Comparison of Two Detection Methods in Thin Layer Chromatographic Analysis of Some Herbicides in a Coastal Savanna Soil in Ghana

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Abstract

o-tolidine plus potassium iodide and photosynthesis inhibition detection methods were investigated for the analysis of three triazine herbicides (atrazine, ametryne, simazine) and two urea herbicides (diuron, metobromuron) in a coastal savanna soil using thin layer chromatography to compare the suitability of the two methods for the study of the herbicides. This was done by spiking 5 g of the soil sample with specific amount of the herbicide standards to generate herbicide-soil concentration of 40.24, 41.46, 40.28, 39.90 and 40.64 μg/g for atrazine, ametryne, simazine, diuron and metobromuron, respectively. Extraction was performed with acetone/hexane mixture (4:1) and the detection limit of each herbicide was then determined. In all, the photosynthesis inhibition method performed better for both the triazine and the urea herbicides, while the o-tolidine plus potassium iodide method was suitable for only the triazine herbicides. With the photosynthesis inhibition method, detectability in the range of 0.004–0.008 ± 0.002 μg/g was attained for the herbicides using the unclean extracts. In the case of o-tolidine plus potassium iodide method, detectability of 0.008–0.406 ± 0.02 μg/g was obtained. With the clean up extracts detectability in the range of 0.025–0.162 ± 0.004 μg/g was obtained using the photosynthesis inhibition method. However, metobromuron was not detected in the cleaned up extracts when o-tolidine plus potassium iodide detection method was used. For the methods described, clean up with SPE cartridge, equipped with C-18, is not critical to obtain the desired results.

Introduction

Herbicides belong to the class of pesticides that are used to control weeds. They are, therefore, also called weed killers. After application of herbicide on target weeds, the active ingradient is gradually lost as a result of breakdown, evaporation and leaching, and the herbicide residue is the amount that remains on the field after application and usage (Afful, 2002). While some herbicides have long residual activity and, therefore, persist in the environment for long time, others have low residual activity and disappear from the environment, or produce low residual concentrations (Walker, 1973).

The residue level of the herbicide in the soil after application is an important factor to be taken into account when assessing their performance as weed killers. There is the need to have information about the duration of phytotoxicity in order to avoid the possibility of damaging a succeeding crop, which may not be tolerant to the herbicide. The environmentalist needs similar informa-tion to assess the impact of the residue on non-target organisms either directly or as a consequence of vegetation changes affecting the ecosystem (Helling *et al.,* 1978). Against this background, it is necessary to study the residue level and the fate of these agro-chemicals after application on the field to have a better understanding of their behaviour on the environment.

Chromatographic methods have success-fully been used for the analysis of pesticide residues in plants, soils, vegetables, water and urine (Abbot *et al.*, 1965). The most important of such chromatographic techni-ques are gas chromatography (GC), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Thin layer chromatography is, however, gaining popularity as an important analytical tool for analysis of pesticides (Yeboah *et al.*, 2003). This is due to the fact that TLC is a fast and simple technique, and offers the opportunity to undertake analysis where there is inaccessibility to instrumentation service personnel, spare parts and continuous electricity supply. These factors make analysis almost impossible with GC and HPLC.

With regard to thin layer chromatographic technique, a number of detection methods are available for the screening of pesticides in environmental samples. Some notable ones are o-tolidine plus potassium iodide method, p-nitrobenzene-fluoroborate method, p-dimethylamino benzaldehyde method, aluminium oxide G-incorporated with silver nitrate method, photosynthesis inhibition method and fungi spore inhibition method (Lowor, 2000). Of these detection methods, o-tolidine plus potassium iodide and photosynthesis inhibition can be used for the study of herbicides. While the o-tolidine plus potassium iodide method is not specific for the study of herbicides, the photosynthesis inhibition method is largely recommended for the analysis of herbicides.

In this study, the suitability of the two detection methods for the analysis of three triazine herbicides (atrazine, ametryne and simazine) and two urea herbicides (diuron and metobromuron), commonly used in Ghana, were investigated in a coastal savanna soil for comparative purposes. The suitability of the detection methods was measured by determining the detection limits of the methods for the herbicides in the coastal savanna soil. The detection limit gives an idea of the lowest practical concentration that can be quantitatively identified and measured in a specific matrix. All the herbicides function by inhibiting photosynthetic electron transport reaction, and they were among 21 herbicides imported into Ghana between 1996 and 2000 for agricultural activities (Gerken *et al.,* 2001).

Materials and methods

Chemicals and reagents

The herbicide standards, which were of 98–99.5% purity, were obtained from Dr Ehrenstorfeer, Gmbh. The other chemicals used in the study were obtained from Merck, Germany and Fluka, Switzerland. They were of analytical grade and were used without further purification.

The TLC detection reagents for the herbicides were prepared as follows:

o-tolidine + potassium iodide (oTKI) reagent was prepared by dissolving 0.5 g of o-tolidine in 100 µl of glacial acetic acid and thoroughly mixed with 10 ml of 20% KI solution. The resultant solution was diluted to 500 ml with distilled water. Photosynthesis inhibition reagent was prepared by mashing 30 g of *Panicum maximum* and 5 g of sea sand in a mortar with pestle, 15 ml of distilled water and 3 ml of glycerine were added, mixed thoroughly, and the liquid squeezed through a knapsack into 50 ml flask. 20 ml of this was added to 13 ml of DCPIP reagent, which was prepared by dissolving 0.1 g of 2, 6-dichlorophenol-indolphenol sodium salt in 250 ml of borax solution, which was also prepared by dissolving 3.325 g of borax in 175 ml of distilled water, and the solution was added to 75 ml of 0.1 *M* HCl.

Soil sampling

Soil samples were taken from a field at Ghana Atomic Energy Commission (GAEC) farm. A sketch map, showing the sampling site is presented in Fig. 1. About 50 m x 50 m plot size on the plot was demarcated for sampling. Soil samples were taken randomly on the demarcated field. Sampling was done with an auger to a depth of 10 cm. Samples were mixed thoroughly, wrapped in aluminium foil and then placed in black polyethylene bags and the ends of the bags taped. In the laboratory, part of the sample was taken a day after sampling for determination of the soil moisture content. The remainder of the sample was sieved with 2-mm mesh-size sieve aperture to remove stones and other debris.

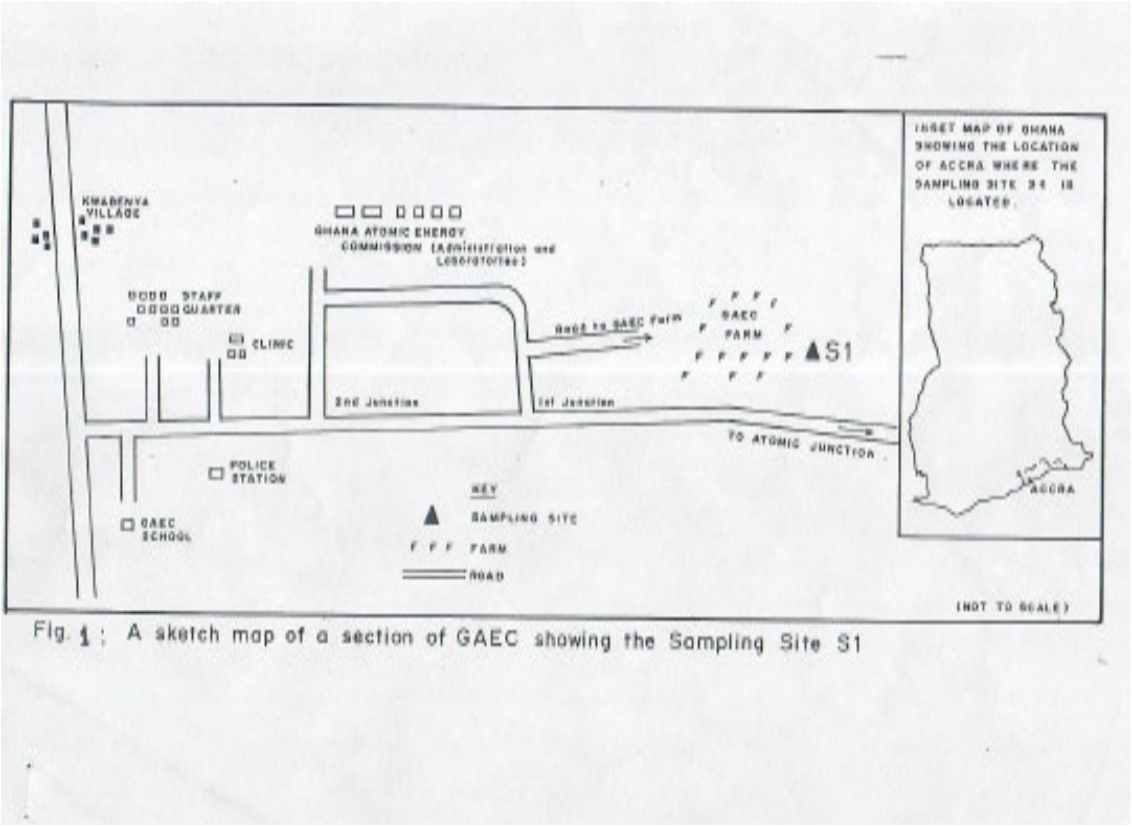

Fig. 1 : A sketch map of a section of GAEC showing the Sampling Site S1

Determination of limit of detection

Spiking of soil samples. 5 g of the soil in five extraction flasks, labeled S_1, S_2, S_3, S_4 and S_5, were spiked with 2 ml of standard herbicide solution of atrazine, ametryne, simazine, diuron and metobromuron, respectively, to generate spiking levels of 40.24, 41.46, 40.28, 39.90 and 40.64 µg/g. The concentrations of the herbicide solutions used for the spiking were 100.59, 103.66, 100.70, 99.75 and 101.59 µg/ml for atrazine, ametryne, simazine, diuron and metobro-muron, respectively. The spiked soil in the extraction flasks was allowed to stand for 30 min before extraction was performed.

Extraction. Extraction was performed by adding 20 ml of acetone/hexane mixture (4:1) unto each of the spiked soil samples and mechanically mixed on a flask shaker for 2 h. Filtration was carried out by use of Whatman No. 42 filter paper. After filtration, the residue was washed three times with 3 ml of the solvent, and the washings were added to the filtrate, which was then dried over anhydrous sodium sulphate (Na_2SO_4). The filtrate was concentrated to dryness by gently blowing in streams of air from a hand dryer. The recovered unclean herbicides were redissolved in 5 ml of acetone and subjected to thin layer chromatographic analysis.

Clean-up of extracts. The extraction procedure was repeated, but this time the unclean filtrates were cleaned by passing the filtrates through SPE cartridge equipped with C-18 as adsorbent, which was earlier preconditioned with 2 µl of acetone/water (1: 9). The cartridge and its contents were dried for 15 min by a vacuum pump. The herbicide was then eluted with 10 µl of acetone to give the clean extracts. The clean extracts were concentrated to 5 µl using the hand dryer and then subjected to thin layer chromatographic analysis.

TLC analysis of extracts. Each herbicide extract (volumes accurately measured in the range of 0.1–5 µl) were spotted using 10 µl micro-syringe on 20 cm × 20 cm already made silica-gel 60 TLC plates, which were earlier activated. The standard of the particular herbicide being investigated was also spotted concurrently. The spotted plates were developed in a TLC tank saturated with 50 µl ethyl acetate used as developing solvent. Saturation was achieved by lining the walls of the tank with filter paper, cut to the size of the tank, and the vapour of the solvent was allowed to saturate the tank for 3 h. The plates were then developed by dipping them into the saturated tank, where the solvent was allowed to rise by capillary action to more than two-thirds of the length of the plates. They were taken out of the tank and the plates allowed to dry, and the spots detected and measured.

Detection and measurement of spots. For the o-tolidine + potassium iodide method, the developed TLC plates were air-dried and placed in a tank saturated with chlorine for 30 sec. The chlorine solution was made by placing a 25-ml beaker containing about 1–2 g potassium permanganate at the bottom of an empty TLC tank and adding a few drops of concentrated HCl to the $KMnO_4$. Excess chlorine was removed in a fume hood after which the plates were sprayed with the oTKI reagent. For the photo-synthesis inhibition method, spots were visualized by spraying the developed plates with the photosynthesis inhibition reagent, and the sprayed plates placed about 20 cm below 60 watt electric bulb for about 2 min. For each analysis the solvent front and the distances moved by the herbicides were measured, and these were used to calculate for the R_f (relative factor) of the herbicides.

Results and discussion

TLC analysis of extracts

The R_f values, which is an identification parameter obtained for the herbicides, are presented in Table 1. The results suggest that in a multi-residual procedure involving a mixture of these chemicals, the silica gel-ethyl acetate system used for the investigation could not be very useful for analysis of atrazine, ametryne and metobromuron, as their spots would overlap and resolution would be difficult. This is because they have R_f values that are close (Table 1), particularly atrazine and ametryne. However, for samples known to contain one of these chemicals, the system could conveniently be used. The R_f values obtained compare favourably with the findings of (Lowor *et al.*, 2000). They reported R_f values of 0.61, 0.61, 0.57 and 0.41 at 32 ^{0}C for atrazine, ametryne, metobromuron and diuron, respectively.

The two detection methods used for the study indicated one spot detected for all the extracts of the soil samples and, in each case, the spot corresponded to the herbicide being investigated with the same R_f as the standard herbicides solution analyzed concurrently. Spots detected with o-tolidine plus potassium iodide method could stay overnight before they disappeared, while spots detected with the photosynthesis inhibition method disappeared within 1 h after detection.

TABLE 1
R_f values of the herbicides at 30.5 ± 2 ^{0}C

Herbicides	*R_f*
Atrazine	0.62 ± 0.004
Ametryne	0.61 ± 0.005
Simazine	0.58 ± 0.006
Metobromuron	0.57 ± 0.005
Diuron	0.42 ± 0.004

R_f values are mean of four replicates.

Detection limits

The detection limit gives an idea of the lowest practical concentration of the herbicide residue or contaminant that can be quantitatively measured and identified in a specific matrix. The results obtained in the study and presented in Tables 2 and 3 suggest the suitability of the methods for the analysis of the herbicides.

TABLE 2
Detection limits (µg/g) of the herbicides using the unclean extracts

Herbicides	*o-tolidine + KI method*	*Photosynthesis inhibition method*

Atrazine	0.008 ± 0.002	0.006 ± 0.001
Ametryne	0.008 ± 0.002	0.004 ± 0.001
Simazine	0.012 ± 0.004	0.006 ± 0.002
Diuron	0.159 ± 0.02	0.004 ± 0.001
Metobromuron	0.406 ± 0.02	0.008 ± 0.002

Note: Values are mean of 3 replicates.

TABLE 3
Detection limits (μg/g) of the herbicides using the cleaned up extracts

Herbicides	*o-tolidine + KI method*	*Photosynthesis inhibition method*
Atrazine	0.048 ± 0.005	0.025 ± 0.003
Ametryne	0.039 ± 0.004	0.017 ± 0.002
Simazine	0.048 ± 0.003	0.025 ± 0.002
Diuron	0.793 ± 0.051	0.024 ± 0.002
Metobromuron	nd	0.162 ± 0.032

Note: Values are mean of 3 replicates;
nd = not detected.

With the unclean extracts, using the o-tolidine plus potassium iodide method of detection, detectability in the range of 0.008 – 0.012 μg/g (Table 2) was achieved for the atrazine, ametryne and simazine (the triazine herbicides), while detectability in the range of 0,159–0.406 μg/g (Table 2) was also achieved for diuron and metobromuron (the urea herbicides). Although the results showed that the o-tolidine plus potassium iodide method is suitable quantatively for both the triazine and the urea herbicides, upon critical comparison, it showed better performance for quantitative study of the triazine herbicides than the urea herbicides. In the case of the photosynthesis inhibition method, detectability was in the range of 0.004–0.008 μg/g (Table 2) for the two classes of the herbicides used in the investigation. Comparing the detectability values obtained for the two detection methods, it is clear that the photosynthesis inhibition method performed better than the o-tolidine + potassium iodide method, and, in general, is more suitable for the screening of the herbicides in the coastal savanna used in the investigation.

The detection limit of 0.025 μg/g (Table 3) obtained for the triazine and simazine compares reasonably well with the findings of Balinova *et al*., 1991. They reported detection limit of 0.02 μg/g for both atrazine and simazine in the study of herbicides residue in the soil using the gas chromato-graphy method.

By comparing the detection limit for the uncleaned and the cleaned extracts, it can be deduced that with the triazine herbicides, their detection limits were reduced by a factor of about 4–6 (compare Tables 2 and 3), on cleaning up the extracts with solid phase extraction cartridge equipped with C-18 as adsorbent. For instance, in the case of atrazine and using photosynthesis detection method, detectability for unclean and clean extracts were 0.008 and 0.048 μg/g, respectively, a reduction of detectability by factor of six after the clean up procedure. In the case of the urea herbicides, detection limit of diuron was reduced by a factor of about 5–6 with the two detection methods, whilst the detection limit of metobromuron was reduced by a factor of about 20 when photosynthesis method was used for its detection. However, metobromuron was not detected on cleaning up the extract when o-tolidine plus potassium iodide method was used for detection (Table 3).

Conclusion

The results suggest that the photosynthesis inhibition method is a better detection method for both the triazine and the urea herbicides, whilst the o-tolidine plus potassium iodide method is suitable for only the triazine herbicides used in the investigation. For the methodology described, clean up with SPE cartridge equipped with C-18 as adsorbent is not critical to obtain the desired result.

Acknowledgement

The authors are grateful to Mr J. Nortenor of the Department of Soil Science, University of Ghana, Legon, and Mr B. Q. Modzinuh of the Department of Chemistry, Ghana Atomic Energy Commission, Kwabenya, for their technical assistance.

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