Ultrasonic Extraction and TLC Determination of Glyphosate in the Spiked Red Soils

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SUMMARY

Pesticides that get into soil bind mostly to its solid phase by physical or chemical processes. In the valley of the Neretva River the use of herbicides, especially of glyphosate is widespread and sometimes uncontrolled.

In this work ultrasonic solvent extraction (USE) followed by thin-layer chromatography (TLC) was applied for determining glyphosate presence in soil. The experiments were conducted with two characterised soil types. The impact of soil composition on extraction efficiency is discussed. Chemical analysis showed that soil 1 contained much more iron and aluminium oxides than soil 2, which was richer in humic substances. Low glyphosate efficiency (ca 44 % in both soils) could be attributed either to its binding to iron and aluminium oxides (soil 1), or to chemisorption on humic macromolecules (soil 2).

KEY WORDS

pesticide, glyphosate; soil, ultrasonic extraction, thin-layer chromatography

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INTRODUCTION

The use of the organo-phosphorous herbicide glyphosate in the Neretva-river basin, is widespread and sometimes uncontrolled. Pesticides that get into the soil mostly bind to its solid phase by physical or chemical processes. It is known that glyphosate, with pK_a values 2.0, 2.6 and 5.6, is a very polar and amphoteric compound with strongly ionised phosphate group. Despite its high water solubility, most papers dealing with the fate of glyphosate in soils emphasize its low mobility as a result of sorption processes in most soils, even those with lower organic and clay content.

Due to these glyphosate characteristics it was necessary to develop a quick and efficient analytical method for determining its presence and possible mobility in the characteristic karstic soils of the Neretva-river basin. In this contribution the procedure of ultrasonic extraction (USE) of glyphosate from the spiked soils and its subsequent determination by thin-layer chromatography (TLC) was developed. In our previous works we have found ultrasonic extraction procedure to be very efficient in pesticide extraction from spiked soils (Babić et al., 1998a and 1998b), and TLC was a very successful separation, detection, and quantification technique for determination of pesticides extraction efficiency (Babić et al., 1998b).

Experiments were conducted on two characterised red soil types: Terra Rosa shallow, very rocky, colluvial and Calcaric Hipogley, anthropogenic soil. Impact of soil composition on extraction efficiency is discussed.

MATERIAL AND METHODS

All solvents were pro analysi grade, supplied by Kemika (Zagreb, Croatia). Silanized silica precoated plates, 20×20 cm (Merck, Darmstadt, Germany) were used for TLC determination. Glyphosate (Figure 1) (Chromos-Agro, Zagreb, Croatia, 62% technical product) was purified before use (Gimsing et al., 2004) and dissolved in redistilled water. Mass concentration of pesticide was $\gamma(\text{glyphosate}) = 0.438 \text{ mg mL}^{-1}$.

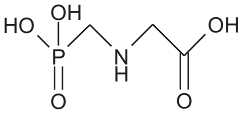

Figure 1. Structure formula of glyphosate

Ultrasonic bath UZ-20R (Iskra, Kranj, Slovenia) was used for extraction of glyphosate. Solvent has been evaporated using a rotary vacuum evaporator R-114/A (Büchi, Switzerland). The samples were applied to the plates as bands by means of TLC applicator Linomat IV (Camag, Muttenz, Switzerland). Camag Video Documentation System in conjunction with the Reprostar 3 (Camag, Muttenz, Switzerland) and 3CCD colour video camera HV-C20 (Hitachi, Japan) was used as the image analysing system.

The soils used in the study were collected from the A horizon at two locations in Neretva river basin: Hum, Mostar (soil 1: Terra Rosa shallow, very rocky, colluvial) and Gabela, Čapljina (soil 2: Calcaric Hipogley, anthropogenic soil).

The soils had never been treated with any agrochemicals. To obtain a representative sample, the sampled soil cores from various points in the vicinity of the sample site were combined. The combined samples were homogenized and appropriately sized subsamples were taken for analysis. The samples were air-dried for 24 hours and sieved through a 2-mm sieve before use.

The prepared soils were characterised using following methods: determination of mechanical composition (Table 1), chemical silicate analysis (Table 2), and X-ray diffraction analysis (Figures 2 and 3). Humic matter was determined using permanganate Kochman method (Škorić, 1965).

According to Atterberg classification texture of soil 1 is heavy clay, and soil 2 is light clay texture (Škorić, 1965).

Freshly spiked soil samples were prepared by adding 1 mL of the standard glyphosate solution to 10 g of dried, sieved and accurately weighted soil. Additional 10 mL of methanol was added to cover the soil particles. The soil suspension was mixed for 60 minutes and then left at room temperature for 24 hours to allow complete solvent evaporation.

Table 1. Mechanical composition of soils (%)

Mechanical category of soil	Soil samples	
	1	2
Coarse sand	3.33	0.45
Fine sand	4.21	35.79
Clay	61.83	25.44
Powder	30.63	38.32

Table 2. Chemical composition of examined soils (%)

	Soil samples	
	1	2
SiO_2	41.80	37.00
Al_2O_3	17.60	6.90
Fe_2O_3	10.96	5.46
CaO	6.63	16.85
MgO	1.05	2.06
TiO_2	1.04	1.60
K_2O	0.52	0.44
Na_2O	0.19	0.20

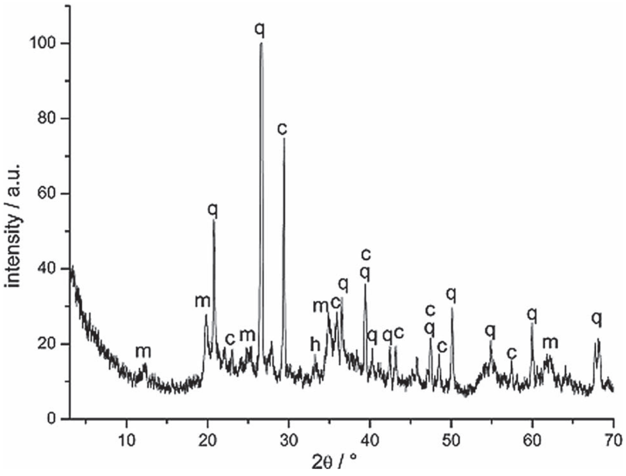

Figure 2.
Mineral composition of soil 1 (m – montmorillonite, q – α-quarc, c – calcite, h – hematite).

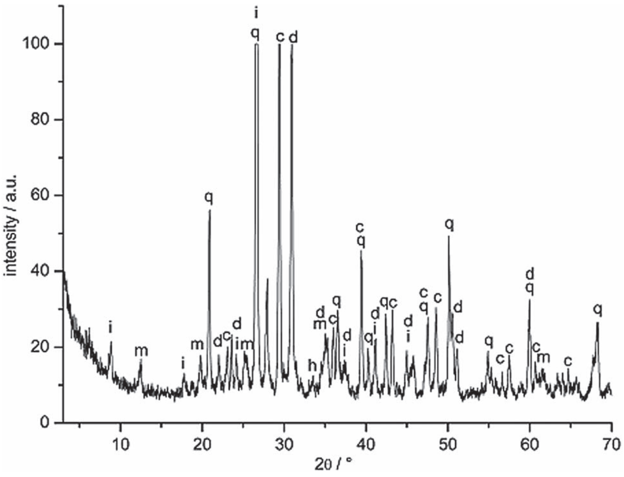

Figure 3.
Mineral composition of soil 2 (m – montmorillonite, i – illite, q – α-quarc, c – calcite, d – dolomite).

According to preliminary results, ultrasonic extraction of glyphosate was performed with 25.0 mL 0.1 mol L^{-1} solution of KH_2PO_4, in one step during 60 minutes.

Extracts were filtered through Whatman 40 filter paper, and the filtrate was evaporated to dryness on a rotary vacuum evaporator at 40°C. The residue of glyphosate extract was dissolved in 2.0 mL of water. These solutions were directly spotted on TLC plate.

The spiking and extraction procedure was performed on three parallel soil samples for each soil type, and an experiment of blank extract (from non-spiked soils) was used as a control.

In TLC determination of glyphosate ethanol/water/acetic acid (6:3.5:0.5, v/v) solvent system was used. Volumes of 5 μL of each standard solution, extracts from the soil samples and blank extract were applied as 10 mm bands to the chromatographic plate. Ascending development was performed at room temperature in a Camag chamber without previous saturation to a migration distance of 8 cm. After development, the plates were air dried. Glyphosate was detected by spraying with 0.3% ninhydrine solution.

Chromatograms were captured under visible light by means of a 3CCD colour video camera. Image acquisition, processing and archiving were controlled via Video Store 2 documentation software. The extended version of Camag Video Scan 1.01 software was used for the quantitative evaluation of TLC chromatograms.

RESULTS AND DISCUSSION

The efficiency of ultrasonic extraction was tested by recovery experiments using simple and fast TLC method. Although column chromatographic techniques with previous extraction procedures predominate in pesticide separation and determination in various soil samples (Baran et al., 2003; Stipičević et al., 2003; Shen et al., 2003; Bouaid et al., 2001; Richter et al., 2003) TLC was found to be excellent screening method because of its simple purification or cleanup procedures.

The ultrasonic solvent extraction is more rapid than the conventional extraction methods, and the solvent consumption is significantly lower. In this work optimal extraction conditions (solvent, extraction time, temperature, number of extraction steps) were determined for glyphosate and both soils, and the efficiency of extraction was compared by recovery experiments. Because of its polarity and amphoteric character it has not been possible to extract glyphosate with a common extraction solvents. Chromatogram of glyphosate standard solution, blank extract and extracts from soil 1 is shown on Figure 4.

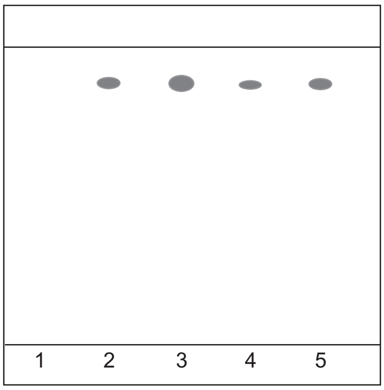

Figure 4.
Chromatogram of glyphosate standard solution (3), blind extract (1) and extracts from soil 1 (2, 4 and 5)

By semiquantitative comparison of spot area of glyphosate standard and extracts calculated by CAMAG image analysing system it can be concluded that extraction efficiency of glyphosate is about 44 % in both soils.

Such a low efficiency confirms its strong binding to examined soils. Recent investigations emphasise its strong adsorption to most soils by binding to layered silicates, aluminium and iron oxides, non-crystalline materials or organic matter.

Chemical analysis of examined soils shows that soil 1 contains much more iron and aluminium oxides than soil 2, which is richer in CaO and MgO as well as humic substances (soil 1- 1.9 %; soil 2 - 4.3 %). Qualitative X-ray diffraction analysis shows very little difference in mineralogical composition of the two soils. Both soils are made of montmorillonite, α-quartz, calcite, dolomite and hematite, and soil 2 also contains some illite.

Binding to clay minerals takes place either through interlayer exchange cations or by hydrogen bonding to the clay surface, and binding to iron and aluminium oxides and non-crystalline materials is facilitated by anion exchange processes. Adsorption to organic matter occurs through hydrogen bonding (Gimsing et al., 2002; Börjesson et al., 2000; De Jonge et al., 2001). It was proven that sorption of glyphosate by the soils is mostly pH dependent. Increasing pH values result in stronger glyphosate sorption because of an increase of reactive amorphous aluminium and iron hydrous oxides (Morillo et al., 2000). Lower pH values lead to the formation of glyphosate species with lower negative charge, which are not easily adsorbed on the negatively charged soil surfaces (De Jonge et al., 2001). The same authors have concluded that the interaction of this pesticide with the soils was mostly related to the content of amorphous iron and aluminium oxides and of organic matter. If metal ions are present in soil, they are already adsorbed onto humic matter, and act as a bridge between the soil and glyphosate, enhancing its adsorption. Complex associations in which metal ions bind to the humic substances also serve as a bridge between humic macromolecules and the phosphate anion in glyphosate structure (Petrović et al., 1999; Petrović et al., 1996).

The difficulties in establishing a simple method for the extraction and determination of such pesticides containing phosphonic group are mainly due to their high solubility in the water, insolubility in organic solvents and favoured complexing behaviour. Many investigators have experienced low and irreproducible recoveries from numerous types of soils. It was made clear that the development of a unified extraction procedure was hampered by the fact that soil composition varies with respect to organic and inorganic matter content.

The results of TLC determination imply that it can successfully serve as a fast screening technique for detection and previous evaluation of efficiency of glyphosate extraction from soils. Low glyphosate efficiency from both soils can be assigned to its binding to iron and aluminium oxides (soil 1), or to chemisorption of humic macromolecules and the amphoteric glyphosate phosphate anion (soil 2). For better quantification recently developed chromatographic methods are recommended (Börjesson et al., 2000; Stalikas et al., 2001). It is not possible to propose a unique glyphosate extraction procedure for soils with various mechanical, chemical and mineralogical compositions. Because of its complex characteristics glyphosate seems to be very interesting for further investigations on real samples.

REFERENCES

Babić S., Petrović M., Kaštelan-Macan M. (1998a). Ultrasonic solvent extraction of pesticides from soil. J. Chromatogr. A. 823: 3-9

Babić S., Kaštelan-Macan M., Petrović M. (1998b). Determination of agrochemical combinations in spiked soil samples. Wat. Sci. Technol. 37:243-250

Baran S., Oleszczuk P. (2003). Determination of s-triazine herbicides in soils of different organic matter content. Chem. Anal. 48(5):817-827

Börjesson E., Torstensson L. (2000). New methods for determination of glyphosate and (aminomethyl)phosphonic acid in water and soil. J. Chromatogr. A. 886:207-216

Bouaid A., Ramos L., Gonzalez M.J., Fernandez P., Cámara C. (2001). Solid-phase microextraction method for the determination of atrazine and four organophosphorus pesticides in soil samples by gas chromatography. J. Chromatogr. A. 939:13-21

de Jonge H., de Jonge L.W., Jacobsen O.H., Yamaguchi T., Moldrup P. (2001). Glyphosate sorption in soils of different pH and phosphorus content. Soil Sci. 166(4):230-238

Gimsing A.L., Borggaard O.K. (2002). Effect of phosphate on the adsorption of glyphosate on soils, clay minerals and oxides. Int. J. Environ. Anal. Chem. 82(8-9):545-552

Gimsing A.L., Borggaard O.K., Bang M. (2004). Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. Eur. J. Soil Sci. 55(1):183-191

Morillo E., Undabeytia T., Maqueda C., Ramos A. (2000). Glyphosate adsorption on soils of different characteristics.: Influence of copper addition. Chemosphere. 40(1):103-107

Petrović M., Kaštelan-Macan M. (1996). The uptake of inorganic phosphorus by insoluble metal-humic complexes. Wat. Sci.Technol. 34(7-8):253-258

Petrović M., Kaštelan-Macan M., Horvat A.J.M. (1999). Interactive sorption of metal ions and humic acids onto mineral particles. Water, Air, and Soil Pollution. 111(1):41-56

Richter P., Sepúlveda B., Oliva R., Calderón K., Seguel R. (2003). Screening and determination of pesticides in soil using continuous subcritical water extraction and gas chromatography-mass spectrometry. J. Chromatogr. A. 994:169-177

Shen G., Lee H.K. (2003). Determination of triazines in soil by microwave-assisted extraction followed by solid-phase microextraction and gas chromatography–mass spectrometry. J. Chromatogr. A. 985:167-174

Stalikas C.D., Konidari C.N. (2001). Analytical methods to determine phosphonic and amino acid group-containing pesticides. J. Chromatogr. A. 907:1-19

Stipičević S., Fingler S., Zupančić-Kralj L., Drevenkar V. (2003). Comparison of gas and high performance liquid chromatography with selective detection for determination of triazine herbicides and their degradation products extracted ultrasonically from soil. J. Sep. Sci. 26(14):1237-1246

Škorić A. (1965). Pedološki praktikum. Sveučilište u Zagrebu, Zagreb

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