders in soil over time were presented in Fig 2. An approximately of four times increase of carbofuran degraders in soil compared to unamended soil suggesting the incorporation of C- and/or N-sources containing in carbofuran and/or agricultural residues into the cells.

Compost (CP) showed a negative effect on carbofuran degradation in soil (half life of 15-28 days) when compared to control (half life of 11days). It was obviously seen that a compost used in this study had the lowest organic carbon i.e, 8.8% (Table 1) implying the lowest source of N compared to other amendments used in this study which might resulted in the longest half-life of carbofuran in soil added with CP. Gibert et al [21] also reported a negative effect of using compost as soil amendment. They found that CP did not improve sulphate degradation in soil. A high amount of lignin present in their compost might inhibit microbial activity and also sulphate degradation ability of microorganisms in soil [21].

Metabolites observed in all treatments were carbofuran phenol and 3-keto carbofuran (data not shown) which suggested hydrolysis and oxidative degradation pathways, respectively.

Table 2: Degradation rate Constants (K) and Half-Lives ($T_{1/2}$) of Carbofuran in Soil and Autoclaved soil amended with agricultural residues

Sample	Percentage of amendment	K_1 day1	$T_{1/2}$ days	$R^{2(a)}$
S	-	0.0630	11	1.00
S + RS	0.5	0.0820	8	0.98
S + RS	1.0	0.0870	8	0.96
S + RS	1.5	0.1230	6	0.98
S + CS	0.5	0.0610	11	0.98
S + CS	1.0	0.0700	10	0.99
S + CS	1.5	0.0650	11	0.98
S + CC	0.5	0.0680	10	0.98
S + CC	1.0	0.0710	10	0.99
S + CC	1.5	0.0760	9	0.99
S + CP	0.5	0.0473	15	0.92
S + CP	1.0	0.0247	28	0.92
S + CP	1.5	0.0259	27	0.93
S + CM	0.5	0.0468	15	0.98
S + CM	1.0	0.0620	11	1.00
S + CM	1.5	0.0657	11	1.00
AS	-	0.0073	95	0.87
AS + RS	1.5	0.0067	103	0.86

(a) = Coefficient of determination for non linear regressions

S = soil; As = Autoclaved soil

Soil microbial Activity

Effect of amendments on soil microbial activity was investigated using dehydrogenase assay and measuring of cumulative CO_2 evolved from soil. Results of dehydrogenase activity indicated that an addition of RS, CS and CC markedly stimulated microbial activity at the early period of incubation (0-7 days) in which dehydrogenase activity was found to be higher than control (Fig. 3 a). Soil added with carbofuran (Fig. 3b) had higher dehydrogenase activity than soil without carbofuran, suggesting an increase of carbofuran degrader activity. These results coincided with the number of carbofuran degraders which was found to increase during incubation (Fig 2).

The highest dehydrogenase activity was found at day 14 in all treatments (Fig. 3b). Rice straw (RS) exhibited the most effective material in stimulating the indigenous microbial population in soil, indicating by the highest dehydrogenase activity (Fig. 3b), which confirmed the shortest half-life of carbofuran in soil amended with RS (Table 2). Organic matter was known as factor affecting microorganisms in soil which can be either an activator or inhibitor when it present in optimum or excessive concentration respectively [9, 22]. Plant residues such as tobacco residue and rice straw were reported to have a stimulation effect on soil microbial activity. Soil added with tobacco residue and rice straw had an increase of dehydrogenase activity up to three to five times than soil without amendment [23].

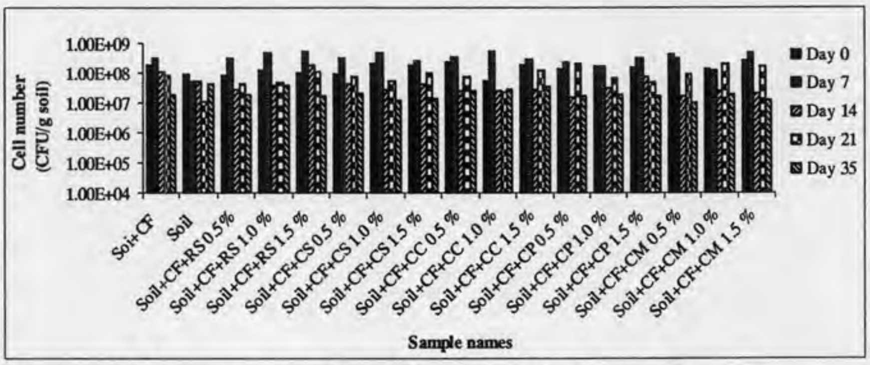

Figure 2: Time course of number of carbofuran degraders

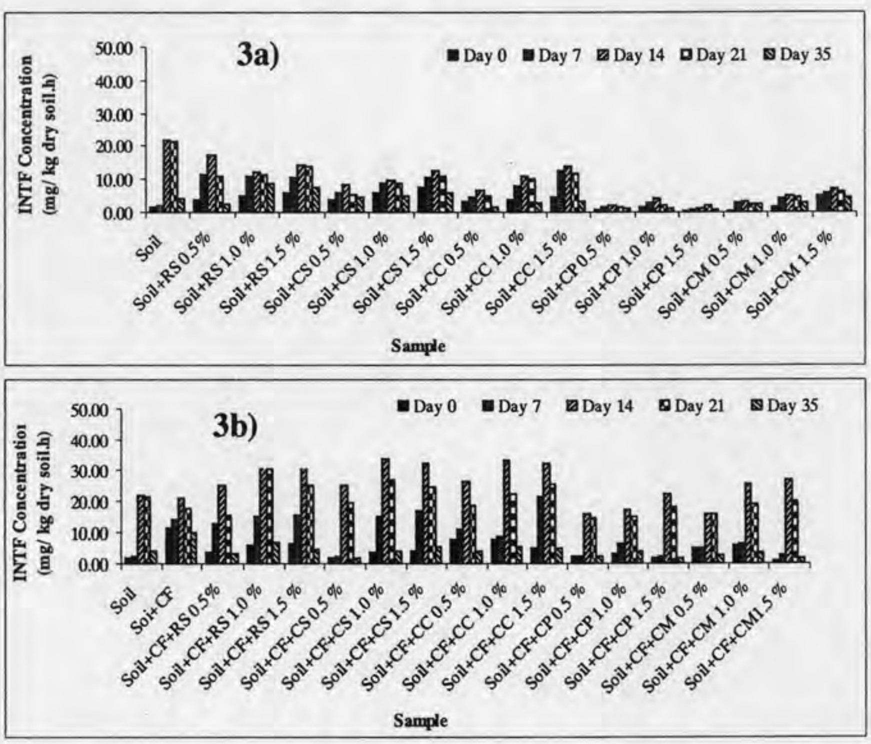

Figure 3: Dehydrogenase Activity in soils: 3a) without CF (control) and 3b) soil with CF at the concentration of 50 mg kg^{-1} dry soil.

Compost (CP) was found to inhibit microbial activity indicated by a decrease of dehydrogenase activity in the soil amended with this material. This might be reason for a decrease of carbofuran degradation efficiency in the soil adding with CP as described in biostimulation experiment. These results coincided with Crecchio [24] who found that microbial activity after soil was amended with compost at a high concentration of 24 ton/ha was inhibited indicated by a reduction of dehydrogenase activity.

Addition of RS, CS and CC at concentrations of 1.0-1.5% resulted in higher cumulative CO_2 than at 0.5% and control (Fig 4 a). This might due to the fact that not only carbofuran was degraded and resulted as the CO2 evolved from the soil but also the organic matter contained in the soil. High percentage of amendments added into the soil could result in a high organic matter in soil which in turn contributed to higher CO2 evolved from the soil contained higher percentage of organic matter than a lower one. Kara [25] also found that RS and tobacco residue added to the soil could stimulate and improve soil microbial activity with an approximately six times increasing of CO_2 production comparing to non-amended soil. CP was found to inhibit microbial activity indicated by a decrease of cumulative CO_2 emitted from the soil amended with this material as the same trend observed in dehydrogenase activity assay (Fig 4a).

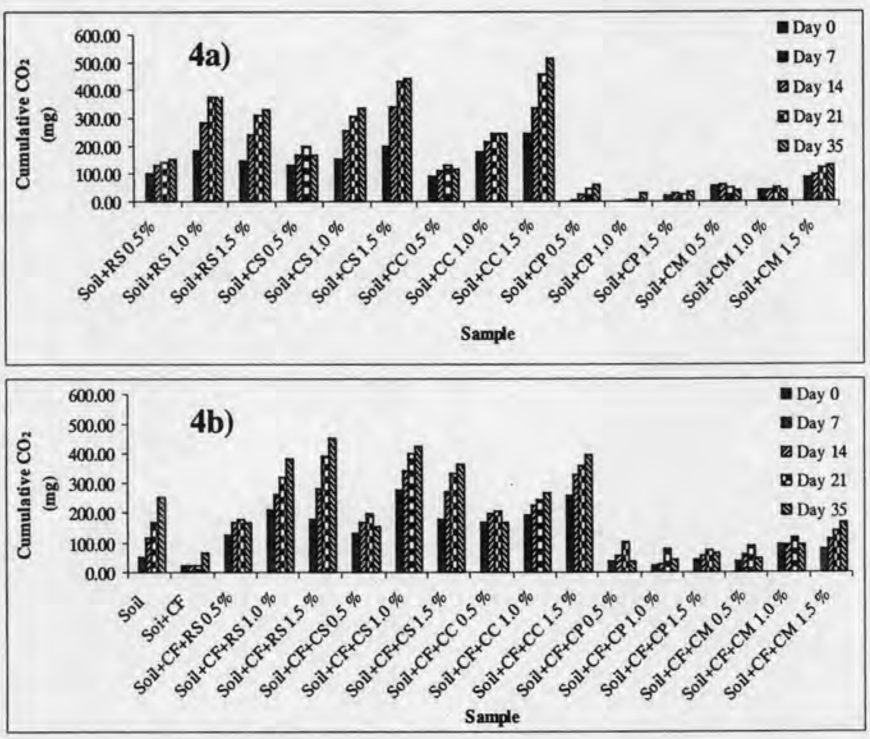

Figure 4: Cumulative CO_2 Evolved from Soils: 4a) without CF (control) and 4b) soil with CF at the concentration of 50 mg kg^{-1} dry soil.

CONCLUSION

In conclusion biostimulaiton of carbofuran degradation in soil by agricultural residues was success RS is the most effective agricultural material for biostimulation of carbofuran indicating by a shortest half-life of carbofuran and highest microbial activity present in the soil amended with this material. Compost was found to inhibit microbial activity resulting in a longer half-life of carbofuran in the amended soil with this material than in the soil without amendment.

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REFERENCES

[1] Ngampongsai, A. 1990. Degradation and residues of carbofuran in soil and sweet corn. [Master Thesis in Agricultural]. Bangkok: The Graduate School, Kasetsart University

[2] EPA. Ground water and drinking water (2003). [online]. Available from: http://www.epa.gov/safewater/ contaminants/ dw_contamfs/carbofur.html

[3] Howard, P. H., 1991.Handbook of Environmental Fate and Exposure Data for Organic Chemicals: Pesticides Chelsea. MI.: Lewis Publishers: 3-15.

[4] Vidali M., 2001.Boremediation and overview. *Pure Appl. Chem.*, 73: 1163-1172

[5] Perez, B. A., Corral, O. L., Linares, L. F., Garcia, F. E., Vazquez, R. R., 2004. Biostimulation of micro-organisms from sugarcane bagasse pith for the removal of weathered hydrocarbon from soil. *Applied Microbiology Letters* 38:373-377.

[6] Barahona, L. M., Vazquez, R. R., Velasco, M. H., Jarquin, C. V., Perez, O. Z., Cantu, A. M., Albores, A. 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. *Applied Soil Ecology* 27: 165-175.

[7] Wong, K. W., Toh, B. A., Ting., Obbard, J. P., 2005. Biodegradation of penanthrene by the indigenous microbial biomass in a zinc amended soil. *Applied Microbiology Letters* 40:50-55.

[8] MIRCEN. Bioconversion of carbohydrate residues in Thailand. nd. [online]. Available from:http://www.unu.edu/unupress/unupbooks/80362e/80362E0a.htm

[9] Zhang, Y., Frankenberger, T. W., 2002. Factors affecting removal of selenate in agricultural drainage water utilizing rice straw. *The Science of the Total Environment* 305: 207-216

[10] Walkley A, Black IA.1934. An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Science*; 29-38

[11] AOAC. 2000. Official method of analysis of AOAC international/William Herwitz, editor. 17th ed. Gaithersburg, Maryland: AOAC International.

[12] Teerakun M., Reungsang A. and Virojanakud W. Phytoremediation of carbofuran in soil. *Songklanakarin J. Sci Technol.*, 26, 171-176 (2004).

[13] Homjan, j., and Leungsaisri, N., 2541. Handbook of soil microbiology laboratory Soil Department: Faculty of agriculture. Khon Kean University.

[14] Von Mersi W, Schinner F. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol Fertil Soils 1991;11:216-20

[15] Dungan, S. R., Kukier, U., Lee, B., 2006. Blending foundry sands with Soil effect on dehydrogenase activity. *Journal of Science of the total environment* 357: 221-230.

[16] Venkateswaru, K., Sethunathan, N., 1979. Metabolism of carbofuran in rice straw amended and Unamended rice straw. *Journal of Environ.Qual.* 8: 365-368.

[17] Harris et al, 1984; Harris, C.R., Chapman, R.A., Harris, C. and Tu, C.M. 1984. Biodegradation of pesticides in soil: rapid induction of carbamate degrading factors after carbofuran treatment. *Journal of Environmental Science and Health* B19: 1-11

[18] Turco, R.F. and Konopka, A. 1990. Biodegradation of carbofuran in enhanced and non-enhanced soils. *Soil Biology and Biochemistry* 22:195-201.

[19] Read D.C.,1983. Enhance microbial degradation of carbofuran and fensulfothion after repeated application to acid mineral soil. *Agr. Eco. Environ.*, 10: 37-46

[20] Morel-Chevillet C., Parekh N.R., Pautrel D. and Fournier J., 1996. Cross-enhancement of carbofuran biodegradation in soil Sample previously treated with carbamate pesticides. *Soil Biol. and Biochem.*, 28, 1767-1776.

[21] Gibert, O., Pablo, J.D., Cortina, J. L., Ayora, C., 2004. Chemical characterization of natural organic substrates for biological mitigation of acid mine drainage. *Water* research 38: 4186-4196.

[22] Bento, F.M., Camargo, F. A.O., Okeke, B. C., Frankenberger, T.W. 2005.Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*. 96: 1049-1055.

[23] Lin, C.H., R. N .Lerch, R. J. Kremer, H. E. Garrett, R. P. Udawatta, and M. F. George. 2005. Soil microbiological activities in vegetative buffer strips and their association with herbicide degradation. AFTA Conference Proceeding:1-9.

[24] Crecchio, C., Curci, M., Pizzigallo, D. R., Ricciuti, P., Ruggiero, P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology& Biochemistry 36:1595-1605

[25] Kara, E. E., 2000. Effects of some plant residues on nitrogen mineralization and biological activity in soils. *Turk J Agric For*.24:457-460.

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