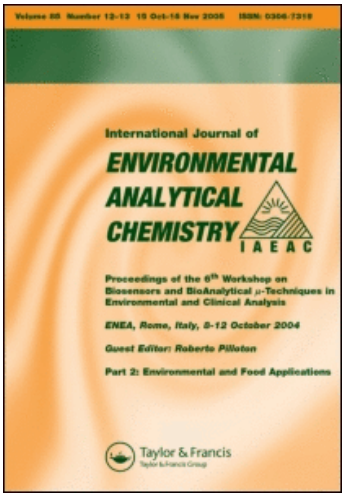

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Analytical Techniques for the Quantitation of Substituted Phenols in Municipal Sludges

John H. Phillips[a]; Matthew Zabik[a]; Richard Leavitt[ab]
[a] Pesticide Research Center, Michigan State University East Lansing, Michigan, U.S.A. [b] Air Products and Chemicals, Inc., Allentown, PA

To cite this Article Phillips, John H. , Zabik, Matthew and Leavitt, Richard(1983) 'Analytical Techniques for the Quantitation of Substituted Phenols in Municipal Sludges', International Journal of Environmental Analytical Chemistry, 16: 2, 81 — 93
To link to this Article: DOI: 10.1080/03067318308078353
URL: http://dx.doi.org/10.1080/03067318308078353

Intern. J. Environ. Anal. Chem., 1983, Vol. 16, pp. 81–93
0306-7319/83/1602-0081 $18.50/0

Printed in Great Britain

Analytical Techniques for the Quantitation of Substituted Phenols in Municipal Sludges

JOHN H. PHILLIPS,† MATTHEW ZABIK, RICHARD LEAVITT

Pesticide Research Center, Michigan State University East Lansing, Michigan 48824, U.S.A.

(Received February 2, 1983; in final form May 18, 1983)

Municipal sewage sludges were characterized for a group of phenols classified as priority pollutants. The development of analytical methodology, including unique problems encountered when sampling, handling and analyzing phenolic compounds, are discussed. Percent recovery data for several sample types, as well as quality control procedures, are included.

Samples were extracted via a liquid/liquid extraction centrifugation technique with dichloromethane. Cleanup was achieved by acid/base partitioning and automated gel permeation chromatography. Reverse phase high pressure liquid chromatography was used for separation of compounds employing an electrochemical detection system for quantitation. Sensitivities in the low picogram range were achieved for phenolic species in biosludge extract.

KEY WORDS: Municipal sludge, substituted phenols, trace environmental analysis, priority pollutants, electrochemical detection.

INTRODUCTION

Sludge is a liquid or semi-solid waste which contains contaminants removed from water during the treatment process. A large percentage of sludge solids consists of bacteria, fungi or other microbes which help to purify the effluent. Sludge management is a

†Current Address: Air Products and Chemicals, Inc., Box 538, Allentown, PA 18105.

problem faced by all municipalities in the United States. Municipal sewage sludges contain many nutrients which are valuable for soil enrichment and, therefore, one of their largest uses has been as a fertilizer in land application. Current methods of sludge disposal include: incineration, landfill, lagooning and land application, the latter being the most economically feasible. Due to mismanagement or lack of proper monitoring of some sludge disposal systems, there has been a history of soil, plant, and ground water contamination from heavy metals, toxic organics and pathogenic bacteria. With the increased input of household chemicals and industrial effluents into municipal sewage systems, the problem of waste disposal has come to the forefront for federal, state and local regulatory agencies. Passage of the Federal Water Pollution Control Act in 1972 has caused a huge increase in the amount of sewage sludge for disposal. In order to manage wastes properly, it is important to know if they contain hazardous materials and, if so, in what levels in order that they may be used safely, detoxified or disposed of via incineration or an approved landfill. For this reason the Michigan Department of Natural Resources and the United States Environmental Protection Agency contracted researchers from Michigan State University to characterize 250 sewage treatment plant sludges.[1] These sludges came from throughout the state and were analyzed for a group of 80 organic priority pollutants and 20 heavy metals. Included in this list were the following phenolic components:

o-Chlorophenol	4,6-Dinitro-*o*-cresol
m-Chlorophenol	2,4-Dinitrophenol
p-Chlorophenol	Hydroquinone
o-Cresol	Pentachlorophenol
2,4-Dichlorophenol	Phenol
2,4-Dimethylphenol	2,4,6-Trichlorophenol

Sludges consist of 5-100% solids which can contain nearly 100% organics, 10–15% of which is solvent extractable material. Typical interferences present in municipal sludges include petroleum fuels, lubricating oils, asphalts, fats, fatty acids and detergents. A rigorous cleanup procedure is required to remove these interferences.

Phenols enter municipal sewage treatment plants via several sources including: coal tars, SRC wastes, industrial processes, residential use of insecticides, herbicides, fungicides, anticeptics,

disinfectants, and metabolites of polychlorinated aromatic hydrocarbons. Phenols can be detected by the human olfactory system in the part per billion range and are readily absorbed through skin, and also readily excreted, if not metabolized.

EXPERIMENTAL

Extraction procedure

Liquid sludge samples with less than 30% solids were routinely basified and extracted three times with one-to-one ratios of dichloromethane (DCM) using centrifugation to break the emulsion. After the base/neutral fraction was separated in this way, the sludges were acidified and extracted with three aliquots of DCM for the phenolic fraction. Solid samples were dried at 30°C and then fifty grams of ground sludge were soxhlet extracted for 24 hours with 200 mLs of DCM. This extract was then passed through an acid/base extraction scheme followed by cleanup via size exclusion chromatography.

Separation by gel permeation chromatography (GPC) was made possible with an Analytical Biochemistry Laboratories automatic gel permeation chromatography unit. Two 2.5×35 cm columns were filled with S-X2 biobeads, effective range 1,000-2,700 M.W. Mobile phase consisted of 66.6% DCM, 33.4% cyclohexane pumped at a flow rate of 5.0 mLs per minute. Prior to chromatographic analysis, samples were reduced in volume to 10 mLs via rotoevaporation and nitrogen purge (Figure 1).

Chromatographic system

The high performance liquid chromatographic system consisted of an Altex pump equipped with an Altex microprocessor-controller and a 10 μl DuPont C-8 Zorbax ODS analytical column. The column was maintained at a constant temperature of 50°C in order to reduce mobile viscosity. For early eluting components, flow rate was held at 0.8 mL/min. with a 3:2 ratio of 0.05 M sodium acetate buffer at pH 3.0 to acetonitrile (Figure 2). For late eluting peaks, flow rate was 1.0 mL/min. with a 4:6 ratio of sodium acetate buffer to acetonitrile as the mobile phase (Figure 3).[2]

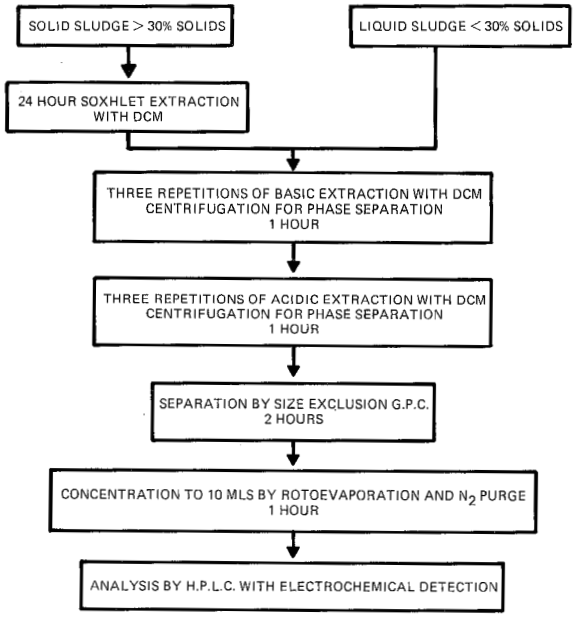

FIGURE 1 Flow diagram of extraction and cleanup procedure for the analysis of phenols in sludge.

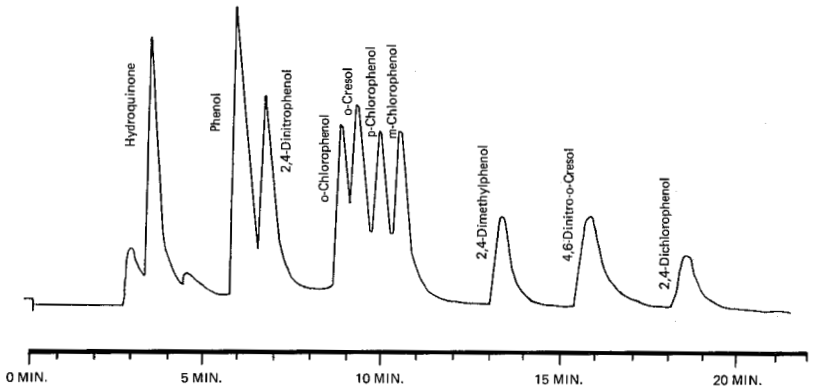

FIGURE 2 Early phenol elutriates by high pressure liquid chromatography using a DuPont C-8 10 μ column 25 cm × 4.6 mm I.D. with a 3:2 ratio of 0.5 M sodium acetate buffer to acetonitrile. Electrochemical detection of 20 pg of each phenolic standard at an oxidation potential of 1.2 volts.

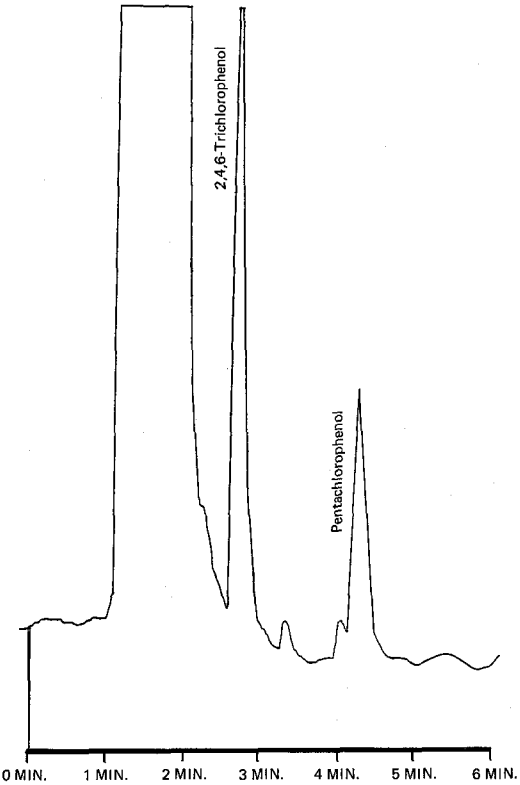

FIGURE 3 Late phenol elutriates by high pressure liquid chromatography using a DuPont C-8 10 μ column 25 cm × 4.6 mm I.D. with a 4:6 ratio of 0.5 M sodium acetate buffer to acetonitrile. Electrochemical detection of 20 pg of each phenolic standard at an oxidation potential of 1.2 volts.

Reagents

All solvents were high purity glass-distilled from Burdick and Jackson, Muskegon, Michigan. Chemicals were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

DISCUSSION

Certain phenols have been shown to be extremely sensitive to bacterial decomposition,[3] while others may be produced in the

sludge stabilization process as noted by Hartenstein.[4] In a brief study, sludge samples were taken from an anerobic and aerobic digester, spiked with selected phenols and stored at 4°C, otherwise unpreserved. At log time intervals, a portion of each sample was analyzed for the phenols of interest. Some of the results are plotted in Figures 4 and 5, clearly demonstrating large fluctuations of phenolic species over time in unpreserved municipal sludges. This emphasizes the dynamic nature of the biota responsible for producing variations in phenolic concentration. To prevent loss or change in the phenolic composition, sludges should be stored at 4°C, acidified (pH = 3.0 H_3PO_4) or basidified (pH = 12.0 NaOH), and have 4 g/L of $CuSO_4$ added to each sample to inhibit biological activity.

Phenolic compounds have been shown by several researchers to photolytically degrade, therefore, samples should not be exposed to direct sunlight but stored in amber containers.[5] When working with trace levels of phenols and glassware that has been activated by a high temperature glass cleaning oven, glassware should be silanized to prevent adsorption onto active sites. When sampling, it may be desirable to make the solution basic in order to place the phenols in their ionized state, so that they will remain in the aqueous solution, rather than being adsorbed onto the glass. Critical temperatures/vapor pressures of phenolic species are quite low, therefore, caution must be maintained when concentrating samples so as not to lose them to the atmosphere. Excessive temperatures, reduced atmospheric pressures or solvent loss may result in partial or complete loss of the analyte.[6]

When compared to conventional liquid/liquid extraction techniques, continuous soxhlet extraction of dried sludge with dichloromethane (DCM) demonstrated superior recovery of phenols under optimum conditions (Table I). Several variables can interact and affect recovery when sludge is dried in preparation for soxhlet extraction (Figure 6). While pH and oven temperature can be regulated, phenol concentration and organic content will be variable. Therefore, continuous liquid extraction was only used for solid samples and a liquid/liquid extraction method adapted from the EPA proposed methods was employed for all liquid samples.[7]

Several methods may be appropriate for the cleanup of interferences in an environmental matrix for the analysis of phenolic components. Among these are acid/base partitioning, separation by

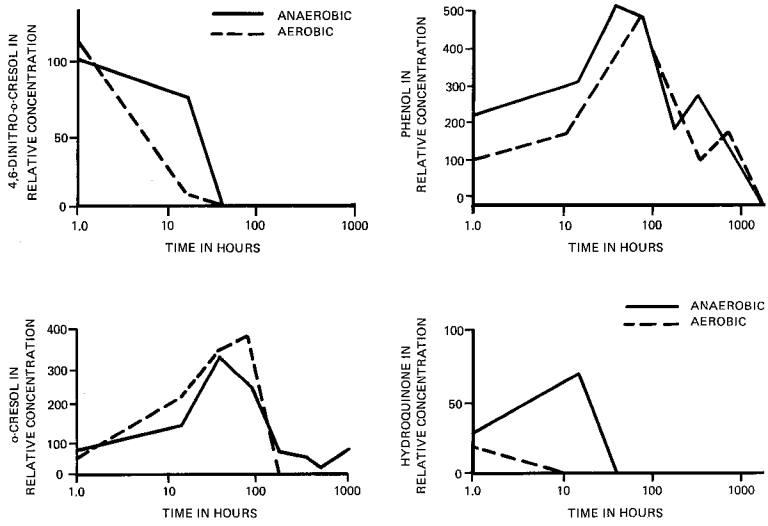

FIGURE 4 Changes in the relative concentration of various phenolic components in unpreserved sludge over time I.

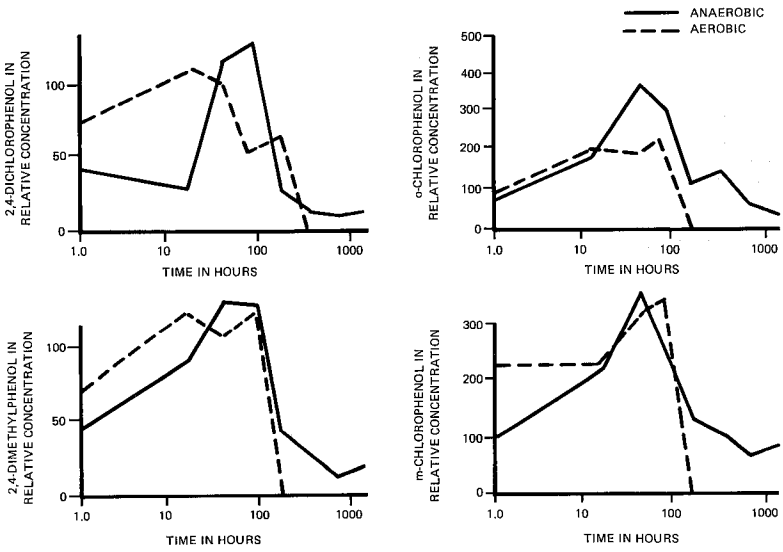

FIGURE 5 Changes in the relative concentration of various phenolic components in unpreserved sludge over time II.

TABLE I

Mean percent recovery of "n" dichloromethane extracted sludges

Compounds	Concentration Range mg/kg Dry Weight	Less than 1% total solids in sludge	From 1–30% total solids in sludge	Greater than 30% solids in sludge
o-Chlorophenol	0.08–90	52	38	81
m-Chlorophenol	0.12–93	85	70	83
p-Chlorophenol	0.10–90	85	56	86
o-Cresol	0.18–180	54	7	66
2,4-Dichlorophenol	0.21–200	92	46	92
2,4-Dimethylphenol	0.09–86	66	41	69
4,6-Dinitro-*o*-cresol	0.20–180	57	11	92
2,4-Dinitrophenol	0.27–500	47	22	72
Hydroquinone	0.14–220	14	31	47
Pentachlorophenol	0.17–8500	87	45	81
Phenol	0.05–290	44	22	24
2,4,6-Trichlorophenol	0.19–1300	91	55	59

(A) $n=32$ Liquid/liquid extractions in duplicate
(B) $n=34$ Liquid/liquid extractions in duplicate
(C) $n=30$ Soxhlet extractions in duplicate

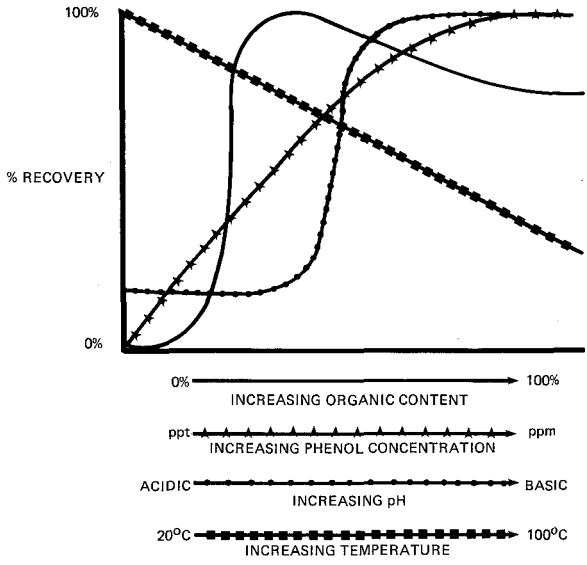

FIGURE 6 General trends for recovery of phenols from oven dried sludge.

size with gel permeation chromatography (GPC), separation by polarity with florisil or silica gel columns, separation by pKa with pH buffer partitioning, separation by affinity to an XAD divinylbenzene polystyrene polymer, or the use of ion-exchangers. Preliminary tests were run with each cleanup technique in order to determine the best method or combination of methods. While every method tested worked well to some extent, only the combination of size exclusion chromatography and solvent partitioning were able to clearly isolate all phenolic species of interest within a unique fraction.[8] Acid/base/neutral partitioning is extremely useful when separating ionic species. The most effective extraction is achieved by choosing an organic solvent with a good solvent strength and a favorable partition coefficient for the compounds of interest.

For separation by GPC, a test standard showed the phenols of interest eluting within a 250 mL range. Phenol was the first compound to elute at the 300 mL fraction and pentachlorophenol the last component to be removed after washing the column with 550 mLs of eluant. This allowed the early eluting large organic polymers, proteins and fatty acids, as well as later eluting small molecule interferences, to be isolated from the phenol fraction. With an increased separation time, phenols can be further separated on the basis of molecular size by simply using a gel with a lower exclusion limit, such as S-X8 biobeads, with an effective molecular weight range under 1,000.

The most frequently employed liquid chromatographic detectors are ultraviolet (UV), fluorescence, refraction index and electrochemical. Fluorescence, UV and electrochemical detectors give good sensitivity, electrochemical being superior for phenols. The spectrophotometric detectors work on the principle that the phenol's benzene ring, a chromatophore, absorbs light energy. The electrochemical or amperometric detector works on the basis that the phenol's hydroxyl group is easily oxidized (Figure 7).[9] When the phenol enters the electrochemical cell, it is oxidized by a carbon paste electrode.[10] The pH of the mobile phase can be critical. For phenols, a pH approximately equal to 4.5 appears to be optimum. One can expect a change in the optimum oxidation potential of 50–60 millivolts per pH unit variation.[11]

Due to the large matrix variability between sludges, duplicates and internal standards of all compounds were randomly run on 20% of

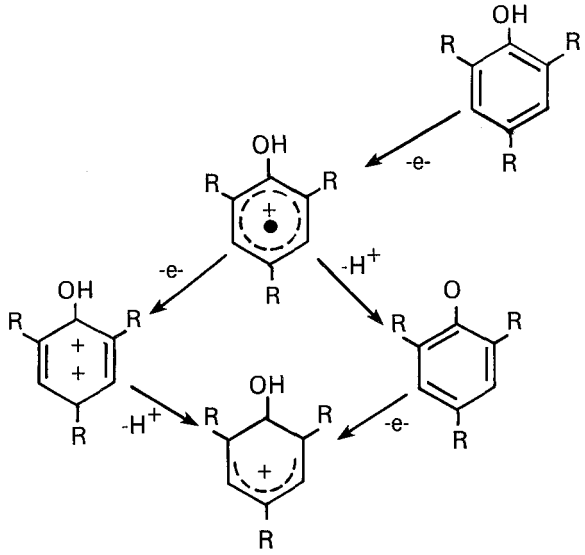

FIGURE 7 Electrochemical oxidation of phenol.

TABLE II

Percent recovery of phenols at the detection limit from 96 unique sludge matrices

Phenols	1-100% Solids Minimum Det. Limit mg/kg	Percent Recovery Maximum	Minimum	Mean	Standard Deviation
o-Chlorophenol	0.03	130.0	0.0	55.0	41.0
m-Chlorophenol	0.03	160.0	11.0	83.0	35.0
p-Chlorophenol	0.03	160.0	13.0	81.0	33.0
o-Cresol	0.03	110.0	0.0	52.0	34.0
2,4-Dichlorophenol	0.03	160.0	3.9	87.0	43.0
2,4-Dimethylphenol	0.03	160.0	3.3	61.0	42.0
4,6-Dinitro-*o*-cresol	0.06	160.0	0.0	56.0	53.0
2,4-Dinitrophenol	0.18	100.0	3.4	44.0	38.0
Hydroquinone	0.07	98.0	0.0	20.0	29.0
Pentachlorophenol	0.03	150.0	52.0	72.0	40.0
Phenol	0.03	83.0	0.0	37.0	25.0
2,4,6-Trichlorophenol	0.06	150.0	0.0	73.0	49.0

the samples to determine percent recovery and maintain quality control (Table II). The large variability in recovery data is typical in trace level analysis of sludges. Several workers have reported recoveries in sludge analysis which range from 0% to over 200%.[12–14] Spikes were introduced into sludges consisting of less than 30% solids prior to extraction via a methanol solution. Sludges with greater than 30% total solids content were spiked with an identical methanol solution previous to continuous soxhlet extraction. Blanks of distilled deionized water were run periodically to check for cross contamination. A stock solution containing all phenols of interest at a concentration of 10,000 ppm was made up every 14 days. Dilutions of this stock solution were made in methanol and methylene chloride every two days for use as internal spikes or external standards, respectively. All standards were stored at 4°C in the absence of light when not in use.

CONCLUSION

The unique character of substituted phenolic compounds requires that special handling and methodology be taken into account. The inherent characteristics of the trace components combined with the complexity of many environmental matrices greatly complicates the tasks of environmental trace analysis. Presented here is an effective method by which to determine the presence of a select group of phenols, classified as priority pollutants, at trace levels in a municipal sludge matrix.

When sampling for phenolics, the following precautions should be taken:

— Use amber glass jars which are organic free and silanized.
— Preserve sample via 4 g/L $CuSO_4$ and store at 4°C.
— Reduce the pH to 3.0 with phosphoric acid.
— Analyze within 7 days.

Extraction of phenolics from a sludge matrix can be achieved using liquid/liquid acid/base/neutral solvent extraction with DCM via centrifugation. Subsequent cleanup of the resulting extract is possible by GPC and unless HPLC is used for component analysis, further cleanup by polarity on a florisil column may be needed. Some gas chromatographic methods may require the derivatization

of phenolic species prior to analysis.[15–17] The most selective and sensitive detector for the determinization of phenols by HPLC was found to be an electrochemical detector. In the oxidation mode, it is capable of detecting phenols at femtogram levels. Limits of detection in biosludge extract sample matrices ranged from 20 pg for most phenolic species to 120 pg for 2,4-Dinitrophenol. The electrochemical detector performed reproductively from day to day with only a slight decrease in sensitivity after several days of continuous service. Glassy carbon electrodes can be easily cleaned even after weeks of heavy service with complex environmental matrices. No calibration time is required between samples, although careful selection of high purity solvents should be made so as to eliminate background interferences upon solvent concentration. Problems with external electrical interferences which plagued earlier electrochemical detectors can be reduced by placing a grounded faraday cage over the analytical cell.

Acknowledgments

The authors wish to acknowledge the technical assistance of Robert Schuetz and Lester Giessel, as well as the laboratory assistants responsible for performing sample workup.

References

1. L. W. Jacobs and M. J. Zabik, "Concentrations of selected hazardous chemicals in Michigan sewage sludges and their impact on land application." A grant from the Michigan Department of Natural Resources, 71-4055, 1980–1981.
2. K. Ugland *et al.*, *J. Chromatogr.*, **83,** 213 (1981).
3. M. J. Carter and M. T. Huston, *Environmental Science and Technology* **12,** 309 (1978).
4. R. Hartenstein, *Science* **212,** 743 (1981).
5. M. J. Zabik, R. A. Leavitt and G. C. C. Su, *Annual Review of Entomology.* **21,** 61 (1976).
6. F. W. Karasek, R. E. Clement and J. A. Sweetman, *Analytical Chemistry,* **53,** 1050 (1981).
7. Environmental Protection Agency, "Analytical procedures for determining organic priority pollutants in sludges." EPA-600/2-80-030 (1980).
8. J. H. Phillips, "The characterization of municipal sludges for thirty-two volatile, semi-volatile and phenolic trace organic components, "M.S. Thesis, Michigan State University, 1981.
9. A. J. Bard and H. Lund, *Encyclopedia of Electrochemistry of the Elements.* (Marcel Dekker, New York, 1978), Volume XI, pp. 272–275.

10. P. T. Kissinger *et al.*, *Clinical Chemistry* **20,** 992 (1974).
11. R. E. Shoup and G. S. Mayer, *Analytical Chemistry* **54,** 1164 (1982).
12. Environmental Protection Agency, "Fate of semivolatile priority pollutants in a wastewater treatment plant." EPA 600/2-81-056 (1981).
13. Environmental Protection Agency, "Fate of priority pollutants in publicly owned treatment works, Pilot Study." EPA-440/1-79-300 (1979).
14. Environmental Protection Agency, "Fate of priority pollutants in publicly owned treatment works." EPA-440/1-80-301 (1980).
15. L. L. Lamparski and T. J. Nestrick, *J. Chromatogr.* **156,** 143 (1978).
16. M. Mattsson and G. Petersson, *Journal of Chromatographic Science* **15,** 546 (1977).
17. N. K. McCallum and R. J. Armstrong, *Journal of Chromatography* **78,** 303 (1973).