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A THIN-LAYER CHROMATOGRAPHIC PROCEDURE FOR THE DETECTION IN SOILS AND WATERS OF HERBICIDE RESIDUES COMMONLY USED IN SASKATCHEWAN

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SUMMARY

A thin-layer chromatographic procedure has been developed for the rapid detection of nineteen herbicides commonly used in Saskatchewan. Thus atrazine, barban, bromoxynil, dalapon, dicamba, diuron, linuron, MCPA, MCPB, MCPP, monuron, picloram, simazine, trifluralin, 2,4-D, 2,4-DB, 2,4-DP, 2,4,5-T and TBA can be collectively detected in soils at the 1 p.p.m. level and in natural waters at the 0.1 p.p.m. level.

INTRODUCTION

The use of thin-layer chromatography is well established for the detection of herbicide residues in soils and waters[1–3], and for the identification of active ingredients in mixed herbicides formulations[4].

In Saskatchewan approximately two dozen different herbicides are recommended for use on agricultural land, and with the severe climatic conditions carry-over of soil-based residues from one growing season to the next is possible. Also with spring run-off the washing of residues into streams and lakes may occur. Therefore it was considered desirable to devise a simple and routine procedure, based on thin-layer chromatography, which would detect the presence of the herbicides used in Saskatchewan both in soils and waters. This procedure could act as a preliminary screen and any residues detected could then be confirmed and the amounts present determined using the appropriate chemical analyses.

The method to be described is for the identification, both in soils and water, of nineteen herbicides used in Saskatchewan.

EXPERIMENTAL

Materials

Herbicides. The common and chemical names of the herbicides used in the studies are listed in Table I. Standard solutions, each containing 1 mg/ml of methanol,

TABLE I

COMMON AND CHEMICAL NAMES OF HERBICIDES USED IN THESE STUDIES

Common name	*Chemical name*
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
Barban	4-chlorobut-2-ynyl N-(3-chlorophenyl)carbamate
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
Dalapon	2,2-dichloropropionic acid
Dicamba	3,6-dichloro-2-methoxybenzoic acid
Diuron	N'-(3,4-dichlorophenyl)-N,N-dimethylurea
Linuron	N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea
MCPA	4-chloro-2-methylphenoxyacetic acid
MCPB	4-(4-chloro-2-methylphenoxy)butyric acid
MCPP	2-(4-chloro-2-methylphenoxy)propionic acid
Monuron	N'-(4-chlorophenyl)-N,N-dimethylurea
Picloram	4-amino-3,5,6-trichloropicolinic acid
Simazine	2-chloro-4,6-bisethylamino-1,3,5-triazine
Trifluralin	2,6-dinitro-N,N-dipropyl-4-trifluoromethylaniline
2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
2,4-DP	2-(2,4-dichlorophenoxy)propionic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TBA	2,3,6-trichlorobenzoic acid

were prepared for all herbicides excepting simazine and atrazine where solutions containing 0.25 mg/ml chloroform were used.

Reagents. These were all of analytical grade.

Soils. Three soil types were used, a heavy clay, a sandy loam and a light sand. In all cases the concentrated herbicide solutions were added to the soils at the required rate using a pipette. The excess solvent was allowed to evaporate and the soils were thoroughly mixed for several min when water was added and the soils moistened to a level intermediate between field capacity and the wilting point. Before extraction the moist soils were allowed to equilibrate for at least 24 h.

Waters. Two natural waters were utilized, one from Wascana Creek which runs through the middle of Regina, and the other from a dug-out at the Regina Research Station. Herbicides were added to these waters at the appropriate level, using a pipette, and the solution equilibrated for at least 24 h prior to extraction.

Thin-layer plates. The glass plates (20 × 20 cm) pre-coated with 250 μ layers of silica gel and containing fluorescent reagent are commercially available from E. Merck, Darmstadt. The plates were developed at 20° and the solvent allowed to run to a height of 12 cm from the origin.

Solvents. (A) Chloroform–acetic acid (190:10); (B) Benzene–hexane–acetic acid (50:100:20); (C) Hexane–acetone (150:45); (D) Chloroform–nitromethane (100:100).

Chromogenic reagent. Bromocresol Green (0.04 g) dissolved in 100 ml ethanol with sufficient 0.1 *N* sodium hydroxide added until the blue colour just persisted.

Extraction procedure

Water samples. A 500 ml aliquot of the water sample was basified with 2 ml 50% aqueous sodium hydroxide and extracted with 3 × 80 ml portions of chloroform to remove the neutral and basic herbicides. The aqueous phase was then

acidified by the addition of 5 ml 12 *N* HCl and shaken with ether (3 × 80 ml) to extract the acidic herbicides. The aqueous solution was then discarded.

The combined chloroform extracts were dried over sodium carbonate and filtered under reduced pressure. After washing the inorganic residue with a further 50 ml chloroform the solvent was removed under *vacuo* at 30° using a rotary evaporator. Chloroform (5 ml) was added to the flask to dissolve the residue and the solution transferred to a small tube. A current of dry warm air was used to reduce the volume of solution to approximately 0.2 ml. This concentrated solution was applied to the origin of a chromatographic plate which was then developed with solvent C. After drying in the oven at 110° for 3 min a second development, at right-angles to the first, was effected with solvent D. The plate was dried at 110° for 5 min and viewed under a short wave UV lamp when the presence of the neutral and basic herbicides could be detected as purple spots on a green fluorescent background. This two-dimensional chromatographic procedure successfully separated atrazine, barban, diuron, linuron, monuron, simazine, and trifluralin. The presence of trifluralin could also be observed visually on account of its yellow colour.

The ether extracts were dried by shaking with sodium sulphate. After filtration and washing the residue with 50 ml ether the solvent was reduced to about 5 ml using the rotary evaporator. The residual solution was transferred to a small tube, the volume reduced to approximately 0.2 ml using a current of dry air, and the solution applied to the origin of a chromatoplate. The plate was developed two-dimensionally, first with solvent A, and then after drying at 110° for 3 min, with solvent B. Finally the plate was dried for 20 min at 110° to remove all traces of acetic acid and sprayed with the chromogenic reagent. Thus bromoxynil, dalapon, dicamba, MCPA, MCPB, MCPP, picloram, 2,4-D, 2,4-DB, 2,4-DP, 2,4,5-T, and TBA appeared as yellow spots on a blue background.

Soil samples. The soils were air-dried at room temperature prior to extraction.

Acidic herbicides. Soil (100 g) was shaken with 250 ml 10% aqueous KCl 0.05 *N*, with respect to NaOH for 30 min. The soil solution was vacuum filtered into 20 ml 4 *N* HCl, after which the acidic herbicides present were extracted with 3 × 80 ml portions of ether. The combined ether extracts were then treated in exactly the same way as were the ether extracts in the preceding section for water samples.

Neutral and basic herbicides. Air-dried soil (50 g) was shaken for 30 min with 125 ml chloroform. After vacuum filtration the residue was washed with a further 50 ml chloroform and the combined extracts evaporated to dryness under reduced pressure. The residue was dissolved in 2 ml chloroform and the solution transferred to a column of 10 g basic alumina containing 15% water. Barban, linuron and trifluralin were eluted from the column with 50 ml carbon tetrachloride while atrazine, diuron, monuron and simazine were eluted using 100 ml ether. The carbon tetrachloride and ether eluates were pooled and evaporated to dryness when the residue was taken up in 5 ml chloroform. This solution was then concentrated, applied to a chromatographic plate and developed two-dimensionally exactly as described in the section on water samples.

RESULTS AND DISCUSSION

Typical chromatograms obtained with herbicide residues extracted from

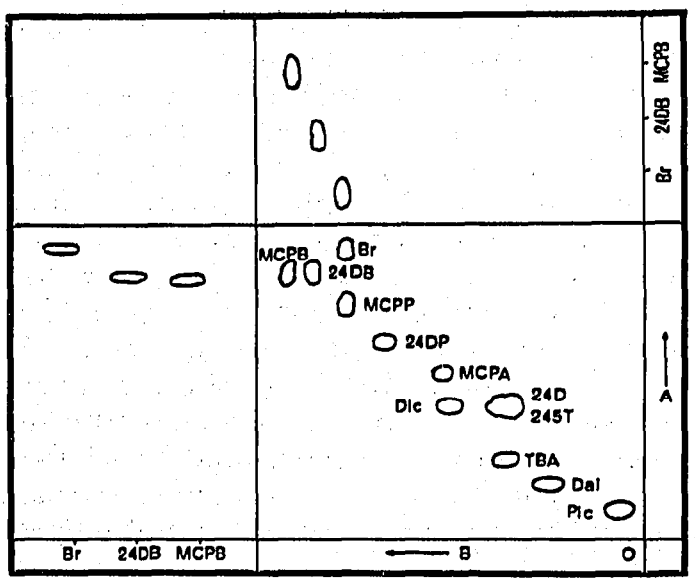

Fig. 1. Two-dimensional chromatogram of acidic herbicides recovered from soils and waters. Adsorbent: silica gel. Mobile solvents: (A) chloroform–acetic acid (190:10); (B) benzene–hexane–acetic acid (50:100:20).

fortified water and soil samples are shown in Figs. 1 and 2. These indicate that a good separation of residues can be achieved with the minimum of cleanup. It is not possible to separate 2,4-D and 2,4,5-T using this procedure as these two herbicides were found to co-chromatograph on silica gel surfaces with a variety of different solvent systems. However, if the presence of either chemical is suspected then gas chromatographic techniques[5] can be used both for separation and estimation purposes.

For the separation of the acidic herbicides a number of solvent systems were

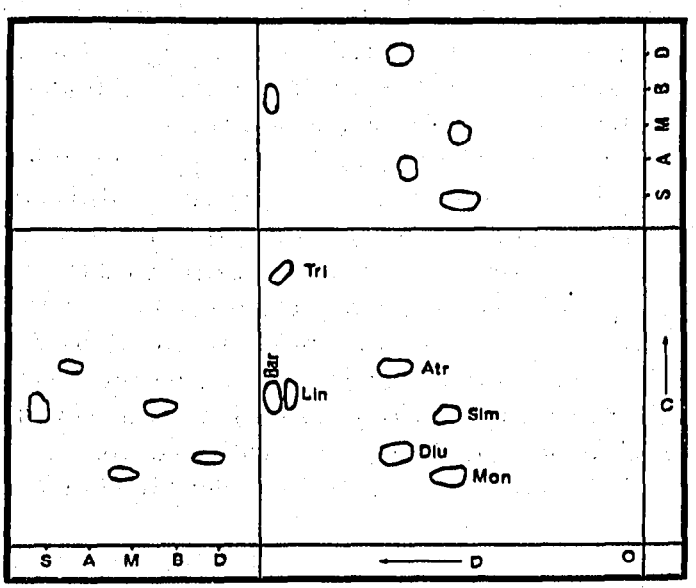

Fig. 2. Two-dimensional chromatogram of neutral and basic herbicides recovered from soils and waters. Adsorbent: silica gel. Mobile solvents: (C) hexane–acetone (150:45); (D) chloroform–nitromethane (100:100).

tried but the chloroform–acetic acid mixture (190:10) (ref. 4), followed by development with benzene–hexane–acetic acid (50:100:20) (ref. 6) resulted in the best separation. The most satisfactory separation of the non-acidic herbicides was achieved using hexane–acetone (150:45) followed by treatment with chloroform–nitromethane (100:100) (ref. 2). The plates were developed at 20° as at higher temperatures the spots became more diffuse and good resolution was not achieved. Bromocresol Green proved to be a very effective chromogenic reagent[7] and the pale yellow spots on a blue background remained visible for several hours. Chromatograms of extracts obtained from samples of untreated soils and water indicated the absence of any acidic materials.

For the identification of residues present, standards were always applied to the edge of the chromatoplate to obtain simultaneous development under identical conditions. Slight variations in R_F values were noted from day to day as has already been reported[2] when acidic solvents are used as mobile phases. However the relative degrees of separation remained similar to those depicted in Figs. 1 and 2.

Several methods were tried for the extraction of the acidic herbicides from soils. The basic KOH solution finally selected gave almost clear aqueous extracts which after acidification, ether extraction and concentration, yielded extracts which were not highly coloured. None of the neutral or basic herbicides were extracted by this solution. For these latter herbicides extraction of the dry soil with chloroform proved satisfactory. As the concentrated chloroform solution was highly coloured a column clean-up stage as described by ZIMDAHL *et al.*[8] was necessary to remove some of the soil extracts.

Using this procedure all nineteen herbicides could be detected at the 1 p.p.m. level in all three soils, which is equivalent to a rate of approximately 0.5 lb./ac. In the two natural waters detection is possible at the 0.1 p.p.m. level. Greater sensitivity could probably be achieved by taking larger quantities of soils and waters, but a more rigorous clean-up would also be necessary. It was found that when 200 g aliquots of soil were analysed, co-extracted soil material resulted in incomplete separation of the herbicides on the developed chromatograms. For all three soils used in these experiments, the soil organic matter content was less than 5%. When a soil containing 8% organic matter was used co-extracted material resulted in very poor separation of the herbicides.

Diallate and triallate are extensively used in Saskatchewan for the control of wild oats but these chemicals are not amenable to detection by this routine procedure. The presence of such residues in waters or soils can easily be checked using simple gas chromatographic analyses[9,10]. Diquat is also used as an aquatic herbicide but thin-layer chromatography is not suitable for ionic compounds. However a satisfactory spectrophotometric method for the determination of such residues in waters has been reported[11].

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