MONITORING OF PESTICIDE RESIDUES IN RIVER NILE WATER FROM EGYPT BY SOLID-PHASE EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROSCOPY

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Ali, Hanaa[1], F.M.; M.A. Osman[1]; T.L. Potter[2] and M.A. Mohamed[1]

ABSTRACT

Solid-phase extraction (SPE), gas chromatography electron capture detector (GC-ECD) and gas chromatography nitrogen-phosphorous detector (GC-NPD) and gas chromatography–mass spectroscopy (GC-MS) were applied for trace-level determination of 20 pesticides in river nile water. Samples were collected from March to October 2003 from different sites located in Greater Cairo, Egypt. First, extraction by on-site solid-phase extraction of 1 liter from field and laboratory spiked and unspiked (raw river water) samples using poly-divinyl benzene-N-vinyl pyrolidine cartridge based sorbent. Next, water extracts were subject of analysis by GC-ECD and GC-NPD. Next, selected samples that were positive to GC-ECD or GC-NPD were analyzed by GC-MS in order to improve the determination of detected pesticides. Recoveries from laboratoy spiked samples were > 85% for 16 of the 20 compounds with % relative standard deviation (% RSD) in the 5 to 10 % range. The lowest recoveries were for aldrin, 52 % and prothiofos, 48 %. A similar trend was observed with p,p-DDE and p,p-DDT values. Field spike results also indicated high % recovery for most of the target compounds. Values were > 85% for 12 of the 20 analytes, as was the case in laboratory spikes, Aldrin, 54 % and prothiofos, 55 % yielded the lowest values. Overall field spike recovery reproducibility was lower since % RSDs were higher, 15-40 %. Overall results indicated a relatively high degree of accuracy and precision could be achieved for most of the target compounds by methods applied in present study.

Key words: Solid-phase extraction, Pesticides residues, Gas chromatography, Mass spectroscopy

INTRODUCTION

The river Nile has been regarded as the life artery of Egypt throughout known Egyptian history. The river has a dominating influence on the economy, culture, and public health. The Nile is the sole source of drinking water for more than 16 million residents of Greater Cairo. Data documenting the levels of pesticide residues in the Nile are needed to assess the water quality. The conduct of such as-

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1- Biochemistry Department, Agriculture Faculty, Cairo University, Giza, Egypt
2- USDA, Agricultural Research Service, Tifton, GA, USA

sessments is consistent with guidelines specified in the most recent version of the FAO international code of conduct on the distribution and use of pesticides **(FAO, 2003)**. These assessments typically include estimates of how much of pesticide active ingredient under a given set of use conditions may reach lakes, streams and rivers used for water supplies **(USGS, 1999)**. A limiting factor in the collection of data to conduct residue analysis studies on the Nile is the need for highly specialized instrumentation to achieve appropriate detection limits. This type of equipments is costly and difficult to maintain and is not readily available in all laboratories in Egypt and other countries. A solution is to collect and extract samples in the field and ship extracts to central laboratories to maintain state-of-the-art residue analysis. One way of doing this is through the use of field-based solid-phase extraction techniques (SPE). A number of studies have shown that SPE is low cost and effective way of concentrating and preserving water samples for pesticide residues analysis **(Senseman *et al* 1993; Johnson *et al* 1994; Senseman *et al* 1995; Ferrer and Barcelo, 1997; Aguilar *et al* 1999; Sabik and Jeannot, 2000; Rodriguez-Mozaz, S. *et al* 2004 and Gonçalves and Alpendurada, 2004)**. A critical quality assurance step in using SPE for pesticide extraction is determining the efficiency of recovery of analytes of interest **(Ferrer and Barcelo, 1999)**. The need for this is based on the fact that the physical and chemical properties of pesticides and adsorbents used in SPE devices vary widely, therefore, development of a universal SPE adsorbent which will quantitatively recover all pesticides from water is an often stated research goal, but development of such universal

devices remains elusive **(Pichon, 2000; Sabik *et al* 2000 and Huck and Bonn, 2000)**.

The main objectives of present study are to assess the performance of a SPE cartridge which contains a new macroporous adsorbent, prepared from a co-polymer, poly-divinyl benzene-N-vinyl pyrolidone as a universal SPE adsorbent for on-site extraction of water samples through preparation and analysis of matrix spikes under field and laboratory conditions and also to determine of levels of selected pesticide residues in raw water in Greater Cairo, Egypt.

MATERIAL AND METHODS

2.1. Chemicals

Pesticide stock solutions of 1000 ppm were obtained from Supelco (Milford, USA). Chromatographic grade of Methanol and Dichloromethane was obtained from Merck (Darmstadt, Germany).

2.2. Sampling

From March to October, 2003, 40 river Nile water samples were collected from 4 different sites around Greater Cairo (Abo Elnomrs, Tahma , Shbermant and El-Ayat). The samples were collected using 10-L glass containers. The samples were filtrated to remove suspended particles, then were split into two equal volumes.

2.3. Spiking of samples

One liter of sample was spiked with aliquots of pesticide mixtures. This included a 3 component mixture of triazine herbicides (atrazine, prometon and

ametryn , and a 6 components mixture of organophosphate insecticides (ethoprophos, disulfoton, methyl parathion, fenchlorphos, chlorpyrifos and prothifos and an 11 component mixture of chlorinated hydrocarbon insecticides (aldrin, gamma-BHC (lindan), *p,p*-DDD, *p,p*-DDE, *p,p*-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin and heptachlor.The target spiking level for each component was 1µg /L^{-1}.

2.4. SPE extractions

Automated SPE was performed with a Supelco manifold extraction unit connected with an oil-less vacuum pump. The cartridges (OASIS HLB 6 ml, 200 mg, Waters, Milford, USA) were conditioned sequentially with 3 ml of methanol and 3 ml of deionized water. One liter of water sample was passed though the cartridge at a flow-rate of 5 ml/min and then washed with 5 ml of deionized water. After extraction, all cartridges were attached for 30 min to a Supelco evaporating unit connected with purified nitrogen gas to eliminate water residues from cartridges. Dried cartridges were wrapped in aluminum foil and shipped to the Southeast Watershed Research Laboratory located in Tifton, GA, USA. Shipments were made using the an express mail service which provided delivery within 3-5 days without freezepacks or other material designed to keep cartridges cold. Immediately after receipt in the USA, the cartridges were sequentially eluted with pesticide residue analysis certified methanol and methylene chloride. The combined eluent volume was reduced to approximately 1 ml by evaporation under a stream of purified nitrogen gas. An additional 10 ml of methanol was added and the entire sample concentrated to 1.00 ±

0.01 g. After transfer to a glass autosampler vial, the sample was fortified with 5 µg of 2-chlorolepine (as an internal standard) and stored in a –20 °C freezer until analysis.

2.5. GC and GC-MS conditions

Gas chromatography was carried out with thermoionic ionization (HP model 6890), an electron capture detector (HP model 5890) and a GC-MS (HP model 5972) was used. The instruments utilized helium as a carrier gas under the following conditions: fused-silica column HP-5 MS (30 m x 0.25 mm, d_f – 0.25 µm), 60°C for 1 min, 60-175°C (4 min) at 6°C/min, 175-240°C (5 min) at 3°C/min, 240-300°C (1 min) at 7°C/min, split-less, temperature of interface was at 270°C, source temperature at 200°C, temperature of injector at 250°C. Electron impact ionization at 70 eV was used. All samples were analyzed in the SIM mode for quantitative analysis of the compounds (Major ions corresponding to the typical fragments of the compounds were selected, and the scan mode was set in the range 70-450 u for confirmation of the spectral data against a real standard and library search.

RESULTS AND DISCUSSION

Summary statistics for percent recovery of 20 compounds from field and laboratory spikes are compiled in Tables (1 and 2). When computing field spike recovery, the response (if any) obtained for each compound in the unspiked portion of each sample was subtracted from responses obtained in corresponding spiked portions. The adjusted value was compared to the target 1 µg L^{-1} nominal spiking rate.

Table 1. Recovery of selected pesticides in selected SPE cartridges after spiking at 1 µg
L^{-1} in distilled deionized water (n=5)

Compound	Class	Instrumental analysis mode	Average % R	Standard deviation
ametryn	triazine	GC-NPD	100	3.9
atrazine	triazine	GC-NPD	110	4.8
prometon	triazine	GC-NPD	110	2.2
chloropyrifos	organophosphate	GC-NPD	88	2.8
disulfoton	organophosphate	GC-NPD	94	3.8
ethoprophos	organophosphate	GC-NPD	106	5.0
fenchlorphos	organophosphate	GC-NPD	91	2.4
methyl parathion	organophosphate	GC-NPD	87	17
prothiofos	organophosphate	GC-NPD	48	4.5
aldrin	chlorinated hydrocarbon	GC-ECD	52	2.7
p,p-DDD	chlorinated hydrocarbon	GC-ECD	90	11
p,p-DDE	chlorinated hydrocarbon	GC-ECD	69	3.7
p,p-DDT	chlorinated hydrocarbon	GC-ECD	76	0.9
dieldrin	chlorinated hydrocarbon	GC-ECD	100	4.7
endosulfan I	chlorinated hydrocarbon	GC-ECD	110	2.1
endosulfan II	chlorinated hydrocarbon	GC-ECD	110	9.9
endosulfan sulfate	chlorinated hydrocarbon	GC-ECD	110	7.9
endrin	chlorinated hydrocarbon	GC-ECD	110	4.1
heptachlor	chlorinated hydrocarbon	GC-ECD	86	4.7
lindane (BHC isomer)	chlorinated hydrocarbon	GC-ECD	110	2.9

% R : % recovery

Table 2. Recovery of selected pesticides on SPE cartridges after spiking at 1 µg L^{-1} in distilled deionized water (n=5)

Compound	Number of results classed as outliers			% Recovery	
	total 10%<%R<130%	high > 130 %	low <10 %	average	SD
ametryn	5	0	5	80	14
atrazine	30	25	5	96	26
prometon	5	0	5	93	19
prometryn	30	5	25	81	36
chloropyrifos	35	25	10	80	29
dichlorvos	15	15	0	64	25
disulfoton	35	35	0	65	29
ethoprophos	30	30	0	95	19
fenchlorphos	40	40	0	89	25
methyl parathion	80	70	10	118	9
prothiofos	5	5	0	55	29
aldrin	45	0	45	54	30
dieldrin	45	35	10	91	30
p,p-DDT	55	0	55	64	29
p,p-DDD	25	20	5	90	20
p,p-DDE	40	15	25	78	19
endosulfan I	5	5	0	88	17
endosulfan II	5	5	0	92	26
endosulfan sulfate	10	10	0	83	31
endrin	20	20	0	91	26
lindane(BHC isomer)	75	75	0	92	12

% R : % recovery

The results for unspiked samples (raw River Nile samples) are presented in Table (3). Recoveries from laboratory spiked samples were > 85 % for 16 of 20 compounds with % relative standard deviation (% RSD) in the 5 to 10 % range (Table, 1). The lowest recoveries were for aldrin, 52 %, and prothiofos, 48%. No explanation is readily available although it is notable that the results were reproducible since the % RSD was < 5%. A similar trend was observed with p,p-DDE and p,p- DDT values. Recoveries were less than quantitative, 68% and 76% respectively, but highly reproducible, % RSD < 5%. Overall results indicated a relatively high degree of accuracy and precision can be achieved for most of the target compounds by SPE extraction. For the 4 compounds which yielded recoveries that were less than quantitative, low % RSD's were observed. Field spike results also indicated a high % recovery for most of the compounds (Table, 2). Values were > 85 % for 12 of 20 analytes. As was the case in the laboratory spikes, aldrin, 54% and prothiofos, 55 %, yielded the lowest values. Overall field spike recovery reproducibility was lower since % RSD's were higher, 15-40 %. Higher % RSDs in field versus laboratory spikes were expected and commonly observed. While field spike results appeared comparable to laboratory spike data, many field values were highly uncertain. This was indicated by the large number of outliers, i.e.samples with % recoveries < 10% or > 130%, observed for many compounds (Table, 2). Among the 20 samples analyzed, 19 of 20 had at least 1 result considered an outlier. Compounds stood out in this regard, the organophosphate, methyl-parathion, and the chlorinated hydrocarbon, lindane. A total 75% of lindane

and 80% of methyl-parathion results were anomalously high, > 130%. One possible explanation was the introduction of coeluting interferences during field extraction. Field blanks were unavailable for analysis. While it appears that uncertainties in % recoveries of methyl-parathion and lindane may have been connected to coextraction and or introduction of interfering compounds, unspiked sample results argued against this. As indicated in table 3, there were only sporadic detections of methyl-parathion and no lindane detections in any of the unspiked samples analyzed. This was the case for nearly all of the 20 used compounds. When residues were detected, levels were uniformly close to analytical limits of detection. The only exception was aldrin. It was tentatively identified in 11 samples by GC-ECD. However, an GC-MS analysis did not confirm results, it is concluded that an interference likely yielded a false positive result, however, further work is required to verify this. Among the field spike samples aldrin % recovery results were also problematic. There were numerous outliers with low % recovery. The opposite was observed with dieldrin suggesting conversion of aldrin to dieldrin in some samples. Dieldrin is an oxidized form of aldrin. Studies have shown that UV irradiation readily catalyzes aldrin-dialdrin conversion **(USEPA, 2000 and 2002)**. This could have occurred when samples, sample solvent extracts or standard spiking solution were exposed to light. Another conversion that may have occurred during analysis was the transformation of p,p- DDT and p,p DDE to p,p-DDD. A possible point where this occurred was during gas chromatographic (GC) analysis. Dechlorination

Table 3. Concentration of selected pesticides in Nile River nile water
samples (n=20)

Compound	Concentration ($\mu g\ L^{-1}$)		
	% detects	minimum	maximum
ametryn	0	0.64	< 0.13
atrazine	0	0.13	< 0.13
prometon	0	<0.13	< 0.13
prometryn	5	0.21	<0.06
chloropyrifos	0	0.13	< 0.13
dichlorvos	0	<0.13	< 0.13
disulfoton	5	0.09	<0.06
ethoprophos	5	0.20	<0.06
fenchlorphos	5	0.20	<0.06
methyl parathion	15	0.49	< 0.13
prothiofos	0	<0.06	<0.06
aldrin	55	1.04	<0.01
p,p-DDT	5	0.09	<0.01
p,p-DDD	0	<0.01	<0.01
p,p-DDE	5	0.33	<0.01
dieldrin	0	<0.01	<0.01
endosulfan I	10	0.21	<0.01
endosulfan II	5	0.11	<0.01
endosulfan sulfate	5	0.08	<0.01
endrin	5	0.09	<0.01
lindane(BHC isomer)	0	<0.01	<0.01

of p,p- DDT with formation of p,p DDE and p,p-DDD is often observed following p,p- DDT injection into heated GC injection ports unless GC systems are carefully deactivated. Even if deactivation is maintained, the conversion can occur if field sample extracts have large amounts of co-extracted materials which are simultaneously injected with target analytes. Injection of p,p- DDT standards alone did not indicate substantial degradation and production of dechlorinated by-products, thus it was concluded that Nile samples likely contained co-extracted materials which promoted p,p-DDT degradation upon injection. Overall results indicated a relatively high degree of accuracy and precision could be achieved for the most target compounds by applied methods in present study.

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رصد متبقيات المبيدات في نهر النيل في مصر باستخدام طريقة الاستخلاص بالطور الصلب والتحليل بجهاز الكروماتوجرافي الغازي والكروماتوجرافي الغازي المتصل بمطياف الكتلة

[10]

هناء فوزي محمد علي[1] – محي الدين على عثمان[1] – توماس بوتر[2] – محمود عبد الحليم محمود محمد[1]

1- قسم الكيمياء الحيوية – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

2- معمل هيئة الزراعة الأمريكية – تفتون – جورجيا – الولايات المتحدة الأمريكية

الاستخلاص بالطور الصلب وجهاز الكروماتوجرافي الغازي المزود بالكاشف من نوع القابض للإلكترونات أو نتروجين فوسفور وجهاز الكروماتوجرافي الغازي المتصل بمطياف الكتلة استخدمت لتقدير المستويات المنخفضة جدا من متبقيات المبيدات في مياه نهر النيل . العينات تم تجميعها خلال شهر مارس إلى أكتوبر 2003 من مناطق مختلفة تقع في القاهرة الكبرى. أولا الاستخلاص بواسطة طريقة الطور الصلب في أماكن تجميع العينات سواء الحقلية(مياه النهر) أو المعملية المضاف أو الغير مضاف إليه مخلوط المبيدات القياسية بحيث يضاف لتر من العينات إلى أعمدة من النوع البولي-داي فينيل بنزين-ن-فينيل بيرو ليدين كمادة ادمصاصية. مستخلصات المياه تم تحليلها بواسطة جهاز الكروماتوجرافي الغازي والنتائج التي أعطت نتيجة إيجابية مع أي من نوعي الكاشف تم تحليلها بواسطة جهـــــاز

الكروماتوجرافي الغازي المتصل بمطياف الكتلة لزيادة كفاءة الكشف عن المبيدات. نسبة الاسترجاع للعينات المعملية المضاف إليها مخلوط المبيدات القياسية كانت اكبر من 85% و ذلك لحوالي من 16 من 20 مركب و بانحراف قياسي نسبي من 5 إلى 10% واقل نسبة استرجاع كانت 52% لبعض المبيدات الكلورينية و48% لبعض المبيدات الفسفورية. بالنسبة لعينات الحقلية المضاف إليها مخلوط المبيدات القياسية كانت نسبة استرجاع عالية لمعظم المركبات المستهدفة و المستويات كانت اكبر من 85% لحوالي 12 إلى 20 مركب . واقل نسبة استرجاع كانت 45% لبعض المبيدات الكلورينية و55% لبعض المبيدات الفسفورية. عموما النتائج دلت علي انه يمكن الوصول إلى درجة عالية نسبيا من الدقة و الحساسية في تقدير المركبات المستهدفة بتطبيق الطرق المستخدمة في هذه الدراسة.

تحكيم: أ.د راجى رياض فرنسيس   أ.د محمد حلمى بلال

1- Meat and Fish Technology Research Department, Food Technology Institute, Agri-culture Research Center, Giza, Egypt