time they had been transferred to pots and moistened with water, the results clearly demonstrate that microbial activity is the major factor in the loss of these insecticides.

The degradation rates in the Cloverdale soil are obviously quite different from the general pattern shown by the other soils. The pH of the Cloverdale soil was significantly lower than that of all other soils and may possibly have affected the degradation rate. The Cloverdale soil was also the only organic soil in the experiment and adsorption onto the larger organic fraction may have contributed significantly to the decreased degradation rate.

LITERATURE CITED

Kaufman, D. D., Haynes, S. C., Jordan, E. G., Kayser, A. J., *ACS Symp. Ser.* **No. 42** (1977).
Williams, I. H., *Pestic. Sci.* **7**, 336 (1976).

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Quantitative Thin-Layer Chromatographic Determination of Br^- Residues in Crops after Soil Treatment by Methyl Bromide

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A procedure is described for the quantitative thin-layer chromatographic determination of Br^- residues in crops grown on soils treated with methyl bromide. The samples are dried and calcinated. The ashes are extracted with water. The Br^- containing solution is spotted on a heated silica gel coated chromatographic plate. The plate is covered with a glass plate just above the spots, sprayed successively with a fluorescein solution and a mixture of glacial HOAc and 30% H_2O_2. After heating, Br^- reacts with fluorescein to form eosin. Bright red spots appear on a yellow background. This reaction is specific for Br^-. After development of the plate, fluorescein, eosin, and small amounts of other brominated products of fluorescein are distinctly separated. The eosin spots are marked and scraped off. The eosin is eluted from the silica gel with distilled water and measured spectrophotometrically at 521 nm. The calibration curve is linear from 10 to 90 ppm Br^-. The results obtained by this method are compared with an X-ray fluorescence spectroscopic technique.

Intensive crop cultures, especially in greenhouses, require a careful control of the soil conditions. Furthermore, to obtain good yields in those cultures it is necessary to eliminate harmful effects of weeds, mold, and eelworms as much as possible. Therefore, the treatment with pesticides, fungicides, and soil disinfectants is practically unavoidable. Their use, however, may give rise to new problems, such as those related to the presence of their residues in the plant.

Among the many soil disinfectants methyl bromide takes an important place because of its efficacy. After application, CH_3Br is rapidly converted to inorganic bromide in the soil and can be accumulated by the plant in that form. Apart from the aspect of public health related to the presence of the bromide, it can also cause phytotoxic action.

For assessing the bromide residues in plant material, there are several methods of analysis. A method based on "neutron activation analysis" has been described by Guinn and Potter (1962), Castro and Schmitt (1962), and Stärk et al. (1971). A promising technique proves to be X-ray fluorescence spectroscopy (Van Cauwenberge and Gordts, 1977). A colorimetric procedure has been developed by Drosihn (1967) and Malkomes (1970) and is based on calcination of the plant material and oxidation of bromide to hypobromite, which reacts, in a buffered medium, with phenol red to form tetrabromophenol blue. A gas chromatographic technique has been proposed by Heuser and Scudamore (1970). In this method the inorganic bromide reacts with ethylene oxide in diisopropyl ether to form 2-bromoethanol which can be determined by gas chromatography.

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However, these methods suffer from certain disadvantages. The first two require a special and costly instrumentation while the third and the fourth techniques are rather cumbersome and time consuming.

In this work it was our purpose to evaluate the possibilities of a technique which is fairly rapid, specific, and easily applicable, by simple means, in the laboratories dealing frequently with pesticide residue analysis. We thought that calcination, separation of the halides with thin-layer chromatography, and, after scraping of the spots, spectrophotometric determination might offer a solution. Separating techniques are described by Gagliardi and Likussar (1965) and Berger et al. (1964). The separation of Br^- was good but the methods proved inadequate for further quantitative work. Feigl (1954) showed that the bromination of fluorescein made a very sensitive and specific spot test. Axelrod et al. (1971) took this as a basis for the fluorimetric determination of bromine in aerosols.

We successfully tried the separation of halides on TLC silica gel plates with fluorescein and glacial HOAc–H_2O_2 as visualizing reagents. After development of the plates they were sprayed with an alcoholic solution of sodium fluorescein immediately followed by spraying with a freshly prepared mixture of glacial HOAc–30% H_2O_2 (50:50). After heating for 5 min at 110 °C, a well-defined red spot of tetrabromofluorescein (eosin) appears on a yellow background. Three bromo-substituted products of fluorescein are also visible with minor intensity. The other halides are not revealed.

Again the background intensity did not allow the quantitative determination. To eliminate this disturbance the color formation was carried out before the separation. For this purpose the TLC plate is covered with a glass plate just above the spots, sprayed with the reagents, heated, and developed. Solvents for the separation of fluorescein and eosin are proposed by Naff and Naff (1963)

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and Rettie and Haynes (1964). We found, however, that a solvent with the composition acetone–1-butanol–concentrated NH_3–distilled water gave better results. When using this solvent eosin, the three bromo-substituted fluorescein products and fluorescein were sharply separated. Following this procedure the quantitative spectrophotometric determination was possible.

EXPERIMENTAL SECTION

Reagents. Only A.R. grade chemicals were used. Standard solutions: (from NaBr) 100, 300, 500, 700, 900 μg/mL of Br^- in distilled water. Glass precoated TLC plates 25 × 25 cm. Silica gel 60, layer thickness 0.25 mm (Merck). Glass TLC precoated plates, 10 × 10 cm silica gel 60, for nano TLC (Merck) spraying reagents: 0.1% Na fluorescein in 50:50 ethanol–distilled water; glacial HOAc–30% H_2O_2 (50:50) mixed immediately before use.

Apparatus. A hot plate having the same dimensions (25 × 25 cm) as the TLC plates was used. When this is not available a metal sheet 1 mm thick can be fixed on an ordinary hot plate. A 50-μL syringe for TLC fitted with a repeating dispenser (Hamilton Co. PB-600-1) was used.

Analysis Procedure. *Sample Preparation.* A hundred grams of plant material is dried overnight (8 h) in a vacuum desiccator at 130 °C. The percentage of dry material is noted. The dried material is then ground to a fine powder by means of an IKA, mill type A 10 (Janke and Kunkel). The samples, approximately 2 g, are exactly weighed in porcelain crucibles (diameter, 5 cm; height 2 cm). To avoid losses of Br^- during the ashing procedure, 5 mL of 0.1 N NaOH is added to the crucibles and the content is carefully mixed with a fine glass rod. After drying in an oven at 110 °C for 1 h, the crucibles are placed in a muffle furnace. The time taken to reach a temperature of 500 °C is 0.5 h. This temperature is then maintained for another 0.5 h. The ashing is incomplete but sufficient and without losses. After cooling in a desiccator the residue in the crucibles is finely crushed and transferred to screw-capped 10-mL tubes with 5 mL of 0.1 N HCl. The tubes are vigorously stirred with an electrical vibrating apparatus. A drop of concentrated H_2SO_4 is added, and the tubes are stirred again. The solution should be acidic (test with litmus paper). If not, another drop of concentrated H_2SO_4 may be added. The tubes are centrifuged at 3000 rpm for 3 min. The clear supernatant liquid is then ready for spotting on the TLC plate.

Calibration Curve. Because rather large quantities (25 μL) of aqueous solutions are to be applied, the heating of the TLC plate seemed to be most appropriate. Therefore it is placed on a hot plate mounted on a "lab-jack" with adjustable height. Precautions must be taken to obtain an even heating of the glass TLC plates, otherwise temperature differences may result in shattering of the plate. The 50-μL syringe fitted with the repeating dispenser is fixed in a clamp above the hot plate. A thin pencil mark is drawn along one side of the TLC plate at a distance of 1.5 cm. The TLC plate is then put on the hot plate and heated at a temperature of ±185 °C (the temperature setting is previously verified with a thermometer placed in a sand bath). The needle of the syringe is placed on the marked line barely touching the plate. A 25-μL sample of the solutions containing, respectively, 100, 300, 500, 700, and 900 μg/mL of Br^- is spotted. The plate is removed and allowed to cool for 5 min on filter paper sheets. The plate is then covered just above the spots with a glass plate and successively sprayed with the fluorescein solution and the freshly prepared mixture of glacial HOAc–30% H_2O_2. The plate is heated in an oven at 110 °C for 5 min and afterwards cooled for 5 min. The plate is placed in a saturated developing tank, lined with filter paper, in the solvent acetone–1-butanol–concentrated NH_3–distilled water (65:20:10:5). The plate is developed for a distance of 12 cm (±35 min).

Table I. Recovery Results of Lettuce Fortified with Br^-

Br^- added, ppm	sample no.	Br^- found, ppm	% recov.	mean value
80	1	77	96	89
	2	74	92	
	3	65	81	
50	1	48	96	90
	2	40	80	
	3	40	80	
	4	52	104	
20	1	17	85	88
	2	21	105	
	3	15	75	

After evaporation of the solvent the red eosin spots and a blank are carefully scraped off. The adsorbent is transferred to screw-capped 10-mL tubes with 5 mL of distilled water. After vigorous stirring with an electrical vibrator the tubes are centrifuged at 3000 rpm for 3 min. Then a sufficient volume of the supernatant liquid is pipetted in 1 × 1 cm cuvettes and measured in a spectrophotometer at 521 nm. The coloration is stable. When working with 100-g samples and when the analytical procedure is followed as for the standard solutions, the values obtained can be read in parts per million. The plot extinction against concentration is linear from 10 to 90 ppm. The linearity could stretch further but 25 μL of the solution containing 900 μg/mL of Br^- is a practical limit for the TLC. When higher concentrations are to be determined, a smaller quantity should be spotted on the plate.

Routine Determination. Before the TLC procedure a screening spot test can be effected. With a pencil a plate for nano TLC is divided in 2 × 2 cm squares. The plate is heated and 10 μL of the 100 μg/mL, the 900 μg/mL of Br^- standard solutions and 10 μL of the sample solutions are spotted. After spraying and heating as previously described, sample solutions for which the color intensity of the spots is inferior to that obtained with the 100 μg/mL of Br^- standard solution can be discarded. From sample solutions for which the color of the spots is more intense than the color of the spot from the 900 μg/mL of Br^- standard solution a smaller quantity should be taken for the proper TLC determination.

Since the calibration curve may vary a little from one determination to another, 25 μL of the 100, 500, and 900 μg/mL of Br^- standard solutions are spotted along with 25 μL of six sample solutions to be measured. The calibration curve for the three known concentrations is drawn, and the corresponding values for the samples are noted.

When 25 μL are spotted on the plate the bromide content in the fresh plant material can be calculated in ppm with the formula:

$$(50 \times X)/w \times D/F$$

where X is the value read from the plot; w, the exact weight of the dry material taken for the analysis (approximately 2 g); and D/F, the ratio dry material/fresh material from the sample (100 g).

RESULTS AND DISCUSSION

To test the validity of the method, standards were prepared from bromide-free plant material (lettuce, examined for bromide content by X-ray fluorescence

Table II. Recovery Results of Lettuce Naturally Contaminated with Br^-

Br^- content (XRFS), ppm	sample no.	Br^- found, ppm	% recov.	mean value
42	1	30.96	73	80.75
	2	35.29	84	
	3	34.20	81	
	4	36.02	85	

Table III. Comparison Tests XRFS-TLC

sample no.	concn of Br^-, ppm	
	XRFS	TLC
	A. Tomatoes	
1	52.0	62.35
2	12.0	11.00
3	67.0	68.63
4	66.0	56.65
5	80.0	85.80
6	26.5	25.64
7	172.5	155.00
8	168.0	180.00
9	137.0	146.20
10	15.5	11.56
	B. Carrots	
1	57.5	44.20
2	102.0	110.20
	C. Lettuce	
1	67.0	69.00
2	52.5	71.00
3	53.5	52.93
4	27.0	21.57

spectroscopy). A known quantity of NaBr was added to this and thoroughly mixed with a high-speed laboratory mill (Janke and Kunkel, Type A 10). Standards containing 80, 50, and 20 ppm Br^- were prepared by successive dilution with blank plant material. Several determinations were carried out on the standards (Table I). When the inherent imperfection of such a "powder dilution", especially at low levels, is taken into account, the recoveries can be considered satisfactory. With a more even Br^- distribution, as in the case of contaminated plant material, the variability is much lower (Table II).

To verify the recoveries from naturally contaminated crops, a sample of lettuce grown on soil treated with methyl bromide was taken. The bromide content was first measured with X-ray fluorescence spectroscopy (XRFS) and then several TLC determinations were made on the same sample. The results were also acceptable (Table II).

Finally a number of samples were analyzed with X-ray fluorescence spectroscopy (XRFS) which is a nondestructive method. The same samples were examined with the described TLC technique and the results were compared (Table III). For tomato samples a statistical evaluation was made and a factor $r = 0.99$, which is a highly significant value, was found. This means that the results of the two methods are very well correlated.

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LITERATURE CITED

Axelrod, H. D., Bonelli, J. E., Lodge, J. P., Jr., *Environ. Sci. Technol.* **5**, 420–422 (1971).
Berger, J. A., Meyniel, G., Petit, J., *C.R. Hebd. Seances Acad. Sci.* **259**, 2231 (1964).
Castro, C. E., Schmitt, R. A., *J. Agric. Food Chem.* **10**, 236–239 (1962).
Drosihn, U. G., Thesis, Hannover, 1967.
Feigl, F., "Spot Tests in Inorganic Analysis", 5th ed, Elsevier, New York, N.Y., 1954, p 246.
Gagliardi, E., Likussar, W., *Mikrochim. Acta*, 765–769 (1965).
Guinn, V. P., Potter, J. C., *J. Agric. Food Chem.* **10**, 232–236 (1962).
Heuser, S. G., Scudamore, K. A., *Pestic. Sci.* **1**, 244–249 (1970).
Malkomes, H. P., Thesis, Hannover, 1970.
Naff, M. B., Naff, A. S., *J. Chem. Educ.* **40**, 534–535 (1963).
Rettie, G. H., Haynes, C. G., *J. Soc. Dyers Colour.* **80**, 629 (1964).
Stärk, H., Süss, A., Trojan, K., *Landwirtsch. Forsch.* **24**, 133–201 (1971).
Van Cauwenberge, P. P., Gordts, L. A., *J. Agric. Food Chem.* **25**, 1000–1002 (1977).

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