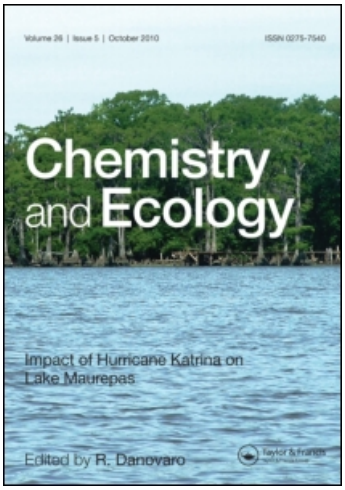

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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To cite this Article Chang, Bea-Ven , Liao, Kao-Tzung , Pan, Tzu-Ming and Yuan, Shaw-Ying(1995) 'Biotransformations of Chlorophenols in River Sediments', Chemistry and Ecology, 10: 1, 105 — 114
To link to this Article: DOI: 10.1080/02757549508035334
URL: http://dx.doi.org/10.1080/02757549508035334

Chemistry and Ecology, 1995, Vol. 10, pp. 105-114
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BIOTRANSFORMATIONS OF CHLOROPHENOLS IN RIVER SEDIMENTS

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(Received 4 Oct, 1993)

The accumulation of chlorophenols, including 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP), from river sediments from southern Taiwan were studied. Through simple or more exhaustive extractions, the results showed that 99% of the samples containing 2,4,6-TCP and PCP could be removed by simple extraction. The concentrations were found to range from non-detectable to 16.60 ngg^{-1} for 2,4,6-TCP and to 25.02 ngg^{-1} for PCP. Partition coefficients (K_p) were 0.71, 0.74 mlg^{-1} for 2,4,6-TCP, 1.35 and 1.41 mlg^{-1} for PCP. Biodegradation by DCP-adapted or unadapted anaerobes in sediment was carried out. During 21 days' incubation, the complete degradation time for 2,4,6-TCP in DCP-adapted anaerobic, unadapted anaerobic, and unadapted aerobic conditions were found to be 9, 10, 12 days for N3 sediment, and 8, 10, 11 days for N6 sediment, respectively; for PCP it was 19 days, without degradation, 14 days for N3 sediment, and 13, 17, 10 days for N6 sediment, respectively. The biodegradable products were identified as 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 3,4,5-TCP, 3,5-DCP, 3-MCP, phenol, methylphenol, and benzoate for PCP, and 2,4-DCP, 4-MCP, phenol, methylphenol, and benzoate for 2,4,6-TCP.

KEY WORDS: biotransformation, chlorophenol, river sediment, sewage sludges

INTRODUCTION

The discharge of industrially derived halogenated organic compounds to the aquatic environment is of great concern, mainly because of their toxicity to man, resistance to degradation, and tendency to bioaccumulation. The transport and concentrations of some chlorophenols in waste, sewage, groundwater, fresh water, and sediments have been investigated extensively by many authors (Valo *et al.*, 1985; Xie *et al.*, 1986; Paasivirta *et al.*, 1988; Suntio *et al.*, 1988; Kawamoto and Urano, 1989; McAllister *et al.*, 1991). Recently, low concentrations of chlorophenols have also been detected in water, soil and sediment samples in southern Taiwan (Chang *et al.*, 1993).

Polychlorinated aromatic compounds are slowly degraded in polluted aerobic zones and may leach into the anaerobic environment. Mikesell and Boyd (1985) observed the reductive dechlorination of PCP in several anaerobic sludges. Mikesell

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and Boyd (1986) also observed that PCP degradation in anaerobic sludges could be enhanced when the microorganisms in the sludge were first adapted for dechlorination by addition of a mixture of monochlorophenols. Bryant *et al.* (1991) observed that in 2,4- or 3,4-DCP adapted sediment communities the lag phase could be eliminated with increased PCP dechlorination. Remberger *et al.* (1986) emphasized the cardinal role of sorption to the sediment phase in determining the fate of chloroquaiacols and chloroguaiacols discharged to the aquatic environment.

The objective of this work is to investigate the relationship between binding and biotransformation of chlorophenols in river sediment. We conducted laboratory studies with natural sediments. Sediment was first adapted with or without DCP, and incubated under various environment-related conditions. Adapted sediment samples were then spiked with 2,4,6-TCP and PCP, and incubated under both aerobic and anaerobic conditions. Analyses were carried out on the water phases to determine the rates of transformation for the substrates and their fate.

MATERIALS AND METHODS

Chemicals

Phenolic compounds including phenol, methylphenol, 3-MCP, 4-MCP, 2,4-DCP, 3,4-DCP, 3,5-DCP, 3,4,5-TCP, 2,3,4,5-TeCP, and PCP were obtained from Supelco Co., U.S.A. All other chemicals were reagents or HPLC grade as needed.

Media

The medium was composed of NH_4Cl 2.7 g, $MgCl_2.6H_2O$ 0.1 g, $CaCl_2.2H_2O$ 0.1 g, $FeCl_2.4H_2O$ 0.02 g, K_2HPO_4 0.27 g, $KHPO_4$ 0.35 g, yeast extract 5.0 g, and resazurin 0.001 g per litre. For anaerobic control, reducing agents L-cysteinium chloride 0.50 g and Na-thioglycollate 0.20 g per litre of solution were added. The medium was first adjusted to pH 7.0, and then sterilized at 121°C for 20 min.

Sediment Collection and Treatment

Sediment samples (N1 to N6) were collected from southern Taiwan. These localities were all in the vicinity of continuous petrochemical works discharge. The sampling was performed on three occasions: August 1992, January 1993, and April 1993. Sediments N1 to N6 were used for assessment, and sediments N3 and N6 were used for sorption and biotransformation studies. These two samples consisted of sand and had a low concentration of organic carbon (Table I). In Taiwan, most rivers are very short and run swiftly in rainy seasons. The sediments have similar characteristics as shown in Table I.

Table I Particle size distribution and organic carbon content analysis of sediment samples

Sediments	Sand (%)	Silt (%)	Clay (%)	Texture	Organic carbon (%)
N3	90	5	5	Sand	0.87
N6	93	2	5	Sand	0.70

Extraction of Chlorophenols in Sediment Samples (Remberger *et al.*, 1986)

Extraction of free compounds

Sediment (20 g, wet weight) was extracted with 50 ml of n-hexane for 15 min. The total extracts were acetylated by mixing them with 0.1 M K_2CO_3 (3 ml), acetic anhydrate (0.5 ml), and then extracted with n-hexane (20 ml).

Extraction of bound compounds

Previously treated sediment was mixed with 10 M KOH (20 ml), and ascorbic acid (1 ml). The sediment was extracted twice again with 0.2 M KOH (5 and 3 ml respectively) for 10 min. All extracts were combined, extracted with n-hexane, and then analyzed by GC-ECD.

Sorption experiments

In a typical experiment, 10 g of soil was suspended in 50 ml of a standard solution, 0.01 M calcium chloride containing a mixture of 3 chlorophenols (concentration ratio: 2,4-DCP;2,4,6-TCP:PCP = 10:10:1). Five standard solutions were made with PCP concentrations of 2, 3, 5, 6 and 8 mgl^{-1} respectively. Experiments were carried out in duplicate. The suspensions were shaken with a rotating shaker at 25°C for 16 h, and then centrifuged at 8,320 g for 15 min. These solutions were then analyzed as described below.

Transformation of chlorophenols by sediments

Experiments were performed with serum bottles under aerobic and anaerobic conditions. For DCP-cross adaptation, 500 g of sampled sediments were added with 1 ml of 2,4 and 3,4-DCP (1 mgl^{-1}) at 7-day intervals and incubated at 30°C for 1 year. For the transformation experiment, 5 g of DCP-adapted or unadapted sediment and 50 ml medium were added in 125 ml bottles capped with butyl rubber stoppers (anaerobic) or aluminum foil (aerobic), and 2,4,6-TCP or PCP added to yield a final concentration of 2 mgl^{-1}. All samples were wrapped in aluminum foil to prevent photolysis of chlorophenolic compounds and then incubated at 30°C in the dark without shaking. For the anaerobic incubation, serum bottles were placed in an anaerobic chamber (Forma Scientific, Model 1025 S/N). As for aerobic incubation, samples were placed in a general growth chamber. All experiments were performed in duplicate. Aqueous samples were taken from all treatment bottles periodically and residual CP concentrations were measured.

Analysis of Chlorophenols

GC methods

Aliquots (3 ml) were used for chlorophenol analysis. 0.5 ml of 1 M K_2CO_3 and 1 ml of acetic anhydride were added, and then the funnel shaken for 2 min. After 20 min 5 ml of n-hexane was added and the funnel was again agitated for 2 min. After the water was drawn off, the n-hexane layer was collected with a new pipette and stored at 4°C. The extracts were analyzed by HP 5890 gas chromatography equipped with electron capture detector, Ultra 2 fused silica capillary column. The column temperature was held at 100°C for 5 min and then increased to 235°C for 5 min. Injector and detector temperatures were set to be 240°C and 300°C respectively. Nitrogen was used as both the carrier and make up gas at the flow rates of 0.6 and 60 $mlmin^{-1}$ respectively.

GC-MASS spectrometry

Samples were acidified to pH < 2 with HCl and extracted twice with dichloroethane. Extracts were analyzed with a GC-MASS (HP GC/MS system 5988A), interfaced with a data system with matching library data base (HP-100 E/Series system). The GC was equipped with a DB-5 capillary column. The column temperature was held at 90°C for 4 min and increased to 280°C and held for 12 min. Helium served as the carrier gas at a flow rate of 1 mlmin ^{1}. The injector temperature was set at 90°C. The impact detector was operated at 70eV and the scanning rate was about 2s ^{1}.

RESULTS AND DISCUSSION

Investigation of Chlorophenols in River Sediment

The accumulative chlorophenols in river sediment continually exposed to industrial discharges in southern Taiwan were studied. With free or bound exhaustive extractions, the results indicated that 99% of samples containing 2,4,6-TCP and PCP could be removed by free extraction. The various concentrations were found in the range from ND (nondetectable) to 16.60 ngg^{-1} for 2,4,6-TCP, and from ND to 25.02 ngg^{-1} for PCP (Table II). The data showed that a substantial concentration of chlorophenol, putatively originating from industrial discharges, may be found in sediment samples.

Sorption Isotherm of Chlorophenols to Sediment

The adsorption data fitted well to linear isotherms over a broad range of water phase concentration. That is $X = K_p.C$, where X denotes the concentration of sorbate on the sediment (μgg ^{1}), C is the equilibrium solution coefficient (μgml^{-1}) and K_p is the partition coefficient (mlg ^{1}). Table III shows that the partition coefficient (K_p) values for 2,4-DCP, 2,4,6-TCP and PCP were in the series of PCP > 2,4,6-TCP > 2,4-DCP. The results show that increasing chlorination level of chlorinated compounds leads to greater sorption by solid particles. This is in agreement with the conclusion of Boyd (1982). The K_p values of Table II were significantly lower

Table II Concentrations (ngg ^{1}) of chlorophenols from river sediments

Chlorophenols	N1	N2	N3	N4	N5	N6
		November, 1992				
2,4-DCP	ND	ND	ND	ND	ND	ND
2,4,6-TCP	ND	ND	16.6	ND	14.09	ND
PCP	ND	ND	4.8	ND	5.29	5.0
		January, 1993				
2,4-DCP	ND	ND	ND	ND	ND	ND
2,4,6-TCP	ND	ND	ND	ND	ND	ND
PCP	25.02	7.41	6.0	4.26	ND	ND
		April, 1993				
2,4-DCP	ND	ND	ND	ND	ND	ND
2,4,6-TCP	ND	ND	ND	ND	ND	ND
PCP	7.48	5.28	9.52	ND	ND	ND

ND: Not detectable

Table III Chlorophenol sorption coefficients for linear isotherms

Chlorophenols	K_p (mlg^{-1})	
	N3	N6
2,4-DCP	0.49	0.55
2,4,6-TCP	0.71	0.74
PCP	1.35	1.41

than Schellengberg's lowest value of 13 mlg^{-1} (1984). The reason for our low K_p values may be due to the low organic carbon content (Table I) of sediment samples. The effect of the organic carbon content of soil and sediment adsorption of non-ionic organic pollutants reported by Karickhoff *et al.* (1979) would justify our results. In other words, a great part (99%) of the chlorophenol could be removed from sandy sediment by simple solvent extraction in the experiment. The magnitude of the bound fractions may, however, be correlated plausibly with demonstration that all of the chlorophenols were less tightly bonded to the sediment.

Transformation of Chlorophenols by Sediment Samples in Various Conditions

Comparison of aerobic and anaerobic conditions for a series of experiments carried out with 2,4,6-TCP are given in Figure 1, and results from similar experiments with

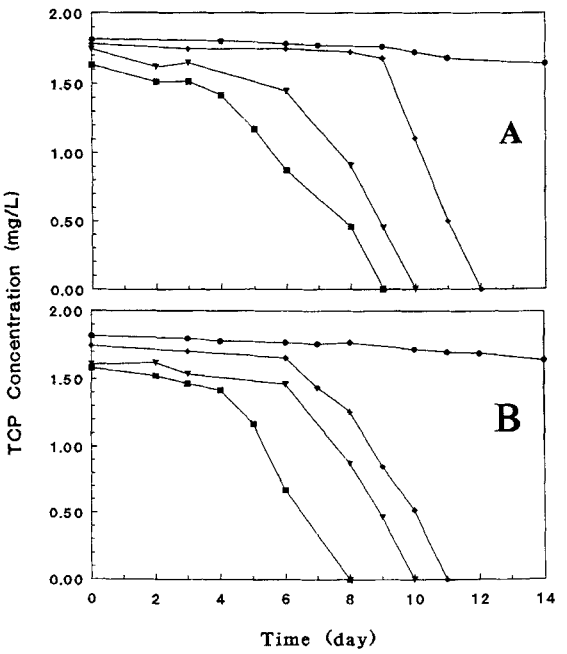

Figure 1 Reductive dechlorination of 2,4,6-TCP for different treatments in N3 sediment (A), and N6 sediment (B) samples. Symbols: —■—, DCP-adapted anaerobic; —▼—, DCP-unadapted anaerobic; —◆—, DCP-unadapted aerobic; —●—, autoclaved control.

PCP are given in Figure 2. The loss of 2,4,6-TCP by DCP-adapted N3 sediment under anaerobic conditions followed by a 3 days' lag time, was complete within 9 days, whereas for unadapted sediment, the lag time and complete dechlorination time were 4 days and 10 days under the same conditions, and 9 days and 12 days respectively under aerobic conditions (Figure 1). It was obvious that adaptation of the sediment to DCP over a period of 1 year resulted in the elimination of the lag period. The same results were obtained with N6 sediment. As for PCP (Figure 2), after 21 days' incubation no transformation was observed for unadapted N3 sediment. For adapted sediment, in contrast, PCP degradation began after a long lag period of 15 days, and completely stopped after 19 days. Different results were found in the case of sediment N6. In anaerobic conditions, PCP was still completely transformed in 17 days after a long lag period (11 days) for unadapted sediment. For adapted sediment, lag time and transformation were reduced to 4 days and 13 days, consistent with the findings of Bryant (1991). In the corresponding experiments under aerobic conditions, both N3 and N6 sediments showed complete transformation at 14 days and 10 days after lag periods of 12 and 7 days, respectively. Samples which had been autoclaved at 121°C for 20 min were unable to bring about any of these transformations. Interestingly, total loss for 2,4,6-TCP was longer for anaerobic than aerobic incubation, but aerobic was longer than anaerobic incubation for PCP. We concluded that in the case of aerobic conditions, controlled in our experiment, the solution was initially aerobic, but became slowly anaerobic after a few days. The change from aerobic to anaerobic conditions possibly favoured microbial consortia which degrade PCP, but not 2,4,6-TCP. Because N3 and N6 sediments were sampled from a possibly

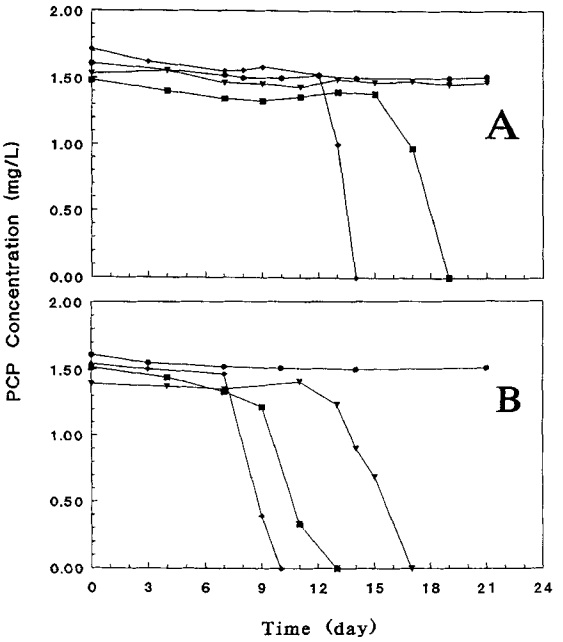

Figure 2 Reductive dechlorination of PCP for different treatments in N3 sediment (A), and N6 sediment (B) samples. Symbols: —■—, DCP-adapted anaerobic; —▼—, DCP-unadapted anaerobic; —◆—, DCP-unadapted aerobic; —●—, autoclaved control.

chlorophenol polluted area as previously reported (Chang *et al.*, 1993), transformation for unadapted sediment had a longer lag period than adapted sediment. This may be due to chlorophenol degradable microbial consortia contained in sediment originating from the sampling source. All our experiments had lag phases before transformation which were significantly different from those previously reported (Chang *et al.*, 1993). This may suggest that consortia inoculum density was insufficient or that our consortia needed more time to adapt before they could degrade chlorophenol. Additionally, at the beginning of the experiment, we used consortia culture liquid without sediment inoculated to the same medium (as described in materials and methods) and incubated under the same conditions. No degradation was observed for either PCP and 2,4,6-TCP after 1 month's incubation. We suggest that sorption by some consortia to the solid phase of the medium could be an important environmental factor for chlorophenol degradation.

The Identification of Intermediate Products of PCP and 2,4,6-TCP

In this experiment, similar intermediates were obtained in DCP-unadapted and adapted anaerobic conditions (Figures 3 and 4). In the extract of the culture supernatant, new peaks of 2,4-DCP, 3,5-DCP, 3,4,5-TCP and 2,3,4,5-TeCP with retention times of 15.1, 15.7, 21.2 and 24.4 min were detected by GC-ECD. The intermediates were identified as phenol, methylphenol, and benzoate by GC-MS (Figure 5). This showed that 2,4,6-TCP and PCP after dechlorination were further degraded by DCP-adapted or unadapted communities. The DCP-adapted

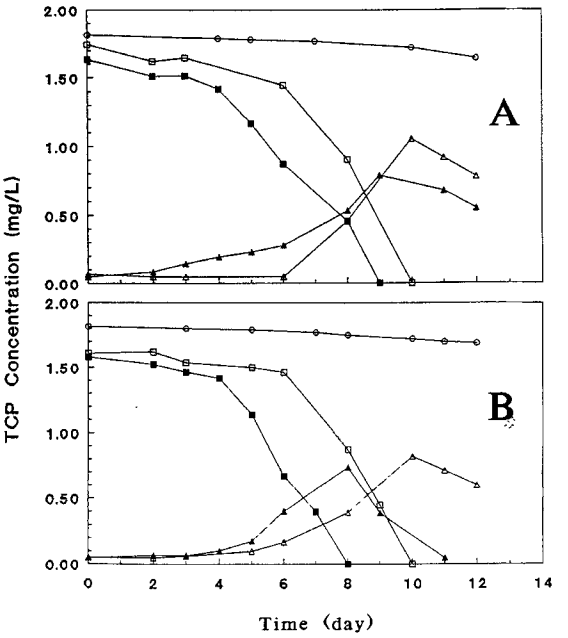

Figure 3 Transformation of 2,4,6-TCP in N3 sediment (A), and N6 sediment (B) samples. Symbols: —■—, 2,4,6-TCP (DCP-adapted); —▲—, 2,4-DCP (DCP-adapted); —□—, 2,4,6-TCP (DCP-unadapted); —△—, 2,4-DCP (DCP-unadapted); —○—, autoclaved control.

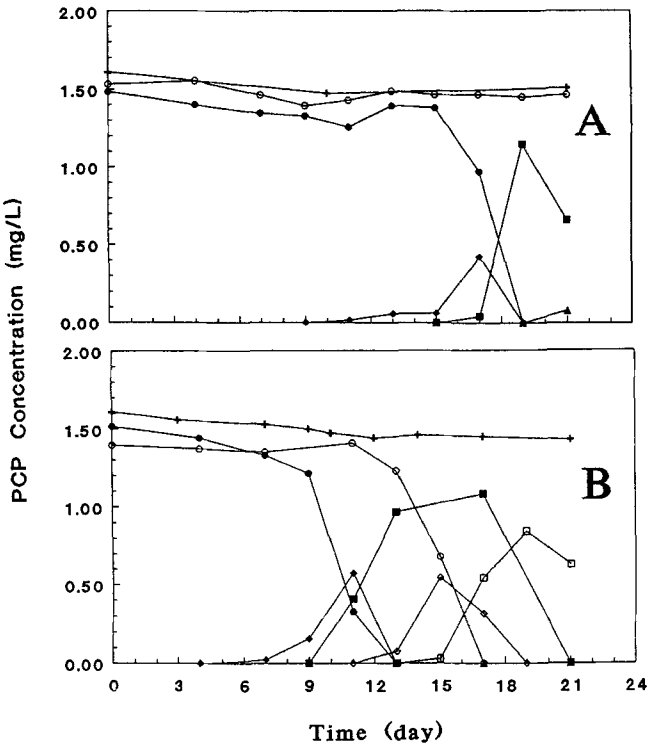

Figure 4 Transformation of PCP in N3 sediment (A), and N6 sediment (B) samples. Symbols: —●—, PCP (DCP-adapted); —◆—, 2,3,4,5-TeCP (DCP-adapted); —■—, 3,4,5-TCP (DCP-adapted); —▲—, 3,5-DCP (DCP-adapted); —○—, PCP (DCP-unadapted); —◇—, 2,3,4,5-TeCP (DCP-unadapted); —□—, 3,4,5-TCP (DCP-unadapted); —△—, 3,5-DCP (DCP-unadapted); —+—, autoclaved control.

communities, consistent with the findings of Bryant (1991), initially removed the ortho-chlorine, and then removed para-chlorine for PCP. The intermediate products were identified as 2,3,4,5-TeCP, 3,4,5-TCP, 3,5-DCP and 3-MCP, phenol, methylphenol, and benzoate for PCP, and 2,4-DCP, 4-MCP, phenol, methylphenol, and benzoate for 2,4,6-TCP. The products of phenol and benzoate were as previously reported by Knoll and Winter (1989), but it was surprising to find the presence of methylphenol, possibly owing to the different microbial consortia and conditions in our studies. This is the first report of such intermediates. The dechlorination of 2,4,6-TCP and PCP showed that degradation rates of all the samples were rather high (Figures 3 and 4). Only PCP was not degraded by unadapted sediment during an incubation of 21 days. Because of climate, the sandy character of river sediments and naturally adapted consortia present in Taiwan, we suggest, on the basis of our samples, that high concentrations of chlorophenols will not be found.

Acknowledgements

This work was supported by grants from the National Science Council, R.O.C. (NSC 82-0421-P-031-002-Z).

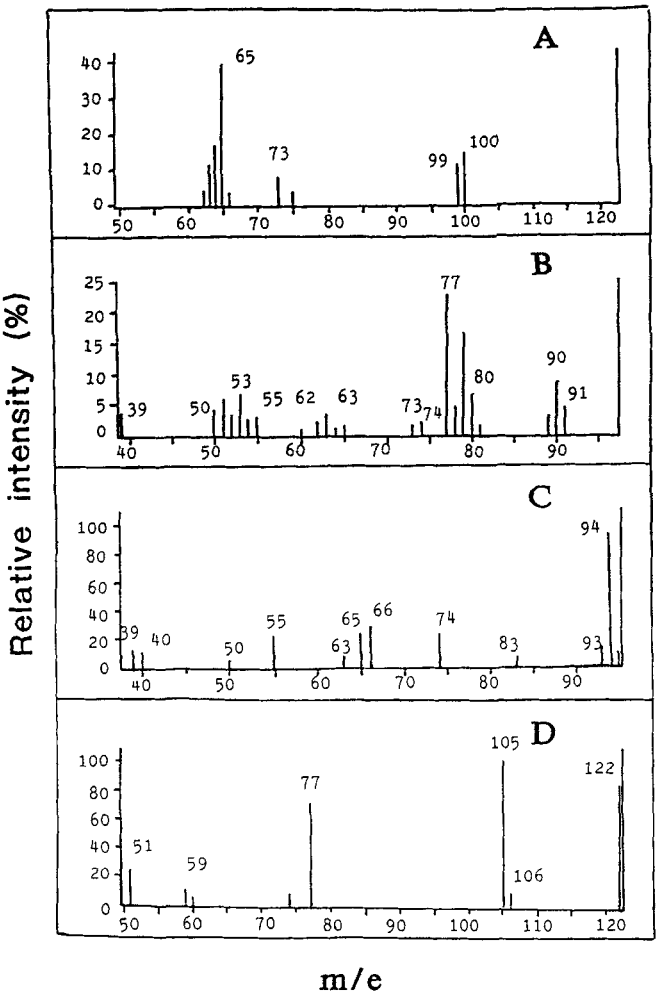

Figure 5 Mass spectra of authentic samples of 4-chlorophenol (A), methylphenol (B), phenol (C) and benzoate (D).

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