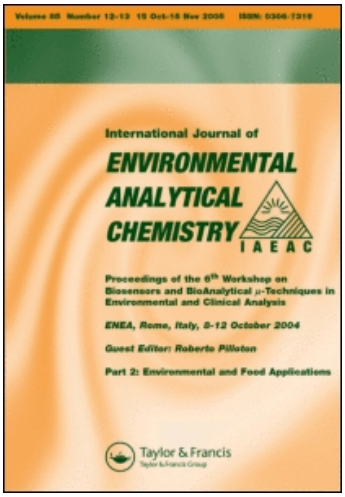

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of acephate and its degradation product methamidophos in soil and water by solid-phase extraction (SPE) and GC-MS

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To cite this Article St-Amand, Annick D. and Girard, Louise(2004) 'Determination of acephate and its degradation product methamidophos in soil and water by solid-phase extraction (SPE) and GC-MS', International Journal of Environmental Analytical Chemistry, 84: 10, 739 — 748
To link to this Article: DOI: 10.1080/03067310410001729600
URL: http://dx.doi.org/10.1080/03067310410001729600

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 10, pp. 739–748, 20 August 2004

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DETERMINATION OF ACEPHATE AND ITS DEGRADATION PRODUCT METHAMIDOPHOS IN SOIL AND WATER BY SOLID-PHASE EXTRACTION (SPE) AND GC-MS

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(Received 28 July 2003; In final form 10 April 2004)

Acephate and its metabolite, methamidophos, are both highly polar organophosphorus pesticides (OPs) and are therefore highly soluble in water, which leads to difficulties when traditional methods of extraction, such as LLE (liquid–liquid extraction), are used. Solid-phase extraction (SPE) is a relatively new, highly versatile method, which has proven successful in many cases that were considered problematic in the past. In this study, several adsorbents (polymeric and silica based) and parameters are considered and modified to obtain maximum recovery. Maximum recoveries for acephate and methamidophos were found to be 90–95% and 85–90% respectively with Oasis HLB cartridges and methylene chloride as the elution solvent. In order to establish applicability and reliability, the matrix effect of several real water and solid (compost and soil) samples was evaluated. A 20–30% diminution of recovery is noted for some samples with a complex matrix containing a high amount of dissolved organic matter.

Keywords: Acephate; Methamidophos; SPE; GC-MS; Water; Polar pesticides

INTRODUCTION

Acephate (*O*,*S*-dimethyl acetylphosphoramidothioate) and its principal metabolite and degradation product, methamidophos (*O*,*S*-dimethyl phosphoramidothioate) are both systemic and contact registered organophosphorus insecticides also used as acaricides. Acephate is more persistent in the environment than methamidophos, but the latter is about 50 times more toxic, its LD_{50} (rat, oral) being 15–18 mg/kg compared to 945 mg/kg for acephate [1–3]. Both structures are presented in Fig. 1.

Although many studies concerning acephate and methamidophos have been realized in the past on fruit and vegetable samples, few have been done successfully on water samples [4–12]. Traditionally, liquid–liquid extraction (LLE) is the preferred method for the extraction of various pesticides from water [13]. It is a very simple and easy technique, but there are some disadvantages. LLE is time costly, generally uses a lot of solvent and is not specific. Moreover, the LLE technique does not do well with

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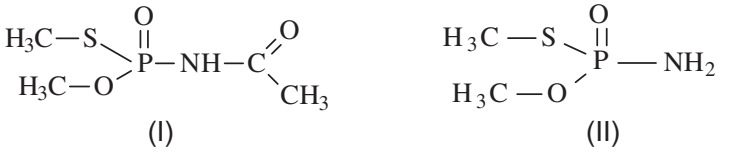
ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310410001729600



(I) (II)

FIGURE 1 Structures of acephate (I) and methamidophos (II).

very polar pesticides, like acephate and methamidophos, which have water solubilities of 650 and 2000 g/L respectively [4,7,13,14]. It should also be noted that their high solubility in water indicates a high potential for these two pesticides to contaminate groundwater [2,15].

Several studies have been done during the past few years on the solid-phase extraction of highly polar organophosphorus pesticides, including acephate and methamidophos, from water, but few were successful. Furthermore, contradictions are found among these investigations [4,5,7,16,17]. One solid-phase extraction study, by Lacorte *et al*., gave recoveries of 24–31% for methamidophos and 125–154% for acephate [15]. Another, by Ingelse *et. al.*, using various conditions and adsorbents, including Oasis HLB, failed to give recoveries higher than 7% for acephate and 2% for methamidophos. The authors then turned to other methods, including direct injection of the treated sample in LC-MS, which gave recoveries of 109–129 % for acephate and 106–132% for methamidophos. However, with fortified samples, relative recoveries were 90–140% for acephate and 96–128% for methamidophos, which demonstrates a noticeable matrix effect [5].

The present study was designed to further investigate the possibility of SPE for the extraction of acephate and methamidophos from various waters and solid samples. Several adsorbents and several parameters (volume and nature of elution solvent, addition of sodium chloride, volume and flow rate of sample) were studied. This extensive list includes not only frequently used commercially available adsorbents but also various parameters that often play a key role during solid-phase extraction. Previous studies dealing with acephate and methamidophos frequently addressed the quantification of several pesticides, and parameters were often optimized for the whole with no or very low recoveries for acephate and methamidophos. In this study only acephate and methamidophos were considered and parameters were optimized for maximum recovery of these two highly polar pesticides from water.

EXPERIMENTAL

Reagents and Materials

HPLC-grade methanol and pesticide-grade acetone, ethyl acetate, acetonitrile, toluene, hexane and dichloromethane were used for the study. Methamidophos and acephate PESTANAL® grade were purchased from Riedel-de Haën. The internal standard used was acenaphtene-d_{10} in pure (neat) solid form obtained from Supelco. Standard solutions of 0.05–10 µg/mL of each analyte and 1 µg/mL of internal standard were prepared in methylene chloride.

The following SPE cartridges were used: Bakerbond C18 (1 mL/100 mg), Bakerbond PolarPlus C18 (3 mL/500 mg), Bakerbond PolarPlus C8 (6 mL/500 mg) and Bakerbond Speedisk H_2O-Philic (3 mL/50 mg) purchased from JT Baker, Empore disk cartridge C2

(1 mL/4 mm) and C8 (1 mL/4 mm) from VWR Canlab, Oasis HLB (3 mL/60 mg) from Waters, Strata X (6 mL/200 mg) from Phenomenex and Chromabond Easy and Chromabond HR-P (3 mL/200 mg) from Fisher Scientific.

Water Sample Preparation

Distilled water samples were prepared daily to limit analyte degradation. Concentrations of analytes used for optimization of extraction parameters were initially 100–150 μg/mL, but were eventually lowered to 0.5–1 μg/mL.

Two parameters were studied: pH and content of sodium chloride of the samples. The pH was adjusted to 3 by adding drops of a diluted phosphoric acid solution (JT Baker). Sodium chloride content was studied to determine the ionic strength effect on the efficiency of extraction; 1 g of ACS grade NaCl (BDH) was added per 5 mL of water sample.

Extraction: SPE Procedure

All extractions were done on a Supelco Visiprep SPE vacuum manifold. Firstly, the SPE cartridges were washed using 3 mL of the elution solvent. The cartridges were then conditioned with 3 mL of methanol and equilibrated with 3 mL of salt water. Immediately after the application of water, 1 mL (5 mL for the optimized method) of sample was extracted under vacuum at a controlled flow-rate of approximately 1 mL/min. The rinsing step, generally recommended by manufacturers, was omitted because this could cause premature loss of the analytes. The cartridges were then allowed to dry under vacuum (2–5 min) and the analytes were eluted with 3 mL of the elution solvent at a flow-rate of approximately 1 mL/min. Finally, pesticide-grade sodium sulfate (Fisher Scientific) was used to remove residual water, and acenaphtene-d_{10} (internal standard) was added to the extracts, which were then subjected to GC-MS analysis. The reproducibility of the efficiency was evaluated by performing triplicate analyses. Consequently, the procedure and parameters were optimized for maximum recovery.

Breakthrough volume was assayed by using different volumes of sample (1–25 mL) and keeping the quantity of analytes constant, allowing assessment of the impact of sample volume on recovery. A sample volume of 100 mL was also briefly considered.

Matrix Effect

Water samples were collected from different sources in and around Moncton, NB, Canada and prepared in the same way as the distilled-water samples (see above). Freshwater samples were collected from Gorge, Halls and Jonathan Creeks, Gorge road spring water, a local tap (Université de Moncton), falling rain, snow and melting snow. The seawater sample was taken from Northumberland Strait in Grande-Digue, NB.

Solid samples (soil and compost) were prepared in a similar way. Approximately 5 g of solid was added to 50 mL of spiked distilled water, equilibrated for 30 min by mixing, and then filtered. A volume of 5 mL collected from the aqueous phase was then extracted as described above. Compost samples were the Tiru company compost (domestic and industrial waste), pig compost (pig manure, wood shavings and mud)

and shrimp compost (shrimp, bovine and poultry manure and peat). Soil samples included black earth, which was bought at McArthur's Nursery in Moncton, NB, and sandy loam soil collected in Cocagne, NB. Solid samples were chosen to represent low to high organic matter content: Tiru compost (46.1%), pig compost (67.3%), shrimp compost (62.3%), sandy loam soil (4.9%) and black earth (70.5%).

Responses collected by GC-MS were used to calculate recoveries, which were then compared to those acquired after extraction and analysis of distilled water samples fortified at the same level (1 μg/mL).

Chromatographic Conditions

An Agilent HP 6890 gas chromatograph equipped with a quadrupole mass-selective detector HP 5973N equipped with a 7683 series automatic liquid sampler was used for all analyses. Injections of 2 μL were made in splitless mode with a split/splitless injector at a temperature of 200°C into a fused-silica capillary column (30 m × 0.25 mm) coated with 0.25 μm chemically bonded HP-5MS phase (5% phenyl methyl siloxane) from Agilent. Helium was used as carrier gas (1.9 mL/min).

The total analysis time on the GC for one run was 16 min and the programming for the oven was: 2 min at 60°C, ramp 10°C/min to 200°C. The GC-MS interface was kept at 300°C and the source at 230°C.

The electron impact ionization method with electron energy of 70 eV was used for data collection. Data for the analytes was collected in single ion monitoring (SIM) mode, but presence of other compounds was verified in scan mode with the help of a spectrum library. The following mass fragments were monitored in SIM mode: methamidophos (94 and 136), acephate (94 and 141), and acenaphtene-d_{10} (80 and 164) and were chosen not for their intensity but rather for their specificity. The dwell times were chosen in reference to the peak width so that 10 data points could be collected per peak, and ranged from 20 to 100 ms. Parameters used for data acquisition were previously optimized to offer maximum sensitivity and a reliable repeat value. Quantification of acephate and methamidophos was done using the internal standard method. The ratios of the peak areas of the analytes and the internal standard were used for quantification.

RESULTS AND DISCUSSION

General Considerations

The first SPE attempts at acephate and methamidophos recovery done with 100 mL sample volume did not yield interesting recoveries and this was linked to the possible low breakthrough volume of the analytes. The volume extracted was therefore lowered from 100 mL to 1 mL and was later adjusted to improve the limits of the method.

Recovery Studies

Cartridges

Two categories of cartridges were considered: silica-based and polymeric. The more traditional silica-based cartridges were eliminated early in the study considering their

low efficiency for the extraction of highly polar analytes from water. Polymeric cartridges, on the other hand, gave significant recoveries even before optimization was undertaken and were usually easier to use. Three cartridges were chosen for the remainder of the study: Oasis HLB, Strata X and Speedisk H_2O-Philic. These adsorbents are made respectively of [poly(divinylbenzene-co-*N*-vinylpyrrolidone)], a modified surface [poly(divinylbenzene)] and [poly(divinylbenzene)].

Sample Preparation

Firstly, the impact of sample pH on efficiency of extraction was investigated at pH 3 and 5 (results are presented in Fig. 2). According to the manufacturers, polymeric adsorbents are stable over a wide spectrum of pH, usually from 1 to 14, but it seems that their efficiency is usually increased at mid-pH (Strata X and Speedisk H_2O-Philic cartridges). However, the efficiency of extraction of both analytes seemed independent of pH with the Oasis HLB cartridges. Finally, a sample pH of 3 was chosen since pesticides are generally more stable in an acidic medium, even though this could represent loss in recovery [17].

Secondly, the ionic strength effect at pH 2–3 was considered (results are presented in Fig. 3). Addition of NaCl was deemed beneficial with the Oasis HLB and Speedisk H_2O-Philic cartridges since recovery improved. This is most likely due to the fact that sodium chloride tends to solvate more efficiently in water than the two analytes, making them less soluble in water and facilitating their interactions with the adsorbent. With the Strata X cartridges a decrease in recovery is noticed, presumably because the interactions between this adsorbent and the analytes are more efficient, causing more retention of the analytes at the beginning of the cartridges. Having demonstrated greater potential, the Oasis HLB and Speedisk H_2O-Philic cartridges were chosen for the remainder of the study and further samples were prepared with sodium chloride at pH 3.

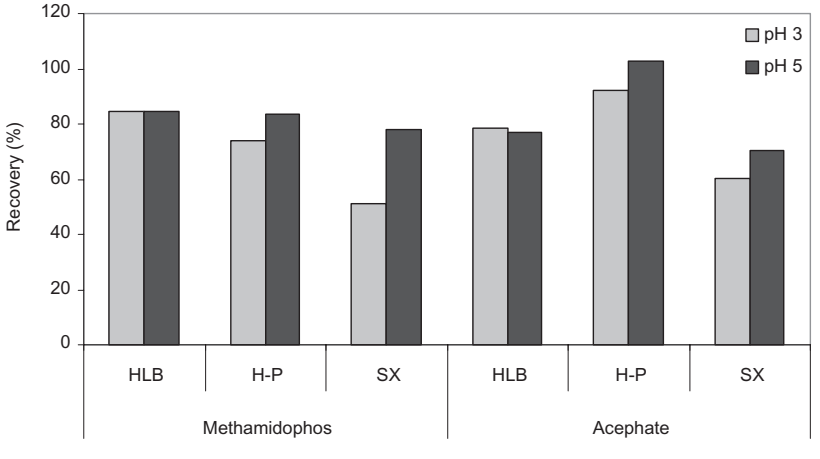


FIGURE 2 Influence of sample pH on recovery of methamidophos and acephate with different cartridges (NaCl 1 g/5mL).

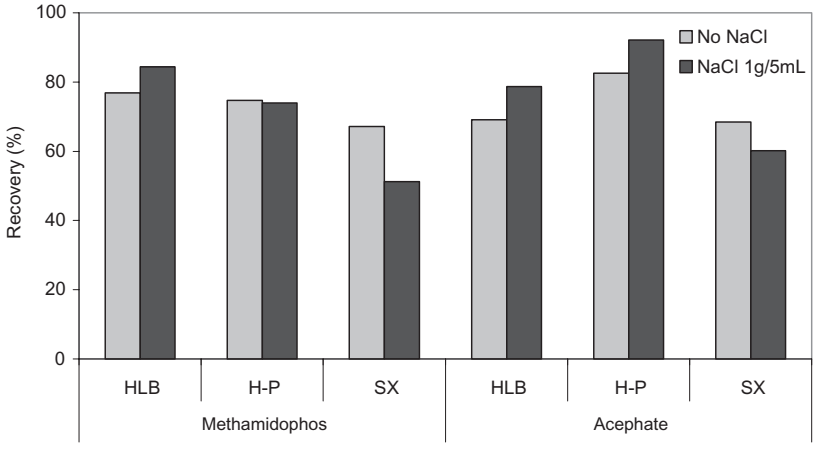


FIGURE 3 Effect of ionic strength on recovery of methamidophos and acephate with different cartridges (pH = 3).

TABLE I Recovery of methamidophos and acephate using three elution solvents

Solvent	*Total recovery* (%)			
	Methamidophos		*Acephate*	
	Oasis HLB	*Speedisk H_2O-Philic*	*Oasis HLB*	*Speedisk H_2O-Philic*
Ethyl acetate	81.9	51.9	71.6	66.3
Acetonitrile	97.2	71.9	72.3	67.8
Methylene chloride	84.4	65.6	78.7	76.0

Elution Solvent

Several elution solvents were considered: methylene chloride, ethyl acetate, acetonitrile, acetone, toluene and hexane. Toluene and hexane were rapidly dismissed because of their low polarity and affinity with the analytes. Acetone was also eliminated since collected fractions were somewhat cloudy, which could be caused by adsorbent dissolution. Investigations with methylene chloride, ethyl acetate and acetonitrile as elution solvents were only performed once and results are presented in Table I. Higher extraction recoveries were obtained with methylene chloride and acetonitrile for acephate and methamidophos respectively for both cartridges. Since fractions collected with acetonitrile were sometimes blurry, different combinations of methylene chloride and acetonitrile were considered as elution solvent, but no significant improvements were noticed. Therefore, the elution solvent methylene chloride and the Oasis HLB cartridges were chosen for the remainder of the study.

Sample Volume

In order to maximize the method's potential, a higher sample volume must be considered, but since the analytes have a great affinity for water compared with the

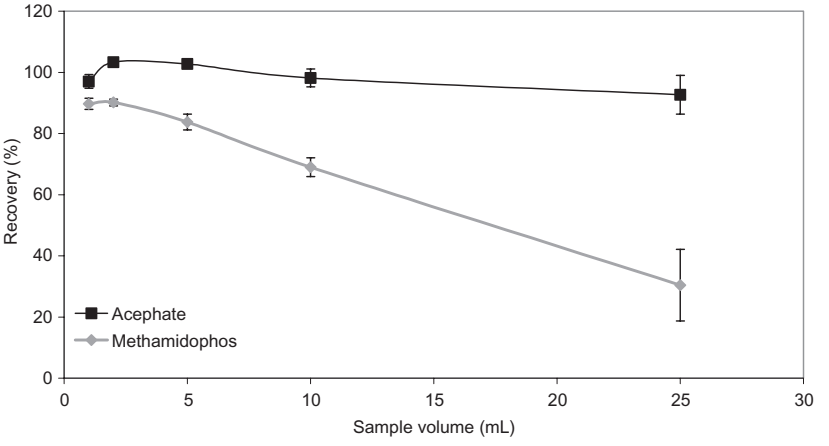


FIGURE 4 Determination of the maximum sample volume for the solid-phase extraction of methamidophos and acephate from water with an Oasis HLB cartridge (3 mL/60 mg) ($N = 3$).

TABLE II Recovery of methamidophos and acephate following an evaporative step using a stream of nitrogen (15–20 min concentration time) ($N = 3$)

Sample	*Methamidophos*		*Acephate*	
	Relative recovery (%)	*Variation coefficient* (%)	*Relative recovery* (%)	*Variation coefficient* (%)
Not concentrated	100.0	1.0	100.0	1.6
Concentrated to ~0.5 mL	75.4	0.8	74.4	5.3
Concentrated to dryness	76.1	7.8	75.6	15

adsorbent used care must be taken not to surpass the breakthrough volume, thus avoiding premature loss of analytes. Therefore, the breakthrough volume was assessed with a sample volume of 1–25 mL. In Fig. 4 it can be seen that the recovery of acephate slowly deteriorates with an increase in sample volume, but is still over 80% at 25 mL. For methamidophos, however, recovery declines rapidly after 5 mL, probably because methamidophos exhibits a higher affinity with water. Therefore, if a joint study of these two pesticides is to be done, a maximum sample volume of 5 mL is recommended for solid-phase extraction. It should be noted that breakthrough volume is dependent on adsorbent mass and, increasing this mass could permit a higher sample volume, which would improve the method's detection limit.

Evaporative Concentration

In order to improve the detection limits of the method, an evaporative step was considered, but it was found that evaporation of solvent under vacuum decreased the recovery for methamidophos by approximately 20%. Another technique, the evaporation of solvent under a stream of nitrogen, did not improve recovery. Results for this technique are presented in Table II. For average recovery, it did not seem to matter whether the extract was concentrated or completely evaporated followed by reconstitution with the

appropriate solvent, although an increase in variation was noted in the latter. After consideration, the evaporative step was eliminated from the procedure.

Quality Control

The linearity of the method was evaluated using the internal standard method following analysis of seven standard solutions prepared in methylene chloride with concentrations ranging from approximately 0.05 to 10 μg/mL of analyte containing 1 μg/mL of internal standard. Linear calibration curves were obtained for both analytes, but quadratic regression curves proved to be more reliable and accurate. In fact, correlation coefficients for quadratic calibration curves for acephate and methamidophos were generally better than 0.999 compared to 0.98 for linear trend curves. Back-calculation of the concentrations of the standards with the constructed curves resulted in deviations usually less than 10%. The instrument limits of detection and quantification were estimated at approximately 10 μg/L and 30 μg/L, respectively, for both analytes.

To investigate the matrix effect, a variety of water samples were collected, fortified at the 1 μg/mL level, extracted and analysed. Blanks of these samples were analysed and showed no measurable contribution. The data obtained is presented in Fig. 5.

A slight suppression was generally observed for both analytes. In most cases, the relative recoveries were between 90 and 110%, demonstrating good method reliability, even in the presence of numerous concomitant species. Results acquired for acephate were as follows: seawater, 95.8%; Gorge Creek, 90.0%; Halls Creek, 90.9%; Jonathan Creek, 96.3%; spring water, 97.3%; tap water, 104.2%; rain, 112.2%; snow, 108.3% and melting snow, 109.5%. For methamidophos, results obtained were: seawater, 78.7%; Gorge Creek, 88.4%; Halls Creek, 80.8%; Jonathan Creek, 101.0%; spring water, 102.8%; tap water, 103.5%; rain, 95.1%; snow, 98.4% and melting snow, 108.7%. Only three samples showed a relative recovery below 90%: seawater, Gorge Creek and Halls Creek.

Two major factors may contribute to this decrease in efficiency of the extraction procedure. Firstly, interactions between the analytes and matrix constituents could

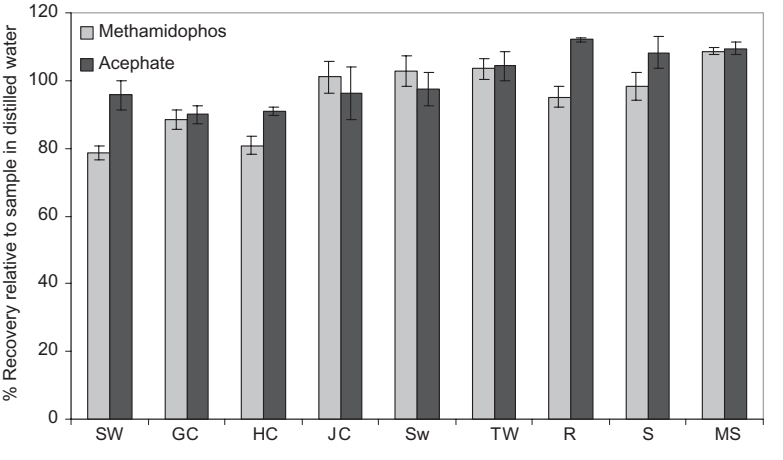


FIGURE 5 Matrix effect for real water samples: Seawater (SW), Gorge Creek (GC), Halls Creek (HC), Jonathan Creek (JC), Spring water (Sw), Tap water (TW), Rain (R), Snow (S) and Melting snow (MS) ($N = 3$).

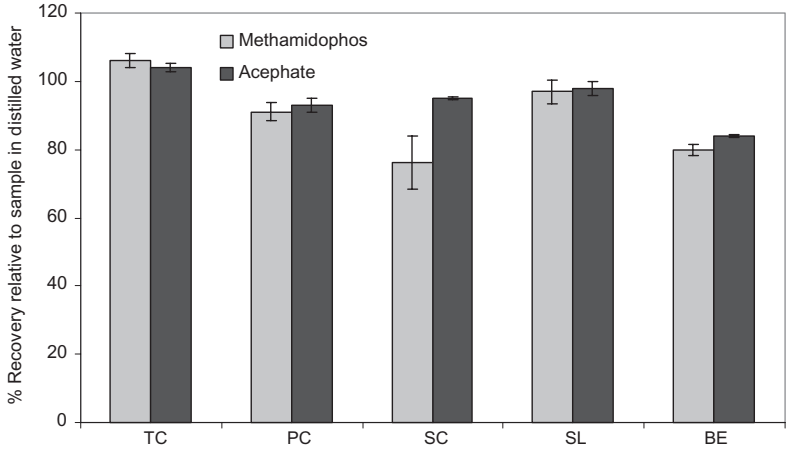


FIGURE 6 Matrix effect for solid samples: Tiru compost (TC), pig compost (PC), shrimp compost (SC), sandy loam soil (SL) and black earth (BE) ($N = 3$).

reduce retention of the analyte on the adsorbent. Secondly, it is possible that some less-polar contaminants could be retained preferentially on the adsorbent, blocking the adsorption sites, and diminishing the retention and recovery of the analytes.

The possibility of using this extraction procedure for acephate and methamidophos in soils and composts was also investigated. As seen in Fig. 6, the matrix effect was generally low, except in the presence of a high content of organic matter (more than 50%). Relative recoveries for acephate were: Tiru compost, 103.9%; pig compost, 93.2%; shrimp compost, 95.0%; sandy loam soil, 97.6% and black earth, 83.7%. For methamidophos these were: Tiru compost, 105.5%; pig compost, 91.3%; shrimp compost, 75.8%; sandy loam soil, 96.6% and black earth, 80.4%. For soils and composts low in organic matter content (Tiru compost and sandy loam soil) the matrix effect is negligible, whereas in those with a high organic content (pig and shrimp composts and black earth) a decrease in the efficiency of the extraction of approximately 10–20% was noticed for both analytes. However, these reductions are relatively small considering the complexity of the matrix, which shows high method reliability. For accurate quantification, the matrix effect should be compensated for.

As mentioned above, interactions between the analytes and the matrix constituents, as well as preferential adsorption of less polar compounds on the adsorbent, could possibly reduce the efficiency of the extraction. Other factors could also be important for solid samples. Presence of microorganisms could promote analyte degradation. Furthermore, metals could complex the analytes diminishing their interactions with the adsorbent. Finally, OPs tend to interact with soil and particulate matter and the analytes may well be lost during the filtration step.

CONCLUSIONS

Solid-phase extraction and gas chromatography–mass spectrometry can be respectively used adequately for the extraction and quantification of acephate and methamidophos in water, soils and compost. Maximum recoveries are 85–90 % for methamidophos

and 90–95% for acephate with Oasis HLB cartridges and methylene chloride elution solvent. Evaporative concentration was proven difficult, showing a 20–30% loss of recovery and was eliminated from the procedure. The method detection limit of 50 μg/L is very promising since not much sample was used and the limit could be improved with increasing adsorbent mass.

Analysis of real fortified water samples revealed relative recoveries of 90–110% for both analytes with coefficients of variation generally below 10%. The matrix effect was more pronounced in three samples (relative recoveries below 90%), which is probably due to a more complex matrix. Furthermore, solid (compost and soil) samples were also considered in this study. Relative recoveries were 90–105% for both analytes with coefficients of variation generally below 10%. A small decrease in recovery is observed with an increase of organic matter content. Nevertheless, the developed method is simple, efficient and reliable, offering minimal variability and good recovery even in the presence of a more complex matrix.

Acknowledgements

The authors thank the National Sciences and Engineering Research Council of Canada and the Faculté des Études Supérieures of l'Université de Moncton for their financial support.

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