

# Pressurized hot water extraction coupled to molecularly imprinted polymers for simultaneous extraction and clean-up of pesticides residues in edible and medicinal plants of the Okavango Delta, Botswana

## Abstract

In this study, an in-cell extraction and clean-up approach, employing pressurized hot water extraction (PHWE) coupled to a molecularly imprinted polymer (MIP) is proposed. The selectivity of PHWE was improved through the use of a chlorophyll MIP (PHWE-MIP) for the determination of organochlorine pesticides residue levels in various edible and medicinal plants of the Okavango Delta, Botswana. The PHWE-MIP method achieved simultaneous extraction and clean-up. PHWE employed an optimal temperature of 260 °C, pressure of 90 bar and flow rate of 1 mL min<sup>-1</sup> in 10 min for the extraction of the pesticides from plants while the MIP selectively overcame the interfering chlorophyll prior to analysis with gas chromatograph coupled to electron capture detector or mass spectrometer (GC-ECD/MS). The results obtained were compared to the QuEChERS Official Method 2007:01 for pesticides residue analysis. The proposed method seems to be nearly fully automated, environmental friendly, selective, simple and quick. Moreover, the recoveries of planar pesticides were improved (93-95%) with relative standard deviations (%RSD) of less than 10%.

## Keywords

Subcritical water extraction • Molecular imprinted polymers • In-cell clean up  
• Sample preparation • Environmentally friendly

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## 1. Introduction

Organochlorine pesticides (OCPs) are of great concern due to their persistence, toxicity, endocrine disrupting effects and bioaccumulation. They are difficult to be metabolized in both biota and environmental matrices [1]. Although the application of most OCPs has been restricted for a considerable period of time in many countries, residues continuously induce a significant impact on the environment and ecosystems [2]. In aqueous and marine environments, OCPs tend to have strong affinities for suspended particulates and accumulate in sediments. The determination of OCP levels in sediments can therefore indicate the level of contamination and bioaccumulation in aquatic organisms and plants.

Plants of medicinal value like crops are susceptible to insect attacks both in the field and storage, and as such pesticides are widely used for their protection [2]. With the ever-increasing use of herbal medicines and the global expansion of the herbal medicines market, safety has become a major concern for both health authorities and the public [3]. Within the overall

context of quality control of herbal medicines, the World Health Organization (WHO) developed general global guidelines for assessing the safety of potentially hazardous substances in herbal medicines, with particular reference to pesticide residues. The maximum residue limit (MRL) recommended by the Codex Alimentarius Commission to be legally permitted in DDT and its metabolites, dichlorvos and hexachlorobenzene are 1.0, 1.0 and 0.1 mg kg<sup>-1</sup> respectively [4].

The monitoring of pesticide residues requires the development of fast, environmentally friendly and effective methods with minimal sample preparation. Traditional sample extraction methods for solid matrices include sonication, soxhlet extraction and ultrasonic extraction. These methods are often time consuming, cumbersome and also require large volumes of non-environmental friendly organic solvents [5]. With the necessity for faster analysis, higher recoveries, possibility of automation and reduced organic solvent usage for newer extraction, techniques such as microwave assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are have been employed [6]. Several studies

have shown that these methods in many cases could be equally or even be more efficient than the traditional methods [7-10].

Pressurized hot water extraction (PHWE), that is, extraction using hot water at temperatures above its boiling point under pressure sufficient to maintain water in the liquid state, has demonstrated its ability to perform more rapid and efficient extraction with minimal consumption of organic solvent [11]. However, when performing extraction at these high temperatures, the selectivity of PHWE is reduced, resulting in co-extraction of untargeted compounds. For instance, in green plants there are high levels of pigments and chlorophyll which often interfere with pesticides analysis [12]. Solid-phase extraction (SPE) utilizes various sorbents such as florisil, silica and alumina as clean-up methods so as to eliminate the interfering substances [13]. SPE cartridges containing C18 [14] and Oasis HLB [15] have been used to isolate pesticides from plant matrices.

A quick, easy, cheap, effective, rugged, and safe (QuEChERS) Association of Official Analytical Chemists (AOAC) sample preparation approach for extraction and clean-up of pesticide residues from multiple classes, was reported as a dispersive form of SPE [16]. In summary, the method uses a single-step buffered acetonitrile (1% HAC) extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ( $MgSO_4$ ) to induce liquid-liquid partitioning. In the clean-up stage of the QuEChERS method, a dispersive solid phase extraction (d-SPE) step is employed. This involves transferring a portion of the acetonitrile extract to a clean-up tube containing a combination of sorbents such as graphitized carbon black (GCB) for the removal of unwanted sample chlorophyll [17]. However, GCB does not remove only chlorophyll. It also retains pesticides with planar structures, resulting in their poor recovery.

Therefore there is a need for a material that is specific for chlorophyll removal without interfering with analytes of interest. Molecularly imprinted polymers (MIPs) are tailor-made functional materials exhibiting specific recognition sites complementary in shape, size and functional groups to the template molecule, which is usually the target in analysis [18]. MIPs are obtained by polymerization of chosen functional and cross-linking monomers around a template molecule. The template-monomer interaction can be through non-covalent interactions, reversible covalent bonds or mixed combinations of the two bonding methods [19]. In recent years, the development of MIPs for solid-phase extraction (MISPE) has been extensively reported in the areas of pharmaceuticals, environmental and food analysis including their use as selective sorbents for the extraction or clean-up of different classes of compounds from various complex matrices [20]. In this study, the selectivity of PHWE was improved through the use of a MIP designed to target the interfering chlorophyll.

## 2. Experimental Procedure

### 2.1 Chemicals and reagents

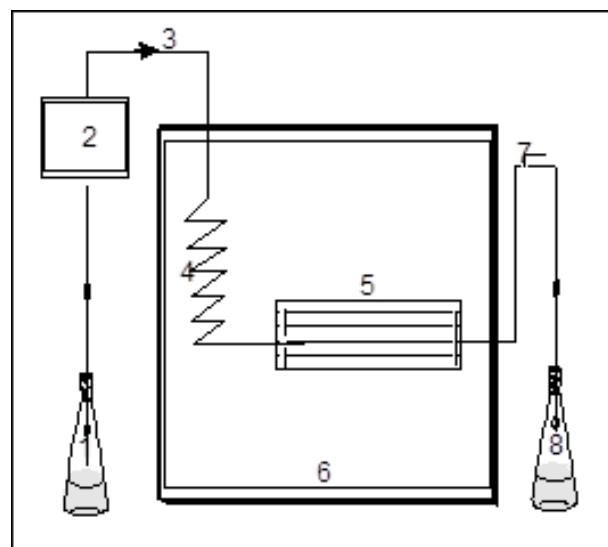
Benzene hexachloride ( $\alpha$ -BHC, 97.9%) and heptachlor (98.5%) were obtained from Supelco (Bellafonte, PA, USA). Aldrin (98.1%), trans-chlordane (98.4%), dichlorodiphenyldichloroethane (4,

4'-DDD, 98.9%), dichlorodiphenylethylene (4, 4'-DDE, 99.5%), 2, 4'-DDE (99.6%), dichlorvos (99.7%), and hexachlorobenzene (HCB, 99.6%) were obtained from Riedel-de-Haën (Seelze, Germany). Individual stock solutions ( $1000 \mu g mL^{-1}$ ) of each pesticide were prepared in acetone and stored in a freezer at  $-20 \text{ }^\circ C$ . HPLC/UV grade acetone and n-hexane were obtained from Ultrafine Limited (London, England). Ultra high purity (UHP) water was generated from a Millipore Alpha-Q system supplied by Millipore (Molsheim, France). Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755 and SampliQ QuEChERS AOAC Dispersive SPE kit, p/n 5982-5058 were supplied by Agilent Technologies Inc. (Santa Clara, CA, USA). The chlorophyll MIP was custom synthesized in our laboratory [21].

### 2.2 Instrumentation

#### 2.2.1 PHWE equipment

All extraction and degradation experiments were performed using in-house built PHWE equipment (Figure 1), featuring a gas chromatographic oven with a maximum temperature of  $350 \text{ }^\circ C$ . Inside the oven, a pre-heater stainless steel coil was present to maintain the programmed temperature, followed by the extraction cell (3 cm in length and 10 mm i.d.) closed with screw caps at both ends, which permitted a continuous flow of water. The screw caps contained stainless steel frits, to ensure that the sample remained inside the extraction cell. A cooler system (made from coiled stainless steel tubing) was used to cool the water from the oven temperature to about  $25 \text{ }^\circ C$ . A restrictor controlled the pressure in the system in order to maintain the extracting water in liquid state. The sample was collected in a glass vial [22].



**Figure 1.** Schematic diagram of laboratory self - assembled PHWE system. 1. Solvent system 2. Pump 3. Flow direction 4. Preheating coil 5. Extraction cell 6. Gas chromatography oven 7. Back pressure regulator 8. Collecting flask.

### 2.2.2 GC Conditions

The determination of OCPs was performed employing an Agilent 6890 gas chromatograph equipped with <sup>63</sup>Ni micro-electron capture detector (GC-ECD). Separation was achieved on a DB-1 column, (30 m × 0.25 mm i.d., and 0.25 μm film thickness). Helium was used as carrier gas at a constant flow of 1.0 mL min<sup>-1</sup> and high purity nitrogen was used as make-up gas (60 mL min<sup>-1</sup>). The temperature program was as follows: initial temperature of 60 °C was held for 2 min, increased to 190 °C at a rate of 5 °C min<sup>-1</sup>, and then increased to 280 °C at a rate of 10 °C min<sup>-1</sup>. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. 1 μL of each sample was injected in the splitless mode.

The MS was operated in electron impact ionization mode with electron energy of 70 eV. The ion source, quadruple and transfer line temperatures were held at 230, 150 and 280 °C, respectively. Target compounds were monitored in selected ion monitoring (SIM) mode.

### 2.3 Sampling and study area

Plant samples (see Table 1) were manually collected and wrapped with aluminum foil between January and September 2010 in the experimental area of the Okavango Delta located in the north east of Botswana, (see Figure 2 for all sampling sites) [23]. Upon arrival at the lab the plants were dried at 40 °C for 72 h in an air recirculation oven, model Hewlett Packard 5890 (Blue Island, IL, USA). The stalks were separated from the leaves,

ground and homogenized using a mortar and a pestle and then kept away from light until analysis.

### 2.4 Sample extraction and clean-up procedures

#### 2.4.1 Pressurized hot water extraction combined with molecularly imprinted polymer clean-up

The preparation of chlorophyll MIP is as detailed by Balokwa *et al* [24]. Extraction and clean-up were performed simultaneously in the same extraction cell. The extraction cell was loaded with 1 g of homogenized plant sample, 1 g of sand, 0.05 g of NaCl and 1 g of a chlorophyll MIP. To optimize the temperature, pressure, flow rate and time required for both extraction and clean-up, the extraction temperatures were varied from 150-260 °C, pressures from 10-100 bar, flow rate from 0.5-1.5 mL min<sup>-1</sup> and extraction time was varied from 5-60 min, with a 5 min preheating and equilibration of the oven. The extracts were collected in a glass

Table 1. Plant samples collected from the Okavango Delta.

Scientific names of plants	Local name (Setswana)	Use
<i>Nymphaea lotus</i>	Tswii	Edible
<i>Cyperus articulatus</i>	Mxowa	Edible/Medicinal
<i>Acalypha filicaulis</i>	Makgonatsotlhe	Medicinal
<i>Cyperus papyrus</i>	Koma	Edible/Medicinal
<i>Phoenix reclinata</i>	Tsaro	Edible

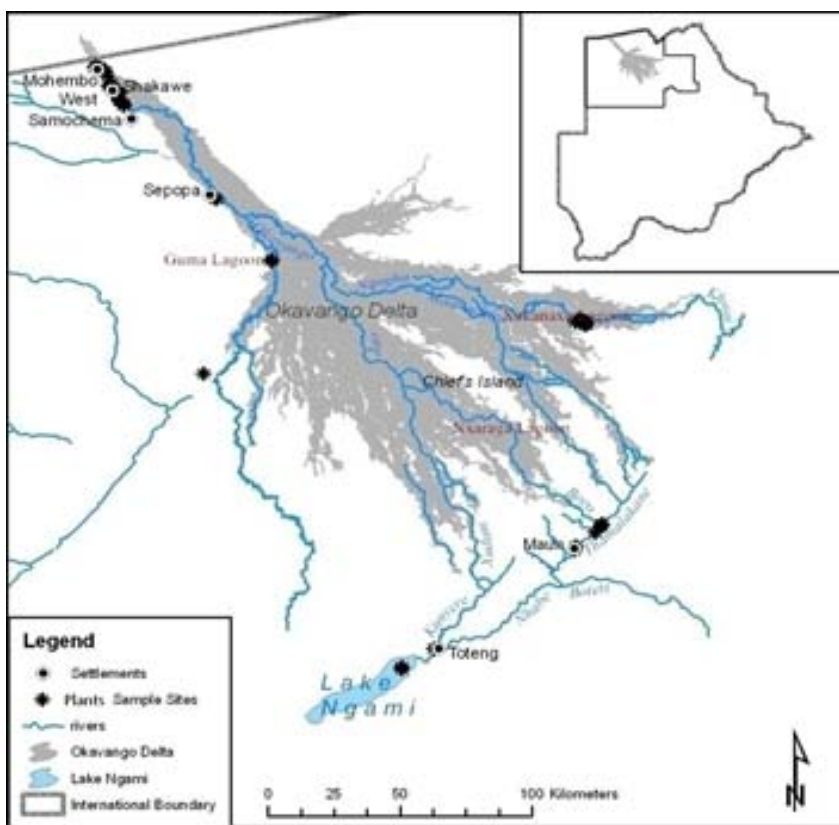


Figure 2. Map of the Okavango Delta showing plants sampling sites.

vial, evaporated at 80 °C under a steady flow of N<sub>2</sub> and then reconstituted in 20 µL of n-hexane prior to GC-ECD and GC-MS analysis.

#### 2.4.2 QuEChERS AOAC Official Method

The European EN Official Method 15662 [16], was modified for this application. 10 g of previously homogenized sample was placed into a 50 mL centrifuge tube (from the SampliQ QuEChERS extraction kit). 15 mL acetonitrile was added to each tube and shaken for 1 min. An Agilent SampliQ QuEChERS extraction salt packet from the kit (p/n 5982-5755) containing 6 g of anhydrous MgSO<sub>4</sub>, and 1.5 g of anhydrous NaOAc was added directly to the tubes. Sample tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted with the entire sample and crystalline agglomerates were dispersed. Sample tubes were then centrifuged at 4000 rpm for 5 min.

#### 2.4.3 Dispersive SPE Clean-up

An 8 mL aliquot was transferred to an Agilent SampliQ QuEChERS dispersive SPE 15 mL tube (p/n 5982-5058), containing 400 mg of PSA and 1200 mg of anhydrous MgSO<sub>4</sub>. The tubes were tightly capped and hand shaken vigorously for 1 min. The tubes were centrifuged at 4000 rpm for 5 min. The aliquot from the extract were dried under N<sub>2</sub>, and then reconstituted in 20 µL of n-hexane prior to analysis by GC-ECD and GC-MS.

A summary of the extraction schemes for the proposed method and the conventional QuEChERS methods was compared (see Figure 3).

### 3. Results and Discussion

#### 3.1 Pesticides detected employing PHWE-MIP

The PHWE-MIP method achieved simultaneous extraction and clean-up of pesticides from medicinal plants in the same extraction cell. PHWE employed an optimal temperature of 260 °C, pressure of 80 bars and flow rate of 1 mL min<sup>-1</sup> in 10 min for the extraction. On the other hand, the MIP performed a successful in-cell clean-up by selectively removing the interfering chlorophyll prior to GC-ECD/MS analysis. A total of nine pesticides were detected when the proposed method was employed in all the medicinal plants investigated. A representative chromatogram of the pesticides detected from *N. lotus* is depicted in Figure 4. All other chromatograms from the rest of the plants showed a similar pattern.

#### 3.2 Pesticides detected employing QuEChERS method

The extraction and clean-up of pesticides from medicinal plants was also achieved employing the QuEChERS method. However, the presence of pesticides with planar structures (HCB, 4,4 DDD, 2,4 DDE and dichlorvos) was significantly reduced relative to the clean-up based on PHWE-MIP method. Only seven pesticides were detected (see Figure 5) from the same samples as the ones evaluated with the PHWE-MIP method. Currently, GCB is employed to remove the interfering chlorophyll during pesticides residue analysis in the dSPE step of the QuEChERS method. While GCB is effective in removing chlorophyll from plant samples, it also removes planar pesticides

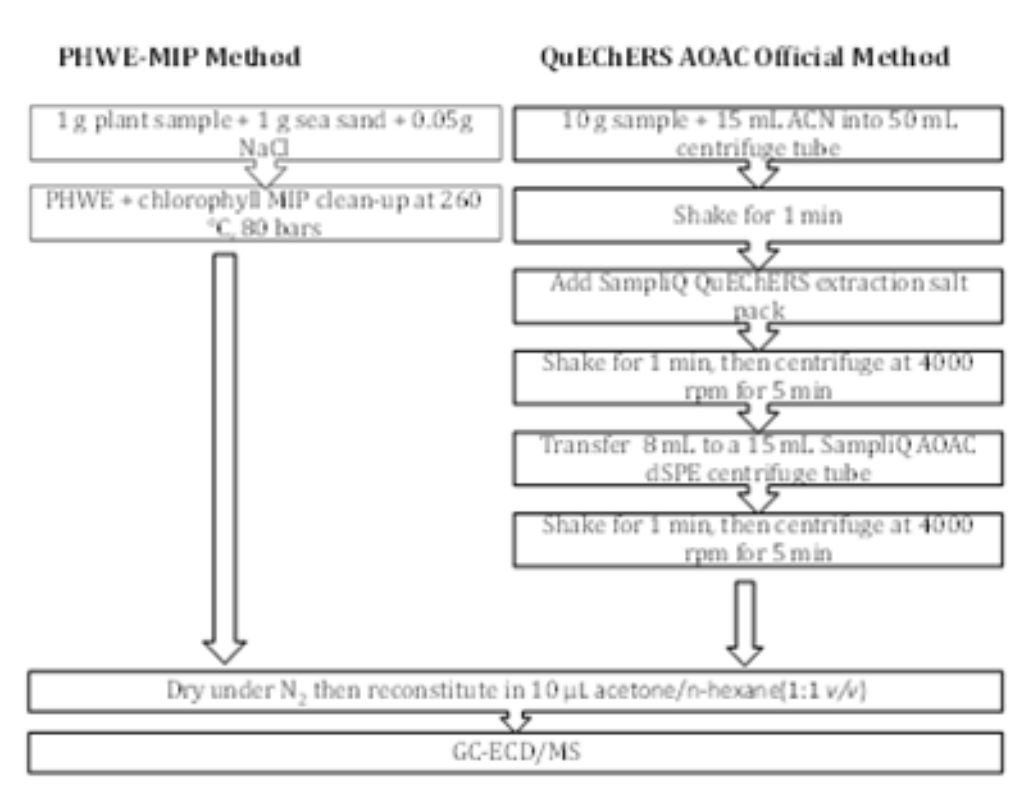


Figure 3. Comparison of the extraction scheme of QuEChERS method with PHWE-MIP.

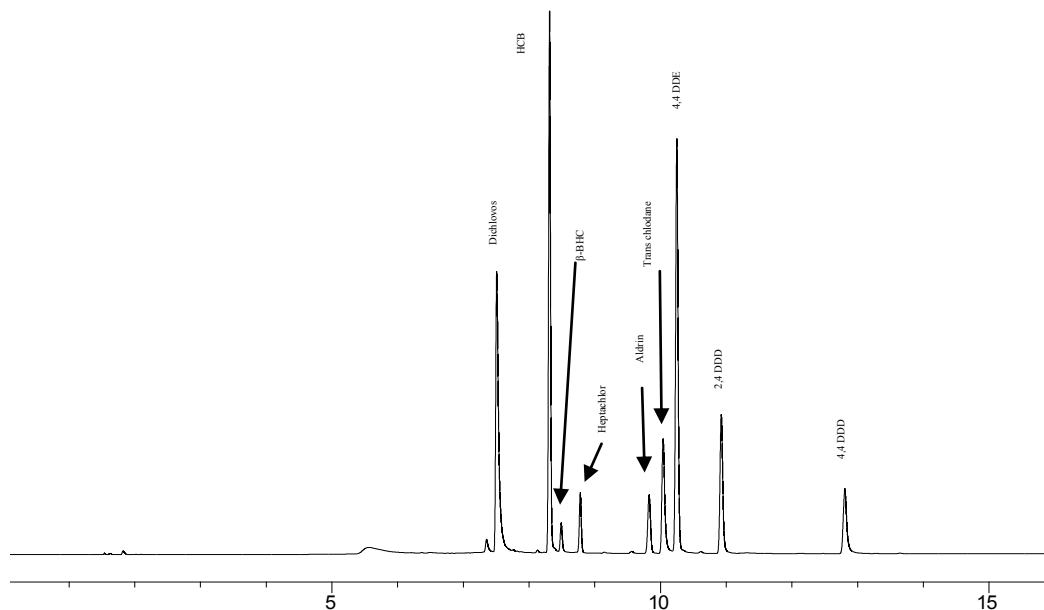


Figure 4. A chromatogram of pesticides detected from *N. lotus* when PHWE was combined with a chlorophyll MIP.

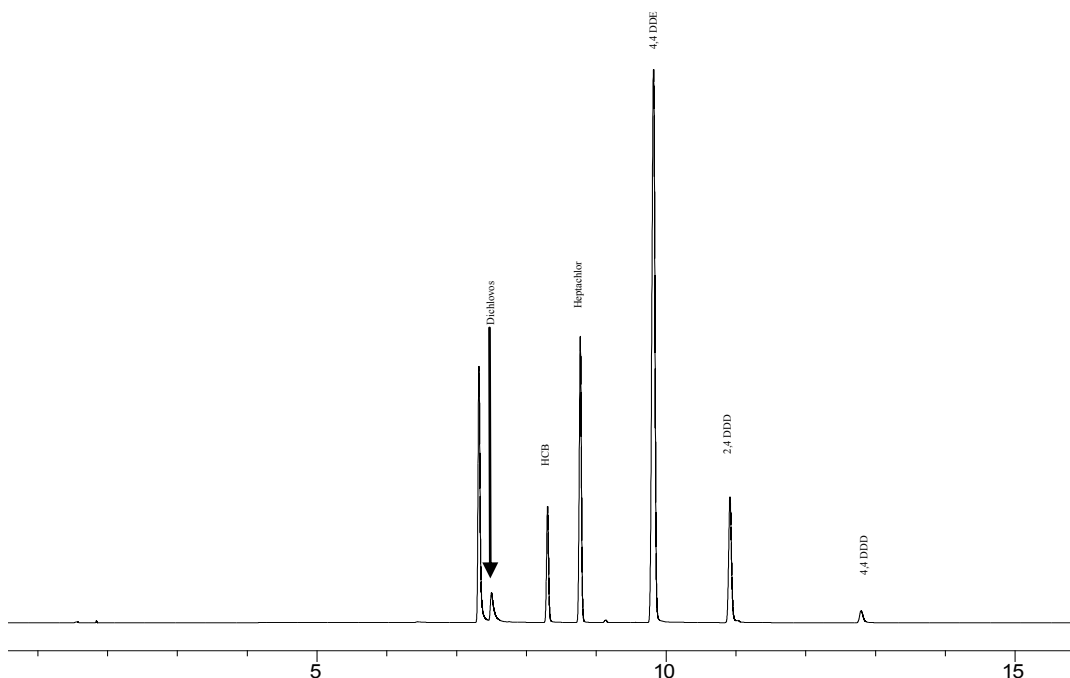


Figure 5. A chromatogram of pesticides detected from *N. Lotus* when QuEChERS method was employed.

### 3.3 Comparison of the proposed PHWE-MIP method with the QuEChERS method

The linearity was determined by the analysis of all plant samples spiked at six levels of concentration, between 0.5 and 2.5  $\mu\text{g kg}^{-1}$ , to obtain the calibration curves. The assays were carried out by GC-ECD. The linearity was evaluated by determining the correlation coefficients, which were all greater than 0.999 for all the plants. The LOD and LOQ of the method

were calculated at 3Sd and 10Sd respectively and they ranged from 0.06-0.3  $\mu\text{g kg}^{-1}$  respectively. Student's t-test was used to statistically compare the recovery and repeatability data of the two methods. At 95% confidence level, the analysis revealed no significant difference between the mean values of both methods. The overall range of recoveries for the various pesticides at 3 different spiking levels (0.1, 0.5 and 1.5  $\mu\text{g kg}^{-1}$ ) for both methods is as summarized in Table 2. However, it

Table 2. Recoveries (%) of pesticides employing both methods for all the plant samples.

	<i>Nymphaea lotus</i>		<i>Cyperus articulatus</i>		<i>Acalpha vilcaulis</i>		<i>Cyperus papyrus</i>		<i>Phonix reclinata</i>	
	QueChERS	PHWE-MIP	QueChERS	PHWE-MIP	QueChERS	PHWE-MIP	QueChERS	PHWE-MIP	QueChERS	PHWE-MIP
4,4'-DDD	58.3	84.7	45.8	78.6	25.8	94.8	45.5	88.6	68.3	85.4
4,4'-DDE	42.9	87.1	36.4	93.1	45.2	89.3	34.6	87.0	58	90.2
Heptachlor	89.2	90.1	91.4	87.2	78.3	78.3	89.9	87.3	58.5	65.6
TransChlodane	78.4	76.4	89.3	89.3	81.3	78.5	87.4	89.3	85.5	78.3
Dichlorvos	25.7	89.6	67.3	89.4	56.3	92.3	67.2	95.3	50.3	86.3
$\alpha$ -BHC	84.9	90.2	89.5	91.4	96.3	91.4	94.6	90.2	94.4	88.4
HCB	17.3	94.6	12.4	89.4	14.7	94.6	0	78.3	28.0	87.4
2,4 DDD	43.7	85.3	33.8	78.2	27.3	89.4	25.0	76.9	26.5	85.4
Aldrin	83.5	89.8	83.2	78.4	78.4	87.4	78.9	79.1	70.3	69.9

RSD <10

should be noted that the recoveries and repeatability of HCB; 4,4 DDD; 2,4 DDD; 4,4 DDE and dichlorvos were significantly reduced in the QuEChERS method compared to the proposed method by a factor of more than half.

This could be due to the fact that GCB has a layered planar structure. The structures of the pesticides are also planar and smaller. This could result in them being trapped between GCB thereby reducing their recovery. Besides reduction in recoveries of pesticides of planar structures (see Table 2), there was more sample handling steps involved when the QuEChERS method was employed compared to the PHWE-MIP (see Figure 3). There is usually loss of analytes and or contamination when there are too many sample handling steps. Moreover, acetonitrile which is very toxic to the environment was employed in the QuEChERS method during the extraction step.

The PHWE-MIP method on the other hand was very selective to the targeted chlorophyll and it utilized only water for extraction, which is environmentally friendly. Furthermore, with the in-cell clean-up approach, there is a possibility for automaton and higher sample throughput and hence better quality of the results. The method was also simple and hence is proposed for the monitoring of pesticides residue analysis in plant material.

### 3.4 Application of PHWE-MIP method to real samples

The proposed method was employed in the analysis of real samples. The levels of the pesticides accumulation in roots and leaves of different plants are presented in Table 3. The results showed a wide variation in the concentrations of the pesticides in different tissues of the various plant species. Generally, it

was observed that the roots accumulated higher concentration levels of the pesticides. This could be due to the fact that the roots were more exposed to the water and sediments which are presumed to contain higher levels of organochlorine pesticides [25]. Among the plant species, *N. lotus* recorded the highest concentration of all the pesticides detected in the root portion. *N. lotus* was more exposed to the water than all other plants since it is aquatic, therefore there could be in direct contact with the contaminated water and sediments. Also, plant and chlorophyll composition can vary according to the season of the year. This may affect the uptake of pesticides by the different parts of the plants.

The plant samples were divided into three regions of sampling sites; the Panhandle, the lower and the upper part of the Delta, indicated on the map (see Figure 6). It was observed that plants from the Panhandle part of the delta contained the lowest concentration levels of the pesticides (0.1 – 0.6  $\mu\text{g kg}^{-1}$ ), while the plants from the lower part of the delta contained the highest concentrations levels (0.8 – 1.1  $\mu\text{g kg}^{-1}$ ). The trend may be due to the direction of flow of the water as well as the low topographic gradient of the Delta which has led to the low flow rates [26]. Low flow rates allow partitioning of the water insoluble components such as pesticides onto suspended matter that subsequently settle to the bottom of the river becoming part of the sediment. Thus pesticides are more likely to be adsorbed onto organic-rich sediment relative to the sandy sediment, characteristic of the Panhandle as reported by Daka *et al.* [27].

In aqueous and marine environments, OCPs tend to have strong affinities for suspended particulates and accumulate in

Table 3. Concentration of pesticides ( $\mu\text{g kg}^{-1}$ ) detected in different plant parts.

Pesticide	<i>Numphea lotus</i>		<i>Cyperus articulatus</i>		<i>Acalypha filicaulis</i>		<i>Cyperus papyrus</i>		<i>Phoenix reclinata</i>	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
4,4 DDD	1.41 ± 0.02	2.47 ± 0.01	0.35 ± 0.03	0.11 ± 0.01	0.53 ± 0.01	0.53 ± 0.71	0.50 ± 0.01	0.56 ± 0.01	0.68 ± 0.05	0.25 ± 0.01
4,2 DDD	0.32 ± 0.01	0.33 ± 0.01	0.16 ± 0.01	0.90 ± 0.01	0.64 ± 0.01	1.06 ± 0.01	0.36 ± 0.01	0.65 ± 0.01	0.58 ± 0.01	0.00
4,4 DDD	0.30 ± 0.01	0.53 ± 0.01	0.26 ± 0.01	0.76 ± 0.01	0.25 ± 0.01	0.60 ± 0.01	0.25 ± 0.01	0.22 ± 0.01	0.58 ± 0.01	0.65 ± 0.01
HCB	0.28 ± 0.01	0.71 ± 0.01	1.69 ± 0.01	0.70 ± 0.01	0.38 ± 0.01	0.50 ± 0.01	0.692 ± 0.01	1.00 ± 0.01	0.85 ± 0.01	0.25 ± 0.01
B-BHC	1.33 ± 0.01	1.39 ± 0.01	0.21 ± 0.01	0.65 ± 0.01	0.61 ± 0.01	0.46 ± 0.01	0.21 ± 0.01	0.60 ± 0.01	0.50 ± 0.01	0.36 ± 0.01
Trans chlordane	0	0	0.33 ± 0.01	0	0.94	0.31 ± 0.01	0.46 ± 0.01	0.50 ± 0.01	0.94 ± 0.01	0.58 ± 0.01
Aldrin	0.35 ± 0.01	0.74 ± 0.01	0.26 ± 0.01	0.80 ± 0.01	0.06 ± 0.01	0.92 ± 0.01	0.36 ± 0.01	0.70 ± 0.03	0	0.14 ± 0.02
Dichlorvos	0.54 ± 0.01	0.32 ± 0.01	0	0.56 ± 0.01	0.54 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0	0.25	0.54 ± 0.01
Heptachlor	0.80 ± 0.01	1.31 ± 0.01	0.23 ± 0.01	0.61 ± 0.01	0.35 ± 0.01	0.23 ± 0.01	0	1.90 ± 0.04	0.20 ± 0.01	0.59 ± 0.01



sediment. The determination of OCP levels in sediments can therefore indicate the level of contamination and bioaccumulation in aquatic organisms and plants. DDT was employed for aerial spraying by health authorities until late 1990 [28]. The presence of DDT metabolites in plant samples at the peripheries of the delta could be due to the fact that the areas act as final catchments for the water. Lake Ngami, for example, is at the receiving end of the delta (see Figure 2), and is not fed by any other water source. The sediments in peripheral areas are rich in organic matter, capable of accumulating considerable quantities of the pesticides by adsorption thereby transferring them to the plants that feed on the water. Alternatively, there could be a subsistent input of pesticides employed on vegetable farming as well as industrial application by the riparian community.

OCPs such as HCB and aldrin are employed in agriculture due to their effectiveness against various pests. OCPs are capable of travelling long distances over a considerable period of time. The possibility of them being employed somewhere within the Okavango River Basin could not be overruled. It should be noted however, that the concentration recovered from all the plants was still lower than MRLs of 0.1-1.0 mg kg<sup>-1</sup> [4], of organochlorine pesticides in solid matrices; EPA method 3545 [29]. However, it is important to understand the factors influencing transport bound contaminants. 2,4 DDD, 4,4 DDD, 4,4DDE, HCB and aldrin were positively confirmed with an MS.

## 4. Conclusions

In this study, the selectivity of PHWE as a sample preparation technique was improved by combining it with another selective sorbent (MIPs) in the same extraction cell. By using this approach, in-cell clean-up was carried out to simplify the extraction of the pesticides, leading to a higher sample throughput and a better quality of the results. The proposed PHWE-MIP showed improved selectivity for the interfering chlorophyll in pesticides residue analysis and was more suitable as a sorbent for SPE than the graphitized carbon black. The method also demonstrated the possibility of combining many sample handling steps in one, thus reducing analysis time. PHWE is also the sustainable method of extraction as it employed water which is cheaper, readily available and pose minimal disposal challenges as compared to the organic solvents used by the other methods.

## 5. Acknowledgements

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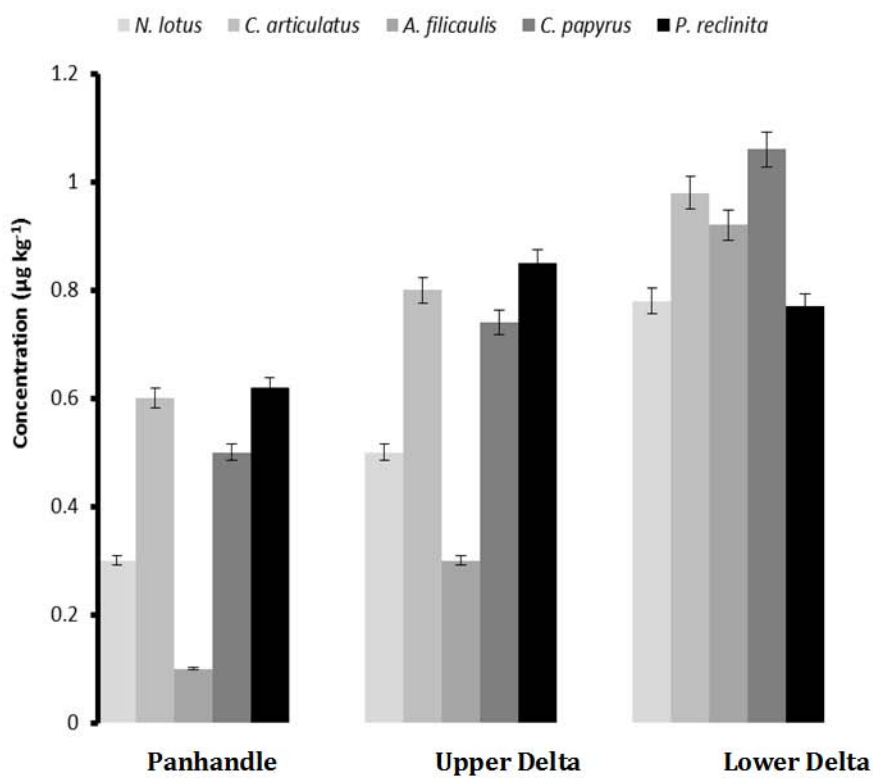


Figure 6. Concentration of 4,4DDE in different plants from all sampling regions. All other pesticides show a similar trend.



## References

- [1] A.L. Chiuchiolo, R.M. Dickhut, M.A. Cochran and H.W. Ducklow, "Persistent organic pollutants at the base of the Antarctic marine food web," *Environ. Sci. Technol.*, 38 (2004) 3551-3557.
- [2] J. Weinberg, Overview of POPs and need for a POPs treaty. Public forum on persistent organic pollutants- the international POPs elimination network, 1998.
- [3] P.K. Gupta, M. Hubbard, B. Gurley and H. P. Hendrickson, "Validation of a liquid chromatography-tandem mass spectrometric assay for the quantitative determination of hydrastine and berberine in human serum", *J Pharm. Biomed. Anal.*, 49 (2009) 1021-1026.
- [4] CODEX alimentarius, ([http://www.codexalimentarius.net/mrls/pestdes/jsp/pest\\_q-e.jsp](http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp))
- [5] X. Gong, S. Qi, Y. Wang, E.B. Julia and C. Lv, "Historical contamination and sources of organochlorine pesticides in sediment cores from Quanzhou Bay, Southeast China" *Marine Pollution Bulletin*, 54 (2007) 1434-1440.
- [6] C. Gonçalves, J.J. Carvalho, M.A. Azenha and M.F. Alpendurada, "Optimization of supercritical fluid extraction of pesticide residues in soil by means of central composite design and analysis by gas chromatography-tandem mass spectrometry", *J. Chromatogr. A* 1110 (2006) 6-14.
- [7] P. Suchan, J. Pulkrabová, J. Hajlová and V. Kocourek, "Pressurized liquid extraction in determination of polychlorinated biphenyls and organochlorine pesticides in fish samples", *Anal. Chim. Acta*, 520 (2004) 193-200.
- [8] L. Chen, L. Ding, H. Jin, D. Song, H. Zhang, J. Li, K. Zhang, Y. Wang and H. Zhang, "The determination of organochlorine pesticides based on dynamic microwave-assisted extraction coupled with on-line solid-phase extraction of high-performance liquid chromatography *Anal. Chim. Acta*, 589 (2007) 239-246.
- [9] C. Gonçalves and M.F. Alpendurada, "Assessment of pesticide contamination in soil samples from an intensive horticulture area, using ultrasonic extraction and gas chromatography-mass spectrometry", *Talanta*, 65 (2005) 1179-1189.
- [10] S. Babić, M. Petrović, M. Kaštelan-Macan, Ultrasonic solvent extraction of pesticides from soil, *J. Chromatogr. A* 823 (1998) 3-9.
- [11] C.C. Teo, S.N. Tan, J.W.H. Yong, C.S. Hew, E.S. Ong, Pressurized hot water extraction, *J. of Chromatogr. A* 1217 16 (2010) 2484-2494.
- [12] M. Anastassiades and S. J. Lehotay, "Fast and Easy Multiresidue Method Employment Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce", *J. AOAC Int.*, 86 (2003) 412- 431.
- [13] A. Hubert, K. Wenzel, M. Manz, L. Weissflog, W. Engewald and G. Schüürmann, High extraction Efficiency for POPs in Real Contaminated Soil Samples Using Accelerated Solvent Extraction, *Anal. Chem.* 72 (2000) 1294-1300.
- [14] Z. Sharif, Y.B.C. Man, N.S.A. Hamid, C.C. Keat, Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using solid phase extraction clean-up cartridges, *J. Chromatogr. A* 1127 (2006) 254-261.
- [15] A.B. Vega, A.G. Frenich, J.L.M. Vidal, Monitoring of pesticides in agricultural water and soil samples from Andalusia by liquid chromatography coupled to mass spectrometry, *Anal. Chim. Acta* 538 (2005) 117-127.
- [16] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, *J AOAC Int* 86 (2003) 412-431.
- [17] S. J. Lehotay, K. Mastovska, A.R. Lightfield, Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables, *J. AOAC Int.*, 88 (2005) 615-629.
- [18] M. Barriada-Pereira, M.J. González-Castro, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, E. Fernández-Fernández, Determination of 21 organochlorine pesticides in tree leaves using solid-phase extraction clean-up cartridges, *J. Chromatogr. A* 1061 (2004) 133-139.
- [19] Sellergren B (2001) In: Sellergren (ed) *Molecularly imprinted polymers. Man made mimics of antibodies and their applications in analytical chemistry B*, Elsevier, Amsterdam, pp 305-322
- [20] E. Concha-Graña, M.I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, E. Fernández-Fernández, D. Prada-Rodríguez, Development of pressurized liquid extraction and cleanup procedures for determination of organochlorine pesticides in soils, *J. Chromatogr. A* 1047 (2004) 147-155.
- [21] C. Baggiani, L. Anfossi L, C. Giovannoli Molecular imprinted polymers as synthetic receptors for the analysis of myco- and phyco-toxins, *Analyst* 133 (2008) 719-730
- [22] B.S Batlokwa, J. Mokgadi, T. Nyokong and N. Torto, Optimal Template removal from Molecularly Imprinted Polymers by Pressurized Hot Water Extraction, *Chromatographia* 73 (2011) 589-593
- [23] D. Adeyemi, J. Mokgadi, J. Darkwa. C. Anyakora, G. Ukpo, C. Turner, N. Torto, Electrospun nanofibers sorbents for pre-concentration of 1,1-dichloro-2,2 bis-(4-chlorophenyl)ethylene with subsequent desorption by pressurized hot water extraction, *Chromatographia* 73 (2011) 1015-1020
- [24] B.S Batlokwa, J. Mokgadi, R. Majors, C. N. Torto, A Novel Molecularly Imprinted Polymer for the Selective Removal of Chlorophyll from Heavily Pigmented Green Plant Extracts prior to Instrumental Analysis, Hindawi Publishing Corporation, *Journal of Chemistry*, Volume 2013, Article ID 540240, 4 pages, <http://dx.doi.org/10.1155/2013/540240>

- [25] L.Mmualefe, Sample preparation for pesticide analysis in water and sediments: A case study of the Okavango Delta, Botswana, Doctoral Thesis, Rhodes University, 2010.
- [26] L.C. Mmualefe N. Torto, P. Huntsman-Mapila, B. Mbongwe, Headspace solid phase microextraction in the determination of pesticides in water samples from the Okavango Delta with gas chromatography-electron capture detection and time-of-flight mass spectrometry *Microchemical Journal* 91(2009) 239-244.
- [27] P.S. Daka, V.C. Obuseng, N. Torto, P. Huntsman-Mapila, Deltamethrin in sediment samples of the Okavango Delta, Botswana, *Water SA* 32 (2006) 483-488.
- [28] M.L.H. Mabaso, B. Sharp, C. Lengeler, Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying, *Trop. Med. Int. Health* 9 (2004) 846-856.
- [29] <http://www.dionex.com/en-us/webdocs/68591-EPA-3545-ASE.pdf>.