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Determination of selected Endocrine Disrupting Pesticides in Water from Mbagathi River, Machakos County, Kenya, using Solid Phase Extraction and Liquid Chromatography Tandem Mass Spectrometry

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Abstract

Prenatal and postnatal exposure to endocrine disrupting pesticides (EDPs) is believed to cause hormonal imbalance in animals. Insufficient data on exposure levels has necessitated the need for assessments to confirm existing EDPs. Solid phase extraction (SPE) is a simple multi-residue sample preparation technique suitable for quick exposure assessments; however, extension of its application in pre-concentration of many EDPs residues in river water is largely unexplored. The method was applied in analysis of Mbagathi River water samples in Machakos County, Kenya. This study aimed to validate a SPE technique for pre-concentration of four suspected EDPs including a triazine (atrazine), a carbamate (carbaryl) and organophosphates (diazinon and dimethoate) in river water for direct liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis. Precision attained was less than 2.6% RSD, while recovery was above 70% for majority of the compounds tested. Low limits of quantification and detection, below 2.5 ng/L and 0.8 ng/L respectively, indicated suitability of the method for trace exposure assessment in river waters. Samples were collected in triplicate from eighteen sampling sites at random along the river during the short rainfall season of mid-September 2019. The selected EDPs detected were in the range of: <LOQ to 3.56 µg/L for atrazine, <LOQ to 1.48 µg/L for carbaryl, <LOQ to 1.9 µg/L for diazinon and <LOQ to 0.82 µg/L for dimethoate. The quantities detected for atrazine, carbaryl, dimethoate and diazinon in a majority of sites were at levels capable of causing significant health effects to human and wildlife.

Keywords: River water, Endocrine Disrupting Pesticides, Liquid chromatography, Solid Phase Extraction, Tandem mass spectrometry

1. Introduction

Pesticides play a significant role in modern agriculture, but their endocrine toxicity to non-target species such as human, wildlife and aquatic organisms has raised widespread concerns in the recent past (Ribeiro *et al*., 2017). Some pesticides are suspected as endocrine disruptors and were listed in the European Commissions (EC) Priority list as well as Endocrine Disruptor Screening (EDS) program tier 1 of Environmental Protection Agency (EPA) (Hrouzková & Matisová, 2012). Increasing evidence shows that long-term exposure to some of these pesticides can disrupt endocrine system (Mostafalou & Abdollahi, 2013), thereby enhancing susceptibility to different types of diseases throughout life (Mnif *et al*., 2011). For instance exposure to atrazine at trace levels has shown reproductive and development dysfunction in frogs (Hayes *et al*., 2006), rat (Giusi *et al*., 2006) as well as humans (Almberg *et al*., 2018). Exposure to carbaryl at trace levels caused reproductive dysfunction in rat (Tange *et al*., 2016), and fish (Toumi *et al*., 2016). Dimethoate also caused reproductive dysfunction at trace levels in mouse (Verma & Mohanty, 2009), and fish (Novais *et al*., 2012). Diazinon showed reproductive dysfunction (Kaleli *et al*., 2017) and development dysfunction in rats (Slotkin *et al*., 2008; Flynn *et al*., 2018; Savy *et al*., 2018), at trace levels. A majority of the suspected EDPs are still under-going human health and ecotoxicological assessment (Bars *et al*., 2012). Consequently, they are still largely unregulated as substances of very high concern (SVHC) (Bars *et al*., 2012) but extensively used for pest control in farms, homesteads and healthcare (Ochilo *et al*., 2018; Marete *et al*., 2021), therefore creating great uncertainty (CropLife, 2015). Environmental exposure levels

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is one of the key risk indicators and plays a critical role in the ongoing hazard based risk assessments of suspected EDPs (CropLife, 2015; Liu *et al*., 2021).

Therefore, appropriate environmental exposure assessment tools in major exposure pathways is critical (Tsimbiri *et al*., 2015). SPE is proving to be a practical and reliable sample preparation technique for LC-MS/MS analysis of emerging pollutants (Kairigo *et al*., 2020; Yao *et al*., 2021; Muriuki *et al*., 2020) such as pesticides (Alder, *et al*., 2006**).** Significant advancements have been made on this technique on sample preparation for pesticide residue analysis. For instance, in a study by (Donato *et al*., 2015) SPE was used to pre-concentrate pesticide residues in drinking water. Another study by (He & Aga, 2019), reported pre-concentration of potential EDCs including hormones and pesticides in surface waters. In a separate study (Lopardo *et al*., 2019), also pre-concentrated various EDCs from water samples. Even though these methods vary slightly, they all used SPE cartridge packed with octadecysilane (C18) sorbent because of high capability to retain wide spectrum of compounds differing in polarities (Lucci & Núñez, 2014). The cartridges were conditioned with methanol and water. The samples were pre-filtered and acidified with organic acid (formic acid) or mineral (phosphoric or nitric acid) to enhance recovery (Donato *et al*., 2015). Loading was performed under gravity at a flow rate ranging from 3 to 10 mL/min. Elution was peformed with polar or non polar solvent or both depending on whether the eluate was directly injected or reconstituted. Acetonitrile, methanol, dichloromethane and ethyl acetate were commonly used. The final eluate was injected in liquid chromatograph coupled with tandem mass spectrometer (LC-MS/MS) for seperation and detection. Majority of these approaches involved solvent exchange through evaporation and reconstitution. Evaporation may lead to lose of some pesticides due to volatilization or decomposition. A SPE method proposed by (Demoliner *et al*., 2010) eliminated evaporation step and instead eluate was directly injected into LC-MS/MS. This approach reduces duration of analysis, making it suitable for routine pesticide residue analysis. Previous SPE-LC-MS/MS methods for analysis of pesticides in water are summarized in Table S1 (Supplementary Information). Lack of sufficient information on environmental exposure potential for individual pesticides especially in African countries (Bornman *et al*., 2015; Horn & Pieters, 2021) including Kenya, has been one of the key impediments to conclusion of the ongoing screening programs such as EDS tier 2 of EPA (Bars *et al*., 2012). Residues of some of these pesticide have been reported in some river waters (Otieno *et al*., 2018; Githinji *et al*., 2019), lake waters (Otieno *et al*., 2015; Kandie *et al*., 2020), soil and vegetable (Ngolo *et al* 2019), (Omwenga *et al*., 2021), honey (Irungu, 2016), soil (Marete *et al*., 2020), tomatoes (Nakhungu *et al*., 2021) and bean pods (Namu, 2016; Kipkemoi *et al*., 2020) in Kenya. However, contamination of Mbagathi River water with suspected EDPs is largely unexplored. Commercial greenhouse horticulture as well as small scale farming is clustered along Mbagathi River, a subsidiary of Athi River and the main river in Mbagathi sub-catchment (Justus & Yu, 2014). Attempts have been made to assess the impact of socio-economic activities in the vicinity of the river, such as contamination with heavy metals (Ratemo, 2018), salinity (Kitheka, 2019), and water balance (Nyika *et al*., 2017), with little information on EDP residues. Therefore the aim of this work was to screen for selected suspected endocrine disrupting pesticides in Mbagathi River and establish their exposure levels. Chemical structures and physicochemical properties of the selected pesticides are summarized in Table S2.

SPE technique proposed by (Demoliner *et al*., 2010), was slightly modified and validated for pre-concentration of four suspected EDPs from river water for direct LC-MS/MS analysis. Post-SPE column ultra-filtration was performed instead of pre-SPE column ultra-filtration and samples were acidified with formic acid instead of phosphoric acid. The method was then applied in analysis of multi-class suspected EDPs including; a triazine (atrazine), a carbamate (carbaryl), and organophophates (dimethoate and diazinon), in river water samples from Mbagathi River in Mbagathi sub-catchment, Machakos County, Kenya.

2. Methodology

2.1 Chemicals and Equipment

Pesticide standards (atrazine, carbaryl, diazinon, dimethoate), as well as HPLC grade solvents (acetonitrile, methanol, formic acid, and pure water), and sorbent SupercleanTM LC-18, Glass fibre filters and Nylon Syringe filters were purchased from Kobian, Nairobi Kenya. LC-MS/MS system (Agilent UHPLC 1290 infinity sense coupled with Agilent G6460C Triple Quadrupole) with Mass Hunter software, pH (WTW pH 3310) and conductivity meter (WTW Cond 3310), vacuum filtration system (Rocker Chemker 300 System) were accessed through collaboration. LC-MS/MS analysis was done at the Ministry of Livestock, Veterinary Laboratory, Nairobi Kenya, while physicochemical analysis was done at the Technical University of Kenya, School of Chemistry and Material Science, Nairobi Kenya.

A stock standard solutions of 1000 mg/L for each pesticide was prepared in solvent (acetonitrile: 1 % (v/v) formic acid in methanol) and stored in amber flask at 4 °C from which 10 mgL^{-1} working solutions was prepared for analysis. A mixture

of working standards of concentration range 0, 0.01, 0.05, 0.1, 0.2 and 1 μgL^{-1} were used to spike the blank and reference samples for determination of precision and accuracy (recovery) respectively, while each series of individual standards were used to determine linearity, range and detection limit.

2.2 Tandem MS conditions

Liquid chromatography (Agilent UHPLC 1290 infinity sense) system coupled with MS/MS (Agilent G6460C Triple Quadrupole) system was used for the analysis. Mobile phase was 1 % (v/v) formic acid in deionized water (solvent A) and 100 % acetonitrile (solvent B). Column used was Agilent Eclipse Plus C18 RRHD, (1.8 µm particle size, 2.1 x 50 mm). Infusion concentrations were 1 µg/L. Flow rate was 300 $\mu Lmin^{-1}$. HPLC mode was gradient elution; 0 – 0.5 min 95 % (v/v) (solvent A), 80 % (v/v) (solvent A) at 4.5 min, 30 % (v/v) (solvent A) at 10 min and 5 % (v/v) (solvent A) at 17 min until 19 min. Total run-time was set to 22 minutes.

Detection was done in Multiple Reaction Monitoring (MRM) mode. The MRM transitions are summarized in Table 1. Ionization was done in positive electron spray ionization (+ESI) mode. Collision gas was nitrogen. Capillary voltage was set to 3.5 kV, cone voltage to 30 V. Source temperature was set to 325 ºC. Total scan-time was set to 22 minutes. Acquisition software was Mass Hunter.

2.3 Method validation

The modified method was validated with respect to linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery) and precision (repeatability) according to EU guidelines, (657/directive/2002). Linearity was determined as regression coefficient of a six level calibration standards of concentrations, 0.01, 0.05, 0.1, 0.25, 0.5 and 1.0 µg L-1 for atrazine, dimethoate, and diazinon and 1.0, 5.0, 10.0, 25, 50 and 100 µgL-1 for carbaryl in acetonitrile/water (acidified). Signal of a set of six replicates of lowest calibration standard (0.01µgL-1) each for atrazine, dimethoate, and diazinon and 1.0 µgL-1 for carbaryl was used to determine limit of detection (LOD) and limit of quantitation (LOQ), where LOD = ($\frac{3.3\sigma}{S}$), while LOQ = ($\frac{10\sigma}{S}$), σ is the standard deviation for the number of determinations and S is the slope of the calibration curve, (Ravisankar *et al*., 2015).

Precision was assumed as the degree of agreement between measurements. Blank samples were spiked in triplicate with 0.05 mL, 0.1 mL and 0.5 mL of mixed working standards to obtain 100 mL of 0.05 µg/L, 0.1 µg/L and 0.5 µg/L fortified blank samples, which were percolated through SPE cartridge and eluate injected into LC-MS/MS. Precision was determined as average three point standard deviation, ($\sigma = \sqrt{\frac{1}{n-1}}\sum(x-\mu)^2$; where n (3) = series of measurements, x is acquired signal (concentration in µg/L) and µ true mean. While relative standard deviation (RSD) was estimated as; $\frac{\sigma}{\mu}$ x 100 %, where σ is standard deviation and µ true mean, (Ravisankar *et al*., 2015).

Accuracy was defined as the degree of agreement between the measured value and the reference value. Three 100 mL aliquots of pre-filtered samples were spiked in triplicate with mixed working standard at 0.05 µg/L, 0.1 µg/L and 0.15 µg/L (dimethoate) and at 0.5 µg/L, 1 µg/L and 1.5 µg/L (atrazine, diazinon and carbaryl).

Both the blank sample and the fortified counterparts were percolated through SPE cartridge, eluates injected in the LC-MS/MS and percent fraction of pesticide recovered determined (Pizzutti *et al*., 2016).

2.4 Sample analysis

Samples were collected from Mbagathi River, Mbagathi sub-catchment Machakos County, Kenya, (GPRS longitude 37.019119 to 36.980088 and latitude -1.44071 to 1.44071), during the short rainfall season of mid-September 2019. GPRS coordinates of sampling sites are recorded in Table S3.

Random sampling was applied. A set of 1000 mL amber glass sample bottles and lids were soaked in warm soapy water, washed with detergent, rinsed with de-ionized water and acetone, and lids lined with aluminium foil. The pH and conductivity of each sampling site were measured using portable pH meter (WTW pH 3310) and portable conductivity meter (WTW Cond 3310) respectively, followed by sample collection. The amber glass sample bottles were filled completely by removing the lids and plunging the bottles to about 30 cm below the water surface and then replacing the lid tightly. In the laboratory, each sample was indexed, acidified to pH 2.5 with formic acid, filtered through 47 mm glass filter, followed by solid phase extraction as shown in figure 1.

EDP residue pre-concentration and clean-up was achieved in one step using SPE cartridges (SupercleanTM LC-18 100 mg, volume 1 mL) conditioned with 1 mL methanol and 1 mL de-ionized water. SPE cartridges were then loaded by percolation of 100 mL pre-filtered (47 mm glass fibre filters) and acidified (pH 2.5 with formic acid) samples at a flow rate of 10 mL per minute using vacuum (Rocker Chemker 300 System). The cartridges were then dried under vacuum for five minutes then washed with 1 mL de-ionized water. Elution was carried out using two aliquots of 500 µL Acetonitrile and 500 µL 1 % (v/v) formic acid in methanol and the combined eluant filtered through 0.22 µm nylon syringe filters into 1 mL autosampler vials. Exactly, 5.00 µL of eluate was directly injected in the

LC-MS/MS.

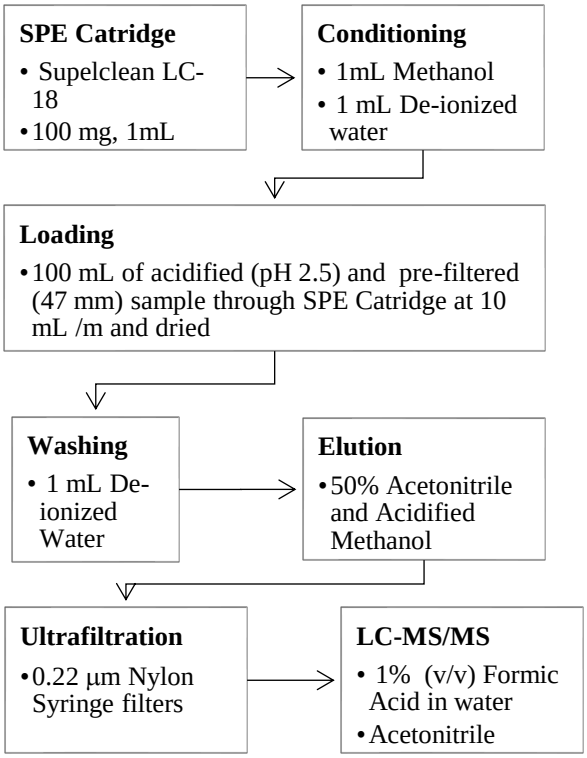

Figure 1: SPE flow chart for EDP residue pre-concentration and clean up.

3. Results and Discussion

3.1 Physicochemical Parameters

Results of physical/chemical parameters tested are summarized in Table S4.TDS ranged from 11 to 805 mg/L while TSS from 286 to 1221 mg/L. A similar study by Kitheka, (2019) in the river during rainy periods, reported TSS of 500 mg/L. High TSS increases the sorption of lipophylic pesticides with high K_{ow} (log 4), (Singh, 2019), while TDS increases the solubility of hydrophilic pesticides with low K_{ow} (<10), (Pereira *et al*., 2016). Given the low K_{ow} values of the selected pesticides; atrazine (-0.97), carbaryl (2.36), dimethoate (0.78), and diazinon (3.81), they have little affinity for sediments and organic matter, hence more distributed in water phase (Pereira *et al*., 2016).

In this study, pH ranged from 6.3 to 8.34. Since dimethoate and carbaryl are neutral and the pH range of the river water was far from pKa values of ionizable pesticides (atrazine and diazinon), pH did not influence their solubility or mobility (Pereira *et al*., 2016). Conductivity ranged from 609 to 1142 (mS/cm) while temperature from 21.2 to 23.9 ^{o}C. Conductivity may be linked to salinity (Kitheka, 2019) which decreases pesticide solubility only to a lesser extent (Pereira *et al.*, 2016). Temperature is one of the factors that affect degradation of pesticides in the environment, however the prevailing temperature range are below decomposition temperatures of the selected pesticides hence do not affects residue availability (Singh, 2019).

3.2 Tandem MS optimization

Ion transitions for each of the selected pesticides and retention times are listed in the Table 1.

Table 1: Retention Times and MRM Transitions

Pesticide	Rt (Min)	M/z +ESI	m/z → m/z	Abund %
Dimethoate	8.21	230	230 → 125	81
			230 → 199	100
Carbaryl	11.63	202	202 → 127	49
			202 → 145	100
Atrazine	12.16	216	216 → 96	34
			216 → 174	100
Diazinon	13.25	305	305 → 153	66
			305 → 169	100

MRM – Multiple Reaction Monitoring, Rt-Retention time, m/z – mass-to-charge ratio and Abund% - Relative abundances.

Molecular ion $[M+1]^+$ was selected as quantifier ion for all the pesticides, where their peak areas were fitted in the regression equation of standard calibration plot for the calculation of concentrations of each pesticide in water sample. Fragmentation patterns (ratio of abundances) as shown in Figure 2, were used for confirmation purposes. These computations were performed using Mass Hunter software. Retention for each pesticide was 8.21 minutes (dimethoate), 11.63 minutes (carbaryl), 12.16 minutes (atrazine) and 13.25 minutes (diazinon). Retention times for pure standards of each of the selected pesticides were compared with those of analyte in the samples to confirm identification, (Donato *et al*., 2015; Lopardo *et al*., 2019; Demoliner *et al*., 2010).

3.3 Method validation

Results of the validation parameters and concentration of each of the selected pesticides are summarized in Table 2 while calibration curves are shown in Table S5 – S8.Chromatograms and mass spectrum of each of the selected pesticides are shown in Figure S1 - S4 (Supplementary Information). The calibration ranged from 0.01 μgL^{-1} to 1 μgL^{-1} for atrazine, dimethoate and diazinon while calibration range for carbaryl

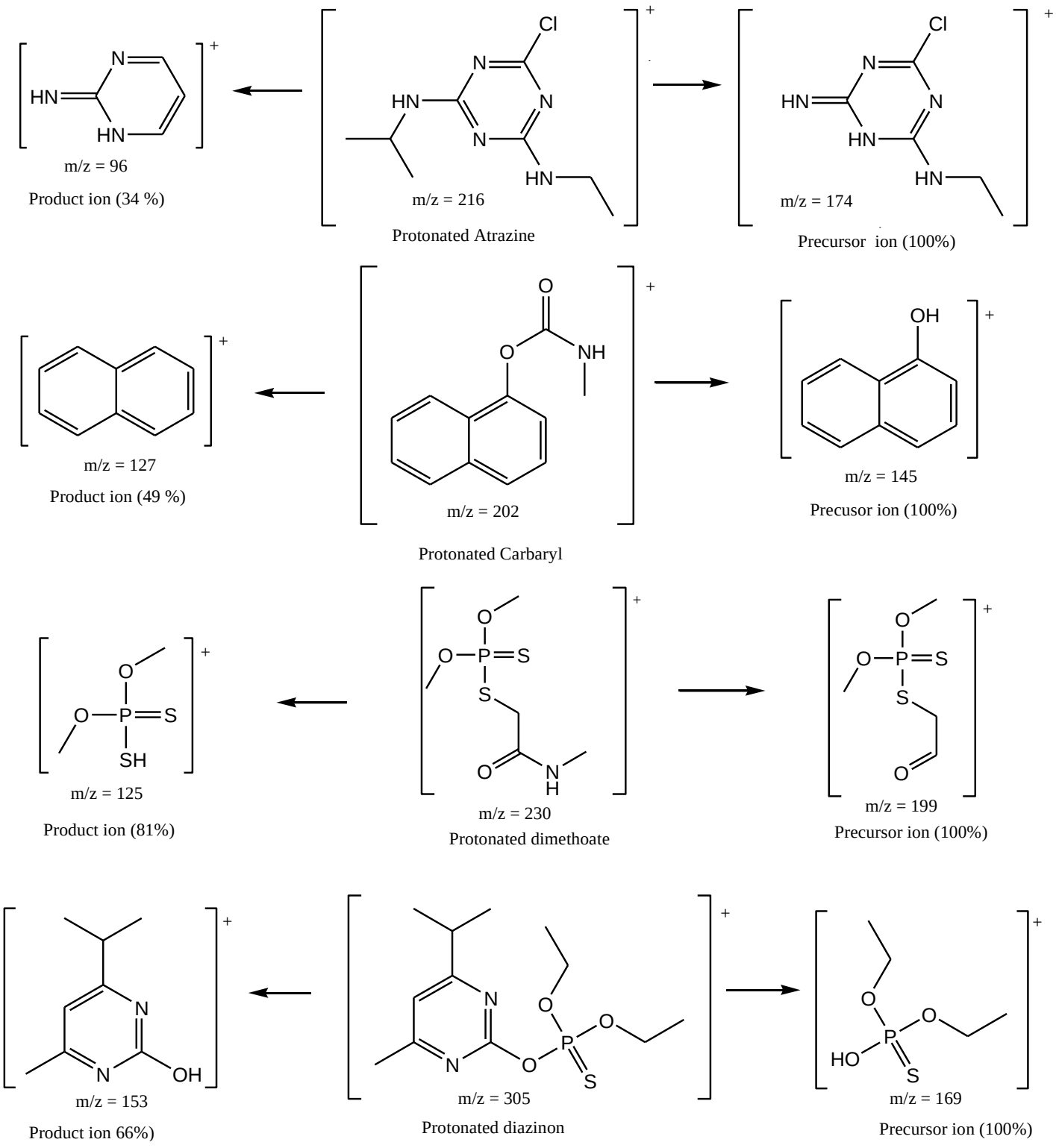

Figure 2: Fragmentations pattern of the selected pesticides

was 1 to 100 μgL^{-1}. A hundred-fold range is sufficient to cover a wide range of typical residue levels. Linearity of the calibration curve determined as a coefficient of correlation for the selected pesticides ranged from 0.996 to 0.999 within the calibration range, was close to the accepted criteria of one, indicating good linearity (Moosavi & Ghassabian, 2010) for the pesticides tested. Limit of detection (LOD) is the minimum concentration of analyte that can be detected reliably while limit of quantification (LOQ) is the lowest concentration of analyte in a sample that can be quantified precisely (Ravisankar *et al*., 2015), and both should cover the expected concentration range (Moosavi & Ghassabian, 2010). Previous studies reported slightly higher LOD and LOQ values, for instance an offline SPE-LC-ESI-MS/MS method developed by (Demoliner *et al*., 2010) achieved LOD and LQD of 2 ng/L and 20 ng/L, respectively for atrazine. Meanwhile, a solid phase extraction and ultra high performance liquid chromatography coupled with quadrupole time of flight (SPE-UHPLC- QTOF) method developed by (Lopardo *et al*., 2019) reported LOD of 0.01µg/L and LOQ of 0.03 µg/L for atrazine.

Table 2: Validation parameters and concentration of selected pesticides in the water samples

P	**R**	**RSD%**	**Recovery %**	**LOD** (ng/L)	**LOQ** (ng/L)	**Conc. (µg/L)**
Dm	0.998	2.4	70 - 80	0.01	0.02	<LOQ to 0.82
Atz	0.999	2.3	91-104	0.43	1.30	<LOQ to 3.56
Dzn	0.999	2.1	89 - 109	0.78	2.4	<LOQ to 1.9
C	0.996	2.5	83 - 102	0.03	0.08	<LOQ to 1.48

(Where, r – coefficient of determination, RSD – relative standard deviation, LOD – limit of detection and LOQ – limit of quantification, P- Pesticide, Atz – Atrazine, C – Carbaryl, Dzn – Diazinon, and Dm –Dimethate)

Meanwhile, a solid phase extraction and ultra-high performance liquid chromatography coupled with quadrupole time of flight (SPE-UHPLC- QTOF) method developed by (Lopardo *et al*., 2019) reported LOD of 0.01µg/L and LOQ of 0.03 µg/L for atrazine. In another study of drinking water by (Donato *et al*., 2015) using SPE-LCMS/MS achieved limit of detection and quantification of 0.15 µg/L and 0.5 µg/L atrazine respectively. A study by (He & Aga, 2019) using SPE-LCMS/MS reported LOD of 1 ng/L for dimethoate, 1.1 ng/L for diazinon, and 0.7 ng/L for atrazine. This study achieved an LOD of 0.01 ngL^{-1} (dimethoate), 0.03 ngL^{-1} (carbaryl), 0.43 ngL^{-1} (atrazine) and 0.78 ngL^{-1} (diazinon), with LOQ of 0.02 ngL^{-1} (dimethoate), 0.08 ngL^{-1} (carbaryl), 1.30 ngL^{-1} (atrazine) and 2.4 ngL^{-1} (diazinon), slightly lower than reported values in the previous methods. In addition these values indicate suitability of the method to detect and quantify trace (ngL^{-1}) residue levels of the selected pesticides relevant in endocrine toxicity.

Precision in terms of RSD for the selected pesticides were 2.3 % for atrazine, 2.4 % for dimethoate, 2.1 % for diazinon and 2.5 % for carbaryl. These values were within the acceptable criteria for precision within 20 % of nominal value (Moosavi & Ghassabian, 2010), and were in proximity to previous studies such as that of (Lopardo *et al*., 2019) which reported precision of 1.6 % for atrazine, and another study by (Donato *et al*., 2015) achieving precision below 19 % for atrazine and carbaryl.

A study by (Lopardo *et al.*, 2019) reported recovery of 102 % for atrazine, while (Donato *et al*., 2015) achieved recovery above 80 % for atrazine and carbaryl. A different study by (He & Aga, 2019) reported recovery of 98 - 106 % for dimethoate, 74 % for diazinon and 99 - 101 for atrazine. In this study the recoveries were: dimethoate was 70 - 80 %, diazinon 89 - 109 %, atrazine 91-104 % and carbaryl 83 - 102 %. The recoveries of the selected pesticides were within the accepted range of 70 to 120 % (Pizzutti *et al*., 2016).

3.4 Sample analysis

Raw data reported in Table S9 were evaluated as shown in Table S10 -S13. Figure 3 and 4 summarize residue levels for each selected pesticide in each site while actual results are recorded in Table S14. Atrazine ranged from <LOQ to 3.56 µg/L, carbaryl ranged from <LOQ to 1.48 µg/L, diazinon ranged from <LOQ to 1.9 µg/L, while dimethoate ranged from <LOQ to 0.82 µg/L.

In a previous study by (Githinji *et al*., 2019), solid phase micro-extraction coupled with Gas chromatography (SPME-GCMS) was used to determined atrazine in water from Likii

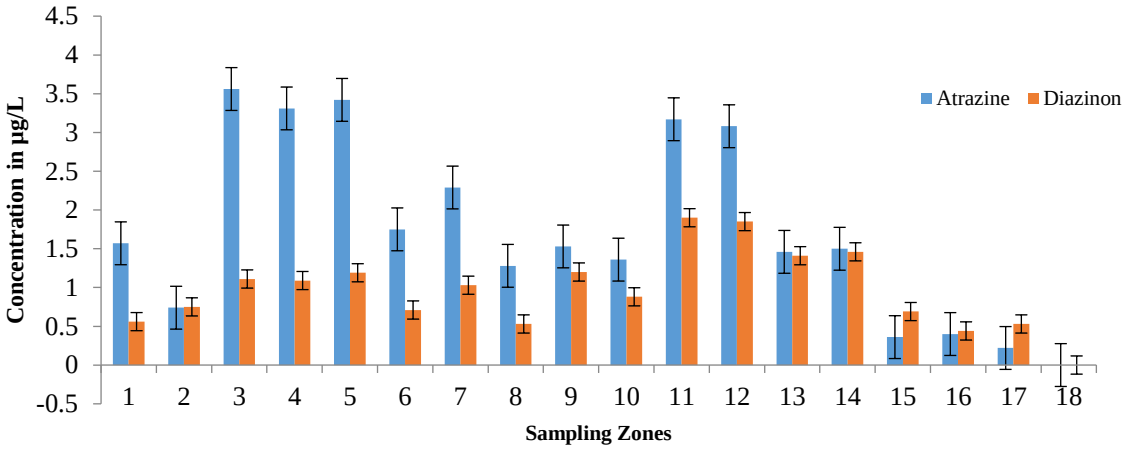

Figure 3: Distribution of Atrazine and Diazinon residues in the water samples

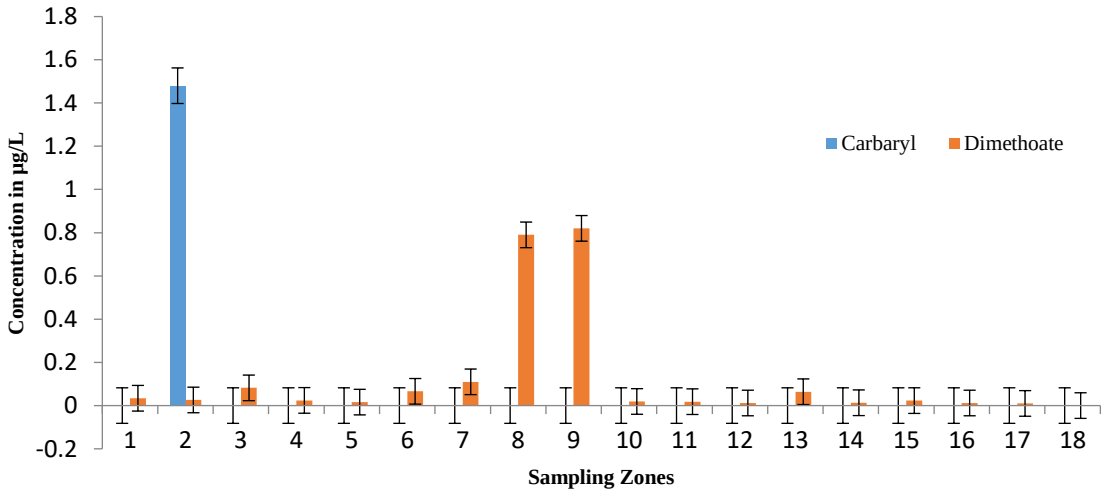

Figure 4: Distribution of Carbaryl and Dimethoate residues in the water samples

river, Laikipia County where up to 76 µg/L of atrazine was reported. In another study by (Otieno *et al*., 2018), atrazine was investigated in water samples from receiving rivers in Nzoia Basin using SPE-HPLC with diode array detector (DAD) where concentration range of 3-140 ng/L was reported. In this study atrazine residue in Mbagathi River ranged from 0.22 µg/L to 3.56 µg/L falling within the previously reported range, however the values exceeded EU MRL of 0.1 µg/L for individual pesticides (98/83/EC). Atrazine is frequently detected in surface waters due to its long half-life in water (168 days) and soil (60 to 75 days), (Singh, 2019). Since reported endocrine effects (Qiu *et al*., 2017; Giusi *et al*., 2006; Rayner, *et al*., 2005), occurred at very low concentrations such as 0.03 µg/L, (Almberg *et al*., 2018) and 0.1 µg/L (Hayes *et al*., 2006), levels of atrazine from this study in sections of Mbagathi River raises concerns of potential endocrine effects to human, wildlife and aquatic organisms.

Carbaryl is rarely reported in water probably due to its short half-life of 4 days in water and 16 days in soil (Singh, 2019), as evident in this study where it was detected only at one site at 1.48 above EU MRL of 0.1 µg/L. In a study by (Irungu, 2016), QuEChERS LC-MS/MS was used to determine carbaryl in honey bee hive products in Rift Valley and central Kenya agricultural zones, where 0.3 µg/L residue level was reported. Compared to concentration levels that caused endocrine effects between 0.05 µg/L to 0.15 µg/L (Tange *et al*., 2016), residue levels of carbaryl in Mbagathi River is a potential endocrine related health risk to wildlife and aquatic organisms.

In this study, dimethoate residue in River Mbagathi occurred in the range of 0.01µg/L to 0.82 µg/L exceeding EU MRL of 0.1 µg/L, in a number of sites. Compared to the exposure doses causing endocrine effects in previous studies (Verma & Mohanty, 2009; Novais *et al*., 2012; Dogan & Can, 2011; Guo & Chen, 2015), the residue levels in this study were lower, hence may not pose endocrine related health risk, however risk due to chronic exposure cannot be ignored.

Previous studies reported presence of diazinon in water from lake Naivasha at a concentration of 33.3 ng/L, (Owuor *et al*., 2015). In this study diazinon residue in sections of Mbagathi River under study ranged from 0.44 µg/L to 1.9 µg/L exceeding EU MRL of 0.1 µg/L. The endocrine effects due to exposure to diazinon was reported at slightly higher concentrations (Slotkin *et al*., 2008; Flynn *et al*., 2018; Savy *et al*., 2018; Katuli *et al*., 2014; ElMazoudy & Attia, 2012) than the observed residue levels in this study, and therefore exposure may not be harmful, although chronic exposure might prove otherwise. Table 3 summarizes some findings of previous studies of selected pesticide residue in sections of Kenya ecosystem.

Table 3: Residue levels in previous studies of selected pesticide residue in sections of Kenya ecosystem

Reference	Method	Matrix	EDP	Residue (Ppb)
(Githinji *et al*., 2019)	SPME-GCMS	Water - Liki River	Atz	76
(Otieno *et al*., 2015)	SPE-HPLC DAD	Water - Lake Naivasha	Dzn	<LOD - to 33.3
		Sediment	Dzn	9.3
(Irungu, 2016)	QuEChERS LC-MS/MS	Honey	Atz	356.7
			C	0.31
			Dm	1.19
			Dzn	1.14
		Pollen	Atz	23.5
			Dzn	4.12
			C	0.4
(Otieno *et al*., 2018)	SPE – UHPLC - MS	Water - Nzoia river	Atrz	3 - 140

(Where, Atz – Atrazine, C – Carbaryl, Dzn – Diazinon, and Dm –Dimethate)

4. Conclusion

Validation results showed that the modified SPE-LC-MS/MS method was reliable, accurate, and reproducible with very low detection and quantification limits, indicating its suitability for trace routine exposure assessment of the selected EDPs in river water. The study also revealed that majority of pesticides tested occurred at levels that raise concerns of potential endocrine effects to living organisms. Similarly risk due to chronic exposure cannot be ignored. In addition, levels of these pesticides in a majority of sites were above EU MCLs for drinking water.

Associated Content

Supporting Information: The Supporting Information is available on the Journal of the Kenya Chemical Society webpage at https://kenyachemicalsociety.org/journals

Disclosure of conflict of interest

The authors disclose no conflict of interest.

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