

Determination of Acid-Extractable Phenols and Organochlorine Pesticides in Laguna Lake by Gas Chromatography–Mass Spectrometry

Maria Cristina A. Dancel, Guinevere D. Ramos, and Fabian M. Dayrit

Department of Chemistry

Ateneo de Manila University

Loyola Heights, Quezon City, Philippines

The presence of eight acid-extractable phenols and sixteen organochlorine pesticides (OCPs) in Laguna Lake water was determined. The acid-extractable phenols which were analyzed included: phenol, 2-chlorophenol, 2-nitrophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, 4-nitrophenol, and pentachlorophenol. The OCPs were as follows: α -BHC, β -BHC, γ -BHC (lindane), δ -BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, 4,4'-DDE, dieldrin, endrin, endosulfan II, 4,4-DDD, endrin aldehyde, endosulfan sulfate, and 4,4-DDT.

Water samples from five different sites in Laguna Lake were collected in October and November 1996, and February and May 1997. The phenols and OCPs were extracted separately by liquid-liquid extraction. Identification and quantification were done by capillary gas chromatography-mass spectrometry (GC-MS).

None of the sixteen OCPs was detected in any of the water samples. On the other hand, phenol and 4-nitrophenol were detected in some samples collected in February and May 1997. These analytes, however, could not be quantified because they were below this study's practical quantification limits (PQL) of 1.8 $\mu\text{g/L}$ for phenol and 1.2 $\mu\text{g/L}$ for 4-nitrophenol. The PQLs were based on the actual method detection limits (MDL) of the acid-extractable phenols and OCPs.

Key words: phenols, organochlorine pesticides, gas chromatography-mass spectrometry

INTRODUCTION

Phenol and substituted phenols are widely used in the production of plastics, dyes, drugs, antioxidants, and pesticides [1-5]. Various studies have shown that they are detrimental to human health when released into the environment. These compounds have been found in wastewater from oil refineries, phenolic resin plants, pulp mill plants, and textile mill plants [6,7].

OCPs are synthetic chlorinated hydrocarbons which were widely used after World War II. In the mid-1960s, detailed studies on the impact of OCPs on the environment revealed that these compounds persist in the environment [8-10] and undergo biomagnification in the food chain [11-14]. Concern was raised over their effects on top predators such as bird and fish, and the contamination of human food supplies. Today, the use of OCPs is banned in most parts of the world.

The identification and quantification of phenols and OCPs in different water matrices remain as important analytical problems. Gas chromatography (GC) using a

variety of detectors is the principal tool used in this analysis [15,16]. In practice, capillary GC, with an electron capture detector (ECD) is used in the analysis of most pesticides because of its high sensitivity. On the other hand, GC-MS has been found to be the most specific method for analytes in complex matrices, although it is less sensitive than ECD. The principal advantage of GC-MS is its unique ability to confirm the identity of a GC-eluting compound based on its mass spectral pattern (m/z) and relative intensities of the ions (ion ratios). This avoids the problems of false GC peak identification which occurs with other detectors [17-20].

In this study, we report on the determination of eight acid-extractable phenols and sixteen OCPs (Fig. 1) in Laguna Lake water using GC-MS. Laguna Lake is the largest freshwater lake in the Philippines and is the second largest in Southeast Asia [21,22]. It has an area of 900 km^2 and an average depth of 2.8 m. The lake is used for aquaculture and receives runoffs from agriculture. It is being considered as a source of water for the residents and industries in Metro Manila. Consequently, the water quality of the lake is a major public concern.

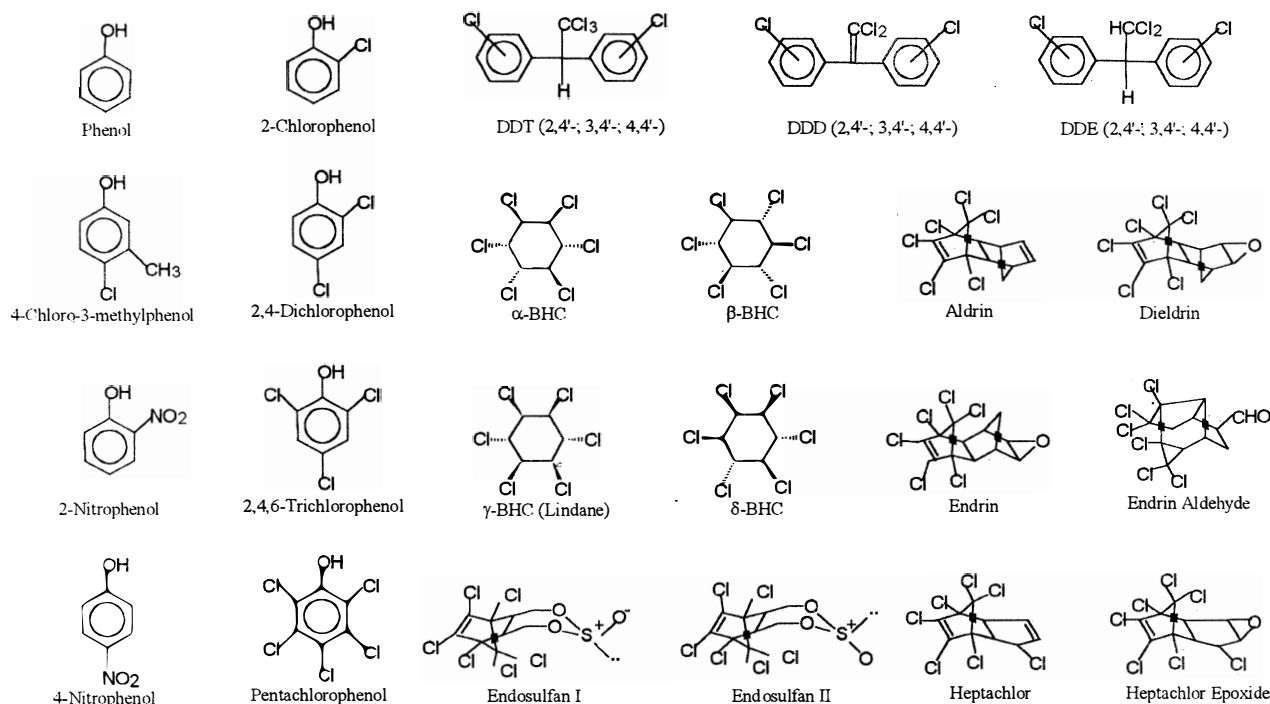


Fig. 1. Structure of acid-extractable phenols and OCPs

EXPERIMENTAL

Solvents and Reagents. Analytical grade solvents from Merck and J.T. Baker were used in the extraction. Standard mixtures of the sixteen OCPs and five acid-extractable phenols were purchased from ChemService. GC-pure 2-nitrophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol were added to the mixture of five phenols, to make a total of eight phenol standards.

Separate phenol and OCP calibration and spike solutions were prepared. 2-Fluorophenol (2-FP) was added as internal standard for the phenol calibration standards and sample extracts, while 2,4,5,6-tetrachloro-*m*-xylene (tCX) was added as internal standard to the final OCP standards and extracts. Fortified samples were also prepared by adding a known amount of standard to 1 L of each sample. Fortified distilled water were also prepared in the same manner.

Equipment and Apparatus. The GC-MS used was a Shimadzu QP 2000A which is equipped with a quadrupole mass analyzer capable of unit mass resolution. Two μL of extract were injected in the splitless mode at 250°C. The GC column was an OV-1 capillary column (50 m length \times 0.2 mm ID \times 0.25 μm film thickness). For the analysis of acid-extractable phenols, the GC oven was programmed as follows: initial temperature of 60°C for 1 min, then heated to 260°C at 25°C/min. The transfer line from the GC to the mass spectrometer was set to 275°C. For the OCP analysis, the GC oven was programmed as follows: initial temperature of 100°C for 1 min, heated to 190°C at 30°C/min, then to 210°C at 15°C/min, and finally to 260°C at 2.5°C/min.

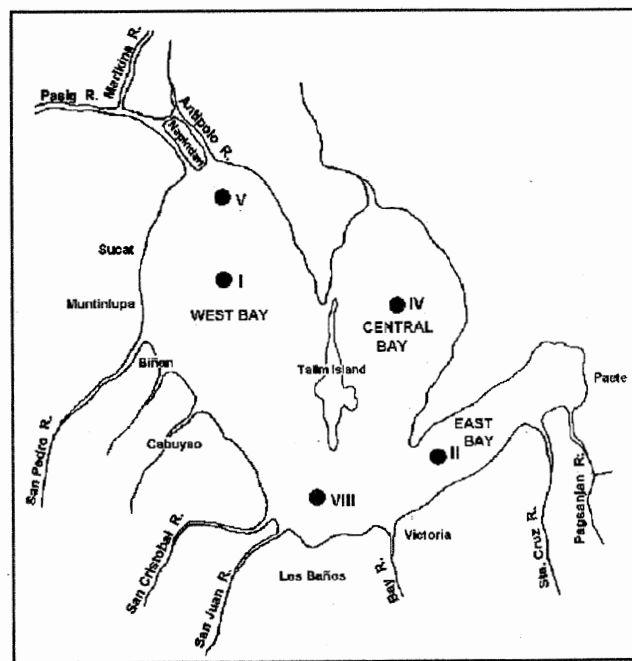


Fig. 2. Location of water sampling stations at Laguna Lake

The mass spectrometer was scanned from m/z 50 to 400 with the ion source temperature at 300°C and the ionization voltage set to 70 eV.

Methodology. Water samples from five different sites in Laguna Lake (Fig. 2) were collected in October and November 1996, and February and May 1997. Sampling was done at

Table 1. Sampling Depths at Different Sampling Stations in Laguna Lake

Station No.	Depth (meter)
I	0.5 and 2
II	0.5 and 2
IV	0.5
V	0.5
VIII	0.5 and 2

different depths (Table 1) using a 3 L Kemmerer sampler. The samples were stored in 4 L amber bottles with teflon-lined covers and stored at 4°C prior to extraction. Extraction of phenols and OCPs was done separately.

Extraction of Phenols. The pH of each 1 L sample was adjusted to less than 2 with 4.5 M sulfuric acid before extraction with DCM. The final extracts were concentrated using a Kuderna-Danish apparatus [23]. The internal standard, 2-FP, was added and the extract was diluted to a final volume of 1 mL.

Extraction of OCPs. Two liters of each water sample were extracted with DCM. The extracts were concentrated by rotary evaporation and solvent-exchanged with hexane. The crude hexane extracts were cleaned up by elution through an alumina column with hexane, followed by ethyl-acetate/benzene (1:1). Each eluant was concentrated, spiked with the tCX internal standard, and diluted to 1 mL.

Quality Control. Quality assurance for both phenols and OCPs was done by preparing one method blank, one sample replicate, and at least two fortified samples for each batch [24]. The method detection limit (MDL) of each analyte for the liquid-liquid extraction was determined using fortified distilled water [25].

Computations. The analyte-internal standard peak area ratios for various concentrations were computed as follows:

$$\text{Peak Area Ratio} = \frac{\text{peak area of analyte}}{\text{peak area of internal standard}}$$

The peak area-ratios were plotted against the concentration. The amount of recovered OCPs and phenols was interpolated from the resulting calibration graphs.

The percent recoveries of OCPs and phenols for the fortified water and MDL extracts were computed using the following formula:

$$\% \text{ Recovery} = \frac{\text{amount of analyte recovered}}{\text{amount of analyte added}} \times 100$$

The standard deviation (S.D.) of recoveries for each analyte was calculated and used to establish its MDL using the following formula:

$$\text{MDL} = t_{(n-1, \alpha=0.99)} \times \text{S.D.}$$

where $t_{(n-1, \alpha=0.99)}$ is the Student's *t* value for 99% confidence level and the appropriate degrees of freedom, *n*-1. For the fortified samples, the S.D. of recoveries from different sampling dates was used to compute the relative standard deviation or %RSD [24,25]. The MDL multiplied by five times gives the PQL [26].

RESULTS AND DISCUSSION

Qualitative Analysis: TICs and EICs

The GC peak generated by the GC-MS is a total ion chromatogram (TIC) which includes all of the mass spectral signals. From the same file, individual ion chromatograms may be extracted. In this study, the extracted ion chromatograms (EICs) were based on the primary ions of each analyte as listed in Table 2. The primary ions were based on experimental spectra and agree with existing literature [23,24,27-32]. Figure 3 shows the TIC and EICs of 2 ppb mixed phenols while Figure 4 shows the TIC and EICs for 1 ppb OCPs. Also shown are the multiplication factors used to adjust the intensity of each EIC.

Table 2 also gives the MDL for the liquid-liquid extraction conducted on fortified distilled water. It is worth noting that the values obtained in this study are lower than any of the reported MDLs using GC/MS in the EPA procedures for chlorinated phenols and OCPs. The MDL multiplied by five times gives the practical quantification limit (PQL) which takes into consideration day to day experimental fluctuations [24,26].

Analysis of Phenols. Water samples taken from Stations II, IV and VIII in February 1997 and from Stations IV, V, and VIII in May 1997 showed peaks with the same retention time as phenol. For example, Figure 5 shows the TIC and EICs of the extract for the sample taken from Station IV in May 1997. To confirm the identity of the sample peak, the ion intensity ratios of *m/z* 94/66 and 94/65 in the sample were compared with those of 2 ppb phenol. The ion intensity ratios (Table 3) confirmed that the sample peak was indeed phenol. The water sample taken from Station II in February 1997 was also confirmed to have 4-nitrophenol.

Analysis of OCPs. The combined use of GC retention time and the EICP was very effective for confirming the presence of the target analytes. For example, in some of the water samples taken in February 1997, the presence of endosulfan I was suspected based on the retention time and EIC of mass *m/z* 195 (Fig. 6). To confirm this, two additional characteristic ions of endosulfan I at *m/z* 237 and 241 were used.

Table 2. Characteristic Retention Times, Mass Ions, MDLs, and PQLs of Acid-Extractable Phenols and OCPs

Analytes	Retention Time, min	Primary Ion	MDL, ppb	PQL, ppb
A. Acid Extractable Phenols				
Phenol (P)	7.4	94	0.37	1.8
2-Chlorophenol (2-CP)	7.6	128	0.84	4.2
2-Nitrophenol (2-NP)	8.9	139	0.96	4.8
2,4-Dichlorophenol (2,4-DCP)	9.2	162	0.85	4.2
4-Chloro-3-methylphenol (4-Cl-3-MeP)	10.2	142	0.74	3.7
2,4,6-Trichlorophenol (2,4,6 TCP)	10.6	196	0.73	3.6
4-Nitrophenol (4-NP)	11.9	65	0.25	1.2
Pentachlorophenol (PCP)	13.8	266	0.64	3.2
B. OCPs				
α -BHC	10.8	183	0.23	1.2
β -BHC	11.1	183	0.18	0.9
γ -BHC (Lindane)	11.4	183	0.20	1.0
δ -BHC	11.6	183	0.19	1.0
Heptachlor	13.8	100	0.16	0.8
Aldrin	15.1	66	0.30	1.5
Heptachlor Epoxide	16.3	353	0.15	0.8
Endosulfan I	17.8	195	0.29	1.4
4,4-DDE	18.4	246	0.11	0.6
Dieldrin	18.8	79	0.10	0.5
Endrin	19.8	67	0.38	1.9
Endosulfan II	19.9	195	0.26	1.3
4,4-DDD	20.1	165	0.17	0.8
Endrin Aldehyde	20.6	67	0.20	1.0
Endosulfan Sulfate	21.8	272	0.19	1.0
4,4-DDT	22.1	165	0.10	0.5

Note: The retention times of the internal standards, 2-fluorophenol (2-FP) and tetrachloro-*m*-xylene (tCX) were 6.8 and 10.1 min, respectively.

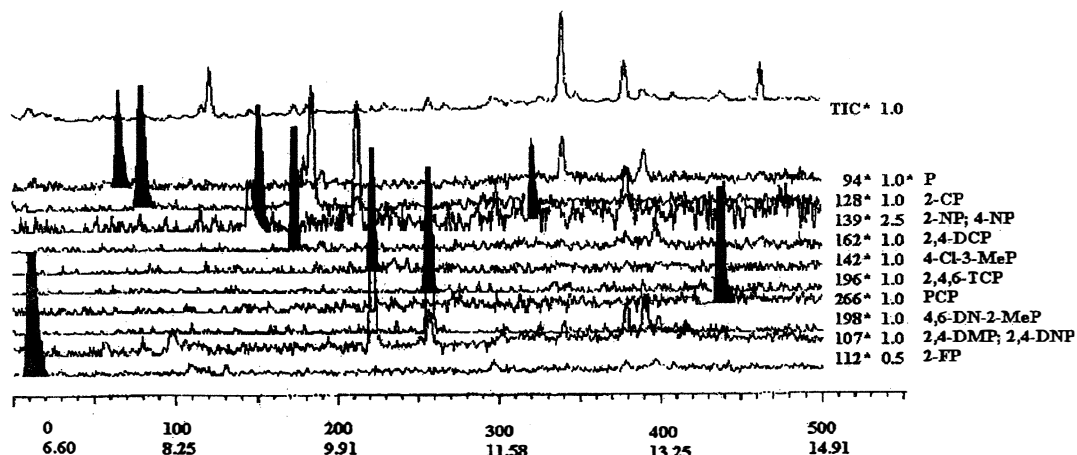


Fig. 3. The total-ion chromatogram (TIC) and extracted-ion chromatograms (EICs) based on primary ions of acid-extractable phenols and internal standard, 2-FP (see Table 2). The concentrations of the phenols and 2-FP are equivalent to 2 ppb and 4 ppb, respectively, in the sample [May 1997].

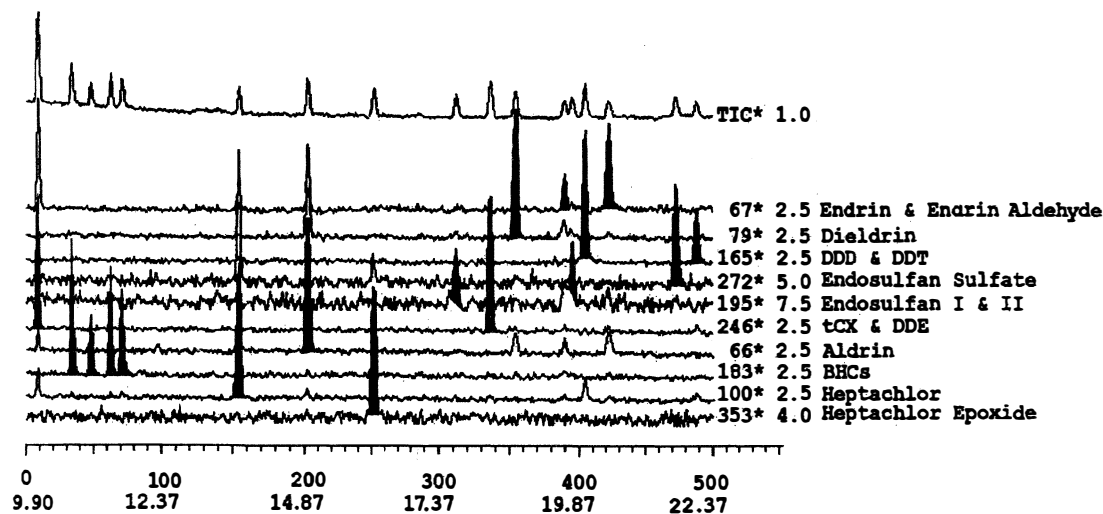


Fig. 4. The total-ion chromatogram (TIC) and extracted-ion chromatograms (EICs) based on primary ions of OCPs and internal standard, tCX (see Table 2). The concentrations of the OCPs and tCX are equivalent to 1 ppb and 2 ppb, respectively, in the sample [February 1997].

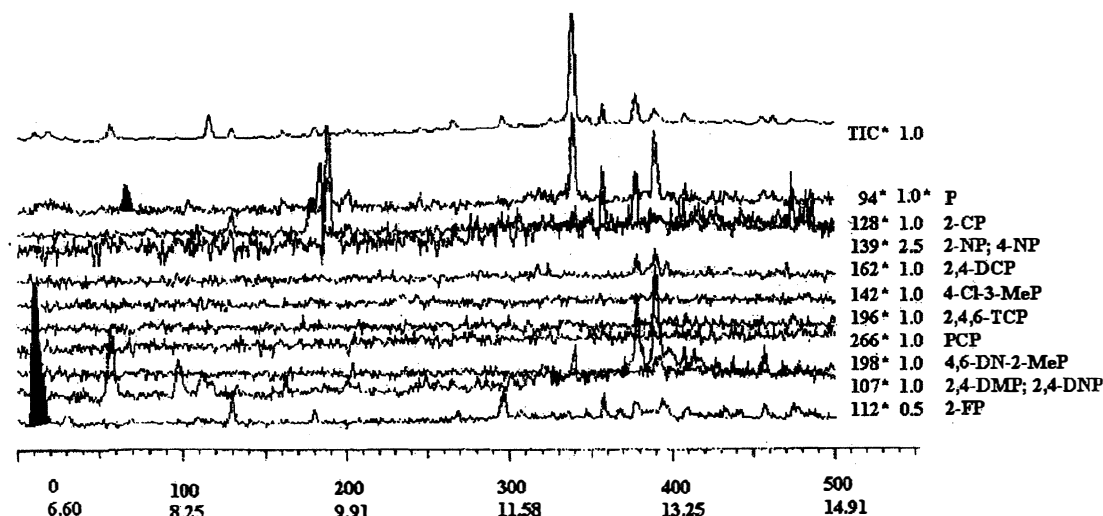


Fig. 5. The TIC and EICs of the extract from Station-IV water sample [May 1997]. The EIC for 94 m/z shows a peak with the same retention time as phenol. The ratios of phenol characteristic mass ions (see Table 3) confirmed that the peak is phenol.

Table 3. Ion Intensity Ratio of m/z 94 against m/z 65 and 66 which Confirmed the Presence of Phenol in Laguna Lake Water

Ion Intensity Ratio	Retention Time (min)	Standard Phenol Concentration		May 20, 1997 Water Samples which Contained Phenol		
		2.0 ppb	4.0 ppb	Station IV	Station V	Station VIII
Phenol						
94/65	~7.70	3.0	4.4	2.3	3.8	2.5
94/66		4.2	2.5	3.4	1.9	3.2

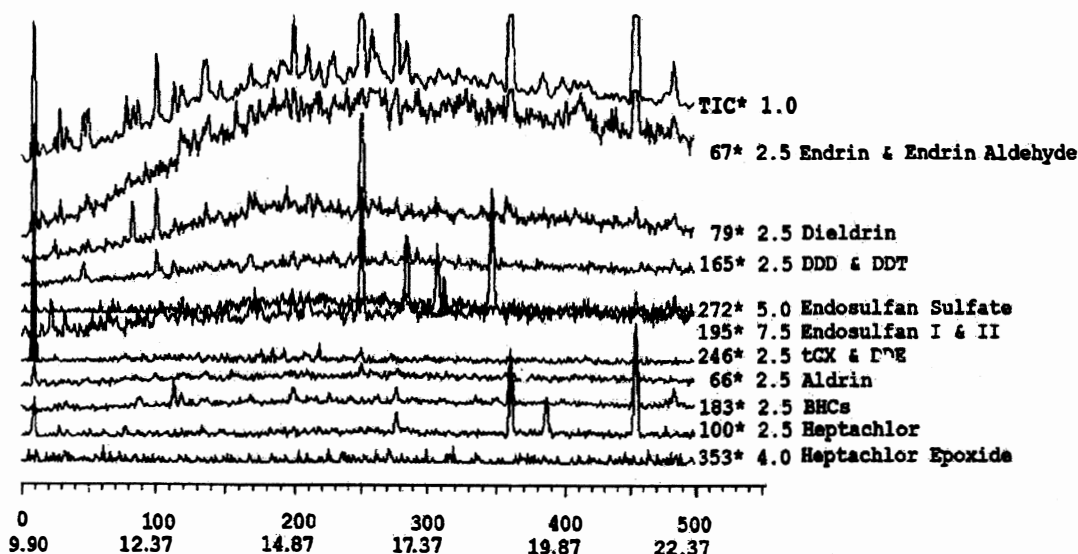


Fig. 6. The TIC and EICs of the hexane extract from Station-VIII water sample [February 1997]. The EIC for 195 m/z shows a peak with the same retention time as endosulfan I (see Fig. 7).

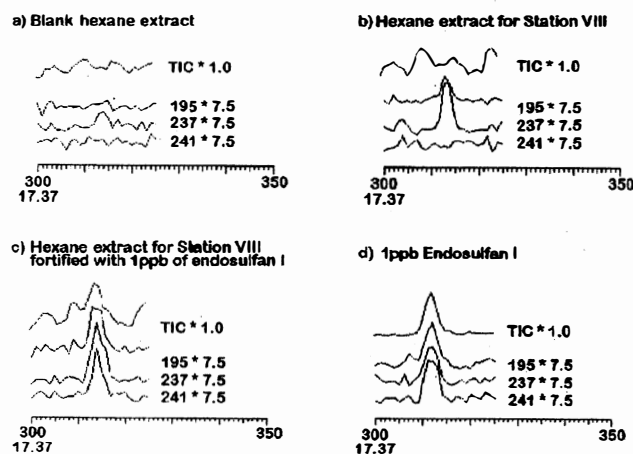


Fig. 7. The EICs of mass m/z 195, 237, and 241 in the (a) blank, (b) sample and (c) fortified sample hexane extracts, for Station VIII water sample [February 1997], were compared against those in (d) standard endosulfan I. The absence of a 241 m/z peak in (b) indicated that the suspected peak is not endosulfan I.

The resulting EICP showed that m/z 237 was also present but m/z 241 was absent (Fig. 7). These results indicate that endosulfan I was not present in the water sample.

Quantitative Analysis: Recoveries and MDLs

Analysis of Phenols. The amount of recovered phenols was computed based on standard addition in distilled water. This method takes into consideration the solubility of phenols in water.

As shown in Table 4, the percent recoveries of 2-chlorophenol, 2-nitrophenol, phenol, 2,4-dichlorophenol, 4-nitrophenol, 4-chloro-3-methylphenol, and pentachlorophenol are all above 70%. A 54% recovery for 4 ppb 2,4,6-trichlorophenol (TCP) was obtained in February 1997. However, recoveries of at least 70% for all eight phenols were achieved for 2 ppb spike in samples taken in May 1997. This indicates that the lower recovery for TCP in February 1997 may be an isolated case.

Analysis of OCPs. Out of the sixteen OCPs, only endrin aldehyde was eluted in the hexane and in the ethyl acetate/benzene eluents. Recoveries in both eluents were added to compute for the total percent recovery. Endosulfan sulfate was eluted only in the ethyl acetate/benzene eluent while the remaining fourteen OCPs were eluted in the hexane extract.

The percent recoveries at the 2 ppb level for α -BHC, β -BHC, δ -BHC, lindane, heptachlor, endosulfan I, 4,4'-DDD, dieldrin, endosulfan sulfate and 4,4'-DDT were always above 70% (see Table 5). Recoveries of at least 70% were also achieved for 1 ppb spike for samples taken in November 1996 and February 1997 which agree with the PQLs reported in Table 2.

On the other hand, the recoveries at the 2 ppb level for aldrin, heptachlor epoxide, endosulfan II, 4,4'-DDE, endrin aldehyde, and endosulfan sulfate were lower than 70% in some instances. Some of these analytes may have been retained in the emulsion layer brought about by suspended particulate matter in the samples. Their respective PQLs were, therefore, not satisfied due to sample matrix interference.

Table 4. Percent Recovery of 4 ppb Acid-Extractable Phenols from Fortified Water Samples

Analyte	Date of Sampling (M/D/Y)			Ave. %R	%RSD
	10/29/96	02/18/97	05/20/97		
Phenol	70	77	103	83	21
2-Chlorophenol	69	87	125	94	31
2-Nitrophenol	--	90	131	111	26
2,4-Dichlorophenol	--	135	110	123	14
4-Chloro-3-methyl phenol	96	117	101	105	10
2,4,6-Trichlorophenol	--	54	100	77	42
4-Nitrophenol	106	97	112	105	7
Pentachlorophenol	88	89	89	89	1

Table 5. Percent Recovery of 2 ppb OCPs from Fortified Water Samples

OCP	Date of Sampling(M/D/Y)				Ave. % R	% RSD
	10/29/96	11/19/96	02/18/97	05/20/97		
α -BHC	117	110	72	62	90	27
β -BHC	129	104	78	83	99	23
γ -BHC (lindane)	118	104	77	70	92	23
δ -BHC	129	69	85	82	91	26
Heptachlor	118	82	63	56	80	28
Aldrin	97	84	64	53	75	20
Heptachlor Epoxide	132	66	76	81	89	30
Endosulfan I	129	104	76	75	96	26
4,4'-DDE	104	83	72	68	82	16
Dieldrin	134	118	82	71	101	30
Endrin	--	--	121	106	114	11
Endosulfan II	--	NR	86	69	78	12
4,4'-DDD	--	106	79	71	85	18
Endrin Aldehyde	--	--	132	73	103	42
Endosulfan Sulfate	NR	NR	96	88	92	6
4,4'-DDT	111	73	85	92	90	16

CONCLUSION

Phenol and 4-nitrophenol were detected in Stations IV and VIII in February and May 1997 water samples, but in levels not quantifiable because they were below their respective PQLs (Table 2). Recommended detection limits for phenol, 2-chlorophenol, 2-nitrophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, 4-nitrophenol, and pentachlorophenol are set to their respective PQLs.

None of the OCPs was detected in any of the water samples from Laguna Lake. Based on actual recoveries and in accordance with their respective PQLs (refer to Table 2), the recommended detection limit for α -BHC, β -BHC, δ -BHC, lindane, 4,4'-DDD, 4,4'-DDT, dieldrin, and endosulfan sulfate in Laguna Lake water is 1 ppb. The recommended detection limit for aldrin, 4,4'-DDE, endosulfan I, endosulfan II, endrin, endrin aldehyde, heptachlor, and heptachlor ep-

oxide in Laguna Lake water is 2 ppb, based on actual spike alone. These seven OCPs were most affected by sample matrix interference.

ACKNOWLEDGEMENT

The authors wish to thank the Laguna Lake Development Authority (LLDA) for their assistance in the sample collection, and the Department of Science and Technology, under the Engineering and Science Education Program (DOST-ESEP), for the MS scholarship grants to MCAD and GDR.

REFERENCES

1. *Agro-Pesticides: Properties and Functions in Integrated Crop Protection*. UN Bangkok (1991).

2. Boyd, S.A., Shelton, D.R., Berry, D., and Tiedje, J.M. *Appl. Environ. Microbiol.* 46(1), 50 (1983).
3. Galceran, M.T. and Jauregui, O. *Anal. Chim. Acta.* 304, 75 (1995).
4. Shreve, N. and Brink, J.A. Jr. *Chemical Process Industries*, 4th ed. (McGraw-Hill Book Company, 1977).
5. Boyd, S.A. and Shelton, D.R. *Appl. Environ. Microbiol.* 47(2), 272 (1984).
6. Abrahamsson, K. and Xie, T.M. *J. Chromatogr.* 279, 199 (1983).
7. Xie, T.M., Abrahamsson, K., Fogelqvist, E., and Josefsson, B. *Environ. Sci. Technol.* 20, 457 (1986).
8. Marty, A.S. *Toxicity of Pesticides to Fish*, Vol. 1 (CRC Press: Boca Raton, Florida 1986).
9. Alloway, B.J. and Ayres, DC. *Chemical Principles of Environmental Pollution* (Chapman & Hall: London, 1993).
10. Giddergs, J.C. *Chemistry, Man and Environmental Change* (Canfield Press: San Francisco, CA, 1973).
11. Day, K.E. *Pesticide Transformation Products: Fate and Significance in the Environment*. L. Sumasundaran and J.R. Coats (Eds.). ACS Symposium Series 459 (American Chemical Society: Washington, DC, 1991).
12. Albaiges, J. (Ed.). *Marine Pollution* (Hemisphere Publishing Corporation: New York, 1989).
13. Xie, T.M., Abrahamsson, K., Fogelqvist, E., and Josefsson, B. *Environ. Sci. Technol.* 20, 457 (1986).
14. Tejada, A.W. *The Philippine Agriculturist* 78(1), 63 (1995).
15. Thomas, Q.V., Stork, J.R., and Lammert, S.L. *J. Chromatogr. Sci.* 18, 583 (1980).
16. *CFR Part 136: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater*. United States. Environmental Protection Agency, EPA Water Resource Center. *Fed. Regist.* 49, 153 (1984).
17. Jongki H., Yunwoo E., Jaeseong R., and Taekjae K. *J. Chromatogr.* 639, 261 (1993).
18. Keith, L.H. and Telliard, W.A. *Environ. Sci. Technol.* 13, 416 (1979).
19. Swallow, K.C., Shifrin, N.S., and Doherty, P.J. *Environ. Sci. Technol.* 20, 136 (1988).
20. Shackelford, W.M. and Cline, D.M. *Environ. Sci. Technol.* 20(7), 652 (1986).
21. *1987 Water Quality Data on the Laguna de Bay and the Tributary Rivers*, Vol. 4 (Published by the Laguna Lake Authority Development).
22. Bacallan, J.J. *Business and Environment*. 3(3), 13 (1997).
23. Clesceri, L.S., Greenberg, A.E., Truselle, R.R., and Franson, M.H. (Eds.). *Standard Methods for the Examination of Water and Wastewater*, 17th ed. (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1989).
24. *Training Manual and Reference Information: Organic Analysis and GC/MS Training Workshop*. ASEAN-Canada Programme on Marine Science (Jakarta, Indonesia, June 6-19, 1993).
25. *Appendix B to CFR Part 136--Definition for the Determination of Method Detection Limit--Revision 1.11*. United States. Environmental Protection Agency; EPA Water Resource Center. *Fed. Regist.* 49, 198 (1984).
26. Eaton, A.D., Clesceri, L.S., Greenberg, and Franson, M. H. (Eds.). *Standard Methods for the Examination of Water and Wastewater*, 19th ed. (American Public Health Association, American Water Works Association and Water Environment Federation, 1995).
27. *Method 604: Phenols*. In Appendix A to CFR Part 136: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. United States. Environmental Protection Agency; EPA Water Resource Center. *Fed. Regist.* 49, 58 (1984).
28. *Method 608: Organochlorine Pesticides and PCBs*. In Appendix A to CFR Part 136: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. United States. Environmental Protection Agency; EPA Water Resource Center. *Fed. Regist.* 49, 89 (1984).
29. *Method 625: Base/Neutrals and Acids*. In Appendix A to CFR Part 136: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. United States. Environmental Protection Agency; EPA Water Resource Center. *Fed. Regist.* 49, 153 (1984).
30. *Method 8270B: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS): Capillary Column Technique*. In Tests Methods for Evaluating Solid Wastes, Physical/Chemical Methods (SW-846), CD-ROM Version 1.0. United States. Environmental Protection Agency (NTIS, US Department of Commerce: Springfield, VA, 1994).
31. *Method 8081: Organochlorine Pesticides and PCBs as Arochlors by Gas Chromatography: Capillary Column Technique*. In Tests Methods for Evaluating Solid Wastes, Physical/Chemical Methods (SW-846), CD-ROM Version 1.0. United States. Environmental Protection Agency (NTIS, US Department of Commerce: Springfield, VA, 1994).
32. Tests Methods for Evaluating Solid Wastes, Physical/Chemical Methods (SW-846), CD-ROM Version 1.0. United States. Environmental Protection Agency (NTIS, US Department of Commerce: Springfield, VA, 1994).