Bull. Environ. Contam. Toxicol. (1987) 39:524–532

Environmental Contamination and Toxicology

Direct Determination of Benzidine in Unaltered Soil Solution by Liquid Chromatography

A. I. Ononye, J. G. Graveel,* and J. D. Wolt

Department of Plant and Soil Science, University of Tennessee, Knoxville, Tennessee 37901-1071

Benzidine (4,4'-diaminobiphenyl) is an aromatic amine which is employed in the manufacture of dyes and paints (USEPA 1979a). It is also commonly used as a stain in biological studies (Gurr 1956; McManus and Mowry 1965). Because this compound has been implicated as a carcinogen (Clayson 1976; USEPA 1980), its presence in the environment is a source of concern. Land disposal of benzidine-containing waste materials is a major source of soil contamination. In previous work, Graveel et al. (1985; 1986) added ^{14}C-labeled benzidine to soils and found that the majority of the ^{14}C was nonextractable. These and similar studies using radiolabels to trace the fate of organic pollutants in soils (Boyd et al. 1984; Berry and Boyd 1985), do not indicate the concentrations of the trace organics which may be supported in soil solution i.e., dissolved in the free water in the soil. Direct determination of a pollutant in soil solution would indicate the actual levels retained in solution for absorption by plant roots and for groundwater contamination. Also, information could be gained on the nature of the pollutant in soil solution and this would be helpful in developing effective cleanup procedures such as those involving enhanced binding of pollutant to soil solid phase (Berry and Boyd 1985; Liu et al. 1987).

A number of methods are available (USEPA 1979b; Riggin and Howard 1979; Rice and Kissinger 1982) for extraction and determination of benzidine (BZ) in various samples, but these do not provide an indication of the levels and exact nature of BZ retained in soil solution. Also, many of these procedures employ solvent extraction which by itself could lead to unknown side reactions (Armentrout and Cutie 1980). In this paper, we report determination of BZ in soil solution without sample pretreatment. Vacuum displacement (Wolt and Graveel 1986) is employed to obtain soil solution while the analysis is performed by high performance liquid chromatography (HPLC) with UV detection. Mass spectroscopy is further used to verify the identity of observed peaks.

*Correspondence and reprint requests.

MATERIALS AND METHODS

Field moist soil samples (12.5% moisture) obtained from B horizons of Fullerton clay (Typic Paleudult) were hand-sieved through a 4 mm pore-size screen and stored moist in loosely-knotted plastic bags.

Benzidine was purchased from Sigma Chemical Company. Solutions were prepared fresh when needed in 0.05% acetic acid (pH 4.5 to 4.7) in distilled-deionized water and then applied to 1 kg moist soil by spraying and mixing to give the required concentration. The initial concentration of BZ applied was in the range of 20 to 50 mg BZ per kg oven-dry soil, and the soil moisture content after addition of BZ solution was usually 17%. This was approximately 30% below the estimated moisture content at field capacity (23.7% at -33 kPa water potential). Amended soils were packed in batches of 50 gm to a volume of 33-34 cm^3 in a 60 cm^3 syringe barrel containing a 25 mm diameter metricel (DM) filter membrane of 0.45 µm pore size and were then incubated at 25°C. Soil solution was obtained diretly from the incubation vessel by vacuum displacement as previously described (Wolt and Graveel 1986). Benzidine in soil solution was monitored over a period of 192 hours starting from 0.5 hours after the addition of BZ solution to soil; three replicates were taken at each sampling. Care was taken to exclude light during mixing, incubation, and displacement of soils. Displaced solutions were immediately analyzed or were refrigerated at 4°C for subsequent analysis.

UV-Visible spectra were obtained on a Beckman DU-7 UV-Visible spectrophotometer.

HPLC was performed with Waters Model 501 pumps equipped with a Model 680 gradient controller and a U6K injector with a 2 mL fixed sample loop, Model 481 UV-Visible detector and Model 740 data module. The analytical column was a reverse phase Nova Pak C_{18}, 3.9 mm x 15 cm, column (Waters Associates). Unless otherwise stated, a 200 µL aliquot of the sample was injected for each run. The mobile phase was 40/60 (v/v) acetonitrile and 0.2 M acetic acid/acetate buffer (pH 4.0).

Mass spectra were obtained on a Finnigan 4000 gas chromatography-mass spectrometer which utilized a DB-5 silica capillary column (30 m long).

RESULTS AND DISCUSSION

The absorption spectrum of BZ was first examined in order to determine the appropriate wavelength to monitor during HPLC analysis. Benzidine has two major absorption bands with λmax around 210 nm and 280 nm, respectively (Figure 1a). The 210 nm peak shows a higher extinction coefficient, but it rapidly decreases and becomes significantly red-shifted with a decrease in solvent pH while the 280 nm peak is relatively unaffected (Figure 1b). The absorption band around 280 nm is due to the aromatic ring of BZ and would be less perturbed by minor transformation or derivatization of BZ.

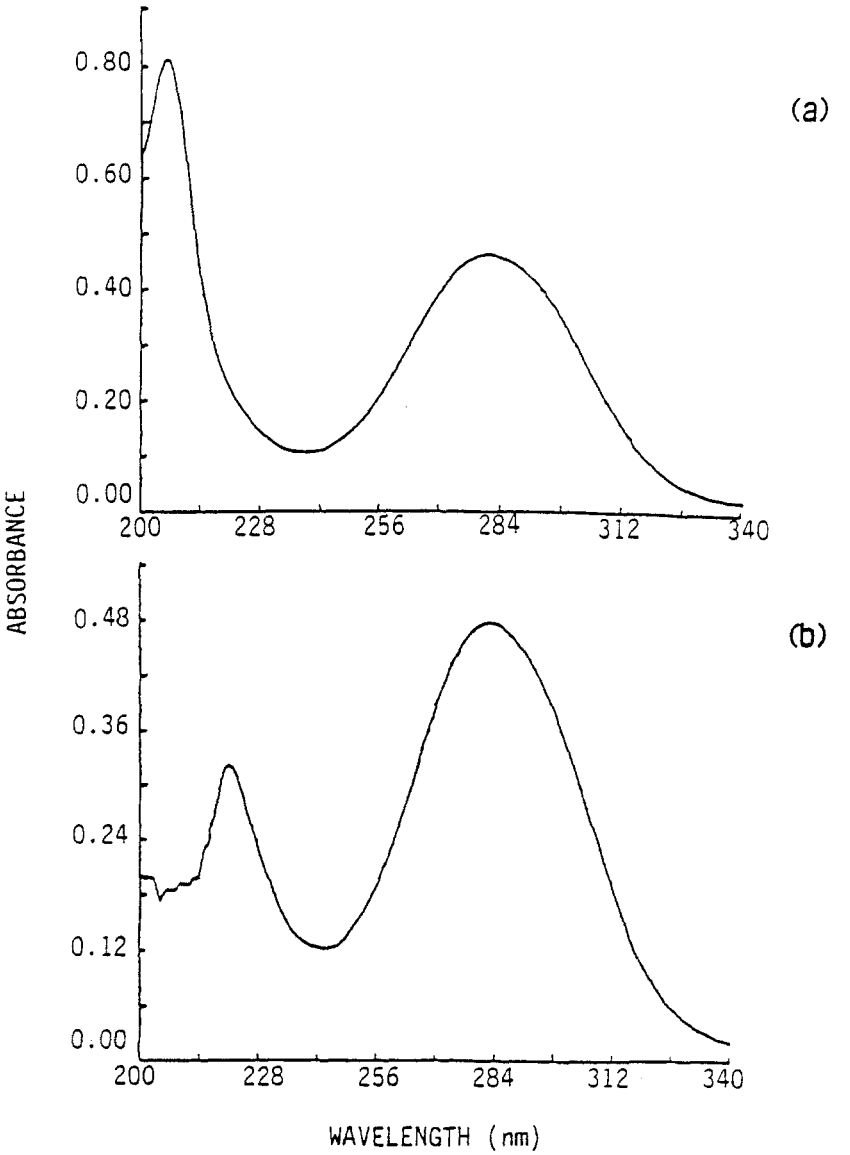

Figure 1. UV-Visible spectrum of benzidine in: (a) 50/50 (v/v) H_2O/CH_3CN; (b) 50/50 (v/v) CH_3CN/0.2M acetate buffer, pH 4.5.

Hence, by monitoring at 280 nm, it would be possible to view the parent compound simultaneously with any derived transient or product. This strategy seems to make the UV-Visible detection very useful in HPLC analysis even though it may not be as sensitive as electrochemical detection (Armentrout and Cutie 1980). Moreover, since eluting species are not modified or transformed by the detection technique, fraction collection could be done and the samples used for further analysis.

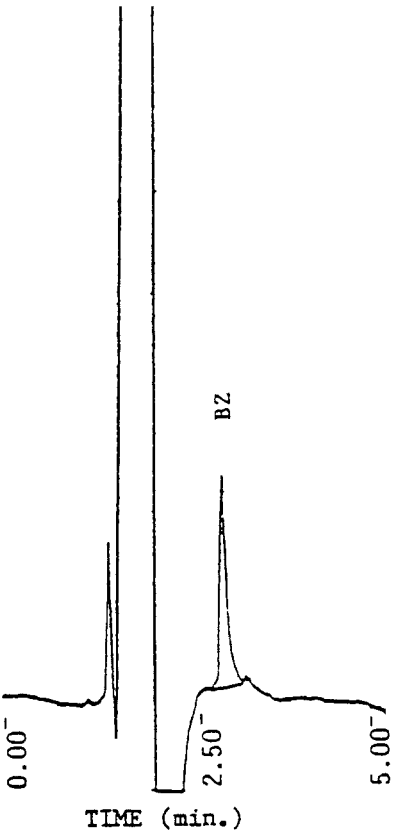

Figure 2. Peak form obtained by HPLC analysis of 5 µg/L benzidine in H_2O. Injection volume 200 µL, mobile phase 40/60 (v/v) CH_3CN/0.2M acetate buffer, pH 4.0, flow rate 0.9 mL/min.

Figure 2 shows the peak form obtained by HPLC analysis for a low standard concentration (5 ug/L) of BZ. The peak corresponding to benzidine is labeled BZ, and in typical experiments, this low concentration could be determined with a signal-to-noise ratio of 10 without any pre-concentration of sample. With sample pre-concentration using C_{18} Sep-Paks, lower concentrations down to 0.1 ug/L are resolvable.

Chromatographic results for a typical incubation experiment starting with 30 mg BZ per kg oven-dry soil are shown in Figure 3. The peaks labeled A and C are due to unknown species present in the background soil solution (see Figure 3c for the control chromatogram).

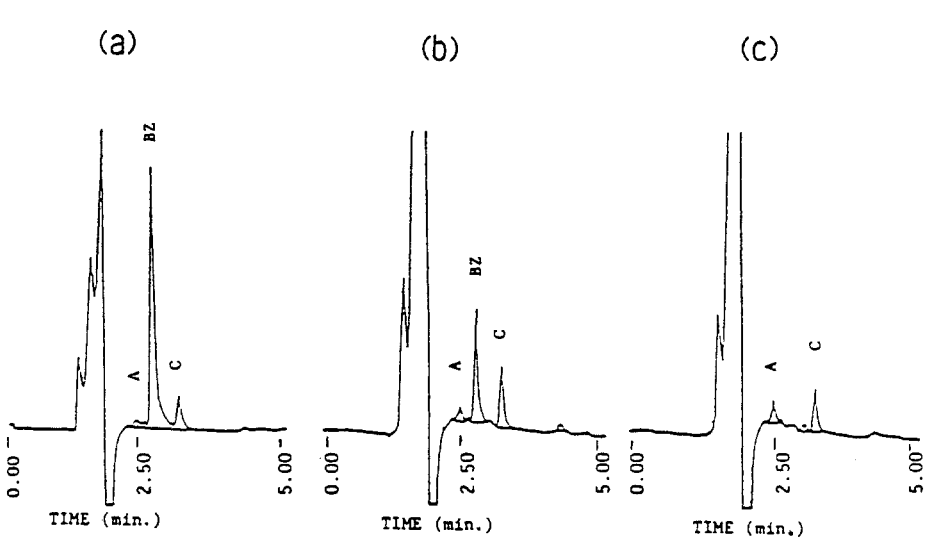

Figure 3. Typical results obtained by HPLC analysis of displaced soil solution from benzidine-amended soils (initial treatment 30 mg benzidine/kg oven-dry soil). Injection volume 100 µL, mobile phase 40/60 (v/v) CH_3CN/0.2M acetate buffer pH 4.0, flow rate 0.8mL/min: (a) 6-hour incubation; (b) 24-hour incubation; (c) control soil solution with no benzidine.

Table 1. Values for the concentrations of benzidine determined by HPLC analysis of displaced soil solutions for initial treatments of (a) 30 mg benzidine per kg oven-dry soil (b) 50 mg benzidine per kg oven-dry soil.

Time of Incubation (Hours)	(a) [BZ] in soil solution (mg/L)	(b) [BZ] in soil solution (mg/L)
0.5	0.128 ± 0.002	-
0.8	-	0.109 ± 0.002
6.0	0.021 ± 0.001	0.056 ± 0.001
12.0	0.017 ± 0.002	0.036 ± 0.001
24.0	0.007 ± 0.001	0.037 ± 0.001
48.0	0.006 ± 0.001	0.027 ± 0.001
96.0	0.007 ± 0.001	0.022 ± 0.001
192.0	0.006 ± 0.001	0.023 ± 0.002

Table 1 gives values of the analytical results obtained for the retention of BZ in soil solution during an 8-day incubation study for two different initial treatments. Three replicates were taken during each sampling, and the precision of the results is quite encouraging; we attribute this to the high uniformity achieved in

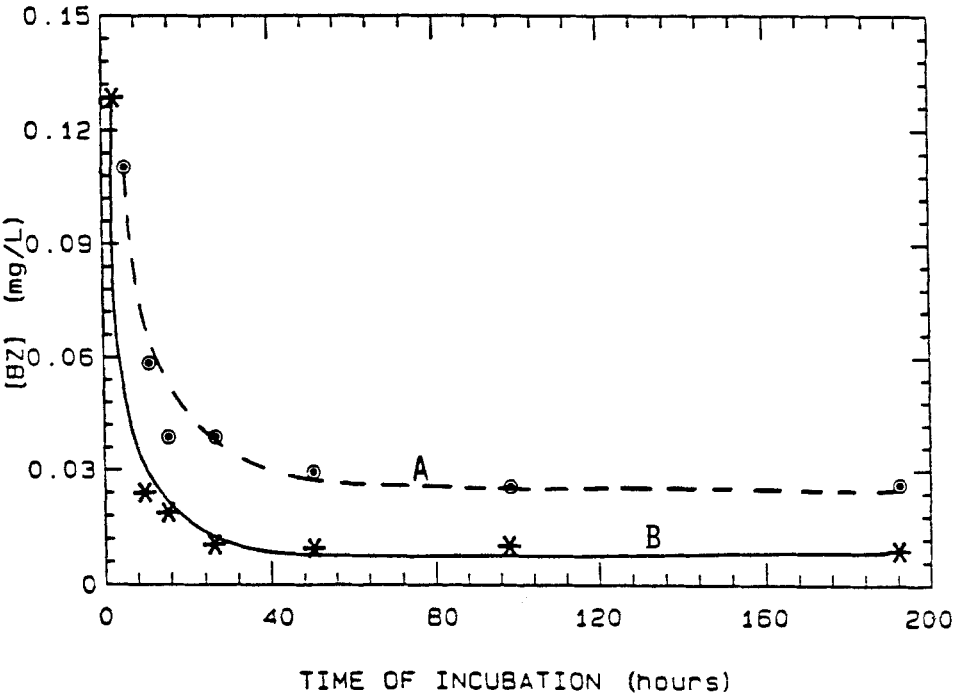

Figure 4. Plot for the retention of benzidine in soil solution. Curves A (broken line) and B (solid line) are for initial treatments of 50 and 30 mg benzidine/kg oven-dry soil, respectively.

mixing the soil with BZ solution and the consistency of the vacuum displacement technique. When plotted (Figure 4), the values in Table 1 indicated that on addition to soil, BZ concentration decreases rapidly within 6 hours and then decreases slowly to low levels within 48 hours after which it remains essentially constant at approximately 0.022 and 0.006 mg/L, respectively, for initial treatments of 50 and 30 mg BZ per kg oven-dry soil. Benzidine concentration of 0.018 mg/L is considered carcinogenic to humans (Sax et al. 1984); therefore despite the immobilization of BZ in the soil solid phase, BZ-contaminated soils are capable of supporting biologically consequential levels of BZ in soil solution.

The identity of the parent benzidine in displaced solutions was further confirmed by its mass spectrum. The mass ion chromatogram of benzidine-treated soil solution (Figure 5a) resembles that of the control soil solution (Figure 5b) except for the peak eluting at about 13.40 minutes in the former; this is due to BZ, and mass analysis gives a parent peak at m/z 184 (Figure 5c), corresponding to the mass of benzidine. No transformation products could be detected in the mass spectral analysis.

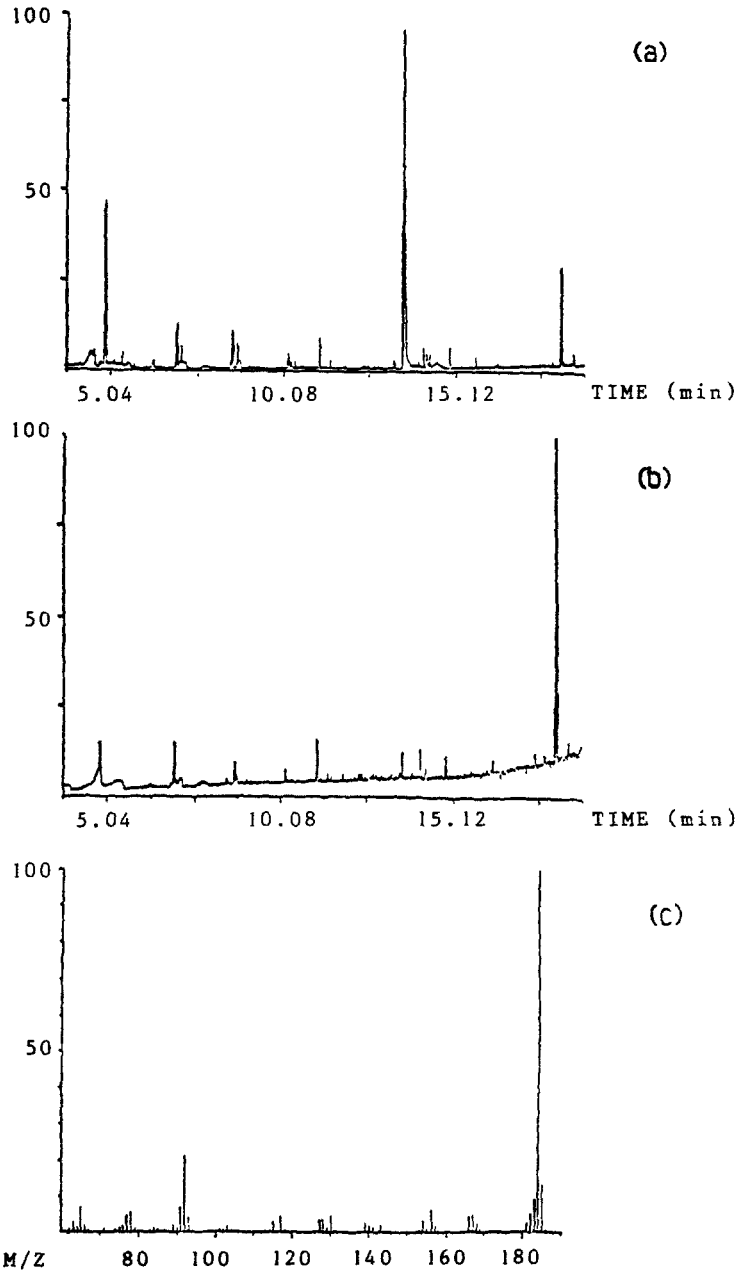

Figure 5. (a) Total ion chromatogram of soil solution from benzidine-amended soil; (b) Total ion chromatogram of control soil solution (untreated); (c) Mass spectrum of benzidine obtained by mass analysis of the peak at 13.40 minutes in (a).

This paper has presented an effective method of obtaining and analyzing unaltered soil solution for the toxic amine benzidine without sample pretreatment. The vacuum displacement technique does not employ solvent extraction and so the problem of side reactions (Armentrout and Cutie 1980) which would otherwise alter the true concentration of BZ in soil solution or obscure observation of any transient peaks is eliminated. HPLC analysis with UV detection was very useful in this study, since adequate sensitivity (5 µg/L) could be achieved with no apparent interference from the numerous species present in soil solution. With the proper choice of column and mobile phase, this approach could be applied to the environmental monitoring of a number of trace organics.

As shown by the HPLC results, no peaks corresponding to transients or transformation products from BZ were detected in this study. Previous studies (Graveel et al. 1985; 1986) have shown that BZ is resistant to microbial decomposition with respect to CO_2 evolution, and that the bulk of the amine remains with the humic acid fraction of soil organic matter. It appears, therefore, that BZ binds directly to the components of humic acid in soil. It may well be that oxidative coupling to the quinonoid groups in humic acid is responsible for this as proposed for other aromatic amines (Parris 1980; You et al. 1982; Saxena and Bartha 1983). We are now carrying out further investigations regarding the probable binding mechanism.

Acknowledgments. We thank Dennis Catalano for his technical assistance with mass spectrometry analyses. This investigation was supported by the Applied Sciences Division of the Center of Excellence in Hazardous Waste Management, University of Tennessee, Knoxville.

REFERENCES

Armentrout DN, Cutie SS (1980) Determination of Benzidine and 3,3-dichorobenzidine in wastewater by liquid chromatography with UV and electrochemical detection. J Chromatogr Sci 18: 370-374

Berry DF, Boyd SA (1985) Decontamination of soil through enhanced formation of bound residues. Environ Sci Technol 19: 1132-1133

Boyd SA, Kao C-W, Suflita JM (1984) Fate of 3,3'-dichlorobenzidine in soil: persistence and binding. Environ Toxicol Chem 3: 201-208

Clayson, DB (1976) Occupational bladder cancer. Prev Med 5: 228-244

Graveel JG, Sommers LE, Nelson DW (1985) Sites of benzidine, α-naphthylamine, and p-toluidine retention in soils. Environ Toxicol Chem 4: 607-613

Graveel JG, Sommers LE, Nelson DW (1986) Decomposition of benzidine, α-napthylamine, and p-toluidine in soils. J Environ Qual 14: 53-59

Gurr E (1956) A practical manual of medical and biological staining techniques. Interscience Publishers, Inc. New York

Liu S-Y, Minard RD, Bollag JM (1987) Soil-catalyzed complexation of the pollutant 2,6-diethylaniline with syringic acid. J Environ Qual 16: 48-53

McManus JFA, Mowry RW (1965) Staining methods: histologic and histochemical. Harper and Row Publishers. New York

Parris GE (1980) Covalent binding of aromatic amines to humates: Reactions with carbonyls and quinones. Environ Sci Technol 14: 1099-1106

Rice JR, Kissinger PT (1982) Liquid chromatography with precolumn sample preconcentration and electrochemical detection: Determination of aromatic amines in environmental samples. Environ Sci Technol 16: 263-268

Riggin RM, Howard CC (1979) Determination of benzidine, dichlorobenzidine, and diphenylhydrazine in aqueous media by high performance liquid chromatography. Anal Chem 51: 210-214

Sax NI, Benjamin F, Fitzgerald JJ, Haley TJ, Weisburger EK (1984) Dangerous properties of industrial materials. Van Nostrand Reinhold Company, New York

Saxena A, Bartha R (1983) Modeling of covalent attachment of chloroaniline residues to quinoidal sites of soil humus. Bull Environ Contam Toxicol 30: 485-491

US Environmental Protection Agency (1979a) Status assessment of toxic chemicals: Benzidine. EPA-600/1-79-210e USEPA Office of Research and Development. Cincinnati, OH

US Environmental Protection Agency (1979b) Guidelines establishing test procedures for the analysis of pollutants, method 605. Federal Register 44: 69489-69491

US Environmental Protection Agency (1980) Preliminary risk assessment: Phase I. Benzidine, its congeners, and their derivative dyes and pigments. EPA-560/11-80-019 USEPA Office of Pesticides and Toxic Substances. Washington, D. C.

Wolt JD, Graveel, JG (1986) A rapid routine method for obtaining soil solution by vacuum displacement. Soil Sci Soc Am J 50: 602-605

You I-S, Jones RA, Bartha R (1982) Evaluation of a chemically defined model for the attachment of 3, 4-dichloroaniline to humus. Bull Environ Contam Toxicol 29: 476-482

Received November 24, 1986; accepted April 3, 1987.