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Natural Adsorbents for Cleaning Fat Matrices in the Quantification of Persistent Organic Pollutants in Fish by GC-MS

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ABSTRACT

This study explores the application of moringa's seed protein (Morp) as a clean-up sorbent on fat matrices such as fish samples for the extraction and quantification of persistent organic contaminants using the GC-MS technique. The study serves as a health risk assessment for the fish consumption in Mozambican open markets. The recovery values of the target compounds were good, ranging from 71 to 110% except Profenophos with 58 to 59%. A good method' precision was observed with %RSD values below 10%. In fish sample' analysis, lindane was obtained at 0.213 ± 0.061 mg/kg on catfish and 0.246 ± 0.070 mg/kg on tilapia, dichlorodiphenyl dichloroethane at 0.195 ± 0.026 mg/kg on tilapia. Both contaminants were above the maximum residue limits set by the Codex Alimentarius at 0.01 mg/kg and 0.2 mg/kg respectively. In addition, 4-Nonylphenol was also detected on three catfish samples ranging from 0.439 ± 0.014 mg/kg to 0.826 ± 0.094 mg/kg. The validation results indicated the applicability of Morp as a sorbent for the quantification of persistent organic pollutants in fat matrices. The results of the health risk assessment suggest the fish consumption from Mozambican open markets is safe.

KEYWORDS

Moringa, Protein, Pesticides, Extraction, QuEChERS

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INTRODUCTION

The use of pesticides in agriculture aim to prevent or reduce crop destruction action of the harmful agents such as insects, weeds, among others.[1] But certain pesticides display low solubility in water, which can lead to their accumulation in different environment compartments like water, were can be absorbed on sediment layer, algae, therefore putting fish and other organisms at risk after exposure.[1,2] In our days low bioaccumulative pesticides like pyrethroids and organophosphates are still used, and their extensive use is also a concern due to their toxicity to some non-target species like fish.[3] After pesticide accumulates in these food item, they are subjected to be transferred to the consumers including the human beings.[4] As a result chronic human health problems, namely malignant tumors, endocrine disorders, reproductive effects and nervous disorders are common on different population age and have been related to the pesticides exposure.[4] Therefore, to assess a food security, fish sample constitute the most important indicators of the aquatic contamination.[3,5] Therefore, studies of pesticide extraction methods have become a fundamental action in controlling food quality. But the determination of residual pesticides in complex matrices, particularly those with high fat and protein contents such as fish, are subjected to many interferences and requires the use of effective extraction methods that can allow high recoveries of the analytes with minimal or absence of matrix interference.[6] For this purpose, various extraction and clean-up techniques have been used in different studies, such as dispersive liquid-liquid extraction (DLLE) with solvents of different polarities,[7-9] gel permeation chromatography (GPC),[3,10] saponification and treatment with sulfuric acid[3,11] and solid phase extraction (SPE) have been used.[12,13] However, the time consumption with manual operations, expensive equipment and high volumes of solvents were identified as disadvantages. To ensure rapid extraction a dispersive solid phase extraction (d-SPE) was developed with the introduction of a fast, easy, cheap, effective, robust and safe method called "QuEChERS" based in PSA as sorbent clean up. Actually, its' modifications have been made to the extraction of pesticides residues in different fat matrices samples such as fish,[9,11,14,15] raw bovine milk,[16] cheese.[17] General modifications of the QuEChERS method are centered on replacing PSA with different sorbents such as Z-Sep+[15] and EMR-Lipid[18] to test their efficiency in removing fat interference on pesticides determination. However, the high cost of these sorbents including PSA has limited research into these compounds in food products, particularly in countries with low financial resources such as Mozambique. Hence the search for the usage of other materials locally available such as proteins from moringa's seeds as sorbents for extracts clean-up, would make extraction costs even more viable. Moringa have been used for various purposes, with greater emphasis on its seeds as they have greater adsorbent capacity, which may be associated with the action of the proteins contained in them such as Mo-CBP3-1, Mo-CBP3-2, Mo- CBP3-3 and Mo-CBP3-4, all of them with coagulant capacities.[19,20] One of the applications of this protein in extract clean-up was demonstrated in a study conducted by[21] on tilapia samples, where the Moringa's seed protein showed good efficiency in removing co-extractives and provided good analytical recovery of the researched compounds. In our previous study[22] the capacity of Moringa's seed protein in cleaning vegetable extracts were observed and the extraction method was successfully validated. Considering these studies, the present study aims to evaluate the possibility of extending the cleaning method using moringa protein, previously validated in our laboratory, to fatty extracts such as fish.

EXPERIMENTAL

Material and methods

In this study, gas chromatograph coupled to the mass spectrometer GC 7820A, MSD 5977B were sourced from Agilent Technologies (Johannesburg, South Africa), freeze drier Labconco, (Johannesburg, South Africa), vortex Thermo Scientific (Osaka, Japan), centrifuge

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ALC 4206 (Italy), weighing balances BM-20 (Osaka, Japan) and Gibertini Europe 500 (Italy). Additionally, analytical standards such as hexachlorobenzene (Ƴ-HCH and ϒHCH) and Chlorpyrifos purchased from Sigma Aldrich (Darmstadt, Germany), diclorodifeniltricloroetano (p,p'-DDT) and 4-Nonylphenol sourced from Pestanal (Johannesburg, South Africa); Cypermethrin, Profenophos and triphenylphosphine 99.0% (a/a) sourced from Pestanal (Darmstadt, Germany) were used. The solvents methanol, acetonitrile (HPLC grade) and glacial acetic acid 99% purchased from Sigma Aldrich (Darmstadt, Germany) were also used. In addition, salts such as Superclean PSA Sigma-Aldrich (Darmstadt, Germany), anhydrous Na_2SO_4 ≥ 99%, batch G3350 sourced from Honeywell (São Paulo, Brazil), NaCl ≥ 98%, batch K53695504150 Merck KGaA (Darmstadt, Germany), moringa protein and acrodisc polyvinylidene fluoride (PVDF) syringe filters (30 mm HPLC syringe filter, PVDF, pore size 0.45µm, injection moulded ring plus print of the membrane type 30162093 sourced from Shimadzu South Africa) were also used.

Preparation of calibration standards

Different concentrations of standards solutions of β-HCH, Ƴ-HCH, Cypermethrin, Chlorpyrifos, dichlorodiphenyldichloroethane (p,p'-DDD), p,p'-DDT, Profenophos and 4-Nonylphenol were prapared. For each standard solution, the solid reagent was weighed and dissolved in HPLC-grade methanol to obtain a concentration of 1000 mg/L (stock solution). Then corresponding volume of each stock solution were pipetted to Eppendorf tubes. To all calibration standards, 100 µL of trimethylphosphine (1 mg/L) was added as internal standard and diluted to a final volume of 1 mL using the blank matrix extract solution (calibration matched matrix). The Eppendorf tubes were vortexed for 3000 rpm for 30 s. The concentrations range for each analyte is presented in Table 2 in results and discuss section.

Method validation

Linearity, limits of detection and quantification

The linearity of the method was evaluated using the correlation factor (R^2) in each calibration curve. The limit of detection (LOD) was determined from successive tests of increasing concentrations until a signal distinct from the baseline was found. The LOD was manually calculated as the analyte concentration corresponding three times the signal-noise ratio and the mass sample was considered in this calculation. The limit of quantification (LOQ) was determined using the equation 1:

$$LOQ = \frac{10 \times LOD}{3} \quad (1)$$

Evaluation of the robustness of the extraction methodology

For this purpose, three of the principal factors which can affect the extraction yield such as extraction rate, solvent mixture composition and extraction time were optimized in our previous publication[22] and similar extraction conditions were applied in this study.

Recovery and repeatability tests of the extraction methodology

For extraction, the modifications on the QuEChERS procedure described in literature[23] were made. Briefly, 10 g of the sample previously freeze dried at –20 °C, 3 atm pressure and crushed in a porcelain mortar were placed in a 50 mL Teflon extraction tube, then 10 mL of ultrapure water were added and 100 µL of trimethylphosphine 10 mg/L solution was added as internal standard in order to obtain a final concentration of 0.1 mg/kg and the mixture was vortexed at 3000 rpm for 1 min. Then, a volume of the standard solution corresponding to the desired concentration was added to the tube and left stand for 30 min, followed by addition of 10 mL of the 1% HAc in ACN: MeOH (90:10) solution. The tube was vortexed at 3200 rpm for 2 min. Then, 1 g NaCl and 4 g Na_2SO_4 previously dried at 110 °C for 3 h were added to the centrifuge tube. The tube was centrifuged at 4000 rpm for 5 min. Then, 2 mL of the supernatant was taken into 15 mL Teflon extraction tube containing 150 mg PSA and 300 mg Na_2SO_4 for PSA clean-up. For moringa's seed protein clean-up, 2 mL of the supernatant was taken into another 15 mL Teflon extraction tube containing 150 mg Morp and 300 mg Na_2SO_4. The tube was vortexed at 3000 rpm for 1 min and centrifuged under the above conditions. The supernatant was filtered into 1.5 mL vials using the acrodisc polyvinylidene fluoride (PVDF) syringe filters, 0.45 µm pore diameter, followed by GC-MS injection.

Application of the validated methodology on real samples extraction

Sample collection

After the validation tests, fish samples were collected from open markets in three different Mozambican provinces: Maputo province (Boane, Matola and Moamba districts) one south of the country, Tete province (Tete city) on center and Nampula province (Larde district) on north. Then, the samples were wrapped with aluminum foil, placed in plastic bags and kept in isothermal boxes containing ice and transported to the Chemistry Department Laboratory at Eduardo Mondlane University. All samples were kept in a freezer below -20 °C until further processing.

Sample preparation e compound extraction

Before analytical extraction the fish samples were rinsed with water and cut into small pieces using metal knives and scissors. Then, from each sampling site, about 400 g of the fish muscle were freeze-dried and later crushed into powder using a mortar and pestle. After that the sample was preserved in aluminum foil until extraction of organic compounds by QuEChERS method. To enhance analytes signals, before extraction, the samples were spiked with analytes standard solutions to final concentration of 0.1 mg/kg. The extraction was performed in QuEChERS procedure described above, followed by injection in GC-MS.

Chromatographic conditions

The gas chromatograph (GC) 7820A coupled to a mass spectrometer (MSD) 5977B, equipped with SH-Rtx-5MS capillary column (30 m x 250 µm x 0.25 µm, -60 to 330 °C, supplied by Agilent Technology, South Africa) were used in all analysis. The injection temperature was maintained at 250 °C. The oven temperature started at 70 °C (5 min), then increased to 190 °C (20 min) at a rate of 30 °C min^{-1}, and finally increased to 300 °C (15 min) at a rate of 20 °C min^{-1}, the total run time was 49.50 min. The injection volume was 1 µL in a splitless mode, helium 99.9997% was used as a mobile phase in a flow rate of 35 mL min^{-1} at 13,265 kPa pressure. The compound identification was performed in SCAN mode, and quantification was performed in selective ion monitoring (SIM) mode, with an ionization energy of 70 eV. For peaks identification, a comparison of the molecular spectra (m/z) for each eluted compound with the spectra of the NIST14 library database was carried out using 80% matching criteria. The quantification of each compound was done using the specific quantification ion mass as per Table 1.

RESULTS AND DISCUSSION

Method validation

The method validation aims to demonstrate objective evidence that a particular proposed method presents requirements for analytical application and ensures the reliability of the results.[24]According to Ribani et al. (2004) and Bhardwaj et al. (2016) the evaluation

Table 1: GC–MS retention times, quantification ions, confirmation ions, and molecular ions used for the identification and quantification of the target analytes

Compound	RT (min) GC-MS	Quantification ion (m/z)	Confirmation ions (m/z)	Molecular ion (m/z)
β-HCH	15.73	109	219; 254	290.8
Ɣ-HCH	15.54	181	219; 254	290.8
Cypermethrin	37.32	163	207; 281	416.3
Clorpyrifos	30.79	197	258; 314	350.6
p,p'-DDD	32.19	165	235; 320	320.0
p,p'-DDT	33.62	235	165; 354	354.5
4-Nonylphenol	17.46	107	77; 220	220.4
Profenophos	31.10	139	97; 208	373.6
Triphenyl phosphine (IS)	35.03	183	108; 262	262.3

IS: internal standard

Table 2: Correlation factor, limits of detection and quantification of target compounds

Compound	Calibration concentration (mg/L)	Calibration Equation	Reference value $R^2 > 0.90$[24]	LOD (mg/kg)	LOQ (mg/kg)
β-HCH	0.067;0.130;0.200;0.250; 0.300	Y= 0.0463x+00048	0.9887	0.005	0.017
Ɣ-HCH	0.067;0.130;0.200;0.250; 0.300	Y=1.4833x-0.0119	0.9971	0.005	0.017
Cypermethrin	0.133;0.200;0.250;0.350;0.450	Y= 0.2171x-0.0015	0.9884	0.02	0.066
Chlorpyrifos	0.125;0.190;0.250;0.350;0.400	Y=1.5474x-0.0005	0.9982	0.01	0.033
p,p'-DDD	0.100; 0.200; 0.300; 0.500; 0.600	Y= 0.1717x-0.0074	0.9945	0.01	0.033
p,p'-DDT	0.02;0.11;0.165;0.220;0.350	Y=1.2775x-0.0188	0.9938	0.005	0.017
Profenophos	0.200;0.250;0.300;0.400;0.550	Y= 0.8349x-0.0283	0.9907	0.02	0.066
4-Nonylphenol	0.067;0.130;0.200;0.250; 0.300	Y=1.3956x-0.0083	0.9999	0.01	0.033

of parameters like selectivity, linearity, detection limit (LOD), quantification limit (LOQ), accuracy, precision and robustness is necessary in method validation study. Hence, in the present work all aforementioned parameters were investigated.[24,25]

Linearity, limits of detection and quantification

The linear correlation factor (R^2), the detection and quantification limits, (LOD) and (LOQ) respectively, for the target compounds are presented in Table 2.

In Table 2, the R^2 values ranging from 0.9834 to 0.9999 are all above the minimum acceptable value $R^2 = 0.90$ indicating good linearity of the analytical method.[24] For linearity evaluation the calibration matched matrix was done. According to the literature, this calibration strategy can improve quantitative results by reducing matrix effects.[26,27] The corresponding calibration curves are presented in the supplementary material (Fig. A1 to A8). The limits of detection ranged from 0.005 to 0.02 mg/kg and the limits of quantification ranged from 0.017 to 0.066 mg/kg.

Comparison of PSA and Morp recoveries

The recoveries and the repeatability of the proposed analytical method were evaluated at three concentration levels by spiking the blank sample with analytical standards of the researched compounds. For each concentration level, five replicates were prepared. The spike was performed in samples of tilapia grown in pesticide free ponds, which was used as a blank matrix. After extraction, the extracts with the same concentration were cleaned with both PSA and moringa's seed protein (Morp) as clean-up sorbents. Then the mean recoveries and precision were determined in both sorbents and the recoveries obtained in both sorbents were statistically compared using the t-student test having the same or different variance.[28] The statistical procedures and formulae used can be found in section A2 in the supplementary materials. The analytical results for the recoveries, precision and t-student test are presented in Table 3.

The experimental "t" values were compared to the critical values (p = 0.05, 2T). The findings suggested no significant differences between the mean recoveries of the proposed moringa's protein (Morp sorbent) and the default QuEChERS extraction methods (PSA sorbent). However, the analytical recoveries differed at one point for the compounds p,p'-DDT, 4-Nonylphenol and profenofos, where the experimental t values were higher than the critical t values. Despite the differences observed at the three fortified concentrations, in general the Morp sorbent is efficient for cleaning fatty extracts since, at most of the fortification concentrations evaluated, the recoveries determined in it were close to those obtained in the PSA sorbent. Its efficiency was demonstrated by the good analytical recoveries observed in all target compounds ranging from 71 to 109% for PSA and 71 to 110% for Morp, except Profenophos in which the recoveries on both sorbents were lesser than 70%. The %RSD values in Morp adsorbent (Table 3) ranged from 0.43 to 9.02%. According to Molina-Ruiz et al. (2014) and Sante (2021) in the validation of an analytical method for the quantification of organic compounds, good recoveries are considered in the range 70 to 120% and the %RSD values below 20%.[29,30] Considering these criteria, the extraction method showed poor analytical recoveries for Profenophos. The lowest recoveries observed on profenophos determination can be related to the strong interactions between Profenophos and both sorbent materials, as some of them can retain target compounds leading to poor recoveries and precision.[31]

Comparison of the current sorbents with others from literature

From Table 4, it can be observed that the Morp sorbent presented satisfactory recoveries comparable to those determined in other studies using other sorbents. These results demonstrate its applicability in cleaning extracts of fat matrices samples like fish.

Table 3: Analytical recoveries and precision of the extraction method

Compound	Conc. (mg/kg)	PSA		Morp		t_{ob}	t_{crit}
		Rec (%)	RSD (%)	Rec (%)	RSD (%)		
	0.50	80	5.18	83	9.02	1.05	4.30
β-HCH	0.15	78	0.92	77	3.70	0.87	4.30
	0.23	71	5.10	71	3.68	0.41	4.30
	0.10	92	1.35	92	1.21	0.14	2.31
ϒ-HCH	0.23	96	0.61	96	5.56	0.05	2.78
	0.275	94	0.49	91	3.00	1.80	2.78
	0.15	85	2.66	86	2.04	0.76	2.31
Cypermethrin	0.35	82	5.25	80	5.27	0.89	2.31
	0.40	78	3.64	82	8.29	1.20	2.31
	0.15	99	1.97	95	5.92	1.70	2.31
Chlorpyrifos	0.25	102	0.37	104	2.44	1.59	2.31
	0.375	75	0.42	77	1.98	1.65	2.31
	0.10	85	2.66	87	5.28	0.76	2.31
p,p'-DDD	0.20	84	7.85	88	6.77	0.81	2.31
	0.30	86	7.64	88	3.20	0.44	2.31
	0.10	83	5.87	84	4.00	0.37	2.31
p,p'-DDT	0.20	86	0.61	74	6.43	**5.80**	**2.78**
	0.30	87	2.55	88	2.41	1.09	2.31
	0.15	109	2.62	110	0.43	0.84	2.78
4-Nonylphenol	0.23	107	0.67	106	3.04	0.90	2.78
	0.275	98	0.54	102	2.35	**3.73**	**2.78**
	0.23	61	4.25	59	6.06	0.88	2.31
Profenophos	0.35	60	0.54	58	1.55	**3.05**	**2.57**
	0.50	60	0.98	58	5.64	0.93	2.78

PSA-primary secondary amine; Morp – moringa protein; t_{ob} – experimental *t*-value; t_{crit} – critical *t*-value

Table 4: Different sorbents used for fatty matrices extracts' clean-up

No	Matrix	Sorbent/sorbent mixture	Recoveries (%)	Reference
1	Fish	3 mL extract +230 mg C18 + 184 mg PSA + 184 mg Z-Sep+	81.4 - 106.6	[15]
2	Fish	1 mL extract + 50 mg PSA	88.1 - 121.2	[9]
3	Colza (Rapeseeds)	6 mL extract + 175 mg EMR-Lipid	70.0 - 116.0	[18]
4	Raw bovine milk	1.2 mL extract + 125 mg PSA + 25 mg Z-Sep + 5 mg Z-Sep+	70.0 - 119.0	[16]
5	Cheese	7 mL extract + 500 mg diatomaceous earth + 150 mg C18	83.1 - 113.6	[17]
6	Fish	3 mL extract +150 mg PSA	70.0 - 115.0	[11]
7	Fish	1 mL extract + 50 mg PSA + 50 mg C18	70.0 – 116.0	[14]

Potential clean-up properties of Morp compared to the commercially available PSA in fish samples matrices

The selectivity of each sorbent was evaluated through the peak resolution and the presence of co-extractive peaks on the chromatogram generated by the blank sample extract, spiked with a mixture of standard solutions containing ϒ-HCH, ϒ-HCH, Cypermethrin, Chlorpyrifos, p,p'-DDD, p,p'-DDT, 4-Nonylphenol, Profenophos and triphenylphosphine. On Figure 1 are presented the chromatograms of the blank sample after Morp clean-up (a) and the chromatograms of the spiked blank sample after PSA (b) and Morp (c) clean-up. In these chromatograms, the selectivity of the PSA and Morp can be observed.

Figure 1 shows that the blank sample used is free of analytes and other interfering compounds, as demonstrated by the absence of peaks at the same retention times as the analytes in the chromatogram (a). Additionally, the two sorbents showed good resolution peaks but different capacity on the co-extractive removal. In this case, the best clean-up efficiency was observed on PSA sorbent displayed on chromatograms (b). On the other hand, Morp sorbent showed to have a strong interaction with all target compounds including triphenyl phosphine used as internal standard, which led to the poor peaks intensity according to the chromatogram (c). According to the literature O'Mahonya et al. (2013), the presence of co-extractives in the analytical extract constitutes analytical interference and can result in an increase or suppression of the analyte signal.[27] In this point of view the poor peaks intensities observed on Morp sorbent clean-up can be related to the remaining fish matrix components in the extract.

Samples analysis results

Persistent organic compounds in fish samples

Following analytical recovery studies and evaluation of the sorbent's efficiency in cleaning extracts, Morp was used as clean-up sorbent in

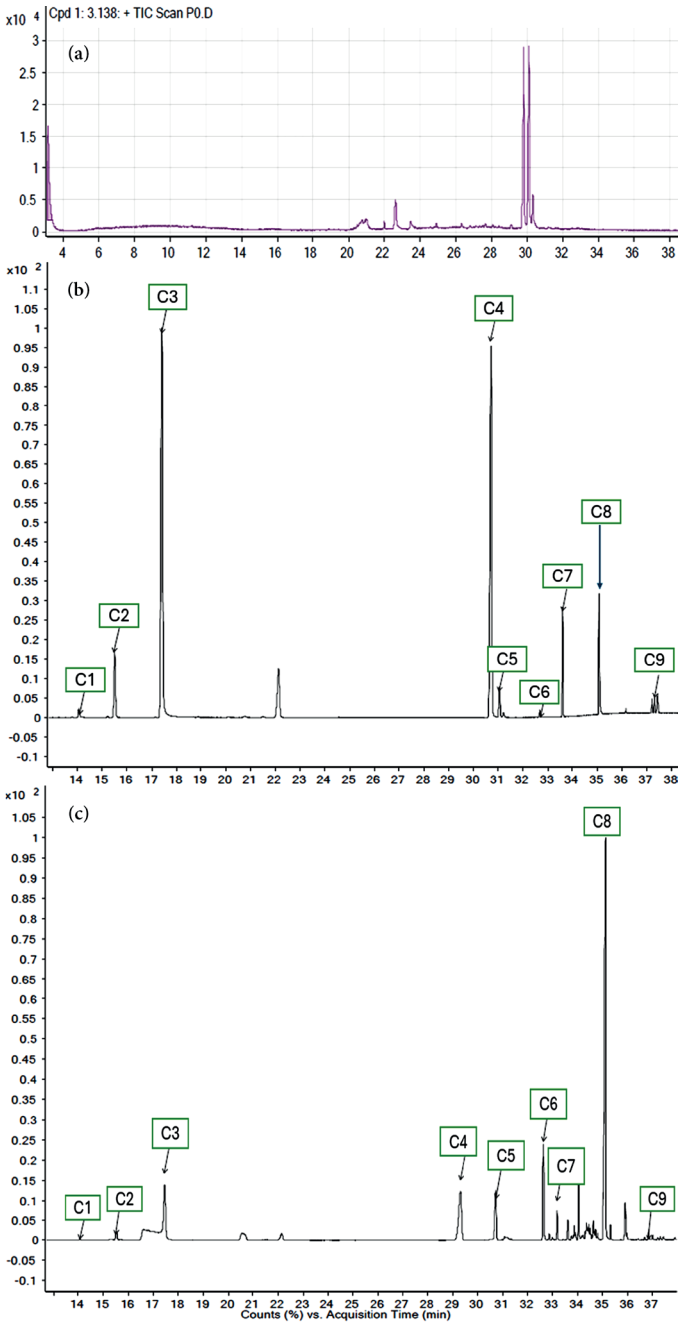


Figure 1: Chromatogram of blank (a), chromatogram of PSA (b) and Morp chromatogram (c) (C1 – ϒ-HCH; C2 – β-HCH; C3 – 4-Nonylphenol; C4 – Chlorpyrifos; C5 – Profenophos; C6 – p,p'-DDD; C7 – p,p'-DDT; C8 – Triphenyl phosphine; C9 – p,p'-Cypermethrin)

fish samples extracts to monitor some contaminants in this food stuff. Often, the concentration of these compounds in the samples is lower when compared to the matrix components. Hence, for their detection it is necessary to spike samples with standard solutions of the target compounds.[32-35] After quantifying the contaminants, the health risk from consuming contaminated fish was also assessed, and the criteria for its estimation can be found in the supplementary materials section A3. The results of the determinations are presented in Table 5.

The use toxic pesticides such as organochlorinated are prohibited in Mozambican agriculture for long, but their residues persist in the environment. Their presence was evidenced by positive detection of these harmful pollutants in some fish samples analyzed in the present study. In this study, ϒ-HCH was obtained in two fish samples, one in a catfish collected in the Larde district (Nampula province) and the other from the tilapia collected in the Tete city (Table 5), at concentrations of 0.213 ± 0.061 mg/kg and 0.246 ± 0.070 mg/kg,

Table 5: Analytical results for fish samples collected in Mozambican open markets

Sample	Concentration ± sp (mg/kg)		
	ϒ-HCH	p,p'-DDD	4-Nonylphenol
BOATL1	nd	nd	nd
BOATL2	nd	0.195 ± 0.026	nd
MOATL1	nd	nd	nd
MOATL2	nd	nd	nd
MATCF1	nd	nd	nd
MATCF2	nd	nd	0.439 ± 0.014
NPTLA1	nd	nd	1.16 ± 0.12
NPTLA2	nd	nd	nd
NPTLA3	nd	nd	nd
NPCFLA1	0.213 ± 0.061	nd	0.826 ± 0.094
NPCFLA2	nd	nd	nd
TeTz1	0.246 ± 0.070	nd	0.678 ± 0.017
TeTz2	nd	nd	nd
TeTz3	nd	nd	nd

BOATL: Boane tilapia; MATCF: Matola catfish; MOATL: Moamba tilapia; NPTLA: Nampula tilapia Larde; NPCFLA: Nampula catfish Larde; TeTz: Tete tilapia Zambeze; nd: Not detected

respectively. Notwithstanding, these values are above the MRL set at 0.01 mg/kg prescribed by Codex Alimentarius Commission (2016).[36] On other study conducted in Republic of South Africa by Nuapia et al. (2016) ϒ-HCH was detected in fish samples at a concentration of 0.1058 ± 0.0052 mg/kg, lower value when compared with the one obtained on the present study.[37]

In addition to ϒ-HCH, another organochlorinated pesticide (p,p'-DDD) was determined in one sample of tilapia fish collected in the Boane district at a concentration of 0.195 ± 0.026 mg/kg (Table 4). Different to ϒ-HCH, the concentrations of p,p'-DDD were below the MRL, which is set at 0.2 mg/kg for fish.[36] But contrary the concentration of p,p'-DDD obtained in the present study is above the concentration range 0.0097 ± 0.0045 mg/kg to 0.0283 ± 0.0058 mg/kg reported in South Korea by Choi et al. (2016) and 0.1027 mg/kg obtained in Turkey by Santini et al. (2024).[1,38]

Organochlorinated pesticides are no longer used in Mozambican agriculture. Therefore, its detection on fish samples is suspected of its illegal use or its low degradation rate in the environment. According to World Health Organization (2003) prohibited pesticides such as DDT are still illegally used in different countries and this is one of the reasons for their detection in the environment.[39] On the other hand, the migratory behavior of fish could also be the reason of these findings which may have been contaminated in neighboring countries such as the Republic of South Africa and the Republic of Zambia, were probably high application of these compounds in the past was common. According to Sudharshan et al. (2012) one of the degradation products of p,p'-DDT is p,p'-DDD, one metabolite with very long lifetime. [40] Hence, the detection of p,p'-DDD may be related to the excessive use of p,p'-DDT in the past and not a recent application.

The 4-Nonylphenol (4-NP) was quantified in two catfish samples, where one was collected in Matola (Maputo province) while the other one was collected in Larde (Nampula province) at concentrations of 0.439 ± 0.014 mg/kg and 0.826 ± 0.094 mg/kg respectively. In addition, 4-NP was also quantified in two tilapia fish samples, one of which collected in the district of Larde at 0.439 ± 0.014 mg/kg and the other one in Tete city at 1.16 ± 0.12 mg/kg (Table 4). However, the 4-NP concentration values on fish samples from this research are within the range reported by Akhbarizadeh et al. (2012) who detected 4-NP with a maximum concentration of 18.80 mg/kg in fish samples collected in Iran. [41] In our research any value of 4-NP MRLs was reported even

Table 6: Assessment of the risk of exposure of adult consumers and children by ingestion of freshwater fish (6.1 g/person. day)

Compounds	ADI (mg/kg bw)	Fish				
		Mean conc. ± sp (mg/kg)	EDI ± sp x10^{-5}	EDI*± sp x10^{-5}	HI ± sp x10^{-3}	HI* ± sp x10^{-2}
β-HCH	0.015[a]	-	-	-	-	-
ϒ-HCH	0.003[a]	0.230 ± 0.023	2.34 ± 0.23	9.35 ± 0.94	8 ± 1	3.1 ± 0.3
Cypermethrin	0.02[b]	nd	-	-	-	-
Chlorpyrifos	0.001[b]	-	-	-	-	-
p,p'-DDD	0.001[a]	0.195 ± 0.026	1.98 ± 0.26	7.93 ± 1.06	20 ± 10	7.9 ±1.1
p,p'-DDT	0.02[a]	nd	-	-	-	-
4-Nonylphenol	-	0.776 ± 0.175	7.89 ±1.75	31.6 ± 7.1	-	-
Profenophos	0.03[b]	nd	-	-	-	-

[a]43; [b]44; ADI – acceptable daily intake; EDI e EDI*- estimated daily intake for adults and children respectively; HI and HI*- health risk index for adults and children; sp – standard deviation

in literature for freshwater fish matrix. 4-NP its emerging endocrine disrupting chemicals, so its MRLs is not yet established for different matrices, except for water where it is set at 0.3 mg/kg.[42]

Consumer health risk assessment

The estimated data of health risk for the Mozambican consumers of the freshwater fish are presented in Table 6.

To assess the health risk to the consumers of contaminated fish, it was considered that they are subjected to the same level of exposure regardless of their age. The HI values ranged from 0.008 ± 0.001to 0.020 ± 0.010 for adults and from 0.031 ± 0.003 to 0.079 ± 0.011 for children (Table 6). All these values were less than one (1). According to the open literature, HI less than 1 reveals that the concentration of that contaminant in the food is below the toxic level for the consumer.[37,43,45] But the long-term consumption of contaminated fish may present a health risk to the consumer due to their ability for bioaccumulation and bio magnification in the organism.

The health risk assessment related to 4-NP was not evaluated because its MRL for fish is not yet set up. But the presence of this compound in food items can put consumer health at risk because toxicological studies associate the consumption of food contaminated by pesticides and 4-NP with various public health problems such as changes in the functioning of the endocrine system, various types of cancer, birth defects, among others.[46-48]

CONCLUSION

The developed method shows good linearity as demonstrated by the linear correlation coefficient (R^2) ranging from 0.9884 to 0.9999. The detection limits (LOD) varied from 0.005 to 0.02 mg/kg and the quantification limits varied from 0.017 to 0.066 mg/kg. The analytical recoveries were also good for all target compounds in the range from 71 to 83% for β-HCH, 91 to 96% for ϒ-HCH, 80 to 86% for Cypermethrin, 77 to 104% for Chlorpyrifos, 87 to 88% for p,p'-DDD, 74 to 88% for p,p'-DDT, 102 to 110% for 4-Nonylphenol, except for Profenophos which the recoveries were lower than the minimum acceptable value of 70%. The method precision was also good with %RSD values ranging from 0.43 to 9.02%. Therefore, the moringa seed protein can be used as an alternative sorbent to the commercially available primary secondary amine (PSA) sorbent for the extract clean-up process in fish matrix. Hence, this study demonstrated the application of moringa seed protein for the determination of all persistent organic compounds evaluated, except Profenophos. The analytical results of the fish samples indicate the presence of ϒ-HCH, p, p'-DDD and 4-Nonylphenol. The evaluation of the health risk assessment for the exposure to these compounds suggests no health risks to the consumers of the fish from the Mozambican open markets, but there is a concern regarding the presence of 4-Nonylphenol as this is an emerging contaminant with potential hazardous effects to the consumers.

SUPPLEMENTARY INFORMATION

The supplementary material can be found in the attached document

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

STATEMENTS ON GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

In this article, the MENDELEY citation and reference tool was used, and the references were subsequently adjusted manually. Additionally, ChatGPT was used to abbreviate the journal names.

AUTHOR CONTRIBUTIONS

Silvestre Lucas Muiambo: sample collection, design, conducting analytical experiments and manuscript writing. Eutério Felizardo Crisino Chaúque and Noor Jehan Gulamussen: research conception, sample collection and manuscript revision. Luke Chimuka and Imasiku Nyambe: research design and manuscript revision. Eric Morifi: technical assistance in conducting analytical experiments and linguistic revision.

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