Bull. Chem. Soc. Ethiop. **2025**, 39(7), 1227-1244.
DOI: https://dx.doi.org/10.4314/bcse.v39i7.1

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X


# RISK ASSESSMENT OF SELECTED ENDOCRINE DISRUPTING COMPOUNDS IN SELECTED RAW FOODSTUFFS SOLD ON OPEN MARKETS IN ZAMBIA

Foster Miyanza[1,2], Eutilério Chaúque[3], Imasiku Nyambe[1], Eric Morifi[2], Heidi Richards[2], and Luke Chimuka[2*]

[1]Integrated Water Resources Management Center, School of Mines, University of Zambia, P.O Box 32379, Lusaka, Zambia
[2]Molecular Sciences Institute, School of Chemistry, University of Witwatersrand, Private Bag X3, Johannesburg 2050, South Africa
[3]Department of Chemistry, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique

(Received March 14, 2024; Revised March 25, 2025; Accepted March 29, 2025)

**ABSTRACT**. The aim of this study was to assess the presence and quantity of selected organic endocrine disrupting compounds (phthalates, DDT (dichlorodiphenyltrichloroethane) metabolites and 4-nonylphenol) in the selected raw foodstuffs (fish and vegetables) sold in open markets and to carry out a health risk assessment of the EDCs. QuEChERS (Quick, Effective, Cheap, Efficient, Rugged and Safe) technique was optimized for extraction and GC-MS (gas chromatography-mass spectrometry) was used for identification and quantification. 4-Nonylphenol and DDT metabolites were not detected in all the samples. The mean levels of DMP (dimethyl phthalate) ranged from 91.05 to 101.76 µg/kg, 77.14 to 123.82 µg/kg, and 85.65 to 98.55 µg/kg for samples from Kitwe, Lusaka and Kabwe, respectively. The mean concentrations of DEP (diethyl phthalate) ranged from 21.46 to 80.69 µg/kg, 63.93 to 161.67 µg/kg and 23.22 to 46.01 µg/kg for samples from Kitwe, Lusaka and Kabwe, respectively. DMP was lowest in tomato in all the towns. DEP was generally higher in spinach. The health risk analysis of DMP and DEP gave the hazard index, HI < 1. Though the health risk parameters for DMP and DEP are within the safety margins, consumers' safety can only be guaranteed after a comprehensive risk analysis of other EDCs.

**KEY WORDS**: Raw foodstuffs, Fish, Vegetables, QuECHERS, DDT, Phthalates

## INTRODUCTION

Endocrine disrupting compounds (EDCs) are superfluous natural or manufactured chemicals that can alter the function of the endocrine system in both humans and animals by either imitating or blocking endocrine activities leading to various adverse effects. The endocrine system regulates hormones responsible for growth, metabolism, reproduction and other vital physiological processes. EDCs can block, mimic or alter the production and breakdown of these hormones [1, 2]. EDCs consists of more than eight hundred different chemical compounds including those that are natural such as phytoestrogens present in an extensive diversity of plants (like soybean genistein and zearalenone) and artificial compounds. EDCs include plasticizers, some heavy metals like lead and mercury, and surfactants (alkylphenols). Preservatives incorporated in personal care products and pharmaceuticals (parabens), brominated flame-retardants; polychlorinated biphenyls (PCBs), organochlorine pesticides (like dichlorodiphenyl-trichloroethane (DDT) with its metabolites), fungicides, organotin compounds, dioxins, various pharmaceuticals and perfluorinated compounds are also classified as EDCs [3-7].

Some of the deleterious effects of EDCs on organisms are that, the EDC may be linked to the increase of learning infirmities, cognitive, severe attention deficit disorder, and brain growth problems. EDCs can also lead to distortions of the body parts like limbs, prostate, thyroid, breast and additional cancers. Feminization of males and/or masculinization of females are some of the sexual development problems caused by EDCs [2, 8].

*Corresponding authors. E-mail: Luke.Chimuka@wits.ac.za

Phthalates are plasticizers that are integrated into polymers so as to increase the plasticity of polymers [9]. They have been extensively utilized in several consumables that include food packaging, cosmetics, building materials, home furnishings, medical supplies, toys owing to their distinctive properties like high strength, good insulation, excellent corrosion resistance, ease of production, and low cost [10, 11]. Phthalates with high-molar weight, like di-(2-ethylhexyl) phthalate (DEHP), butylbenzyl phthalate (BBzP), and blends of di-n-octyl phthalates (DnOP), have been applicable as plasticizers in flooring, medical tools, and food packaging materials. On the contrary, lighter phthalates including diethyl phthalate (DEP), dimethyl phthalate (DMP), and dibutyl phthalate (DBP), are chiefly added to make-ups and some personal-care products as fixatives, solvents, as well as adhesives [12]. Due to lack of covalent bonds linking the phthalates to their parent materials, significant leaching of phthalates and volatilization may transpire leading to pollution of the environment and consequently universal exposures in people [12, 13].

DMP was reported [14] to modify the conformation of DNA by attaching to sperm coding DNA. DEP is the one of the most broadly used phthalate as a fixative and denaturant in personal-care products. Early (antenatal) exposure to DEP may lead to alterations in anogenital distance and male sperm factors [15]. DEP has been shown to induce gastrointestinal toxicities including toxicity of the cardiovascular organs. Some enzymes of the muscle and liver have shown substantial variations in their activities on exposure to DEP. DEP has also been reported to worsen metabolic diseases like diabetes [16].

4-Nonylphenols exist as a consequence of biodegradation of an extensively utilized set of nonionic surfactants, known as the nonylphenol ethoxylates. These surfactants are famous of being tenacious, noxious, and estrogen active [17]. Although the global use of DDT has diminished, its tenacity in the atmosphere has occasioned unrelenting human exposure. Further, DDT is still applied in many parts of the world, particularly places where the risk of contracting malaria is high [18]. DDT is famous for adversely influencing reproductive development through disruption of manifold endocrine paths. The chief metabolite of DDT is DDE (4,4'-dichlorodiphenyldichloroethylene) and is far more tenacious than the original compound and hence DDE is still present in the environment at low levels. These metabolites, DDE and DDD (4,4'-Dichlorodiphenyldichloroethane), consist of similar physical and chemical characteristics to DDT [19].

The QuECHERS (Quick, Effective, Cheap, Efficient, Rugged and Safe) extraction technique has been optimized, developed and applied for the determination of various pesticides, pharmaceuticals and veterinary drugs. QuECHERS has been applied in various food matrices including olives, teas, chamomile, meat, eggs, fruits and vegetables [20-25] since its introduction in 2003. In this study, QuECHERS technique was used for the extraction of EDCs from the food matrices. GC-MS was employed for identification and quantitation of selected EDCs. GC-MS is a powerful analytical detection and quantitation tool for various organic pollutants in different matrices due to its high separation efficiency, low detection limit, enhanced selectivity and identification abilities [26].

The majority of the Zambian population lives in cities/towns and depend on foodstuffs sold on open market whose quality and level of EDCs are not known. Therefore, the aim of this study was to assess the quantity of selected organic EDCs (phthalates, DDT (dichlorodiphenyl-trichloroethane) metabolites and 4-nonylphenol) in the selected foodstuffs (tilapia, Lake Tanganyika Sardine or simply sardine throughout the text, also commonly known as kapenta) and vegetables (tomatoes, Chinese cabbage, cabbage, rape, cassava leaves, spinach) sold in open markets and to carry out a health risk assessment of the EDCs. We also recorded the detected phthalates that were not quantitatively assessed. The study was conducted in cities/towns because that is where major open markets are concentrated with a lot of industrial activities taking place. To the best of our knowledge, no such study has been conducted anywhere in Zambia. Therefore, this study also serves a pilot study.

## EXPERIMENTAL

*Sample collection and preparation*

Fifty samples were collected and prepared as reported by Miyanza *et al.* [27]. The samples included fish (sardine, tilapia) tomato and vegetables (rape, cabbage, cassava leaves, spinach, Chinese cabbage) from open markets of Kitwe (Chamboli, Chisokone and Nakadoli markets), Kabwe (Kamanda and Mine markets) and Lusaka (Baulen, Mtendere and Soweto markets) towns.

*Reagents and chemicals*

The target organic EDCs standards used namely DEP (CAS No 84-66-2), DMP (CAS No 131-11-3), 4-nonyl phenol (CAS No 104-40-5), DDE (CAS No 72-55-9) and DDD (CAS No 72-54-8) were bought from Merck (Johannesburg, South Africa). All the standard EDCs were over 95% pure. Both stock and standard reagents were made using methanol. Acetonitrile and acetone were also obtained from Merck (Johannesburg, South Africa). Analytical grade $MgSO_4$ was bought from Merck (Johannesburg, South Africa). For cleanup, PSA was acquired from Merck (Johannesburg, South Africa). Nitrogen gas (99.999%) was used for blowing and evaporation of the solvent to the needed volume of 2 mL.

*Preparation of the stock solution*

Individual EDC solutions were prepared from standards in methanol. Solutions of 1000 mg/L of each compound were prepared in 25 mL volumetric flasks by dissolving 25 mg of each standard compound in the separate flasks that were filled with methanol to the mark. The stock solutions were stored at -20 °C. The working standard solution of the five compounds was made by taking out 100 µL of every solution made into a 10 mL volumetric flask and diluting to the mark with methanol. The final concentration of each endocrine disrupting compound in the 10 mL flask is 10 mg/L. It is from this final concentration that a 1 mg/L standards solution was made and other standards for calibration curve. These solutions were kept at 4 ℃ of the refrigerator until analysis.

*Apparatus and instruments*

The apparatus included 50 mL Teflon tubes, 50 mL centrifuge tubes polypropylene, spatula, 10 mL volumetric flasks, 25 mL volumetric flasks, 0.22 mm pore size filters polytetrafluoroethylene (PTFE) and 2 mL vials all from Merck (Johannesburg, South Africa). The instruments included the gas chromatography (GC) 7890A (Agilent Technologies, DE, USA) equipped with an electron capture detector (ECD) with a WCOT fused silica capillary column (30×0.25 mm ID, 0.25 m film thickness), a LECO Gas chromatography-mass spectrometry (GC-MS) with a capacity for a GC×GC equipped with a time of flight-MS (TOFMS) detector 7890B (LECO Corp., St Joseph, MI, USA), GVM-AS variable speed Vortex mixer (Sigma-Aldrich, Johannesburg, South Africa) and Electronic balance (220 g x 0.1 mg) (Sigma-Aldrich, Johannesburg, South Africa).

*GC-ECD conditions*

The 7890A GC (Agilent Technologies, DE, USA) that was equipped with an electron capture detector (ECD) with a WCOT fused silica capillary column (30 x 0.25 mm ID, 0.25 µm film thickness) was used to optimize the QuECHERS method. The GC-ECD conditions were selected to get the best separation. The GC and the detector response conditions were attuned to equal the response and relative retention times. Analytical conditions were: capillary column coated with ZB-5 (30 m*0.25 mm, 0.25 µm film thickness). The carrier gas used was nitrogen (99.999%) with

flow rate of 1.2 mL/min. The temperature of the oven was set to run from 60 °C (5 min) to 150 °C at a rate of 10 °C/min (1 min), was further increased to 200 °C at a rate of 30 °C/min (3 min) then lastly to 300 °C at a rate of 15 °C/min for 10 min. The injector temperature was set to split less mode (injected volume of 10 µL) and held at 300 °C and ECD temperature was 250 °C.

*GC×GC/TOFMS conditions*

A GC-MS with GC x GC capacity equipped with a TOF/MS detector 78790B (LECO Corp., St Joseph, MI, USA) was used to identify and quantify selected EDCs in samples. A 7683 series injector was used. The software used for analysis was ChromaTOF®. The following were the MS conditions: transfer line temperature 320 °C; multiplier voltage 1450 V and ion source temperature 250 °C. The injector with set temperature vaporization and working in solvent-split style was used. The injected volume was 10 µL with 50 mL/min split flow and injection time of 0.50 min with 100 mL/min injection flow. The temperature of the oven was programmed with first temperature set at 50 °C then increased to 150 °C with 10 °C/min rate followed by a ramp-up to 300 °C at a rate of 5 °C/min. The carrier gas used was helium at a flow rate of 1 mL/min. The ion trap mass detection was run in full scan mode from 50 to 500 amu (atomic mass unit).

*QuECHERS extraction method*

For extraction, QuEChERS technique was performed following the modified process stated by Rawn *et al.* [23]. Normalized samples without detected EDCs were employed for recovery studies, including the preparation of standards that matched the matrix for calibration. A 0.7 g sample was weighed into each polypropylene centrifuge tube, then spiked with 200 µL and 500 µL of 1000 µg/L of a standard mixture of EDCs. The spiked samples mixtures were left to equilibrate for 30 min. Then 6 mL of 99.8% methanol was added followed by vortexing for 1 min. The salting-out step was followed by addition of 0.5 g sodium chloride (NaCl) and 1 g of anhydrous ($MgSO_4$), which was followed by vortexing for 1 min and then centrifuged for 5 min at 4000 rpm. After centrifuge, supernatant was transferred into another polypropylene centrifuge tube to clean-up with 100 mg $MgSO_4$ and 75 mg primary/secondary amine (PSA). Vortexing of the solution for 30 s was done and then centrifugation for 5 min at 4000 rpm, the volume of extract adjusted to 2 mL using nitrogen gas flow and then filtered using a 0.22 mm PTFE into 2 mL vials and injected into the GC-ECD (electron capture detector) and/or GC x GC/TOFMS (time of flight-MS) for analysis. All the samples were prepared in triplicate.

*Preparation of calibration curves*

From the 10 mg/L standards solution prepared earlier, a standard solution of EDCs of different concentration ranging from 0.2 mg/L to 1.0 mg/L was prepared and used for determination of calibration curve. The calibration curves were linearly fitted from GC-ECD/GC-MS.

*Statistical analysis and method validation*

Descriptive data analysis was done using Microsoft Excel 2016 Software with alpha level of significance kept at 0.05. For method validation, the limit of detection (LOD), limit of quantification (LOQ), linearity and percentage (%) recoveries of spiked samples were determined. The calculation of LOD and LOQ were as below:

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

where σ is the standard deviation of triplicate measurements and S is the slope of the calibration curve.

The % recoveries were calculated as:

$$\%\ Recovery = \frac{C_O - C_i}{C_O} \times 100 \quad (3)$$

where $C_O$ is concentration of analyte in spiked sample and $C_i$ is the concentration of analyte in samples that were not spiked.

*Health risk estimation*

The non-cancer risk to consumers was estimated according to the methods recommended by Wang *et al.* [28]. The estimated daily intake (EDI) was estimated using the formula:

$$EDI = C \times \frac{IR}{Bw} \quad (4)$$

where C is concentration of particular food, $B_w$ is body weights of 70 kg for adults and IR is rate of food consumption for fish and vegetables in Zambia is 0.06 kg/person/day [27]. The hazard quotient was calculated as:

$$HQ = \frac{EDI}{RfD} \quad (5)$$

where RfD is defined as the daily maximum permissible level of contaminants; 10 mg/kg/day and 0.8 mg/kg/day for DMP and DEP, respectively [29]. The hazard index larger than 1 indicates health risk associated with the EDC detected in the food sample [31].

*The hazard index*

The hazard index (HI), being the summation of target hazard quotients of different chemicals was calculated as shown in the following equation:

$$HI = \sum \mathrm{HQ} = \mathrm{HQ\ DMP} + \mathrm{HQ\ DEP}. \quad (6)$$

where, ∑HQ is the sum of hazard quotients of pollutants. HQ DMP and HQ DEP are the hazard quotients for dimethyl phthalate and diethyl phthalate, respectively. If ∑HQ < 1, the population is not at risk. If ∑HQ ≥ 1, the population is at risk [31].

## RESULTS AND DISCUSSION

*Quality assurance*

The chromatogram of target EDCs as obtained from GC-ECD after method optimization is shown in Figure 1. The peaks and their retention times were used to identify the peaks for the target EDCs. Figures 2 and 3 show the chromatograms of the spiked samples of rape and fish, respectively. The rape chromatogram was chosen to represent vegetable samples and that of fish to represent fish samples.

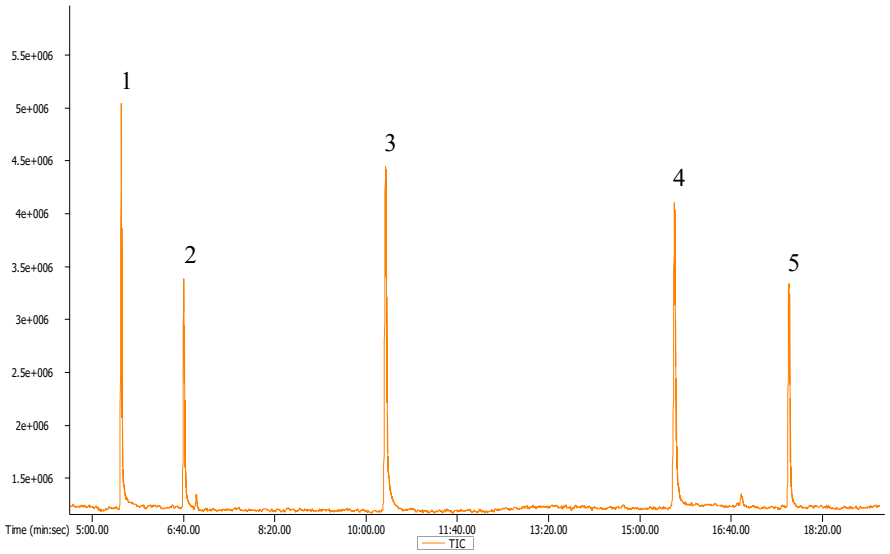



Figure 1. GC-EDC Chromatogram of EDCs standards. The target compounds are in the following order: (1) dimethylphthalate, (2) diethylphthalate, (3) 4-n-nonylphenol, (4) 4,4'-dichlorodiphenyldichloroethylene (DDE) and (5) 4,4'-dichlorodiphenyldichloroethane (DDD).

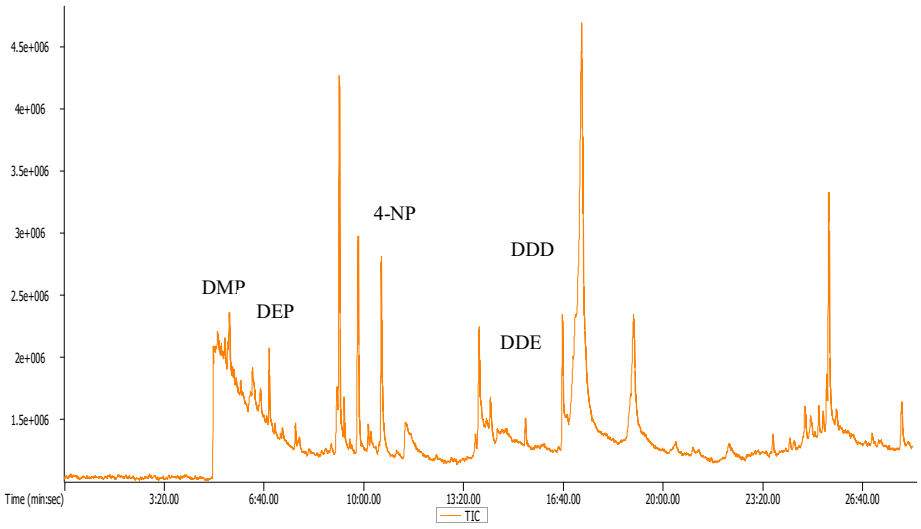



Figure 2. GC×GC/TOFMS chromatogram of spiked rape sample.

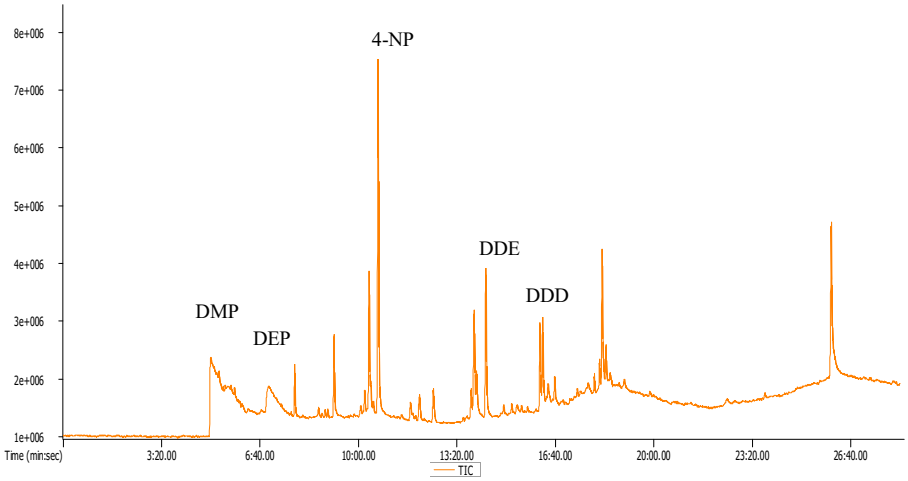



Figure 3. GC×GC/TOFMS chromatogram of spiked fish sample.

The method sensitivity is conveyed by LOD, LOQ and linearity ($R^2$) as indicated in Table 1. The LOD and LOQ of the procedure were both evaluated based on the lowest content of the residues in every sample matrix that could be measured reproducibly. The LOQ was taken to be ten times that of the LOD. The values for the precision of the method are expressed as percent relative standard deviation (RSD, n=3) and linearity ($R^2$). Analyses of blanks was done for every batch of six samples analyzed.

Table 1. Limit of detection (LOD in µg/kg), limit of quantification (LOQ in µg/kg), RSD (%), linearity ($R^2$) and retension times ($T_R$ in mm:sec.msec.

| Analyte | LOD | LOQ | RSD | Calibration equations | $R^2$ | $T_R$ |
|---|---|---|---|---|---|---|
| Dimethyl phthalate | 1.61 | 16.1 | 2.26 | Y = 111218x - 3.65622e6 | 0.9918 | 05.30.0 |
| Diethyl phthalate | 1.18 | 11.8 | 1.37 | Y = 53984.8x - 384387 | 0.9967 | 06.35.0 |
| 4-n-Nonyl phenol | 2.89 | 28.9 | 2.25 | Y = 138749x - 8.91953e6 | 0.9944 | 10.43.6 |
| 4,4'-DDE | 1.12 | 21.2 | 2.95 | Y = 70952.4x - 1.3399e6 | 0.9939 | 15.23.7 |
| 4,4'-DDD | 1.77 | 17.7 | 5.63 | Y = 2465.27x - 239113 | 0.9372 | 16.38.3 |

Samples that were fortified with 200 µL and 500 µL of 1000 µg/L concentration of a mixed standard solution containing the EDCs are shown in Table 2. The standard deviation and percent recoveries were calculated in triplicate. For the analysis of EDCs, accuracy and recovery of 70-120% is considered acceptable [31, 32]. The procedure can be applied for assessment of the selected EDCs in food samples under study. The recovery percentages in spiked samples ranged from 74.23 to 86.02% for tomatoes; 76.47 to 91.09% for fish; 72.06 to 74.26% for cassava leaves; 81.36 to 86.16% for spinach; 84.20 to 91.27% for cabbage; 79.63 to 82.20% for rape, and 73.75 to 84.38% for Chinese cabbage. Recoveries for 4-n-nonylphenol, DDE and DDD were not included because they were not detected in original samples.

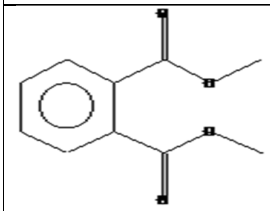
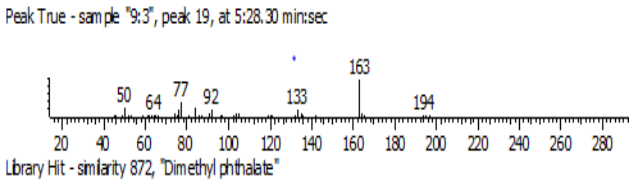
Table 2. Analytical recoveries (%) ± SD of endocrine disruptors (EDCs) in food samples at 200 µL and 500 µL fortification with 1000 µg/L standard mixture of the EDCs.

| Sample | Dimethyl phthalate | | Diethyl phthalate | |
|---|---|---|---|---|
| | 200 µL | 500 µL | 200 µL | 500 µL |
| Tomatoes | 75.23 ± 0.26 | 86.02 ± 0.19 | 74.23 ± 0.20 | 76.02 ± 0.29 |
| Fish | 76.47 ± 0.18 | 76.89 ± 0.21 | 86.27 ± 0.18 | 91.09 ± 0.11 |
| Cassava leaves | 72.06 ± 0.06 | 74.26 ± 0.43 | 72.86 ± 0.26 | 73.46 ± 0.13 |
| Spinach | 84.29 ± 0.22 | 81.36 ± 0.17 | 85.37 ± 0.29 | 86.16 ± 0.14 |
| Cabbage | 86.28 ± 0.46 | 91.27 ± 0.33 | 84.20 ± 0.61 | 91.27 ± 0.13 |
| Rape | 79.87 ± 0.12 | 82.20 ± 0.08 | 79.63 ± 0.12 | 80.20 ± 0.28 |
| Chinese cabbage | 73.75 ± 0.28 | 75.09 ± 0.31 | 75.09 ± 0.09 | 84.38 ± 0.27 |

*Identification of phthalates, 4-nonylphenols and DDT metabolites*

Identification of phthalates was achieved by GC-MS. The fragmentation patterns of identified phthalates are shown in Figure 4. The distribution of the identified phthalates in food samples is shown in Table 3. DMP was only detected in tomatoes and fish for Kitwe town, and only in rape and fish for Kabwe town. For Lusaka town, DMP was detected in tomatoes, fish and cassava leaves. DEP was not detected in cabbage, cassava leaves and Chinese cabbage for Kitwe town. For Kabwe town, DEP was detected in all the samples except fish. Only sardine, rape and cassava leaves did not have DEP detected in them among samples from Lusaka town. DBP was only detected in two samples from Kitwe town, cassava leaves and Chinese cabbage. Three samples from Kabwe, fish, sardine and cabbage, did not test positive for DBP while only three samples from Lusaka, fish, spinach and cabbage had DBP. DAP was identified in only four samples from Kitwe; tomatoes, cabbage, cassava leaves and Chinese cabbage. Only one sample, cassava leaves, from Kabwe had DAP in it as well as one sample, Chinese cabbage, from Lusaka. Spinach, rape, cabbage and cassava leaves were the only samples from Kitwe in which DEHP was identified.

For samples from Kabwe, DEHP was not detected in tomatoes, fish and sardine. DEHP was identified in only two samples, sardine and spinach, from Lusaka. DIOP was only identified in three samples, spinach, cassava leaves and Chinese cabbage, among samples from Kitwe; no sample from Kabwe was positive for DIOP, and only in one sample, cabbage, was DIOP detected among the samples from Lusaka. HTDP was identified in fish, rape and cassava leaves for Kitwe samples. Among samples from Kabwe, HTDP was only identified in sardine, rape and cabbage. For samples from Lusaka, only cabbage gave a positive result for HTDP. DEHP was the most frequently detected phthalate followed by DBP, which was followed by HTDP, then DAP and DIOP was least abundant. 4-Nonylphenols and DDT metabolites were not detected in all the samples by mass-spectral library screening.

| Name/structure | Fragmentation pattern |
|---|---|
|  Dimethyl phthalate |   |

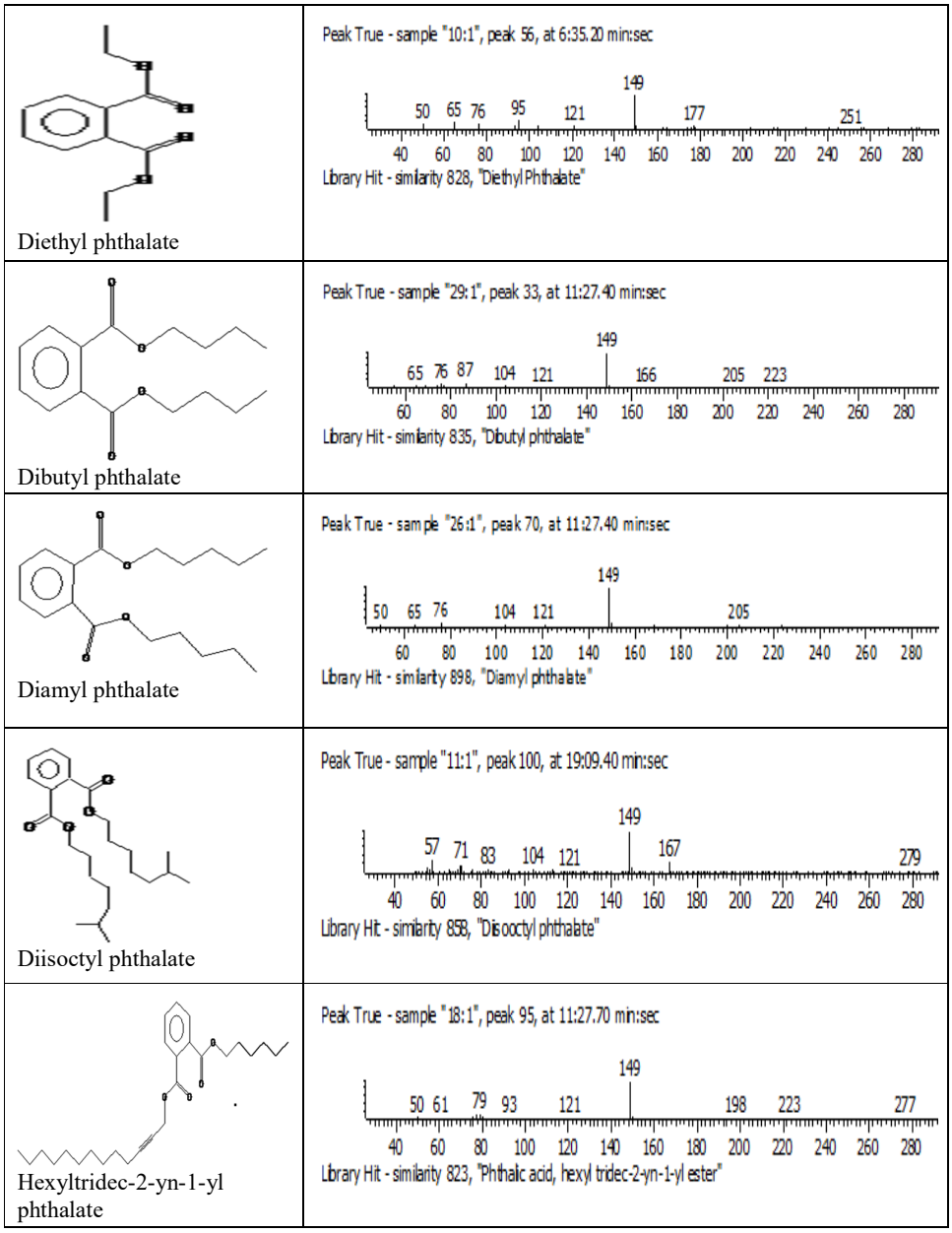



Figure 4. Structures and fragmentation patterns of the identified phthalates

Table 3. Phthalates identified by GC/MS in the samples under study.

| Town | Sample | DMP | DEP | DBP | DAP | DEHP | DIOP | HTDP | NP | DDE | DDD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kitwe | Tomatoes | √ | √ | Nd | √ | Nd | Nd | Nd | Nd | Nd | Nd |
| | Fish | √ | √ | Nd | Nd | Nd | Nd | √ | Nd | Nd | Nd |
| | Sardine | Nd | √ | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Spinach | Nd | √ | Nd | Nd | √ | √ | Nd | Nd | Nd | Nd |
| | Rape | Nd | √ | Nd | Nd | √ | Nd | √ | Nd | Nd | Nd |
| | Cabbage | Nd | Nd | Nd | √ | √ | Nd | Nd | Nd | Nd | Nd |
| | Cassava leaves | Nd | Nd | √ | √ | √ | √ | √ | Nd | Nd | Nd |
| | Chinese cabbage | Nd | Nd | √ | √ | Nd | √ | Nd | Nd | Nd | Nd |
| Kabwe | Tomatoes | √ | √ | √ | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Fish | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Sardine | √ | √ | Nd | Nd | Nd | Nd | √ | Nd | Nd | Nd |
| | Spinach | √ | √ | √ | Nd | √ | Nd | Nd | Nd | Nd | Nd |
| | Rape | Nd | √ | √ | Nd | √ | Nd | √ | Nd | Nd | Nd |
| | Cabbage | √ | √ | Nd | Nd | √ | Nd | √ | Nd | Nd | Nd |
| | Cassava leaves | √ | √ | √ | √ | √ | Nd | Nd | Nd | Nd | Nd |
| | Chinese cabbage | √ | √ | √ | Nd | √ | Nd | Nd | Nd | Nd | Nd |
| Lusaka | Tomatoes | √ | √ | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Fish | √ | √ | √ | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Sardine | Nd | Nd | Nd | Nd | √ | Nd | Nd | Nd | Nd | Nd |
| | Spinach | Nd | √ | √ | Nd | √ | Nd | Nd | Nd | Nd | Nd |
| | Rape | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Cabbage | Nd | √ | √ | Nd | Nd | √ | √ | Nd | Nd | Nd |
| | Cassava leaves | √ | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| | Chinese cabbage | Nd | √ | Nd | √ | Nd | Nd | Nd | Nd | Nd | Nd |

A mark shows presence of specific phthalate; Nd = not detected, DMP = dimethyl phthalate, DEP = diethyl phthalate, DBP = dibutyl phthalate; DAP = diamyl phthalate; DEHP = di-(2-ethylhexyl) phthalate; DIOP = diisooctyl phthalate, HTDP = hexyl tridec-2-yn-1-yl phthalate, NP = nonylphenol, DDE = dichlorodiphenyldichloroethylene, DDD = dichlorodiphenyldichloroethane.

*Quantification of selected EDCs in food samples*

The results of the concentration of selected EDCs in the food samples from three towns are shown in Table 4. However, 4-n-nonyl phenol, 4,4'-DDE and 4,4'-DDD were not detected (Table 3) in all the samples. Therefore, these three EDCs were omitted on the results table and in the subsequent discussion.

Table 4. Mean concentrations of EDCs (µg/kg) in foods per dry weight.

| City/Town | Sample | DMP | DEP |
|---|---|---|---|
| Kitwe | Tomatoes | 91.05 ± 1.45 | 63.27 ± 8.92 |
| | Fish | 101.76 ± 1.99 | 49.91 ± 0.51 |
| | Sardine | Nd | 21.42 ± 17.98 |
| | Spinach | Nd | 22.36 ± 4.36 |
| | Rape | Nd | 80.69 ± 3.85 |
| | Cabbage | Nd | Nd |
| | Cassava leaves | Nd | Nd |
| | Chinese cabbage | Nd | Nd |
| Kabwe | Tomatoes | 77.14 ± 0.03 | 82.93 ± 5.54 |
| | Fish | Nd | Nd |
| | Sardine | 92.85 ± 0.24 | 135.77 ± 9.60 |
| | Spinach | 105.34 ± 0.36 | 161.67 ± 0.26 |

| | | | |
|---|---|---|---|
| | Rape | Nd | 143.14 ± 0.77 |
| | Cabbage | 123.82 ± 0.38 | 131.26 ± 2.90 |
| | Cassava leaves | 90.16 ± 1.10 | 91.17 ± 0.21 |
| | Chinese cabbage | 92.38 ± 1.42 | 63.93 ± 0.33 |
| Lusaka | Tomatoes | 85.65 ± 2.08 | 25.08 ± 3.07 |
| | Fish | 98.55 ± 0.40 | 23.99 ± 9.98 |
| | Sardine | Nd | Nd |
| | Spinach | Nd | 23.22 ± 17.22 |
| | Rape | Nd | Nd |
| | Cabbage | Nd | 25.66 ± 0.19 |
| | Cassava leaves | 95.65 ± 7.34 | Nd |
| | Chinese cabbage | Nd | 46.01 ± 2.71 |
| | | | |

Nd = not detected, DMP = dimethyl phthalate, and DEP = diethyl phthalate.

The mean concentrations of the quantified EDCs are summarized in Table 4. For Kitwe open markets, DMP was only detected in tomato and fish with concentrations of 91.05 μg/kg and 101.76 μg/kg respectively. The mean concentrations of DEP ranged from 21.46 μg/kg in sardine to 80.69 μg/kg in rape. The sequence of DEP mean concentrations was as follows: sardine < spinach < fish < tomatoes < rape. The least concentration recorded for DEP was recorded in sardine as 21.46 μg/kg.

For Kabwe open market, the mean concentrations of DMP ranged from 77.14 μg/kg in tomatoes to 123.82 μg/kg in cabbage. Only two sample types, fish and rape, had no DMP detected. This was contrary to the results recorded for Kitwe town open market where only two samples had DMP detected in them. The sequence for DMP concentrations is as follows: tomatoes < cassava leaves < Chinese cabbage < sardine < spinach < cabbage. The lowest concentration of DMP was found in tomato samples just like was the case with samples from Kitwe open market. However, this concentration of DMP was the lowest in samples from all cities. The highest concentration of DMP was also recorded for Kabwe town open market when compared to other two towns. There was no DEP detected in the fish samples. However, DEP was detected in the other samples with mean concentrations between 63.93 μg/kg in Chinese cabbage to 161.67 μg/kg in spinach. The mean concentration sequence was given as Chinese cabbage < tomatoes < cassava leaves < cabbage < sardine < rape < spinach. The concentrations here recorded were higher than those recorded for both Kitwe town open market and Lusaka town open market.

As Table 4 shows, DMP was only detected in three samples from Lusaka open markets; tomatoes with mean concentration of 85.65 μg/kg, cassava leaves with mean concentration of 95.65 μg/kg and fish with mean concentration of 98.55 μg/kg. It was interesting to note that the lowest concentration of DMP was recorded in tomato samples, which was consistent with the results from the other two open markets of Kitwe and Kabwe towns. The lowest concentration as well as the highest concentration recorded here were lower than those recorded for samples from Kitwe open market were. The mean concentrations of DEP ranged from 23.22 μg/kg in spinach to 46.01 μg/kg in Chinese cabbage. The concentration sequence was as follows: spinach < fish < tomatoes < cabbage < Chinese cabbage. These results are much comparable to those recorded for Kitwe town open market except for the highest concentrations. The highest concentration of 46.01 μg/kg was much lower than the concentration of 80.69 μg/kg recorded under Kitwe open market.

The most probable sources of these phthalates are; irrigation with wastewater and use of contaminated soils for vegetables, industrial discharges into the aquatic environment and food contact with phthalates in the air since foodstuffs are sold on the open in open markets. Sewage aquaculture is one potential source of phthalates with wastewaters being used for culturing fishes [33]. Application of pesticides may have contributed to the presence of phthalates, especially DEP, in vegetables. Phthalate leachate from garden hoses is another source of contamination [34].

For statistical analysis of data, single factor ANOVA was used with post-hoc analysis in Microsoft Excel 2016 version. For post-hoc analysis, we used the Bonferroni correction. For DMP mean concentrations, there was not a substantial variance for three towns, $p > 0.05$. However, one-way ANOVA for the mean concentrations of DEP showed a significant difference, $p < 0.05$. The post-hoc analysis indicated insignificant difference in the levels of DEP for samples from Kitwe town open market and Lusaka town open market, $p > 0.0167$. The variances in the levels of DEP were significant in samples from Kitwe open market and Kabwe open market, and samples from Kabwe open market and Lusaka open market, $p < 0.0167$.

*A comparison of our results with other similar studies*

Findings from similar studies are presented in Table 5. Our study reports no detection of DDT metabolites. However, 4,4'-DDE and 4,4'-DDD were detected and quantified by other studies in cabbage from Pretoria, South Africa; Kinshasa, Democratic Republic of Congo [31] and Cape Town, South Africa [35]. The two metabolites were also identified in fish from Pretoria, South Africa; Kinshasa, Democratic Republic of Congo, Eastern Lake Tanganyika, Tanzania and River Po, Italy [31, 36, 37] and spinach from Cape Town, South Africa [35]. Just like in my study, 4-n-Nonylphenol was not detected in the study conducted by Lu *et al.* [38] from Florida, United States of America. In studies conducted by She et al. [39] from local Supermarkets, China; [37] from River Po, Italy and [40] from Pearl River Delta, South China, 4-n-nonylphenol was detected and quantified in cabbage, fish, Chinese cabbage and spinach. A concentration of 4-n-nonylphenol of less than 3.3 µg/kg in fish from Kalamazoo River, Michigan, United States of America was reported [41].

Levels of 210 µg/kg in tomatoes from 10 cities that include Shenyang, Beijing, Shougaung, Xianyang, Siyang, Haimen, Nanjing, Changshu, Fuzhou and Kunming in China were reported by Chen *et al.* [42] and 140 µg/kg in cabbage from North China Plain was reported [43]. There results were higher than what we report in this study because the highest amount we recorded was 123.82 µg/kg. A mean concentration of 100 µg/kg was reported in fish from Virginia Beach, Pakistan [44] which is comparable to the findings in this study. Other studies reported lower concentrations of DMP in fish from Hong Kong Market, China [45], Asan Lake of Korea [46] and Chinese cabbage from Nanjing City, China [28].

Table 5. Mean concentrations of dimethyl phthalate (DMP), diethyl phthalate (DEP), 4-n-nonylphenol (4-n-NP), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD) in foods as reported by other studies (µg/kg).

| Country/sample | Reference | DMP | DEP | 4-n-NP | 4,4'-DDE | 4,4'-DDD |
|---|---|---|---|---|---|---|
| Tomatoes | | | | | | |
| China | [42] | 210.00 | 315.00 | - | - | - |
| Florida, USA | [38] | - | - | Nd | - | - |
| Zambia | This study (Kabwe) | 77.14 | 82.93 | Nd | Nd | Nd |
| Zambia | This study (Kitwe) | 91.05 | 63.27 | Nd | Nd | Nd |
| Zambia | This study (Lusaka) | 85.65 | 25.08 | Nd | Nd | Nd |
| Cabbage | | | | | | |
| China | [43] | 140.00 | 60.00 | - | - | - |
| China | [47] | 3.36 | 1.18 | - | - | - |
| China | [39] | - | - | 21.59 | - | - |
| South Africa | [31] | - | - | - | 106.65 | 95.67 |
| South Africa | [35] | - | - | - | 11.60 | 12.50 |
| D.R. Congo | [31] | - | - | - | 81.05 | 61.05 |
| Zambia | This study (Kabwe) | 123.82 | 131.26 | Nd | Nd | Nd |
| Zambia | This study (Kitwe) | Nd | Nd | Nd | Nd | Nd |
| Zambia | This study (Lusaka) | Nd | 25.66 | Nd | Nd | Nd |
| Fish | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| South Africa | [31] | - | - | - | 125.78 | 105.74 |
| D.R. Congo | [31] | - | - | - | 90.09 | 73.52 |
| China | [45] | 1 | Nd | - | - | - |
| Pakistan | [44] | 100.00 | 123.00 | - | - | - |
| Korea | [46] | 3.30 | 4.90 | - | - | - |
| Tanzania | [36] | - | - | - | 100.10 | 35.42 |
| Michigan, USA | [41] | - | - | <3.30 | - | - |
| Italy | [37] | - | - | 3.60-26.80 | 17.30-2902.00 | 3.60-98.20 |
| Zambia | This study (Kabwe) | 77.14 | 82.93 | Nd | Nd | Nd |
| Zambia | This study (Kitwe) | 101.76 | 49.91 | Nd | Nd | Nd |
| Zambia | This study (Lusaka) | 98.55 | 23.99 | Nd | Nd | Nd |
| Chinese cabbage | | | | | | |
| China | [28] | 14.00 | 52.00 | - | - | - |
| China | [40] | - | - | 5.30 | - | - |
| Zambia | This study (Kabwe) | 92.38 | 63.93 | Nd | Nd | Nd |
| Zambia | This study (Kitwe) | Nd | Nd | Nd | Nd | Nd |
| Zambia | This study (Lusaka) | Nd | 46.01 | Nd | Nd | Nd |
| Spinach | | | | | | |
| South Africa | [35] | - | - | - | 10.50 | 10.10 |
| China | [40] | - | - | 6.41 | - | - |
| Zambia | This study (Kabwe) | 105.34 | 161.67 | Nd | Nd | Nd |
| Zambia | This study (Kitwe) | Nd | 22.36 | Nd | Nd | Nd |
| Zambia | This study (Lusaka) | Nd | 23.22 | Nd | Nd | Nd |

Nd = not deteced. Hyphen means not investigated. Results were just reported as mean concentrations, without ± SD, in order to suit all results as most of the authors just reported mean concentrations.

The mean concentrations of DEP reported by Chen *et al.* [41] in tomatoes from the 10 cities in China as aforementioned and [44] in fish from Virginia Beach, Pakistan were higher than what we report in this study. The concentration of 60 μg/kg in cabbage from the North China Plain as reported by Yan *et al.* [43] is lower than what we have reported for cabbage samples from Kabwe Town but higher than in samples from Lusaka City. A concentration of 52 μg/kg of DEP in Chinese cabbage from Nanjing City, China, was recorded [28] that is lower than our result for Chinese cabbage samples from Kabwe Town but higher than in samples from Lusaka City. Other similar studies from Eastern, China [47] and Asan Lake of Korea [46] reported concentrations of DEP, which are lower than our findings. Fish samples from Hong Kong Market, China were reported to have no DEP detection in them [45]. Studies that report the EDCs of interest for this study in rape, cassava leaves and sardine are limited.

*Health risk assessment*

The EDI of all the foodstuffs did not exceed the tolerable intakes as proposed by Wang *et al.* [28]. The average daily intake for DMP were calculated to be 0.0825 μg/kg/day, 0.0840 μg/kg/day and 0.0797 μg/kg/day for Kitwe, Kabwe and Lusaka, respectively. The average daily intake for DEP were recorded as 0.0406 μg/kg/day, 0.0913 μg/kg/day and 0.0244 μg/kg/day for Kitwe, Kabwe and Lusaka, respectively. All the reported risk parameters were calculated for adults only. The hazard quotients and the hazard indices of all the foods in all the three towns were below the threshold of 1 (Table 6). The hazard indexes recorded for DMP were 0.0165, 0.0239 and 0.0502 for Kitwe, Lusaka and Kabwe, respectively. For DEP, the hazard indexes were 0.1772, 0.2710 and 0.9110 for Lusaka, Kitwe and Kabwe. If HI < 1, the population is not at risk. If HI ≥ 1, the population is at risk [28]. The HI value (0.9110) for DEP in samples from Kabwe was close to the threshold of 1. However, these results indicate that, for DMP and DEP contamination, there is no non-carcinogenic risk associated with consumption of the foods under study by the local

consumers. The total phthalate risk can best be ascertained if all the phthalates are analyzed, which phthalates were identified in the foodstuffs (Table 3). The HI values calculated in this study are comparable to other studies. The HI value of 0.17 for adult individuals was calculated for phthalates in vegetables from China [42]. Another study reported the HI value of 142.2 for Hong Kong residents, a value way higher than the recommended threshold of 1 [45].

Table 6. Mean concentrations of EDCs (μg/kg), estimated daily intake (EDI) (μg/kg/day), hazard quotient (HQ) and hazard index (HI) for non-carcigenic risk for adults.

| | | Dimethyl phthalate | | | Diethyl phthalate | | | HI |
|---|---|---|---|---|---|---|---|---|
| Town | Sample | Conc. | EDI | HQ | Conc. | EDI | HQ | ΣHQ |
| Kitwe | Tomatoes | 91.0500 | 0.0780 | 0.0078 | 63.2700 | 0.0540 | 0.0680 | 0.0760 |
| | Fish | 101.7600 | 0.0870 | 0.0087 | 49.9100 | 0.0430 | 0.0530 | 0.0620 |
| | Sardine | Nd | - | - | 21.4200 | 0.0180 | 0.0230 | 0.0230 |
| | Spinach | Nd | - | - | 22.3600 | 0.0190 | 0.0240 | 0.0240 |
| | Rape | Nd | - | - | 80.6900 | 0.0690 | 0.0860 | 0.0860 |
| | Cabbage | Nd | - | - | Nd | - | - | - |
| | Cassava leaves | Nd | - | - | Nd | - | - | - |
| | Chinese cabbage | Nd | - | - | Nd | - | - | - |
| | Sum of hazards for all foods | | 0.0825 | 0.0165 | | 0.0406 | 0.2540 | 0.2710 |
| Kabwe | Tomatoes | 77.1400 | 0.0660 | 0.0066 | 82.9300 | 0.0710 | 0.0890 | 0.0950 |
| | Fish | Nd | - | - | Nd | - | - | - |
| | Sardine | 92.8500 | 0.0790 | 0.0079 | 135.7700 | 0.1200 | 0.1500 | 0.1500 |
| | Spinach | 105.3400 | 0.0910 | 0.0091 | 161.6700 | 0.1400 | 0.1700 | 0.1800 |
| | Rape | Nd | - | - | 143.1400 | 0.1200 | 0.1500 | 0.1500 |
| | Cabbage | 123.8200 | 0.1100 | 0.0110 | 131.2600 | 0.1100 | 0.1400 | 0.1500 |
| | Cassava leaves | 90.1600 | 0.0770 | 0.0077 | 91.1700 | 0.0780 | 0.0980 | 0.1100 |
| | Chinese cabbage | 92.3800 | 0.0790 | 0.0079 | 63.9300 | 0.0550 | 0.0680 | 0.0760 |
| | Sum of hazards for all foods | | 0.0840 | 0.0502 | | 0.0913 | 0.8650 | 0.9110 |
| Lusaka | Tomatoes | 85.6500 | 0.0730 | 0.0073 | 25.0800 | 0.0210 | 0.0270 | 0.0340 |
| | Fish | 98.5500 | 0.0840 | 0.0084 | 23.9900 | 0.0210 | 0.0260 | 0.0340 |
| | Sardine | Nd | - | - | Nd | - | - | - |
| | Spinach | Nd | - | - | 23.2200 | 0.0190 | 0.0250 | 0.0250 |
| | Rape | Nd | - | - | Nd | - | - | - |
| | Cabbage | Nd | - | - | 25.6600 | 0.0220 | 0.0270 | 0.0270 |
| | Cassava leaves | 95.6500 | 0.0820 | 0.0082 | Nd | - | - | 0.0082 |
| | Chinese cabbage | Nd | - | - | 46.0100 | 0.0390 | 0.0490 | 0.0490 |
| | Sum of hazards for all foods | | 0.0797 | 0.0239 | | 0.0244 | 0.1540 | 0.1772 |

*Limitations of the study and future outlook*

Information for the exposure of humans to EDCs through ingestion of food and environmental matrices in Zambia is uncommon. In the current study, only few representative foodstuffs (vegetables and fish) were used for approximation of exposure to humans. In addition, only two phthalates were considered for exposure assessment. However, straight exposure from consummables (e.g. drugs and cosmetics, toys and cleaning materials) were unaccounted for possibly leading to underestimation of total daily intake of EDCs. Moreover, the present study only focused on three towns of Zambia, and the approximations might be differing to a large degree per dissimilar geographical location. Nonetheless, this study presents a case, stressing a scenario of EDCs exposure in the three towns, Kabwe, Kitwe and Lusaka, of Zambia. Therefore, high-quality evaluations is needed for approximating present state of human exposure to a number of EDCs. Furthermore, a holistic risk assessment that includes age and gender differences need to be measured.

## CONCLUSION

The EDCs 4-n-nonylphenol and DDT metabolites were not detected in all the samples in this study. Though DDT is still being used in some areas, these results could indicate its non-use or usage that does not affect the food environment in the study areas. The general population in the study areas is free from the health effects of these EDCs. On the other hand, phthalates were identified in most of the foodstuffs. DMP was identified and quantified in two samples from Kitwe open market, three samples from Lusaka open market, and was not identified in only one sample from Kabwe open market. However, substantial difference in the levels of DMP among the three towns was not found. DEP was not identified in three samples for Kitwe and for Lusaka open markets while it was not identified in only one sample from Kabwe open market. The mean concentrations of DEP showed no significant difference for samples from Kitwe and Lusaka open markets. However, a significant difference in the mean concentrations does exist between samples from Kitwe and Kabwe open markets, and samples from Kabwe and Lusaka open markets with samples from Kabwe town having higher concentrations in both cases. Though the health risk parameters for DMP and DEP are within the safety margins, consumers' safety can only be guaranteed after a comprehensive risk analysis of other EDCs.

## ACKNOWLEDGEMENTS

This work was funded by the National Science and Technology Council of Zambia under the trilateral collaborative agreement grant no. NSTC/101/6/8, and National Research foundation of South Africa under the trilateral collaborative agreement grant no. 118473.

## REFERENCES

1. Diamanti-Kandarakis, E.; Bourguignon, J.P.; Giudice, L.C.; Hauser, R.; Prins, G.S.; Soto, A.M.; Zoeller, R.T.; Gore, A.C. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr. Rev.* **2009**, 30, 293-342.
2. Sanderson, J.T. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. *Toxicol. Sci*. **2006**, 94, 3-21.
3. Basheeru, K.A.; Okoro, H.K.; Adekola, F.A.; Abdus Salam, N. Speciation and quantification of organotin compounds in Lagos harbour, Nigeria. *Int. J. Environ. Anal. Chem*. **2022**, 102, 8250-8269.
4. Okoro, H.K.; Fatoki, O.S.; Adekola, F.A.; Ximba, B.J.; Snyman, R.G. Spatio-temporal variation of organotin compounds in seawater and sediments from Cape Town Harbour, South Africa using Gas Chromatography with Flame Photometric Detector (GC-FPD). *Arab. J. Chem*. **2016**, 9, 95-104.
5. Okoro, H.K.; Ige, J.O.; Iyiola, O.A.; Pandey, S.; Lawal, I.A.; Zvinowanda, C.; Ngila, J.C. Comprehensive reviews on adverse health effects of human exposure to endocrine-disrupting chemicals. *Fresenius Environ. Bull*. **2017**, 26, 4623-4636.
6. Okoro, H.K.; Pandey, S.; Ogunkunle, C.O.; Ngila, C.J.; Zvinowanda, C.; Jimoh, I.; Lawal, I.A.; Orosun, M.M.; Adeniyi, A.G. Nanomaterial-based biosorbents: Adsorbent for efficient removal of selected organic pollutants from industrial wastewater. *Emerg. Contam.* **2022**, 8, 46-58.
7. Nigatu, G.; Hussen, A. Obsolete pesticide residue level analysis and toxicological risk evaluation from Hadya zone dump site, Ethiopia. *Bull. Chem. Soc. Ethiop*. **2022**, 36, 329-337.
8. Sharma, R.P.; Schuhmacher, M.; Kumar, V. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. *Environ. Int.* **2017**, 99, 1-14.

9. Jeddi, M.Z.; Janani, L.; Memari, A.H.; Akhondzadeh, S.; Yunesian, M. The role of phthalate esters in autism development: A systematic review. *Environ. Res.* **2016**, 151, 493-504.
10. He, Y.; Wang, Q.; He, W.; Xu, F. The occurrence, composition and partitioning of phthalate esters (PAEs) in the water-suspended particulate matter (SPM) system of Lake Chaohu, China. *Sci. Total Environ.* **2019**, 661, 285-293.
11. Chi, Z.; Zhao, J.; Li, W.; Araghi, A.; Tan, S. In vitro assessment of phthalate acid esters-trypsin complex formation. *Chemosphere* **2017**, 185, 29-35.
12. Serrano, S.E.; Braun, J.; Trasande, L.; Dill, R.; Sathyanarayana, S. Phthalates and diet: A review of the food monitoring and epidemiology data. *Environ. Health* **2014**, 13, 1-14.
13. Cao, X.L. Phthalate esters in foods: Sources, occurrence, and analytical methods. *Compr. Rev. Food Scie. Food Saf.* **2010**, 9, 21-43.
14. Chi, Z.; Wang, D.; You, H. Study on the mechanism of action between dimethyl phthalate and herring sperm DNA at molecular level. *J. Environ. Sci. Health B* **2016**, 51, 553-557.
15. Swan, T.S.H.; Main, K.M.; Liu, F.; Stewart, S.L.; Kruse, R.L.; Calafat, A.M.; Mao, C.S.; Redmon, J.B.; Ternand, C.L.; Sullivan, S.; Teague, J.L. Study for future families Research. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ. Health Persp.* **2005**, 113, 1056-1061.
16. Ghorpade, N.; Mehta, V.; Khare, M.; Sinkar, P.; Krishnan, S.; Rao, C.V. Toxicity study of diethyl phthalate on freshwater fish Cirrhina mrigala. *Ecotoxicol. Environ. Saf.* **2002**, 53, 255-258.
17. Okoro, H.K.; Basheer, K.A.; Victor, O.A.; Orimolade, B.O.; Ngila, J.C. Development of a method for estimating the concentration of endocrine phenolic compounds in surface water using HPLC technique. *J. Kenya Chem. Soc*. **2017**, 10, 76-83.
18. van den Berg, H.; Manuweera, G.; Konradsen, F. Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. *Malar. J.* **2017**, 16, 1-8.
19. Guan, Y.; Wang, J.; Ni, H.; Zeng, E.Y. Organochlorine pesticides and polychlorinated biphenyls in riverine runoff of the Pearl River Delta, China: Assessment of mass loading input source and environmental fate. *Environ. Pollut.* **2009**, 157, 618-624.
20. Cunha, S.C.; Lehotay, S.J.; Mastovska, K.; Fernandes, J.O.; Beatriz, M.; Oliveira, P.P. Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives. *J. Sep. Sci*. **2007**, 30, 620-632.
21. Huertas-Pérez, J.F.; Arroyo-Manzanares, N.; Havlíková, L.; Gámiz-Gracia, L.; Solich, P.; García-Campana, A.M. Method optimization and validation for the determination of eight sulfonamides in chicken muscle and eggs by modified QuEChERS and liquid chromatography with fluorescence detection. *J. Pharm. Biomed. Anal*. **2016**, 124, 261-266.
22. Lozano, A.; Rajski, Ł.; Belmonte-Valles, N.; Ucles, A.; Ucles, S.; Mezcua, M.; Fernandez-Alba, A.R. Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *J. Chromatogr. A*. **2012**, 1268, 109-122.
23. Rawn, D.F.K.; Judge, J.; Roscoe, V. Application of the QuEChERS method for the analysis of pyrethrins and pyrethroids in fish tissues. *Anal. Bioanal. Chem*. **2010**, 397, 2525-2531.
24. Sinha, S.N.; Vasudev, K.M.; Rao, V.V. Quantification of organophosphate insecticides and herbicides in vegetable samples using the ''Quick Easy Cheap Effective Rugged and Safe'' (QuEChERS) method and a high-performance liquid chromatography-electrospray ionisation–mass spectrometry (LC-MS/MS) technique. *Food Chem.* **2012**, 132, 1574-1584.
25. Wilkowska, A.; Biziuk, M. Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chem*. **2011**, 125, 803-812.
26. Tolcha, T.; Gomoro, K.; Megersa, N. SALLE combined with LD-DLLME for pesticides analysis in sugar and soil samples. *Bull. Chem. Soc. Ethiop*. **2021**, 35, 1-16.
27. Miyanza, F.; Ramalepe, T.; Monyai, M.; Chaúque, E.; Nyambe, I.; Chimuka, L. Determination and risk assessment of heavy metals in raw foodstuffs sold from open markets

in Zambia; A comparison of Kabwe, Kitwe, and Lusaka towns. *Int. J. Environ. Health Res.* **2024**, 34, 1566-1579.
28. Wang, J.; Chen, G.C.; Christie, P.; Zhang, M.Y.; Luo Y.M.; Teng, Y. Occurrence and risk assessment of phthalate esters (PAEs) in vegetables and soils of suburban plastic film greenhouses. *Sci. Total Environ*. **2015**, 523, 129-137.
29. Ai, S.; Wang, X.; Gao, X.; Xu, Q.; Li, J.; Liu, Z. Distribution, health risk assessment, and water quality criteria of phthalate esters in Poyang Lake, China. *Environ. Sci. Europe* **2023**, 35, 1-14.
30. Kortei, N.K.; Heymann, M.E.; Essuman, E.K.; Kpodo, F.M; Akonorb, P.T.; Lokpor, S.Y.; Boadi, N.O.; Ayim-Akonor, M.; Tetley, C. Health risk assessment and levels of toxic metals in fishes (*Oreochromis noliticus* and *Clarias anguillaris*) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety. *Toxicol. Rep.* **2020**, 7, 360-369.
31. Nuapia, Y.; Chimuka, L.; Cukrowska, E. Assessment of organochlorine pesticide residues in raw food samples from open markets in two African cities. *Chemosphere* **2016**, 164, 480-487.
32. Gonzalvez, W.; Armenta, S.; Cervera, M.L.; De La Guardia, M. Elemental composition of seasoning products. *Talanta* **2008**, 74, 1085-1095.
33. Barse, A.V.; Chakrabarti, T.; Ghosh, T.K.; Pal, A.K.; Jadhao, S.B. Endocrine disruption and metabolic changes following exposure of Cyprinus carpio to diethyl phthalate. *Pest. Biochem. Physiol.* **2007**, 88, 36-42.
34. Wang, Y.; Zhu, H.; Kannan, K. A review of biomonitoring of phthalate exposures. *Toxics* **2019**, 7, 21-30.
35. Olatunji, O.S. Evaluation of selected polychlorinated biphenyls (PCBs) congeners and dichlorodiphenyltrichloroethane (DDT) in fresh root and leafy vegetables using GC-MS. *Scie. Rep.* **2019**, 9, 538-549.
36. Mahugija, J.A.M.; Nambela, L.; Mmochi,A.J. Determination of Dichlorodiphenyltrichloroethane (DDT) and metabolites residues in fish species from eastern lake tanganyika. *S. Afr. J. Chem*. **2018**, 71, 86-93.
37. Viganò, L.; Mascolo, G.; Roscioli, C. Emerging and priority contaminants with endocrine active potentials in sediments and fish from the River Po (Italy). *Environ. Sci. Pollut. Res*. **2015**, 22, 14050-14066.
38. Lu, J.; Wu, J.; Stoffella, P.J.; Wilson, P.C. Analysis of bisphenol A, nonylphenol, and natural estrogens in vegetables and fruits using gas chromatography−tandem mass spectrometry. *J. Agric. Food Chem*. **2013**, 61, 84-89.
39. She, Y.; Wang, J.; Zheng, Y.; Cao, W.; Wang, R.; Dong, F.; Liu, X.; Qian, M.; Zhang, H.; Wu, L. Determination of nonylphenol ethoxylate metabolites in vegetables and crops by high performance liquid chromatography–tandem mass spectrometry. *Food Chem.* **2012**, 132, 502-507.
40. Chai, Q.Y.; Huang, H.J.; Lu, H.; Mo, C.H.; Zhang, J.; Zeng, Q.Y.; Tian, J.J.; Li, Y.W.; Wu, X.L. Occurrence of nonylphenol and nonylphenol monoethoxylate in soil and vegetables from vegetable farms in the Pearl river delta, South China. *Arch. Environ. Contam. Toxicol*. **2012**, 63, 22-28.
41. Kannan, K.; Keith, T.L.; Naylor, C.G.; Staples, C.A.; Snyder, S.A.; Giesy, J.P. Nonylphenol and nonylphenol ethoxylates in fish, sediment, and water from the Kalamazoo River, Michigan. *Arch. Environ. Contam. Toxicol*. **2003**, 44, 77-82.
42. Chen, N.; Shuai, W.J.; Hao, X.M.; Zhang, H.C.; Zhou, D.M.; Gao, J. Contamination of phthalate esters in vegetable agriculture and human cumulative risk assessment. *Pedosphere* **2017**, 27, 439-451.
43. Yan, L.; Guanhua, H.; Lei, Z.; Hua, G.; Chunhua, L.; Hang, Z.; Honglu, L. Phthalate esters (PAEs) in soil and vegetables in solar greenhouses irrigated with reclaimed water. *Environ. Sci. Pollut. Res*. **2020**, 27, 22658-22669.

44. Munshi, A.B.; Karim, N.; Shaukat, S.; Hashmi, D.; Boardman, G.D.; Flick, G.J. Toxicity of phthalate esters in fish and shellfish from virginia beach using matrix solid phase dispersion (MSPD) and GC-MS. *J. Chem. Soc. Pak*. **2013**, 35, 1463-1471.
45. Cheng, Z.; Xiang-Ping, N.; Hong-Sheng, W.; Ming-Hung, W. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. *Environ. Int.* **2013**, 57-58, 75-80.
46. Lee, Y.M.; Lee, J.E.; Choe, W.; Kim, T.; Lee, J.Y.; Kho, Y.; Choi, K.; Zoh, K.D. Distribution of phthalate esters in air, water, sediments, and fish in the Asan lake of Korea. *Environ. Int.* **2019**, 126, 635-643.
47. Jianteng, S.; Lili, P.; Daniel, C.W.T.; Zhiheng, L.; Lizhong, Z.; Xiangdong, L. Phthalate esters and organochlorine pesticides in agricultural soils and vegetables from fast-growing regions: a case study from eastern China. *Environ. Sci. Pollut. Res*. **2018**, 25, 34-42.