Punjab Univ. J. Zool., Vol. 14, pp. 127-139, 1999

ISOLATION AND CHARACTERIZATION OF PENTACHLOROPHENOL DEGRADING BACTERIA FROM INDUSTRIAL WASTES*

SYED SHAHID ALI, ZAINAB SAMAD AND ABDUL RAUF SHAKOORI

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: Effluent samples collected from tanneries of Kasur and paper industry Lahore were spread on agar plates containing glucose deficient M9 media in which the pentachlorophenol (PCP) was added as the only carbon source. Four bacterial strains CB17 to CB20 were isolated and on the basis of physical and biochemical characterization identified as belong to genus *Listeria* sp., *Lactobacillus* sp., *Pseudomonas* sp. and *Xanthomonas* sp., respectively. The pure cultures of these isolates were prepared and their tolerance for PCP was determined upto 12 μg/ml. The minimum inhibitory concentration (MIC) for CB17 was 10 μg/ml, for CB18 it was 11 μg/ml, for CB19 and CB20 it was 12 μg/ml., Optimum growth conditions of these isolates were determined. The optimum temperature for the growth of all these isolates was 37°C. The optimum pH for CB17 strain was 6.0, for CB18 and CB19, 5.5 and for CB20 it was 7.0. Growth curves for these isolates were prepared. They showed log and stationary phases. The span of log phase was 8, 8, 12 and 24 hours in 4 strains, respectively. The bacterial isolates were also tested for resistance against some heavy metals i.e., Cu^{+2}, Cr^{+6} and Pb^{+2}. The MIC for Cu^{+2} was 175, 350, 400 and 275 μg/ml, for Pb^{+2} it was 100, 200, 500 and 250 μg/ml and for Cr^{+6} 200, 275, 175 and 225 μg/ml respectively for these strains. All isolates showed resistance to antibiotics such as urixin, erythromycin, cefazolin and SXT. The MICs of these isolates against PCP proved that these insecticide degrading bacteria although, can be used for the purpose of environmental cleanup but they showed relatively, moderate potential for degradation of PCP.

Key words: Decontamination, bioremediation, biodegradation, polychlorophenols, chlorinated phenols, microbial degradation, *Lactobacillus* sp., *Listeria* sp., *Pseudomonas* sp., *Xanthomonas* sp.

INTRODUCTION

Huge amount of chemicals are being used in the form of insecticides, herbicides, bactericides, rodenticides, dyes' food additives, cosmetic products, pharmaceuticals and fertilizers (Winter and Street, 1992; Carrara *et al.*, 1993; Conning, 1993; Francis, 1994; Lin and Ho, 1994; Timbrell, 1995). The most dangerous pollutants are pesticides and industrial compounds such as halogenated aliphatics, polychlorinated biphenyls, chlorinated phenols and polycyclic

0079-8045/99/0001-0127 $ 03.00/0

aromatic hydrocarbons (Nichols *et al.*, 1995; Orser, 1996; Chauhan *et al.*, 1998; Livingston *et al.*, 1998; Rawlings *et al.*, 1998).

The three main groups of insecticides, which are being used these days, are organophosphorous (OP), organochlorine (OC) and pyrethroids which are extremely toxic to non-target animals in both acute and chronic studies (Shakoori *et al.*, 1982, 1992; Shakoori and Haq, 1987; Ali *et al.*, 1988, 1997; Ahmad *et al.*, 1995; Ali and Shakoori, 1996, 1998, 1999; Ali and Mir, 1998). Organochlorine compounds are serious pollutants in the environment. They become concentrated, instead of dispersal, in food chain. They are not easily degraded into their metabolites (Focardi *et al.*, 1986; Masud and Hasan, 1992; Winter and Street, 1992; Redetzke and Applegate, 1993). Their toxicity is being increasingly reported in terrestrial, marine and freshwater animals including fish, birds, dairy animals and other mammals. After entering into the system, variable quantity of these pesticides and thus metabolites are ultimately accumulated in various tissues of animals including man.

Pentachlorophenol (PCP) is one such compound used as wood preservative fungicide, bactericide, molluscicide, herbicide and insecticide etc. During last several decades large quantities of PCP have found their way into the environment, resulting in significant environmental contamination at many wood processing sites (McGinnis *et al.*, 1994; Hryhoxzuk *et al.*, 1998). It is very stable compound with high cummulative potential in animals, plants and food chains. Significant amounts of this compound have been reported in human serum, adipose tissue and urine (Feit *et al.*, 1998; Osman *et al.*, 1998; Petroske *et al.*, 1998). PCP is even found in people not occupationally exposed to it or not living in PCP treated log houses (Jorens *et al.*, 1991). The acute pentachlorophenol intoxication is dangerous for the life. Chronic PCP exposure may increase the incidence of several diseases e.g., immunodeficiency, blood disorders, reproductive abnormalities, tissue lesions, mutagenicity and malignancies (Vasarhelyi *et al.*, 1993; Gichner *et al.*, 1998; Hryhorczuk *et al.*, 1998; Gerhard *et al.*, 1999; Umemura *et al.*, 1999). Acute and chronic poisoning may occur by dermal absorption, inhalation or ingestion. Chronic poisoning occurs mainly in saw mill workers or people living in log houses treated with PCP - containing wood protection formulations. PCP poisoning may be classified systematically into effects on the skin metabolism, the haematopoietic tissues, the respiratory systems, central and peripheral nervous systems, the kidney and gastrointestinal tract (Jorens and Schepens, 1993).

Increasing public concern over the contamination of the environment with industrial wastes has elicited considerable research into methods of removing them from soil and water (Kirk *et al.*, 1995; Karamanev *et al.*, 1998). Microbial degradation is of considerable interest. In such experiments investigation generally follow the disappearance of polychlorinated organic compounds by dehalogenation, oxidation, reduction, hydrolysis, ring cleavage by condensation or conjugate formation (Nakatsugawa and Morelli, 1976; Gibson and Skett, 1993; Parkinson, 1996). Microorganisms are the scavengers in nature, responsible for recycling most natural and synthetic waste materials into harmless or non-toxic compounds, when faced with an increasing array of synthetic compounds such as various compounds manufactured by chemical industries for common use. Thus such xenobiotic compounds tend to accumulate in the nature exerting their toxic effects on animals and humans upon their entry into the body, mostly as the part of food chain.

In present study an attempt is being made to isolate and characterise some indigenous bacteria which may prove beneficial for degradation of highly persistent chlorinated compound, the pentachlorophenol from contaminated soil and water.

MATERIALS AND METHODS

Collection of samples and bacterial isolation

The waste water samples to isolate useful bacteria were collected in sterilized bottles from waste effluents of paper industry, Lahore, and tanneries in Kasur.

Media used for bacterial screening

Three type of media were used for bacterial isolation i.e., M9 Agar medium, LB (Luria Bertani) Agar medium and LB broth medium (Maniatis *et al.*, 1982).

Pollutant used and its preparation

The technical grade insecticide, fungicide i.e., pentachlorophenol (PCP) was used for these studies. The stock solution was prepared by dissolving 10 mg PCP/ml acetone. This stock solution was added @ 50 μl (=0.5 mg) per 100 ml of M9 medium.

Screening of PCP resistant bacteria

Waste effluent samples (50 μl) were spread on the plates containing M9 medium in which PCP was added as the only carbon source. Two types of control media were used. First with glucose only and second without glucose and insecticide. Four bacterial types successfully grew at plates containing M9 media in which the toxicant PCP was added as the only carbon source @ 5 μg/ml. For pure culture single colony was picked and streaked on LB agar plates. These strains were stored as glycerol stocks.

Study of PCP resistance of isolates

The recovered bacterial strains were further tested for determination of minimum inhibitory concentration (MIC). The PCP concentrations used for this purpose were 6, 8, 10 and 12 μg/ml of medium.

Identification and characterization of isolates

Various physical, morphological and biochemical tests were performed to determine the major biochemical activities of the isolates, which are later on used for their identification. They were also characterized for their optimum growth conditions. All these strains were cultured in different media with the determination of optimum conditions of temperature, pH, inoculum size etc. They were also checked for resistance against some heavy metals and antibiotics.

Study of optimum growth conditions of isolates

For determination of optimum growth conditions, for each isolate, two parameters i.e., temperature and pH were considered. Following procedure was adopted to find out the optimum temperature at which the strains showed maximum growth.

For each isolate 12 LB broth culture tubes were prepared for each temperature. These tubes were autoclaved and after cooling incubated at 37°C for checking contamination. Fresh culture (50 μl) of each isolated strain was inoculated in each tube and incubated at 25°C, 30°C, 37°C and 42°C for 8 hours. The light absorbance which is a measure of growth was noted at 600 nm wavelength using Hitachi 2000 double beam spectrophotometer. The values were plotted in graphical form and the peak of graph indicated the optimum temperature of strain.

Determination of optimum pH of isolates

To study the optimum pH for maximum growth of bacterial strains, following procedure was adopted.

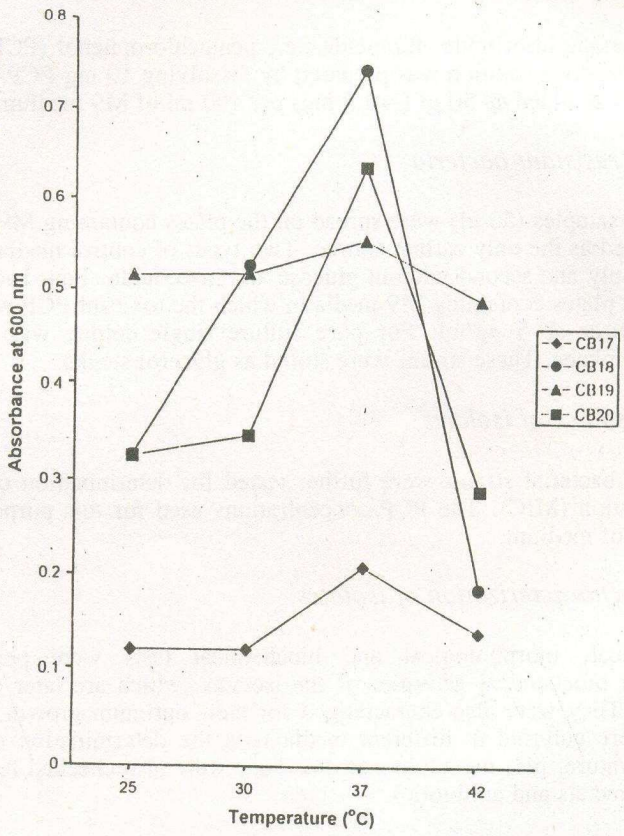

Fig. 1: Graph showing the optimum temperature for growth of four bacterial isolates.

LB broth (5 ml), taken in triplicate, each with 9 tubes was used for each isolate. The pH in each set was adjusted at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 of 1M HCl and 1M NaOH solutions. All test tubes were autoclaved cooled and inoculated with fresh pure culture of isolates, followed by incubation in shaking water bath for 8 hours at 37°C to check the growth of each bacterial isolated. The pH showing highest optical density was considered as the optimum pH of that isolate.

Preparation of growth curves of isolates

To determine the growth curves of each isolate, 39 test tubes with 5 ml LB broth in each, were used. These were adjusted at their optimum pH, and were autoclaved and incubated at 37°C overnight. Fresh bacterial culture (50 ml) was inoculated in each tube and incubated in shaking water bath at 37°C for 10 hours consecutively and then after 12, 24 and 36 hours. Their light absorbance was measured and graph was plotted.

Antibiotic and heavy metal resistance of isolates

The resistance of all isolates was also checked against toxic heavy metals like Cu^{+2}, Cr^{+6} and Pb^{+2}. The starting minimum concentration was 300 μg/ml, 1 mg/ml and 100 μg/ml of LB broth. These concentrations were successively increased or decreased to determine MIC depending upon the results obtained with each isolate.

For checking antibiotic resistance, sensitivity discs of four antibiotics (urixin, erythromycin, SXT and cefazolin) were placed separately in 4 LB agar plates, which were inoculated previously with 4 different bacterial isolates. These were incubated overnight. Clear area around the antibiotic discs indicated the sensitivity of isolates for that particular antibiotic. While the growth of isolates around antibiotic disc indicated its resistance.

RESULTS

The four PCP tolerant isolates, recovered from industrial waste samples were tested for various characteristics. These include the evaluation of minimum inhibitory concentration (MIC), heavy metal resistance and antibiotic resistance. Their optimum growth and growth conditions were determined. The isolates were also characterized physically, morphologically and biochemically for their identification. Four bacterial isolates which grew successfully in PCP containing M9 agar media were designated as CB-17, CB-18, CB-19 and CB-20.

Minimum inhibitory concentration (MIC) of isolates

The four bacterial isolates slightly differ in MIC against PCP which was 10 μg for the MIC of PCP for CB-17 was 10 μg/ml, for CB-18 it was 11 μg/ml and for CB-19 and CB-20, 12 μg/ml. The MIC values indicated that these four isolates have almost similar potential as far as PCP degradation is concerned.

Characterization of isolates

Different physical and morphological characteristics shown by the four isolates have been shown in Table I, while biochemical characterization is mentioned in Table II. On the basis of these tests, the isolates CB-17 to CB-20 were identified as *Listeria* sp., *Lactobacillus* sp., *Pseudomonas* sp. and *Xanthomonas* sp., respectively.

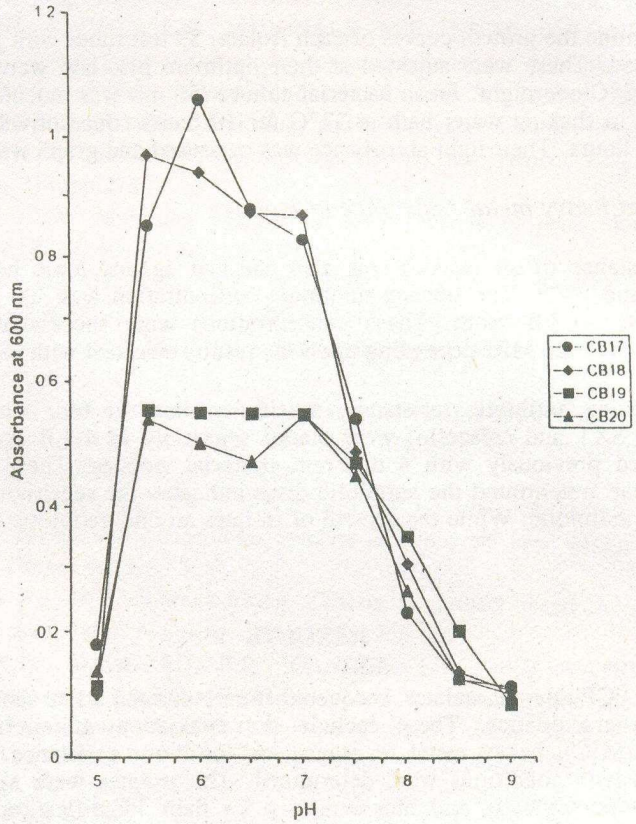

Fig. 2: Graph showing the optimum pH range for growth of four bacterial isolates.

Optimum growth conditions and growth curves

For the study of optimum growth conditions, these isolates were cultured at different temperatures and pH. The mean optimum temperature for the growth of all four isolates was 37°C (Fig.1), while the optimum pH for CB-17 bacterial isolate was 6.0, for CB-18 and CB-19, 5.5 and for CB-20 it was 7.0 (Fig.2). The growth curves, using light absorbance of the media, for CB-17 to CB-20 were prepared. They clearly

showed log phases (Fig.3).

Cross heavy metal resistance of isolates

The resistance of above four bacterial strains were checked against different heavy metals i.e., Cu^{+2}, Cr^{+6} and Pb^{+2}. The MIC shown by CB-17 was 175 μg/ml for copper, 200 μg/ml for chromium and 100 μg/ml for lead. The MIC for CB-18 was higher i.e., 350 μg/ml for copper, 275 μg/ml for chromium and 200 μg/ml for lead. CB-19 showed 400 μg/ml MIC for copper, it was 175 μg/ml for chromium and 500 μg/ml for lead. CB-20 showed highest MIC 275 μg/ml for copper, 225 μg/ml for chromium and 250 μg/ml for lead.

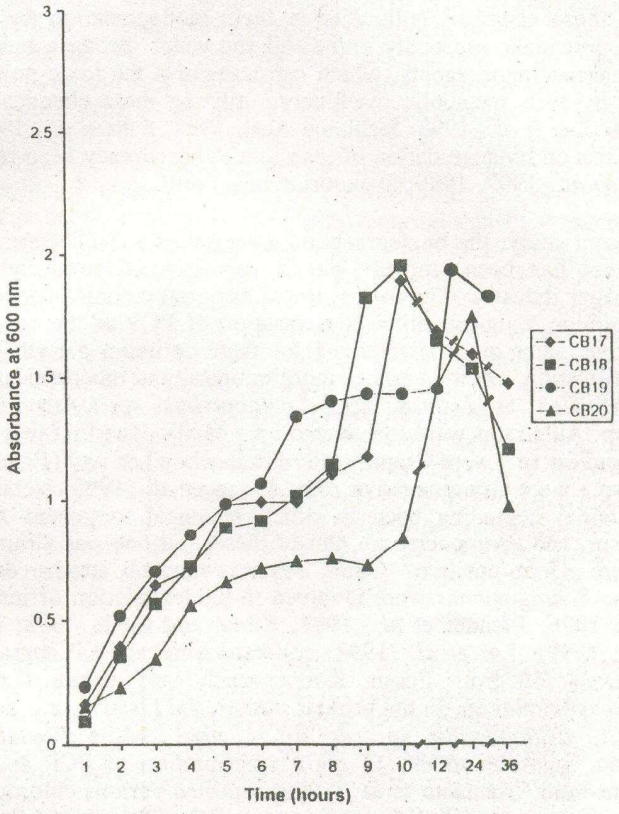

Fig. 3: **Graph showing the normal growth curves for isolated four bacterial strains under optimum conditions of temperature and pH.**

Resistance against antibiotics

The bacterial isolates were tested for resistance against different antibiotics i.e., erythromycin, cefazolin, urixin and SXT. CB-17 showed sensitivity for erythromycin, urixin and SXT while it was resistant to cefazolin. CB-18 and CB-19 were resistant to all four antibiotics and CB-20 showed resistance against urixin and cefazolin while sensitive to erythromycin and SXT.

DISCUSSION

Chlorinated hydrocarbons are notorious for their ability to induce genotoxicity, mutagenicity and carcinogenicity (Saracci *et al.*, 1991; NTP working group, 1993; Pitol and Dragan, 1996; Umemura *et al.*, 1999). In fact persistence and long stability of their molecules in the ecosystem is responsible for this behaviour. The remedy from the destruction of these chemical pollutants is their biodegradation by microorganisms having specific potentials, especially in the soil and water. Bacteria and fungi have been proposed as bioremediation agents, which can transform the toxic pollutants into non-toxic products by their metabolic machinery, utilizing these chemicals for their own metabolic needs (Lee *et al.*, 1998; Stahl and Aust, 1998; Fahr *et al.*, 1999; Tayal *et al.*, 1999). The studies on biodegradation of insecticides has already been reported from this laboratory (Ali *et al.*, 1997, 1999; Shakoori *et al.*, 1999).

In the present study, the biodegradation potential of some bacterial isolates against pentachlorophenol has been studied, which were isolated from industrial waste of tanneries and paper industry. The four types of bacterial colonies appeared/ survived in the media containing 5 μg/ml initial concentration of PCP as the only carbon source. The isolates were, later on, characterized for their optimum growth conditions along with their identification. On the basis of morphological and biochemical characterization they were identified as *Listeria* sp., *Lactobacillus* sp., *Pseudomonas* sp. and *Xanthomonas* sp. All strains were rod shaped i.e., bacilli. The first two isolates (*Listeria* sp. and *Lactobacillus* sp.) were Gram-positive rods while last two (*Pseudomonas* sp. and *Xanthomonas* sp.) were Gram-negative rods. Farah *et al.* (1999) isolated 4 endosulfan (an OC insecticide) degrading bacteria which belonged to genera *Planococcus* sp., *Marinococcus* sp., and *Acetobacter* sp. Out of these, last one was Gram-negative, while other two were Gram-positive. Other reports showed strains of *Pseudomonas, Flavobacterium, Sphingomonas* were involved in biodegradation of insecticides (Orser, 1996; Pfender, 1996; Pfender *et al.*, 1997; Saboo and Gealt, 1998; Lo *et al.*, 1998; Schmidt *et al.*, 1999). Lee *et al.* (1998) collected a novel PCP degrading strain from PCP contaminated soil from Pusan, Korea which can tolerate 1 mg PCP/ml and identified as *Pseudomonas* sp. In the present studies, the *Listeria* sp., showed the growth up to 10 mg/l, *Lactobacillus* sp., up to 11 mg/l, while *Pseudomonas* sp. and *Xanthomonas* sp. grow up to the 12 mg/l concentration of PCP as the only carbon source in the medium. Mannisto *et al.* (1999) reported various chlorophenol degrading bacteria from a long term polluted groundwater. Polychlorophenol degrading capacity has been found in isolates of *Nocardioides, Pseudomonas, Ralstonia, Flavobacterium* and *Caulobacter* previously not known to degrade these compounds. According to this study a Gram-positive isolate was sensitive to PCP with tolerance level of 5 mg/l. Isolates belonging to Cytophaga/ Flexibacter Bacteroides phylum shown to tolerate 25-63 mg/l concentrations of chlorophenols. The various strains of *Sphingomonas* sp., were also reported to degrade chlorinated phenols (Rutgers *et al.*, 1998; Mannisto *et al.*,

1999). Buitron *et al.* (1996) isolated and characterized the microorganisms responsible for degradation of a mixture of phenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol in terms of their degradation rate which, according to this study, was sequential with phenol and 4-chlorophenol degraded first followed by 2,4-dichlorophenol and then 2,4,6-trichlorophenol. It has also been reported that presence of highly chlorinated compounds in the mixture, increase the degradation of less chlorinated compounds significantly (Lu *et al.*, 1993).

The optimum temperature for growth of all four isolates was 37°C. It has been observed that at 45°C the first PCP dechlorination steps were very rapid but later dechlorination occurred with difficulty. Below 15°C the dechlorination was very slow (Juteau *et al.*, 1995). These findings were also true for our studies in the present experiment in which less growth was observed below 15°C. The pH of the media has also profound effect on the biodegradation potential of the microorganisms. The *Listeria* sp., in the present study showed successful growth at pH 6, *Lactobacillus* sp., and *Pseudomonas* sp., at pH 5.5, while *Xanthomonas* sp., at pH 7. According to Sloff *et al.* (1991) the mobility of the chlorophenols in soil is dependent on the characteristics of the compound and those of the soil. It can vary from extremely mobile to almost mobile. Whether a chlorophenol occur in a water phase as neutral hydrophobic compound or as a soluble phenolate anion, depends on the pka of the chlorophenol concerned as well as pH of its aquatic environment. In environmentally relevant pH ranges between 6-8, the solubility increases sharply. The *Pseudomonas* sp. and *Xanthomonas* sp., showed almost stable growth zone in pH range 5.5-7.0. The *Lactobacillus* sp., also behaved almost similarly in the above range. Rutgers *et al.* (1998) reported that PCP exhibited stronger toxicity at low pH working with *Sphingomonas* sp., strain P-5. The reults suggest that increase in environmental pH, may reduce the risk of chlorophenol toxicity. It has been observed that for successful biodegradation system, a suitable combination of physical and chemical factors is required for each organism under which that can work efficiently. The growth curves of the isolates prepared using optimum growth conditions, showed log and stationary phases. No lag phase appeared because the isolates have already passed this during 24 hours pre-inoculation incubation at 37°C. It has also been reported that lag phase prior to PCP biotransformation increase with decrease in temperature. The log phase of 72 hours was observed at 31°C (Cole *et al.*, 1996). In this study, under present set of conditions *Listeria* and *Lactobacillus* showed 12 hours log phase while *Pseudomonas* sp. and *Xanthomonas* sp., showed 24 and 30 hours log phase respectively. Similar studies by other workers indicated variable response of isolated bacteria against PCP (Lee *et al.*, 1998; Stuart and Woods, 1998; Schmidt *et al.*, 1999).

Keeping in view the MIC of PCP and various heavy metals against these isolates, it can be concluded that these isolates can be used for the purpose of environmental clean-up. As far as comparative biodegradation potential is concerned these isolates showed moderate capacity to degrade PCP. There is a need to extend these studies for screening of microorganisms having better biodegradation potential.

REFERENCES

AHMAD, F., ALI, S.S. AND SHAKOORI, A.R., 1995. Sublethal effects of Danitol (fenpropathrin) a synthetic pyrethroid, on Chinese grass carp, Ctenopharyngodon idella. Folia biologica (Krakow), 43: 151-159.

ALI, S.S. AND MIR, S.S., 1998. Cypermethrin toxicosis in the chicks of domestic fowl, Gallus domesticus: haematological studies. Punjab Univ. J. Zool., 13: 179-189.

ALI, S.S. AND SHAKOORI, A.R., 1996. Short and long term toxicity of DDT in albino rats: Biochemical effects in liver. Punjab Univ. J. Zool., 11: 7-24.

ALI, S.S. AND SHAKOORI, A.R., 1999. Hepatotoxicity of lindane (gamma-HCH) in albino rats: short and long term studies. Pakistan J. Zool., 31: 175-185.

ALI, S.S., ALI, N.S. AND SHAKOORI, A.R., 1988. Biochemical alterations induced by short term feeding of endrin on various blood components of albino rats. Proc. Pakistan Congr. Zool., 8: 101-112.

ALI, S.S., ARSHAD, M. AND ALI, T., 1997. Malathion induced haematoxicity in chicks of fowl, Gallus domestricus. Punjab Univ. J. Zool., 12: 127-137.

ALI, S.S., HAQ, R., KHALIQ, M. AND SHAKOORI, A.R., 1997. Use of ultraviolet spectrophotometry for determination of insecticides and aromatic hydrocarbon pollutants. Punjab Univ. J. Zool., 12: 31-34.

ALI, S.S., ZIA, N.A. AND SHAKOORI, A.R., 1999. Cypermethrin and heptachlor degrading bacteria from soil and water samples of industrial wastes. Folia biologica (Krakow), 47: (accepted).

BUITRON, G., GONZALE, A., BALLY, D., ASANO, T., BHAMIDIMARRI, R., CHINNK, GRABOW, WOK, HALL, E.R., OHGAKI, S., ORHON, D., MILBURIN, PURDON, C.D. AND NAGLE, P.T., 1996. Characterization of microorganisms from an acclimated activated sludge degrading phenolic compounds water Quality International 96 Parts 3: Modelling of activated sludge processes. Microorganisms in activated sludge and biofilm processes. Anaerobic biological treatment. *Water Sci. Technol.*, **34**: 289-294.

CARRARA, M., CIMA, L., CERINI, R. AND CARBONARE, M.D., 1993. An *in vitro* method for assessing potential toxicity of cosmetic products. *J. Toxicol.*, **12**: 3-13.

CHAUHAN, S., BARBIERI, P. AND WOOD, T.K., 1998. Oxidation of trichloroethylene, 1,1-dichloroethylene and chloroform by toluene/o-xylene monooxygenase from *Pseudomonas stutzeri* OX1. *Appl. Environ. Microbiol.*, **64**: 3023-3024.

COLE, J.D., WOODS, S.L. AND BRICKER, T.R., 1996. Pentachlorophenol reductive dechlorination in an interceptor trench. Temperature effects. *Ground Water*, **34**: 784-790.

CONNING, D.M., 1993. Toxicology of food and food additives. In: *General and Applied Toxicology* (eds. B. Ballantyne, T. Marrs and P. Turner), Basingstoke.

ECOBICHON, D.J., 1996. Toxic effects of pesticides. In: *Casarett and Doull's Toxicology - The Basic Science of Poisons* (eds. C.D. Klaassen, M.O. Amdur and J. Doull), Fifth Edition. McGraw-Hill, pp.643-689.

FAHR, K., WETZSTEIN, H.G., GREY, R. AND SCHLOSSER, D., 1999. Degradation of 2,4-dichlorophenol and pentachlorophenol by two brown rot fungi. *FEMS Micribiol. Lett.*, **175**: 127-132.

FEIL, V.J., HUWE, J.K. AND HAKK, H., 1998. Dioxins in beef, milk and forage. Fedrip Database, National Technical Information Service (NITS).

FOCARDI, S., FOSSI, C., LEONZIO, C. AND ROMEI, R., 1986. PCB, Congeners, hexachlorobenzene and organochlorine insecticides in human fat in Italy. *Bull. Environ. Contam. Toxicol.*, **32**: 644-650.

FRANCIS, B.M., 1994. *Toxic substances in the environment. An Overview of Environmental Toxicology*. John Wiley & Sons, New York.

GERHARD, I., FRICK, A., MONGA, B. AND RUNNEBAUM, B., 1999. Pentachlorophenol exposure in women with gynecological and endocrine dysfunction. *Environ. Res.*, **80**: 383-388.

GIBSON, G.G. AND SKETT, P., 1993. *Introduction to drug metabolism*, 2nd Edition. Chapman and Hall, London.

GICHNER, T., WAGNER, E.D. AND PLEWA, M.J., 1998. Pentachlorophenol-mediated mutagenic synergy with aromatic amines in *Salmonella typhimurium*. *Mutat. Res.*, **420**: 115-124.

HRYHORCZUK, D.O., WALLACE, W.H., PERSKY, V., FURNER, S., WEBSTER, J.R. Jr., OLESKE, D., HASELHORST, B., ELLEFSON, R. AND ZUGERMAN, C., 1998. A morbidity study of former pentachlorophenol-production workers. *Environ. Hlth. Perspect*, **106**:

JORENS, P.G. AND SCHEPENS, P.J.C., 1993. Human pentachlorophenol poisoning. *Hum. Exp. Toxicol.*, **12**: 479-495.

JORENS, P.G., JANSSENS, J.J., VAN, TICHELEN, W.J., VAN, PAESSCHEN, W., DE, DEYN, P. AND SCHEPENS, P.J., 1991. Pentachlorophenol concentrations in human cerebrospinal fluid. *Neurotoxicology*, **12**: 1-7.

JUTEAU, P., BEADET, R., McSWEEN, G., LEPINE, F. AND BISAILLON, J.G., 1995. Study of the reductive dechlorination of pentachlorophenol by a methanogenic consortium. *Can. J. Microbiol.*, **41**: 862-868.

KARAMANEV, D.G., CHAVARIE, C. AND SAMSON, R., 1998. Soil immobilization: new concept for biotreatment of soil contaminants. *Biotechnol. Bioeng.*, **57**: 471-476.

KIRK, T.K., LAMAR, R.T. AND HAMMEL, K.E., 1995. Development of bioremediation technologies that are based on the xenobiotic-degrading abilities. *National Technical Information Service (NTIS)*.

LEE, S.G., YOON, B.D., PARK, Y.H. AND OH, H.M., 1998. Isolation of a novel pentachlorophenol-degrading bacterium, *Pseudomonas* sp., Bu34. *J. Appl. Microbiol.*, **85**: 1-8.

LIN, J.K. AND HO, Y.S., 1994. Hepatotoxic actions of dietary amines. *Toxicology and Ecotoxicology News*, **1**: 82-87.

LIVINGSTONE, D.R., 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, **120**: 43-49.

LO, K.V., ZHU, C.M. AND CHEUK, W., 1998. Biodegradation of pentachlorophenol by *Flavobacterium* species in batch and immobilized continuous reactors. *Environ. Technol.*, **19**: 91-96.

LU, C.J., TSAI, Y.H., BHAMIDIMARRI, R., LI, X. AND LIU, S., 1993. The effects of a secondary carbon source on the biodegradation of a recalcitrant compounds. Development and waters pollution control in Asia. *Water Sci. Technol.*, **28**: 97-101.

MANIATIS, T., FRITSCH, E.F. AND SAMBROOK, J., 1982. *A Laboratory Manual*. Cold Spring Harbor Laboratory, USA, pp.249-254.

MANNISTO, M.K., TIIROLA, M.A., SALKINOJA-SALONEN, M.S., KULOMAA, M.S. AND PUHAKKA, J.A., 1999. Diversity of chlorophenol-degrading bacteria isolated from contaminated boreal groundwater. *Arch. Microbiol.*, **171**: 189-197.

MASUD, S.Z. AND HASAN, N., 1992. Pesticide residue in food stuff in Pakistan. Organochlorine, organophosphorous and pyrethroid insecticides in fruits and vegetables. *Pakistan J. Sci. Ind. Res.*, **35**: 499-504.

McGINNIS, D., DUPONT, R.R., EVER, H.K. AND STLAURENT, G., 1994. Evaluation and management of field soil pile bioventing systems for the remediation of PCP contaminated surface soils. *Environ. Technol.*, **15**: 729-739.

NAKATSUGAWA, T. AND MORELLI, M.A., 1976. Microsomal oxidation and insecticide metabolism. In: *Insecticide Biochemistry and Physiology* (ed. C.F. Wilkinson), pp.114.

NICHOLS, J.W., LARSEN, C.P., McDONALD, M.E., NIEMI, G.J. AND ANKLEY, G.T., 1995. Bioenergetics-based model for accumulation of polychlorinated biphenyls by nesting tree swallow, *Tachycineta bicolor. Environ. Sci. Technol.*, **29**: 604-612.

NTP WORKING GROUP, 1993. Toxicology and carcinogenesis studies of pentachloroanisole in F344/N rats and B6C3F1 mice (gavage studies). *Nat. Toxicol. Prog. Technical Report-Series* 414, pp.278.

ORSER, C.S., 1996. Molecular analysis of dehalogenation of pentachlorophenol. *National Technical Information Service (NTIS) Project #9304553.*

OSMAN, A.M., POSTHUMUS, M.A., VEEGER, C., VAN-BLADEREN, P.J., LAANE, C. AND RIETJENS, I.M., 1998. Conversion of pentahalogenated phenols by microperoxidase-B/H202 to benzoquinone-type products. *Chem. Res. Toxicol.*, **11**: 1319-1325.

PARKINSON, A., 1996. Biotransformation of xenobiotics. In: *Casarett and Doull's Toxicology - The Basic Science of Poisons* (eds. C.D. Klaassen, M.O. Amdur and J. Doull), Fifth Edition. McGraw-Hill, pp.113-186.

PETROSKE, E., ZAYLSKIE, R.G. AND FEIL, V.J., 1998. Reduction in polychlorinated dibenzodioxin and dibenzofuran residues in hamburger meat during cooking. *J. Agric. Food Chem.*, **46**: 3280-3284.

PFENDER, W.F., 1996. Bioremediation bacteria to protect plants in pentachlorophenol-contaminated soil. *J. Environ. Qual.*, **25**: 1256-1260.

PFENDER, W.F., MAGGARD, S.P., GANDER, L.K. AND WATRUD, L.S., 1997. Comparison of three bioremediation agents for mineralization and transformation of pentachlorophenol in soil. *Bull. Environ. Contam. Toxicol.*, **59**: 230-237.

PITOL, H.C.III AND DRAGAN, Y.P., 1996. Chemical carcinogenesis. In: Casarett and Doull's Toxicology, the Basic Science of Poisons (eds. C.D. Klaassen, M.O. Amdur and J. Doull), 5th Edition, McGraw-Hill, New York.

RAWLINGS, N.C., COOK, S.J. AND WALDBILLIG, D., 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J. Toxicol. Environ. Hlth.*, **54**: 21-36.

REDETZKE, K.A. AND APPLEGATE, H.G., 1993. Organochlorine pesticides in adipose tissue of persons from EI Paso, Texas. *J. Environ. Hlth.*, **56**: 25-27.

RUTGERS, M., VAN-BOMMEL, S., BREAURE, A.M., VAN-ANDEL, J.G. AND DUETZ, W.A., 1998. Effect of pH on the toxicity and biodegradation of pentachlorophenol by *Sphingomonas* sp. strain P5 in nutristat culture. *Environ. Toxicol. Chem.*, **17**: 792-797.

SABOO, V.M. AND GEALT, M.A., 1998. Gene sequences of the pcpB gene of pentachlorophenol-degrading *Sphingomonas chlorophenolica* found in non-degrading bacteria. *Can. J. Microbiol.*, **44**: 667-675.

SARACCI, R., KOGVINAS, M., BERTAZZI, P.A. *ET AL.*, 1991. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. *Lancet*, **338**: 1027-1032.

SCHMIDT, L.M., DELFINO, J.J., PRESTON, J.F. 3rd AND ST-LAURENT, G. 3rd., 1999. Biodegradation of low aqueous concentration pentachlorophenol (PCP) contaminated groundwater. *Chemosphere*, **38**: 2897-2912.

SHAKOORI, A.R. AND HAQ, R., 1987. Effects of starvation on heptachlor toxicity in rabbits. Biochemical changes in blood. *Folia Biol.*, **35**: 33-42.

SHAKOORI, A.R., ASLAM, F., SABIR, M. AND ALI, S.S., 1992. Effect of prolonged administration of insecticide (Cyhalothrin/Karate) on blood and liver of rabbits. Folia biologica (Krakow), 40: 91-99.

SHAKOORI, A.R., NAWAZ, M., ALI, S.S. AND HAQ, R., 1999. A pesticide degrading bacterium isolated from soil samples and its possible use in environmental decontamination. Proc. Pakistan Acad. Sci., 36: 5-10.

SHAKOORI, A.R., RASUL, Y.G. AND ALI, S.S., 1982. Effects of dieldrin feeding for 5 months on the albino rats. Biochemical and histological changes in liver. *Pakistan J. Zool.*, **14**: 191-204.

SLOFF, W., BREMMER, H.G., JANUS, J.A. AND MATTHIJSEN, A.J., 1994. Integrated criteria document chlorophenols. National Institute for Public Health and Environmental Protection, pp.154.

STAHL, J.D. AND AUST, S.D., 1998. Use of fungi in bioremediation. *Reviews in Toxicology.*, **2**: 189-194.

STUART, S.L. AND WOODS, S.L., 1998. Kinetic evidence for pentachlorophenol-dependent growth of a dehalogenating population in a pentachlorophenol and acetate-fed methanogenic culture. *Biotechnol. Bioeng.*, **57**: 420-429.

TAYAL, A.K., DAS, L. AND KAUR, I., 1999. Biodegradation of pentachlorophenol (PCP) by white rot fungal strains screened from local sources and its estimation by high-performance liquid chromatography. *Biomed Chromatogr.*, **13**: 220-224.

TIMBRELL, J.A., 1995. *Introduction to toxicology*, 2nd Edition. Taylor & Francis, London, pp.167.

UMEMURA, T., KAI, S., HASEGAWA, R., SAI, K., KUROKAWA, Y. AND WILLIAMS, G.M., 1999. Pentachlorophenol (PCP) produces liver oxidative stress and promotes but does not initiate hepatocarcinogenesis in B6C3F1 mice. *Carcinogenesis*, **20**: 1115-1120.

VASARHELYI, B., BLAZOVICS, A. AND FEHER, J., 1993. The place of pentachlorophenol in general medicinal practice. *Orv. Hetil.*, **134**: 2643-2652.

WINTER, S. AND STREET, B., 1992. Organochlorine compounds in the three step terrestrial food chain. *Chemosphere*, **24**: 1765-1774.

(Received: July 20, 1999)