DETERMINATION OF 2,4-D AMINE IN SOILS USING ANION EXCHANGE MEMBRANES.

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Key words: 2,4-D, anion exchange membranes, soil

SUMMARY

A new method for the quantitative determination of 2,4-D in soils by use of anion exchange membranes with GC detection was developed. Preliminary investigation of ion exchange properties of pure 2,4-D acid on the membranes revealed that when a suitable solvent system is used, a quantitative recovery of 2,4-D acid can be achieved. Linear relationships between 2,4-D acid removed by the membrane and 2,4-D concentration in solutions, soil suspensions and soils were obtained within the range tested. The developed method was successfully applied for the determination of 2,4-D amine from a commercial formulation on soil surfaces. The method was tested in two concentration ranges representing a typical farm spraying application rate and a spill. The relationship between amount of 2,4-D amine detected by membranes and the spike level on the soil surface was linear for both concentration ranges. The applicability of the method was examined for a degradation study of 2,4-D amine after a spill. The low detection limit and the simplicity of the procedure make this method very suitable for 2,4-D determination in soils.

INTRODUCTION

The determination of pesticides in soils usually consists of several steps: pesticide extraction, sample cleanup and determination by GC or HPLC (Smith et al. 1989, Gutemman et al. 1964, Greer and Shelton 1992). Contaminating organic compounds are often co-extracted and may interfere with chromatographic analysis. Therefore, frequently sample cleanup can be complicated and tedious.

Ion exchange resins have been used for extraction of ionic species from variety of materials. The advantage of using ion exchange extraction over a solvent extraction is that it is more specific for compounds under investigation and thus the sample requires less or no purification before chromatographic determination. Use of ion exchange and non polar resins for extraction of pesticides has been reported in the literature. It was observed that many pesticides can be easily adsorbed and desorbed from non polar resins (Gasta and Olness 1992, Junk et al. 1976, Rees and Au 1979, Sundaram et al. 1979). Ion exchange resins were found to adsorb pesticides well but showed poor desorption characteristics (Basta and Olness 1992, Storherr and Burke 1964, Grover and Smith 1974).

The goal of this work was to examine anion exchange membranes for the determination of 2,4-D acid and 2,4-D amine in soils. 2,4-D acid is ionized in alkaline solutions while 2,4-D amine is ionized in water solutions. Therefore both can be isolated from soil and other materials by an ion exchange process. The advantage of ion exchange membranes over ion exchange resins in bead form is that they are very easy to use and many samples can be prepared at the same time (Schoenau and Huang 1991, Qian et al. 1992) while resins require columns and larger

volumes of solvents.

Our work was divided into two parts. In part (1) the exchange of 2,4-D acid on the anion exchange membranes was studied. Different solvents systems were examined for adsorption and desorption of 2,4-D acid to and from the membranes in solutions. Next, extraction of 2,4-D acid from soil suspensions was evaluated using previously selected solvents. Also, the applicability of the developed method for the determination of 2,4-D acid by anion exchange membranes in soil burial experiments was examined. In part (2) removal of 2,4-D amine by membranes from soil surface was investigated within two different concentration ranges simulating a typical farm spraying of 2,4-D amine and a 2,4-D amine spill on the soil. The developed method for 2,4-D amine determination on soil surface by membranes was applied to a degradation study of 2,4-D amine after a spill.

EXPERIMENTAL

Instrumentation:

GC analysis of methylated 2,4-D was performed on a Hewlett Packard model HP5790(A) gas chromatograph equipped with a flame ionization detector. A glass column (2.1m x 4mm ID) was packed with 5% DC200 on Chromosorb W AW DMCS 100/120 mesh. The following temperature conditions were found optimal for the determination of methylated 2,4-D: oven=220°C, detector=250°C, injector=245°C. Nitrogen at a flowrate of 40 ml/min was used as a carrier gas. Air and hydrogen flowrates were set at 300 and 30 ml/min. The freshly packed column was conditioned at 240°C for 24 hrs. The injected volume was 5 μl at a sensitivity setting of $8x10^{-10}$ a.f.s. The retention time of methylated 2,4-D was only 2.8 min (see Fig.1) making the GC monitoring of membrane elution process very fast and efficient.

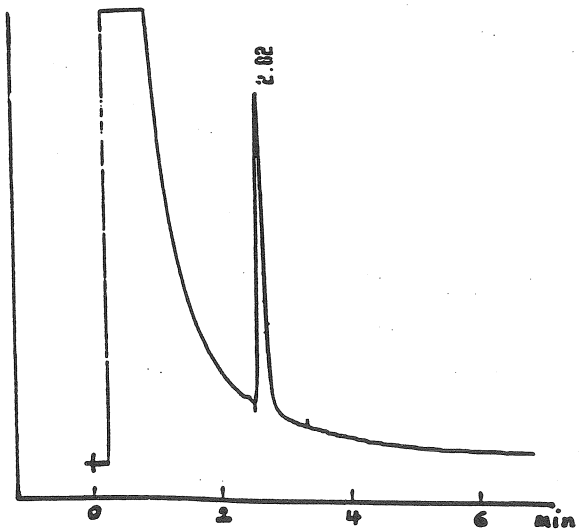

Fig.1. Gas chromatogram of methylated 2,4-D. 5ul of 50ppm standard injected.

Materials and methods:

Anion exchange membranes from BDH were used. New membranes were washed 5 times with 0.5M HCl and regenerated to bicarbonate form by washing 5 times in 0.5M $NaHCO_3$ (pH adjusted to 8.5 with 0.5g NaOH/liter). Regenerated membranes were stored in deionized water.

Boron trifluoride methanol (BF_3-MeOH) from Supelco was used for methylation of 2,4-D. All solvents for extraction and derivatization of 2,4-D were Reagent Grade.

For part (1) of our investigation, 2,4-D acid was obtained from Sigma. Standard solutions for GC analysis were prepared in 0.5M $NaHCO_3$ (pH adjusted to 9 with 1.5g NaOH/liter) in the range of 25 to 150 ppm. One ml of each solution was transferred to a small vial, acidified with 50% H_2SO_4 till pH 3 was achieved (or till no CO_2 bubbles appeared). Three ml of diethyl ether were added, the sample shaken and after layer separation the ether layer was withdrawn and transferred to another vial. The ether extraction was repeated with 2ml portion of ether. Ether fractions were combined, evaporated under a gentle stream of nitrogen, 1ml methanol added and sample subjected to derivatization.

One ml solution of 2,4-D eluted from the membranes was transferred to a vial, methanol evaporated under a gentle stream of nitrogen and sample treated the same way as standard i.e. acidified, extracted with ether, evaporated, methanol added and sample derivatized.

Methylation was carried out by adding 0.5ml BF_3-MeOH reagent to 1ml methanolic solution of 2,4-D and heating in a water bath for 15 min. The vials were allowed to cool, 1ml of 5% Na_2SO_4 and 1ml of hexane were then added. After shaking the vials vigorously, layers were allowed to separate and 5 μl of hexane layer containing esterified 2,4-D were injected into GC.

In part (1) of our study, the membranes (18cm^2) were placed in centrifuge tubes containing 25ml of 2,4-D acid solution in 0.5M $NaHCO_3$ (pH adjusted to 9) and shaken overnight on a mechanical shaker. Next, the membranes were removed and the following solvents were tested for efficiency of 2,4-D elution from the membranes: 0.5M NaOH, 0.5M Na_2CO_3, 0.25M Na_2CO_3 + 0.125M $NaHCO_3$, 0.25M Na_2CO_3 + 20% MeOH, 0.25M Na_2CO_3 + 0.25M Na_2SO_4 and 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ +20% MeOH. Membranes were shaken overnight with 25ml of each solvent on a mechanical shaker. 2,4-D content was determined in the solution left after membrane removal and in membrane eluate in order to monitor the rate of adsorption and desorption of 2,4-D to and from the membrane. For soil suspensions, 2g soil were mixed with 25ml of 2,4-D solutions and shaken overnight with the membranes (9cm^2). The membranes were transferred to 25ml of 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH and shaken overnight. The rate of adsorption and desorption of 2,4-D was monitored by GC. The membranes were also buried in the soil contaminated with 2,4-D. Contaminated soils were prepared by adding 2,4-D acid directly or in a methanol solution, evaporating the methanol then further mixing the soil. Each membrane (9cm^2) was buried in 70g of contaminated soil, the soil saturated to field capacity with 0.5M $NaHCO_3$ (pH 9), and left overnight. Membranes were removed, washed with deionized water and shaken with 25ml of 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH overnight on a mechanical shaker.

In part (2) of our study 2,4-D amine was a commercial formulation from IPCO containing 470g 2,4-D amine in 1 liter. Standard solutions for GC analysis were prepared by diluting the original formulation to yield solutions of 2,4-D amine in the range of 29.5ppm to 118ppm. One ml of each solution was extracted and methylated the same way as described in part (1).

To simulate either a farm application of 2,4-D amine or a 2,4-D amine spill on the soil surface, 2,4-D amine solutions were sprayed onto soil contained in 10x10cm plastic trays. Determination of 2,4-D amine was achieved by placing membranes onto the soil surface. To ensure a complete contact of the membrane surface with soil, a beaker filled with water was placed on top of each membrane and the membrane was gently pushed down. The surrounding soil area was wetted with water. For a typical farm application of 2,4-D amine (commercial

formulation diluted 100 times and applied at a rate of 100liters per 1 hectar representing an equivalent of $0.47x10^{-3}g/100cm^2$), 5ml solutions containing 2,4-D amine in the range of $0.12 \times 10^{-3}g$ to $1.41 \times 10^{-3}g$ were sprayed evenly onto 10 x 10cm soil surface. For a 2,4-D amine spill (commercial formulation diluted 50 times) 10ml solutions containing 2,4-D amine in the range of $2.35 \times 10^{-2}g$ to $9.40 \times 10^{-2}g$ were sprayed onto 10 x 10cm soil surface.

To work out the most efficient conditions for 2,4-D amine determination on the soil surface within both concentration ranges, size of membranes, time the membrane remained on soil surface (contact time) and volume of membrane eluate taken for GC analysis were varied. The following were tested for a farm spraying concentration range: 7 and $16cm^2$ membranes at a contact time of 1, 3, 8 hrs and overnight with 15 and 25ml membrane eluate used for GC determination. Variables tested for a simulated spill included 2 and $7cm^2$ membranes, contact time of 5, 10, 15min, 1hr and 1, 5, 10ml membrane eluate taken for analysis. After removing membranes from soil surface and rinsing off soil particles with deionized water, all membranes were shaken with 25ml of 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH overnight on a mechanical shaker. Sample treatment was the same as in part (1) except for the volume of diethyl ether used for extraction. Larger volumes of membrane eluates taken for GC analysis required larger amounts of ether which were adjusted accordingly.

Using the selected optimal conditions for 2,4-D amine determination on soil surface, degradation of 2,4-D amine within the spill concentration range was studied from 0 to 10 days. Fresh membranes were placed on the soil surface each day of testing. Areas where membranes had been previously applied were marked so that the measurements would reflect the actual 2,4-D amine residue on the soil surface. Trays containing contaminated soil were kept covered throught the entire period of testing to avoid drying of the soil.

RESULTS AND DISCUSSION

Part (1): Optimization of ion exchange parameters.

2,4-D acid forms salts in alkaline solutions and becomes soluble at pH 9-10. Therefore, for an ion exchange process to take place, 2,4-D had to be in alkaline solution when exposed to the membrane and eluted from the membrane. 0.5M $NaHCO_3$ (pH 9) was found suitable for 2,4-D acid solubilization.

Six different solvent systems were tested for efficiency of 2,4-D elution from the membranes. As seen in Table 1, the best results were obtained using 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ mixed with 20% MeOH. It has been reported that 2,4-D adsorbs onto the ion exchange materials but cannot be desorbed (Basta and Olness 1992, Storherr and Burke 1964, Grover and Smith 1974). We found that by adjusting solvent composition a consistent 25-30% rate of desorption can be achieved. Percentage adsorption from solutions onto the membrane was between 76-80% using $18cm^2$ membranes (see Table 2). A series of solutions in the range of 25ppm to 150ppm in 0.5M $NaHCO_3$ (pH 9) was prepared and extracted by membranes. As seen in Fig.2, a linear relationship between 2,4-D concentration as determined by the membrane and 2,4-D concentration in solution was obtained showing that ion exchange membranes can be used as an indicator of 2,4-D contamination.

Table 1. Concentration of 2,4-D acid (ppm) eluted from the membrane by different solvents.

Original concentr. (ppm)	0.5M NaOH	0.5M Na_2CO_3	0.25M Na_2CO_3 + 0.125M $NaHCO_3$	0.25M Na_2CO_3 + 20% MeOH	0.25M Na_2CO_3 + 0.25 Na_2SO_4	0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH
50	0	0	2.2	3.2	1.7	3.4
100	3.0	3.0	6.7	9.0	4.0	11.0
150	6.0	6.8	15.3	13.6	10.5	24.0

Table 2. Concentration of 2,4-D acid (ppm) left in solution after membrane removal.

Original concentration (ppm)	Left in solution concentration (ppm)	% adsorption
50	10.0	80
100	24.1	76
150	31.2	79

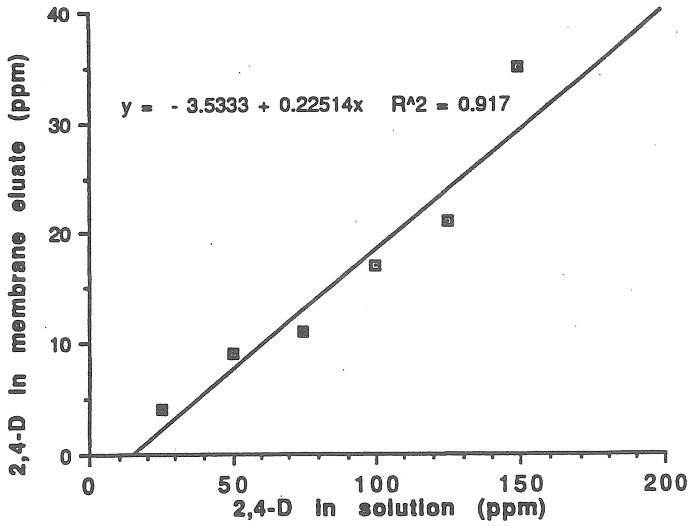

Fig.2. Relationship between 2,4-D acid (ppm) detected by anion exchange membranes (18cm²) and 2,4-D concentration in solution (ppm).

Used membranes were washed up to 5 times with 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH and each time the amount of 2,4-D eluted from the membranes was monitored by GC. Shaking times longer than overnight did not increase the amount of 2,4-D eluted. However, with each new portion of the elution solvent more 2,4-D was washed out of the

membranes. After 5 washes 75-85% of 2,4-D was recovered. Further washes would probably clean the membranes, however the use of new membranes for reliable results is recommended for each test.

Therefore, for soil suspension work smaller size new membranes were used ($9cm^2$). Soil was mixed with 2,4-D solutions in 0.5M $NaHCO_3$ (pH 9) in the range of 25 to 150ppm. Percentage adsorption onto the membranes was 65-75%, somewhat lower than previously determined in solutions due to the fact that smaller membranes were used and probably due to some retention of 2,4-D on soil particles. Percentage desorption of 2,4-D from the membranes was the same as determined earlier. Fig.3 shows the relationship between 2,4-D concentration detected by the membranes and 2,4-D concentration in soil suspension. It was linear up to 100ppm 2,4-D in soil suspension. It levelled off at higher concentrations indicating that the membranes became saturated with 2,4-D at 100ppm and above.

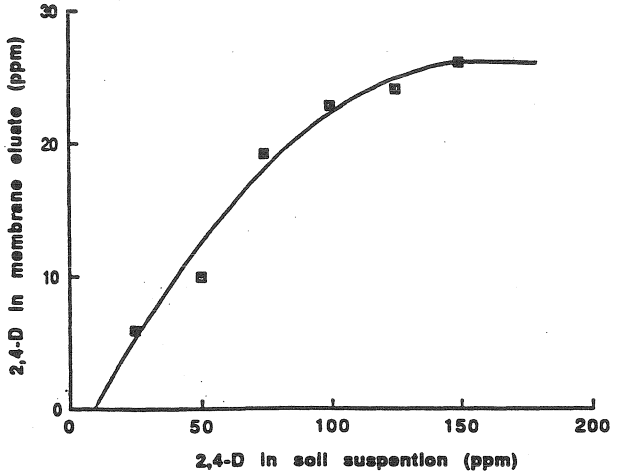

Fig.3. Relationship between 2,4-D acid (ppm) detected by anion exchange membranes ($9cm^2$) and 2,4-D concentration in soil suspension (ppm).

A wide range of 2,4-D acid contamination in the soil from $4x10^{-6}$ to $4x10^{-2}$g/g soil was examined when membranes were buried in the soil. When soil was spiked with 2,4-D in MeOH solution, concentration as low as $8x10^{-6}$g/g soil was detected using the developed method of membrane burial in the soil. When 2,4-D pellets were added directly to the soil, the detection limit was somewhat higher i.e. $24x10^{-6}$g/g soil probably due to less uniform distribution of 2,4-D within soil particles. The relationship between 2,4-D removed from the soil by the membranes and 2,4-D spike level in soil was linear over the entire range tested (see Fig.4).

Part (2): Detection of 2,4-D amine on soil surfaces.

2,4-D amine salt is soluble in water. Therefore after the membrane was placed on the soil surface it was wetted with water not with 0.5M $NaHCO_3$ (pH9) as was required for 2,4-D acid in part (1). However, for it to be extracted from the membrane and solubilized the same solvent system as for 2,4-D acid: 0.25M Na_2CO_3 + 0.125M $NaHCO_3$ + 20% MeOH was required. By varying membrane size, contact time and volume of membrane eluate used for GC analysis, the most suitable conditions for 2,4-D amine determination were found. As seen in the example illustrated in Fig. 5 for farm spraying concentration range, the amount of 2,4-D amine removed by the membrane from the soil surface was linear with the soil surface spike level and it increased with the increased contact time. For this example, $7cm^2$ membranes were used and

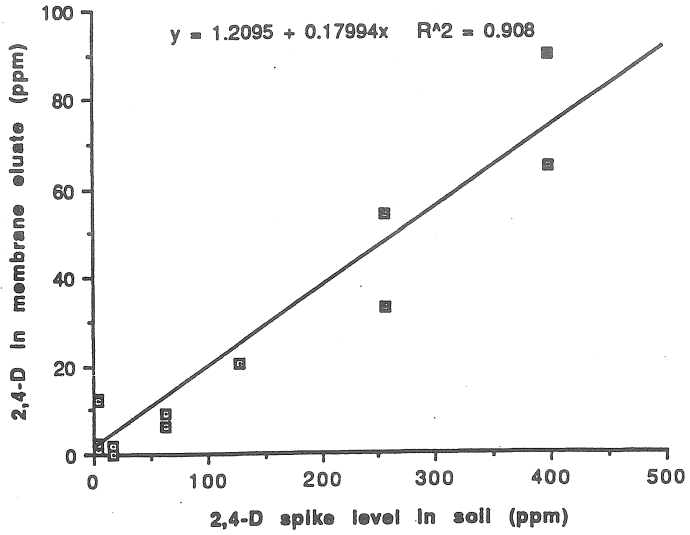

Fig.4. Relationship between 2,4-D acid detected by anion exchange membranes (9cm²) and 2,4-D spike level in soil.

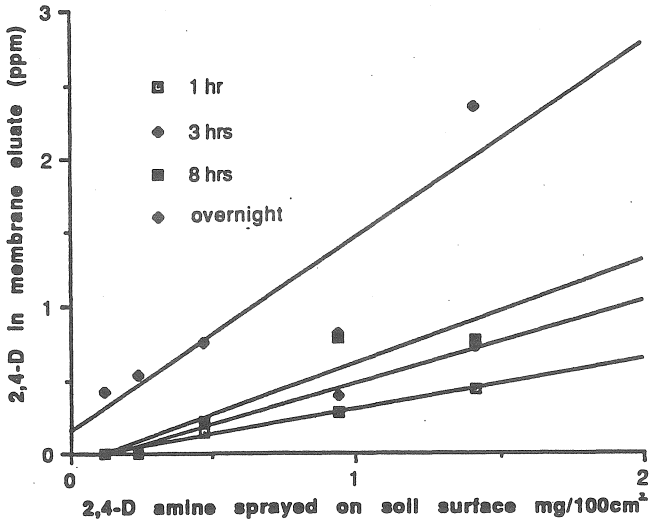

Fig.5. Relationship between 2,4-D amine detected by membranes and the soil surface spike level at different contact times for a farm spraying concentration range (7cm² membranes used and 15ml membrane eluate taken for GC analysis).

15ml of membrane eluate were taken for GC analysis. It was also found that the amount of 2,4-D amine removed by the membrane from the soil surface increased when larger membrane was used, and that the sensitivity of the GC analysis improved with increased volume of membrane eluate. Similar relationships were obtained for all combinations of membrane size, contact time and volume of membrane eluate. With the preference for shorter contact times, the following conditions were selected for fast, reliable and sensitive 2,4-D amine determination on soil surfaces: in a farm spraying concentration range a 3hr test using 16cm² membranes and 25ml membrane eluate taken for GC analysis; because of a much higher 2,4-D concentration in a spill concentration range a contact time as short as 10min using small 2cm² membranes and 10ml membrane eluate taken for GC analysis were found optimal. Using these parameters standard curves were constructed for both concentration ranges (see Fig. 6 and 7). Both showed linear relationship between amount of 2,4-D amine detected by the membrane and spike level on the

soil surface.

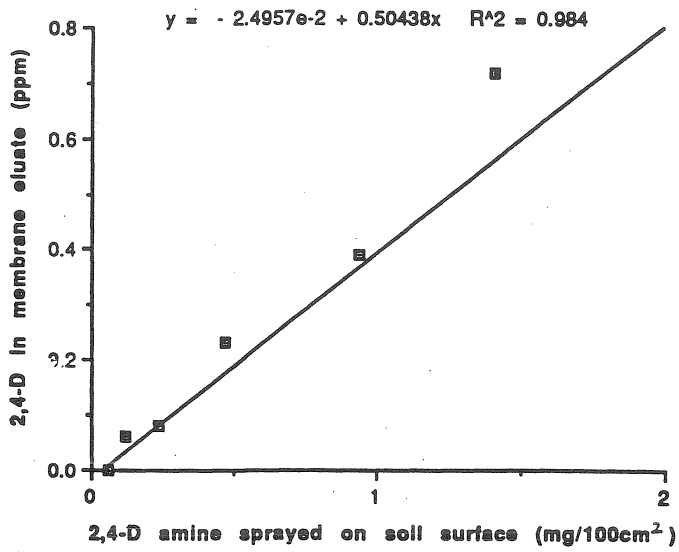

Fig.6. Standard curve for 2,4-D amine determination within a farm spraying concentration range (3hr contact time, 16cm² membranes, 25ml membrane eluate taken for GC analysis).

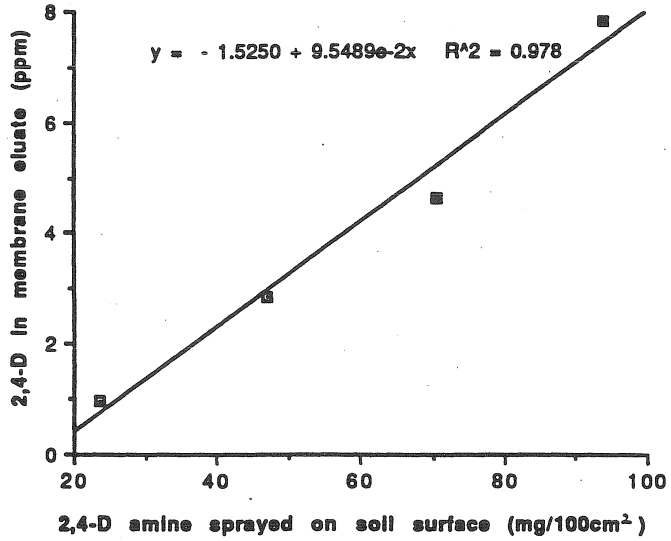

Fig.7. Standard for 2,4-D amine determination within a spill concentration range (10min contact time, 2cm² membranes, 10ml membrane eluate taken for GC analysis).

The detection limit of ca. 0.06kg/ha found for this method is close to 0.03kg/ha, the value quoted by Smith et al.(1991). However, if needed it can be improved by increasing any of the variables discussed above i.e. membrane size, contact time and volume of membrane eluate taken for GC analysis. Further improvement of the detection limit can be achieved with the use of electron capture detection.

The present study demonstrated that the anion exchange membranes are a useful tool for 2,4-D determination in soils. To further examine the applicability of the method, degradation of 2,4-D amine within a spill concentration range was investigated. As seen in Fig. 8 at the lowest concentration tested (2.35×10^{-2}g/100cm²) no 2,4-D amine was detected on the soil surface by membranes after 5 days while 10 days were required for 2,4-D amine at the highest concentration tested (9.40×10^{-2}g/100cm²) to be degraded to a near zero level.

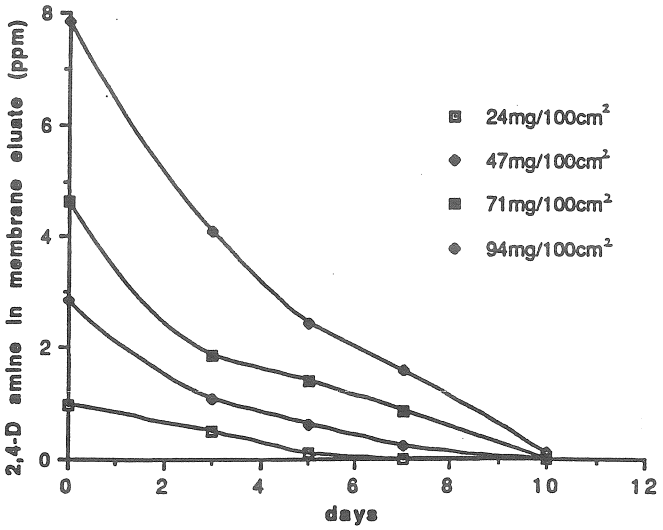

Fig.8. Degradation curves of 2,4-D amine as determined using anion exchange membranes.

It is known that 2,4-D is rapidly degraded in the soil by soil microorganisms (Smith 1989). When a different chemical form of herbicide other then acid is applied, it undergoes a fast hydrolysis or dissociation to the phenoxyalkanoic ion prior to biological breakdown. The rate of 2,4-D breakdown depends on variety of factors such as soil type, temperature, moisture, pH, 2,4-D formulation, concentration and repeat treatments (Smith 1989); however, they were not investigated. Our goal was to demonstrate that the developed method can be applied for monitoring 2,4-D amine degradation. Our results are in good agreement with results of persistance studies carried out in a variety of Saskatchewan soils under laboratory conditions at 20°C and 85% of field capacity (Smith et al. 1991). Values reported for the half-life ranged from <7 to 20 days.

CONCLUSIONS

The low detection limit and linearity between detected and added amount of 2,4-D to soil over a wide range of concentration make the method of 2,4-D determination using ion exchange membranes very attractive. It should be emphasised that the method is very fast and simple and does not require any special skills. Membranes are simply placed in contact with moist soil after 2,4-D application. After membrane removal from the soil, the remaining steps i.e. 2,4-D elution from the membranes and GC analysis is performed in an analytical laboratory. Because of the purity of the methylated membrane eluate injected onto GC, the column performance was excellent throught the entire investigation and the column packing did not have to be replaced.

ACKNOWLEDGEMENTS

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