Method development for extraction and determination of the presence and quantity of Endocrine Disrupting Chemicals in selected food items sold in South African open markets

By

Thapelo Ramalepe

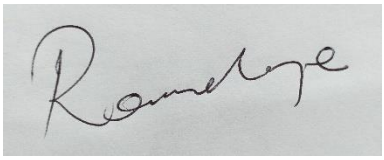
A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

Johannesburg 2022

Declaration

I, Thapelo Ramalepe,

declare that this dissertation titled “Method development for extraction and determination of the presence and quantity of Endocrine Disrupting Chemicals in selected food items sold in South African open markets” is my own. It is submitted for the degree of Master of Science at the university of the Witwatersrand, Johannesburg. It has not been submitted before at any other university. Where I have consulted the published work of others, this is always clearly attributed. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this dissertation is entirely my own work.



……

Date: 11 May 2022 at Johannesburg

Dedication

My dear Mother,

For every support I have received from you all my life, I beg to dedicate this MSc degree in your name as an expression of gratitude.

Your one and only son,
Thapelo Ramalepe.

Acknowledgements

Everything considered, thank God Almighty for providing me with the opportunity, strength, and patience to take part in this trilateral project until satisfactory completion. Without his blessing, this opportunity would have instead gone over my head.

There are many people to thank for their help with this research and the production of this dissertation. Firstly, Special gratitude to my supervisor and advisor Prof. Luke Chimuka for his ardent academic and financial support throughout the project. His profound guidance and advice have been very fruitful. I take pride in extending my special gratitude to my co-supervisors: Dr. Heidi Richards and Prof. Hlanganani Tutu. Dr. Richard's valuable encouragement and strict timekeeping in the form of progress reports has made me keep track of my progress from the initial days of my project. Furthermore, I would like to take this opportunity to thank my colleagues in the environmental chemistry team especially Dr Yannick Nuapia Belo and Dr Bisratwongel Tegegne for their input, perspective, and interest on this research topic.

Extension of thanks to Mr. Thapelo Mbhele (a technician in the MS lab at WITS) and Ms. Mokgaetsi Andelina Monyai for the instrumental training and making me a competent GCxGC MSTOF and ICP-OES instrument operator. Also Dr. Yannick for making me GC-ECD competent, especially the software (chemosphere version B.04.03) troubleshooting and hardware configuration.

My gratitude would not be complete without mentioning my mother, Mrs. Selinah Ramalepe as my great source of motivation and strength. Many thanks for her blessings and wishes to enroll and complete this degree. Finally, to my good friends and office mates, the *MSc elite squad*: Lindelwa Ndhlovu, Sabrina Khoosal and Jessica Tsakani Mhlongo, thank you for putting up to me and listening to me ramble for two years about *Moringa*!

It is a great pleasure to thank all of the above people for making this dissertation possible.

Abstract

Exposure to Endocrine Disrupting Chemicals (EDCs) has potential carcinogenic and mutagenic effects to human health. Endocrine Disrupting Chemicals are contaminants that have the potential to disrupt the processes of natural body hormones that enables the organism to respond normally to its environment. The primary source for EDC exposure is through the consumption of foodstuff like contaminated fruits, vegetables, fish, and beef. In fruits and vegetable farms, most EDCs originate from the fertilizers applied to the soil. The EDCs are constantly being leached from the soil environment and released into rivers and other aquatic ecosystems. Eventually, they make their way into the lipid membranes and further into the muscles of fish via the process of bioaccumulation. For beef, some of the EDCs bioaccumulates within their muscles when the cattle (cow or bull) drink the contaminated river water when grazing. When humans consume the foodstuff contaminated with the EDCs, the EDCs are passed onto their body muscles via the process of biomagnification. Due to the EDC toxicity in humans from from diet, it is essential to investigate their levels in foodstuff like fruits, vegetables, fish and beef muscle.

On the other hand, bioeconomy is an interesting research field which is gaining a lot of attention. Currently, there is considerable need to maximize the use of plant based biomass in analytical chemical processes. One such source is the use of *Moringa Oleifera (M.O)* seed protein bio sorbent which can be extracted, purified, and applied for a greener and low-cost sample clean-up. The aim of this study was to optimize the QuEChERS method using Primary secondary amine (PSA) sorbent and then comparing it with *Moringa Oleifera* protein biosorbent sorbents and other sorbents like Hemp cellulose, activated carbon sorbent for the extraction of selected organic EDCs in foodstuff. The optimization was done using chemometrics called Response Surface Methodology (RSM). A Design of Experiment (DoE) was used to investigate the effect of the sample mass (0.5-3 g), centrifuge speed (3400- 4000 rpm) , time (5-20 mins), mass of $NaCl$ and $MgSO_4(1-3\ g)$, and solvent extraction volume (5-10 mL). Firstly, a screening on the aforesaid parameters was done using an L8 (3 levels) linear model. Thereafter, a Central Composite Orthogonal (CCO) approach was used to

optimize the QuEChERS technique for maximum extraction of the selected EDCs. The analysis was done using Gas chromatography – Electron capture detection (GC-ECD) and 1 dimensional gas chromatography mass spectroscopy time of flight (1D GCxGC MSTOF). The PSA method presented optimal values of 3 g of sample, 150 mg PSA, 4000 rpm for 6 mins centrifuge conditions, 2 g NaCl, 2 g $MgSO_4$ extracted in 10 mL methanol, respectively. The PSA method presented recoveries between 74.1 $\pm$ 1.12% and 107.4 $\pm$ 0.88%. Furthermore, the *Moringa Oleifera* method presented recoveries between 76.2 $\pm$ 0.85% and 105.1 $\pm$ 2.24%. Limits of detection and quantification ranged between 0.16 to 1.77 $\mu g\ kg^{-1}$ and 6.1 to 17.7 $\mu g\ kg^{-1}$with RSD values $\leq$ 13.32 ,respectively. From this investigation, the *Moringa Oleifera* seed protein bio sorbent is a promising alternative to the synthetic PSA and other sorbents like cellulose, C18 and activated carbon in clean-up of food samples using QuEChERS approach as it was more selective towards target compounds

The optimal *Moringa Oleifera* method was then applied to thirty-eight different samples from Johannesburg (CBD Open markets, City deep main market and a farm along Hennops river) and Pretoria (Open markets and Tshwane main market) including fruits and vegetables, fish and beef samples. The maximum detected concentrations of six organic Endocrine Disrupting Chemicals extracted (Dimethyl phthalate, Diethyl phthalate, 4-n-Nonylphenol, 4,4'-DDT, 4,4'-DDD and 4,4'-DDE) were 65.93 $\pm$ 9.80, 111.61 $\pm$ 11.27, 115.15 $\pm$12.18, 101.17 $\pm$ 9.65, 114.7 $\pm$20.76 and 105.45 $\pm$ 18.33 $\mu g\ kg^{-1}$, respectively. WHO regulation comparison of the determined concentrations revealed that consumers staying in the two cities are not at risk of cancer from ingestion of the studied organic EDCs in the detected levels.

Secondly, the study aimed at extracting and determining the levels of inorganic Endocrine Disrupting Chemicals (selected metals) which are toxic at certain doses. The same thirty-eight samples collected for organic analysis were digested using Anton Paar microwave digestion system using 10% nitric acid and hydrogen peroxide. Thereafter, the total metal concentration analysis was done with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The selected metals of interest from the totals were Aluminum

(Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Arsenic (As), Lead (Pb), Manganese (Mn), Selenium (Se) and Zinc (Zn). The results show that the mean metal concentrations for Johannesburg foodstuff is not significantly higher ($P > 0.05$) than the mean concentration for Pretoria foodstuff. However, the two samples from the Hennops river farm (Rape and Spinach), exhibited the highest metal concentrations among all samples. Most of the metal concentrations were generally higher than the limits set by the World Health Organization (WHO), which alerts critical endocrine disrupting issues associated with the two cities under study. However, to critically conclude this regard, future work for thorough carcinogenic risk assessments must be established in terms of Daily limit Intake of Metals (DIM) and Hazard index (HI).

Presentation of MSc work at conferences and PostGraduate Workshops

RAMALEPE, T., CHIMUKA, L., RICHARDS, H. & TUTU, H.

1. Chromatography society of South Africa: Postgraduate student workshop 2021- **Oral presenter**

Ms Teams online conference
Prize: Overall second place for best presenter

Title: Comparison of *Moringa Oleifera* seed protein bio sorbent to synthetic traditional sorbents for QuEChERS clean-up followed by analysis using GC-ECD and GCxGC MSTOF confirmation.

2. 12^{th} Annual cross Faculty Postgraduate symposium 2021 – **Oral presenter**

Ms Teams online symposium

Title: Method development for extraction and determination of Endocrine Disrupting Chemicals in South African open market foods.

Table of Contents

Abbreviations

BHC	**:** Beta-Hexachlorocyclohexane
CRM	**:** Certified reference material
DDD	**:** Dichlorodiphenyldichloroethane
DDE	**:** Dichlorodiphenyldichloroethylene
DDT	**:** Dichlorodiphenyltrichloroethane
EDC	**:** Endocrine disrupting chemicals
EDI	**:** Estimated daily intake
EPA	**:** Environmental protection agency
FTIR	**:** Fourier-transform infrared spectroscopy
GC-ECD	**:** Gas chromatography – Electron capture detection
GC-FID	**:** Gas chromatography – Flame ionization detector
GCXGC	**:** Gas chromatography coupled with secondary Gas chromatography
GC-MS	**:** Gas Chromatography- Mass spectroscopy
ICP-MS	**:** Inductively coupled plasma mass spectrometry
ICP-OES	**:** Inductively coupled plasma optical emission spectrometry
JHB	**:** Johannesburg
LOD	**:** Limit of detection
PFE	**:** Pressurized fluid extraction

PHWE	: Pressurized hot water extraction
PPB	: Parts per billion
PPT	: Parts per trillion
PSA	: Primary secondary amine
PTA	: Pretoria
QuEChERS	: Quick, Easy, Cheap, Effective, Rugged and Safe
RSD	: Relative standard deviation
SFE	: Supercritical fluid extraction
TOF	: Time of flight
UAE	: Ultrasound-assisted extraction
WHO	: World health organization

List of Figures

List of Tables

CHAPTER 1: INTRODUCTION

Endocrine Disrupting Chemicals are now detected everywhere: the food we eat, the water we drink and the soil we plant in. This chapter details the background information on Endocrine Disrupting Chemicals, their origin, toxicity and extraction.

1.1 Background

Natural bodily hormones bind to their respective receptors via a lock and key mechanism (Alon 2021). For instance, each body hormone (key) in the endocrine system fits exactly into its receptor (lock), resulting in desirable cellular responses (Sanford, 2019; Okpashi et al., 2019). Endocrine Disrupting Chemicals (EDCs) are chemicals that mimics this normal hormone binding to bring about undesirable cellular responses (Autrup et al., 2020; de Coster and van Larebeke, 2012; Yilmaz et al., 2020). Examples of this include the mimicking of melatonin hormone in the pineal gland by carbaryl and carbofuran insecticides, mimicking of prolactin and oxytocin in the pituitary gland by Bisphenol A and the metal cadmium (Cd)(Steinmetz et al., 1997), mimicking of insulin and glucagon in the pancreas by phthalates and the metal Selenium (Se)(Rotondo and Chiarelli, 2020). Some EDCs are reported to completely discontinue the production of the bodily hormones and thus prevent any cellular response (Newbold *et al.*, 2007; Heindel *et al.*, 2015).

The main source of EDCs in human bodies is through eating contaminated foodstuff (Sharma et al., 2021). Further, contamination by Endocrine Disrupting Chemicals in foodstuff is classified into two forms (Nuapia et al., 2016; Kopittke et al., 2019). More commonly, it is by long-term contamination of organic and inorganic EDCs in ppb and ppt levels. This occurs via the diffusion of the EDCs into the foodstuff before they reach the markets (Torretta et al., 2018; Vikrant et al., 2018). Less commonly, it is by short-term contamination of highly concentrated organic and inorganic EDCs, which occurs via accidental release of the contaminants from its source. They are detected in ppm or higher levels (Nuapia et al., 2016) meaning that they are more potent. In general, the foodstuff contamination can occur during the farming and cultivation stage, during transportation of the foodstuff to the main markets and during the handling of the foodstuff in open markets (Greenberg, 2017; Borsellino et al., 2020; Pais et al., 2020). As a result of the effects of the foodstuff contamination in open markets, there was a 2019 appeal signed for the immediate removal of raw fruits and vegetables in some open markets which contain harmful preservatives. Most were coated with cancerous chemicals to increase their product life span in the markets (Delhi High Court,

2019). These chemicals include some Endocrine Disrupting Chemicals (EDCs), which can be organic and inorganic.

Organic EDCs are volatile or semi-volatile chemicals that disrupt the endocrine system to cause toxic effects (Chen et al., 2019; Lauretta et al., 2019). Organic EDCs include most Persistent Organic Pollutants (POPs), some pesticides and some preservatives found in foodstuff including fruits and vegetables (Vlassi et al., 2020) which are mostly man made meaning their quantities are controlled (Petrovic et al., 2002). Some man-made EDCs are plasticizers and pesticides (Ingerslev et al., 2003; Ripamonti et al., 2018; Sabir et al.,2019). Examples include DDT and its metabolites (van Cauwenbergh et al., 2020; Jaacks et al., 2019), bisphenol A (BPA), dioxins, monomethyl phthalate, dimethyl phthalate, and diethyl phthalate. Their target organs are the brain and prostate glands (Nesan et al., 2018; Rosenfeld and Cooke, 2019).

Another group of EDCs receiving some attention is inorganic EDCs. They are metals such as Hg, As, Pb, Cd, Cu, Cr, Fe, Mn, and Al. The metals have a tendency of bioaccumulating within an organism (Nascimento et al., 2018; Paschoalini et al., 2019). This results in chronic toxicity over time within the host (Elgawad et al., 2020). These metals have been found in fish samples (El-Moselhy et al., 2014; Gusso-Choueri et al., 2018; Javed and Usmani, 2019; Keshavarzi et al., 2018; Korkmaz et al., 2019; Maurya et al., 2019), water samples (Bhuyan et al., 2017; Chaturvedi et al., 2018; Mirzabeygi et al., 2017), beef muscle (Inobeme et al., 2018; Adzitey et al., 2018; Chaturvedi et al., 2018; Kasozi et al., 2018; Duman et al., 2019; Oloruntoba and Nathaniel, 2019) and fruits and vegetable samples (Shaheen et al., 2016; Gupta et al., 2018; Manzoor et al., 2018; Zwolak et al., 2019; Afonne and Ifediba, 2020; Cooper et al., 2020; Moyo et al., 2020; Altarawneh, 2021). Due to the increasing population size in South Africa and anthropogenic causes, the quantity and toxicity of these Endocrine Disrupting Chemicals are expected to increase over the years (Wooding et al., 2017; Horak et al., 2021).

Toxicity of both groups of EDCs at certain doses causes health related issues. In fish, this may result in decreased fertility and egg production in females or lead to reduced gonad size or feminization of genetic male fish (Langston, 2020; Olaniyan and Okoh, 2020; White et al., 2017). In humans, the toxicity results in abnormal reproductive processes (Rehman et al., 2018; Rahman et al., 2021), and the development of testicular and prostate cancer, even at a trace concentration of $1ng\ kg^{-1}$ (Gaudriault et al., 2017; Kaushik and Bhartiya, 2018; Kubincová et al., 2019). Developmental exposures of EDCs have also been hypothesized to be a leading factor for the increasing incidences of breast cancer in industrialized countries (Perrot-Applanat et al., 2018; Rodgers et al., 2018). For the developing embryo and infants, exposure can cause a delay in their early development and later reproduction, causing neurological and immune system defects (Mezcua et al., 2012). Further, the toxicity can give rise to complications such as cancerous tumors, obesity, diabetes, hypertension, attention deficit hyperactivity disorder (ADHD) and other developmental disorders (Shoaff et al., 2020; Street et al., 2018). The extent of the diseases varies depending on the level of EDC exposure (Parkkinen et al., 2018). These levels can be determined directly from human samples such as blood, breast milk and adipose tissue (Nuapia et al., 2016) , and they can also be measured indirectly from foodstuff consumed in relation to the everyday food intake (FAO/WHO, 1999) using different extraction and analysis techniques.

There are already various analytical techniques for the extraction of organic Endocrine Disrupting Chemicals in food including QuEChERS, Soxhlet extraction, Ultrasonic Assisted Extraction (UAE), Pressurized Fluid Extraction (PFE), Pressurized Hot Water Extraction (PHWE) and Supercritical Fluid extraction (SFE) (Cloutier et al., 2017; Rasul, 2018). A conventional Soxhlet extraction has been used for the extraction of EDCs with acceptable recoveries (Cloutier et al., 2017). However, the limitation with this technique is that it is time consuming as the reflux must be done for 24 hours, and it requires large amount of solvent which has negative disposal effects on the environment (Juhaimi et al., 2018; Hirondart et al., 2020a; Zhang et al., 2018). Ultrasonic Assisted Extraction technique overcomes the Soxhlet extraction disadvantages of time consumption and large volume usage; however, its limitation is the repeatability of the method as the ultrasound energy that transfers from the

ultrasonic bath to the sample container is dependent on the properties of the bath and as a result, a probe is needed to control the ultrasound frequency and amplitude for precise repeatability (Nuerxiati et al., 2019; Wu et al., 2020). The elevated temperatures used in Pressurized Fluid Extraction may degrade unstable Endocrine Disrupting Chemicals (Marcon et al., 2019; Yang et al., 2019). Also, the rate of diffusion of the solvent to the sample increases as the solvent volume is increased, which is not ideal when the solvent volume is increased above the optimized amount (Casella et al., 2021). QuEChERS extraction technique is preferred for the study because it limits the extraction labour intensities and uses less solvent to produce optimal recoveries (Campêlo et al., 2021; Montemurro et al., 2021). This chosen technique is quick, easy, cheap, effective, rugged, and safe to use (Anastassiades et al., 2003; Lehotay et al., 2010; Golge et al., 2018; Santana-Mayor et al., 2019).

Lastly, sustainable and Green Chemistry Principles (GCPs) in the development of chemical processes enable us to protect the environment and conserve energy (Keijern et al., 2019). One example is the profound research gap in moving from synthetic to greener biomaterials in the analysis of EDCs when using QuEChERS as the sample preparation technique. A commercial primary secondary amine (PSA) adsorbent is used at present, but very costly. A possible cheaper and greener alternative is the *Moringa Oleifera* seed protein biosorbent which can be applied as a QuEChERS sample clean-up sorbent.

This project aimed to explore the use of *Moringa Oleifera* seed protein as a clean-up biosorbent as a PSA alternative in QuEChERS extraction technique of selected EDCs in fish muscle, beef muscle, fruits and vegetables sold in open and main markets in South Africa from Johannesburg and Pretoria. Furthermore, the QuEChERS extraction technique was optimized using chemometrics, a Response Surface Methodology. A linear L8 model and a quadratic central composite design model were employed to investigate and optimize the speed of centrifuge, time of centrifuge, amount of salt, volume of solvent and type of solvent. The optimized method and parameters were applied to quantify different real food samples taken from selected open markets in Johannesburg and Pretoria, along with a farm by

Hennops river. This was done to provide evidence on the status of Endocrine Disrupting Chemicals in these areas. Further samples were microwave extracted for the extraction of selected metals followed by ICP-OES analysis. The levels are then compared to regulatory bodies, FAO/WHO.

1.2 Problem Statement

Endocrine Disrupting Chemicals have been known to disrupt the normal human health and well-being in South Africa (Horak et al., 2021a). Monitoring of EDC levels in South African open market foodstuff is very poor (Farounbi and Ngqwala, 2020). This is because many sellers and hawkers in the open markets don't know the main sources of their fruits and vegetables as they stock from different sources every day, so the levels of EDCs present are very difficult to track and attribute.

A vast number of residents in Johannesburg and Pretoria depend on foodstuff sold in open markets as they are cheap, healthy, locally owned and most are available for consumption on-the-go. Many open markets in JHB and PTA operate on unsanitary and busy roads. This makes it easier for the pollution particulates and chemicals emitted from the vehicles to make their way into the food items sold there (Nuapia et al., 2016). More important, EDC contaminated foodstuff has been known to cause endocrine related diseases all over the world, South Africa included. This ranges from minor and controlled effects to more chronic effects (Kabir et al., 2015). This widens the research gap in finding the effects of endocrine disruption in South Africa. Also, much of environmental risk assessment has focused on water and soil related pollution but very little on foodstuff. Thus, data on the presence and quantity of Endocrine Disrupting Chemicals is mostly available for water and wastewater but not in food related studies. A continued monitoring of EDCs levels in foodstuff in South Africa is essential to supply more evidence of EDCs contamination to the government to revise legislations and policies for consumer safety.

Moreover, Aluminum salts and synthetic polymers are currently used as solutions in water purification and extract cleaning sorbents. The cost, health, and environmental impacts of these make the processes less green and unsustainable. Hence, it is of vital importance to

develop and optimize the type and amount of cleaning sorbents by keeping the process as green as possible as opposed to fossil fuel driven economy. The use of the protein extracted from *Moringa Oleifera* seeds has a fast separation and enhanced removal efficiency advantage over synthetic sorbents (Amuanyena et al.,2019). This study aims to test the *Moringa Oleifera* seeds protein as a QuEChERS clean-up bio sorbent.

CHAPTER 2: LITERATURE REVIEW

This chapter provides literature review on:

- The characteristics of organic Endocrine Disrupting Chemicals
- Target organic Endocrine Disrupting Chemicals.
- Sources of Endocrine Disrupting Chemicals in foodstuff
- Toxicity of organic EDCs
- Limits and regulations of EDCs concentration by WHO/FAO
- *Moringa Oleifera* seed protein bio sorbent
- Different extraction techniques for organic EDCs extraction
- Analytical instruments for detection of EDCs
- Heavy metals
- Freeze-drying technique for sample sublimation

2.1 Introduction: Endocrine Disrupting Chemicals (EDCs)

The United States Environmental Protection Agency (US EPA) lists Endocrine Disrupting Chemicals under groups of heterogeneous chemicals with concerning health and environmental effects (Harding et al., 2006). This is because they have the potential to cause cancer in humans and also cause negative effects in the aquatic environment (Harding et al., 2006; la Merrill et al., 2020; Lauretta et al., 2019) . The primary source of Endocrine Disrupting Chemicals to humans is through the diet (Li et al., 2018; Sharma et al., 2021b), hence the popular phrase "*you are what you eat*" (D'Souza and Bhattacharya, 2019; Petta et al., 2020),originally came up by Jean Anthelme Brillat-Savarin, 1926.

Pesticides are one class of EDCs (Encarnação et al., 2019). Commonly used pesticides include 4,4'-DDT and its metabolites, which has already been reported as endocrine disruptors (Guarino et al., 2021; Jaacks et al., 2019). The pesticide 4,4'-DDT was once used as a random pesticide from the 1940s in the protection of malaria and other insect-borne diseases. It was also used in the protection of crops in the agricultural sectors, thus being introduced into the environment (Li et al., 2018; Malusá et al., 2020) . Because it is hazardous in nature, it was disqualified and later banned as a pesticide, although it remains active in some countries today (Davis, 2019; Wolmarans et al., 2021). 4,4'-DDT is accumulated in the fat, until it is converted to usable forms of bodily energy (Tawar et al., 2021). Once reaching toxic levels, it causes immune-suppression, reproductive and behavioral effects, like delays in immune responses in children (Marziali et al., 2017; Pavlikova et al., 2020). One study reported that *utero* exposure of 4,4'-DDT is linked to breast cancer later in life which debates the safety of 4,4'-DDT(Cohn et al., 2019). It is thus essential to thoroughly investigate 4,4'-DDT and its metabolites and its toxicity thereof.

2.2 The characteristics of organic Endocrine Disrupting Chemicals

4,4'-DDT is a very persistent organochlorine pesticide and has a half-life of approximately 15 years in soil (Zhang et al., 2018) . During this period, its metabolites in the soil environment are 4,4'-DDD and 4,4'-DDE, which are also persistent and have comparable endocrine disruption properties as 4,4'-DDT (Guan et al., 2009). It functions by opening sodium ion gates in the neuron of an insect, which is usually fatal (Boussahel et al., 2009). It

has since been banned for agricultural use in North America, although it is still commonly used to treat malaria in some countries (Ayejuyo et al., 2008). 4, 4'-DDD is a 4, 4'-DDT metabolite and is chemically and structurally similar, although it is generally less toxic to humans than the parent pesticide (Golfinopoulos et al., 2003). BHC (Lindane) is another organic group which has been used both as an insecticide and as a pharmaceutical treatment for lice (Ghahvechi Khaligh et al., 2021; Hassan et al., 2020). In humans, it affects the nervous system, liver and kidney (Seth et al., 2005). It is currently unclear whether BHC is an endocrine disruptor or not although it has been reported to cause neurological effects in humans (Meyer et al., 2014). WHO classifies BHC as moderately hazardous (WHO, 2000). In 2009, the agricultural use of BHC was also banned amid the Stockholm convention on Persistent Organic Pollutants, though it was exempted to be used as a second-line pharmaceutical treatment for lice (WHO, 2009). WHO and FAO have reported high levels of these metabolites in vegetables and fish consumed by many people in Africa (WHO, 2000).

Organophosphorus pesticides are another class of pesticides which are predominantly used worldwide in agriculture (Nakata et al., 2002). Farm workers are thus the target population susceptible to exposure to organophosphorus pesticides, which results in increased risk of development of respiratory symptoms as compared to the normal population. Example of this class is chlorpyrifos, which is used in pest control (Ventura et al., 2019; Xiao et al., 2021).

Some aquatic animals, like fish, are an essential constituent in the human diet (Muncke, 2009). In aquatic environments, some EDCs are very persistent (Bai and Acharya, 2019; Carnevali et al., 2018). They tend to bioaccumulate and biomagnify for long periods of time because they are lipophilic and make their way easily cross the lipid membrane of fish species (Diao et al., 2017; Liu et al., 2015). These further find their ways into the muscles of the fish (Casanova et al., 2011). They have longer half-lives in sediments, soils, and biota (Pojana et al., 2007). The physical and chemical characteristics of target EDCs are given in table 2.1.

2.3 Target organic Endocrine Disrupting Chemicals

2.3.1 Dimethyl Phthalate

Dimethyl phthalate (Molecular weight 194.19 g mol^{-1} $CAS\ number$ $131-11-3$) is an organic Endocrine Disrupting Chemical which is a methyl ester of phthalic acid (Kaur et al., 2021; Şolpan and Mehrnia, 2018). Dimethyl Phthalate is used in many ways, including, but not limited to plasticizers, solid rocket propellants and insects repellents (Kaur et al., 2021). Its structure is given in figure 2.1.

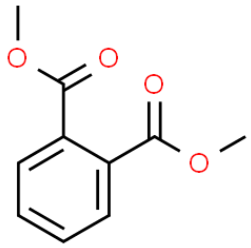


Figure 2.1 chemical structure of dimethyl phthalate

Its toxicity effect depends on the mode and extent of exposure. Acute or short-term exposure via inhalation causes eye and nose irritation (Rodrigues et al., 2019; Tse et al., 2021). Chronic or long-term effect are still being studied and no information is confirmed for human beings. For animal species, a study by the United States Environmental Protection Agency reported light effects on growth and on the kidney as a result of chronic exposure to the endocrine disruptor (Jeong et al., 2020; Weaver et al., 2020). Human beings are exposed to dimethyl phthalate by eating contaminated food, including fruits and vegetables sold in open markets, as many are rinsed and cleaned with contaminated water (Hou et al., 2021; Wang et al., 2018). Other sources of exposure include drinking contaminated water and using hemodialysis tubes which are made up of the chemical (Das et al., 2021). Workers in factories that manufacture the chemical are also at a risk of contact with the pesticide (Ruiz et al., 2018).

Table 2. 1 Physical and chemical properties of target EDCs.

Compound	Number of rings	MW ($g\ mol^{-1}$)	Water Solubility ($mg\ L^{-1}$)	B.P (℃)	$Log\ P$	Vapor pressure
Dimethyl phthalate	1	194.2	4.3	284	1.11	0.01 mmHg at 20 ℃
Diethyl phthalate	1	222.2	1080	302	2.18[1]	2.1×10^{-3} mmHg at 20 ℃
4-Nonylphenol	1	220.35	1.1	293-297	5.76[1]	8.18×10^{-6} mmHg at 20 ℃
4,4'-DDD[2]	2	320.05	0.05	305	5.18	1.60×10^{-7} torr at 20 ℃
4,4'-DDT[2]	2	354.5	0.025	Decomposes: 185-187	5.18	1.35×10^{-6} torr at 25 ℃

1. *Itokawa H et al; Chem Pharm Bull 37: 1619-21 (1989)*

2. (Agency for Toxic Substances and Disease Registry, 1995)

All the chemical and physical properties of the chemicals were referenced from PubChem database library, 20

2.3.2 Diethyl Phthalate

Diethyl Phthalate (Molecular weight 222.24 g mol^{-1}, CAS number 84-66-2) is produced in industry via a reaction between phthalic anhydride and ethanol and a sulphuric acid catalyst (H_2SO_4) (Khadka et al., 2020). Its structure is given in figure 2.2.

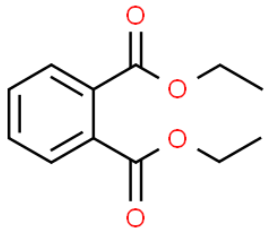


Figure 2. 2 Structure of diethyl phthalate

Diethyl phthalate is used as a plasticiser in a wide variety of consumer products such as cellulose ester plastic films and cosmetic packaging (WHO, 2003). It is reported as a constituent in sixty seven cosmetic formulations, including bath oils and salts, eye shadow, toilet waters, perfumes and other fragrance preparations (Khalid and Abdollahi, 2021) . Both as a short and long term effect, Diethyl Phthalate is likely to undergo biotic (environmental) biodegradation (Prasad, 2021; Zhang et al., 2019). However, it is unlikely to undergo abiotic degradation processes such as hydrolysis, oxidation, and photolysis (Min et al., 2021). To add, Diethyl phthalate is not likely to biomagnify through the food-chain (Cheng et al., 2021).

Dimethyl phthalate is introduced in the environment as a result of its manufacturing in industry and also during its disposal (US EPA). Environmental releases are mainly from water and soil as a result of leaching from landfills. Furthermore, dimethyl phthalate may enter the atmosphere via combustion reactions of plastics (Rajmohan et al., 2019; Velis and Cook, 2021).

Biodegradation of diethyl phthalate in soil occurs as a series of sequential steps, similar to all phthalate groups. It degrades into phthalic acid when it comes in contact with water (Wang et al., 2018). Phthalic acid further converts to monoethyl phthalate when more water

molecules is added. This is called the hydrolysis of dimethyl phthalate chains (Lu et al., 2020; Ozaki et al., 2017; Shariati et al., 2021; Zhang et al., 2018).

In soil, diethyl phthalate has a half-life if 0.75 days at 20℃ and thus not expected to persist in the environment (Billings et al., 2021; Deng et al., 2021). Humans are exposed to diethyl phthalate mainly by eating the seafood contaminated with this type of pesticide (Kuzukiran et al., 2018; Talley et al., 2020). Also, exposure is by eating fruits and vegetables that were washed with contaminated water or contaminated with diethyl phthalate leached from packaging materials (Lestido-Cardama et al., 2020; Talley et al., 2020). Also, human exposure can come from breathing contaminated air (Demirtaş et al., 2020; Szewczyńska et al., 2020). Direct levels of diethyl phthalate in humans can be detected in adipose tissue samples (Jeong et al., 2019).

2.3.3 4-Nonylphenol

4-Nonylphenol (Molecular weight 220.35 $g\ mol^{-1}$ CAS number 104-40-5) is an estrogenic disruptor (disrupts the normal female reproductive processes and secondary sex characteristics) (Sheikh, 2020). It is found in detergents and is known to contaminate food and drinking water (Study and Ferguson, 2021; Sukuroglu et al., 2021). In the environment, it is a toxic contaminant and when released into the environment, it is very persistent and over time, it can be broken down into its metabolites like alkylphenol, which can also disrupt the endocrine system (Lalonde and Garron, 2021; Mahalakshmi et al., 2020; Wilkinson et al., 2017). The structure of 4-Nonylphenol is given in figure 2.3. It is widely used to make lubricating oil additives, plasticizers, fungicides, and plastics for industrial, agricultural and domestic (Anh et al., 2019; Liu et al., 2020).

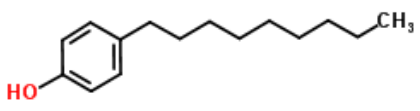


Figure 2.3 Structure of 4-Nonylphenol

Workers in factories that manufacture the chemical may inhale 4-Nonylphenol vapors (Chmielewski et al., 2021; Darbre, 2018). The general population are exposed to 4-Nonylphenol primarily by ingesting food contaminated with the chemical, by drinking contaminated water or by dermal contact with the chemical (Chung, 2021; Zhou et al., 2020). People consmuning open markets food are also exposed to traces of the chemical as some originate from the farms where the samples were harvested (Aworh, 2021; Kapeleka et al., 2020). Most fruits and vegetables are also washed and rinsed with contaminated water unknowingly.

4-Nonylphenol has been detected in human breast milk, urine, and blood samples (Sukuroglu et al., 2021). Several studies have reported that 4-Nonylphenol increases the risk of autoimmune diseases such as inflammatory bowel disease (Malaisé et al., 2020; Popescu et al., 2021). Data on the effect of 4-Nonylphenol on infertility, birth defects and cancer are not available. Since 4-Nonylphenol is hydrophobic, it can easily accumulate in humans, where it will display a wide range of toxic effects (Paolella et al., 2021; Study and Ferguson, 2021; Tato et al., 2018). 4-Nonylphenol tends to bioconcentrate to a notable degree in aquatic species (Graca et al., 2021). The bioconcentration factors (BCFs) are measured by the US Environmental Protection Agency in some fish at alarming levels. 4-Nonylphenol has been detected in twenty-five tissues of fish in descending order of concentration:bile, liver, kidney, fat, gill, heart, and muscle (Mohamed et al., 2019).

2.3.4 4,4'-Dichlorodiphenyltrichloroethane

4,4'-Dichlorodiphenyltrichloroethane (4,4'-DDT) (Molecular weight 354.49 g mol^{-1}, CAS number 50-29-3) is a synthetic organochlorine endocrine disruptor. It was banned in several countries in the early 1970's due to its ecological properties in the environment. However, in some countries it is used for the control of insect-transmitted diseases such as yellow fever, malaria and others (Burgos-Aceves et al., 2021; Chandra et al., 2021; Maia et al., 2020; Olatunji, 2019). It was designed as a Persistent Organic Pesticide (POP) by the governing council of the united nation in 1997 (Islam et al., 2018). It is persistent in the environment depending on temperature and is resistant to complete degradation by microorganisms and by light (Bilal et al.,2019; Gaur et al., 2018). At tropical climates, 4,4'-DDT is less persistent

than in temperate conditions (Ding et al., 2021). The structure of 4,4'-DDT is given in figure 2.4.

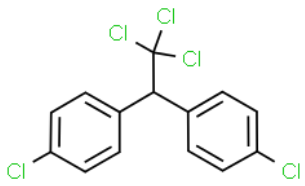


Figure 2.4 Structure of 4,4'-DDT.

4,4'-DDT is readily absorbed onto sedimens and soils, which serve as long term exposure mediums (Jin et al., 2019). It has a very strong affinity to be absorbed onto surfaces and so it persists on soil particles (Ahmed et al., 2018). It is readily taken up by organisms that drink contaminated water and from food. In fish, it is directly absorbed from the water medium (Abbassy et al., 2021; Topal and Onac, 2020). Due to its hydrophobicity, it retains in the fatty tissue of an organism (Jackson et al., 2017; Onal et al., 2017; Yamashita et al., 2021). Organisms in higher trophic levels are more supsciptible to 4,4'-DDT exposure. It can be transported around the world in the bodies of animals, in air and in the ocean (Ghani et al., 2017; Tsygankov, 2019). Humans are thus exposed to 4,4'-DDT through air, water and food (El-Nahhal, 2021; Mazlan et al., 2017). In a vast majority of South African population, they are exposed to the chemical via ingestion of fruits and vegetables sold in open markets (Aworh, 2021).

2.4 Sources of Endocrine Disrupting Chemicals in Foodstuff

Fruits and vegetables can accumulate toxic chemicals from the environment they grew in (Nuapia et al., 2016). This occurs via deposition of the contaminants from the soil to the plant surface (Shahawi et al., 2010). It is general knowledge to wash fruits and vegetables bought from local markets before consumption, to decrease the chances of direct chemical transfer into the human body(Wanwimolruk et al., 2015), however washing samples does not remove 100% of the contaminants as some penetrate within the samples. The sources of these soil

particulates are affected by mining activities (Cobb et al., 2000), atmospheric deposition and other emission sources in agriculture (Hu et al., 2017; Nuapia et al., 2015). These chemicals find their way into the human immune system by inhalation, ingestion and skin contact (Oduntan et al., 2014).

Moreover, environmental pollution is another source of toxic organic chemicals that end up in food (Gallo et al., 2018; Seltenrich, 2015). This type of pollution is caused by manufacturing facilities which deals with cement, ceramic and brick production, among others (Mostafa and Peters, 2017; H. Zeng et al., 2020). The elements of great concern range from more hazardous Ag, As, Au, Cd, Cr, Cu, Hg, Pb, Sb and Zn to less hazardous Ga, La, Nb, Sr and Zr (Borghesi et al., 2016; Kanduč et al., 2019; Lazareva et al., 2019; Nuapia et al., 2018) . Emission sources can also be anthropogenic, caused by human activities (Sobota et al., 2015; Streets et al., 2017). They include origin sources such as petroleum refineries, gas gasification, agricultural and refuse combustion, automobiles ad vehicles (Ben-Iwo et al., 2016).

2.5 Bioaccumulation of organic Endocrine Disrupting Chemicals

The potential of bioaccumulation of this class of chemicals followed by the level of toxicity they possess continues to be an important environmental concern in South Africa and throughout the world (Liu et al., 2017). EDCs tend to spread and distribute globally through various pathways including the atmosphere, streams, rivers, oceans, and many others (Hochella et al., 2019; Kumar and Mukherji, 2017); hence EDCs distributed in some regions of the world can travel and end up in areas far from their point source (Liu et al., 2017). The potential of the long-range travel of these compounds has caused legislation bodies globally to take urgent global actions to eliminate their use and discharge into the environment (Environment Canada, 1995; Geyer et al., 2000).

2.6 Toxicity of target organic Endocrine Disrupting Chemicals

Toxic elements cause negative effects to humans (Mahurpawar, 2015; Sun et al., 2014). The cause ranges from cumulative to non-cumulative effects, which vary based on dose, exposure duration, route of pathway and the state of health of consumers (Evans et al., 2015; Moreira et al., 2015). Cumulative effects occur after repeated exposure by a toxic substance, while

non-cumulative effects may be caused by rapidly absorbable and rapidly eliminated toxic substances (Ambwani et al., 2018; Duffus et al., 2007). Contamination by organic compounds is a serious problem, as most of them are carcinogenic and mutagenic agents (Cheng et al., 2016; Prathyusha et al., 2016). Exposure to small concentrations of EDCs was reported to be associated to pathogen resistance, chronic toxicity, and endocrine gland malfunction (Braun, 2017; Kabir et al., 2015). The Endocrine Society and the European Commission has shown evidence for adverse effects of the EDCs on reproduction, thyroid functioning, metabolism, obesity and brain functioning (Diamanti-Kandarakis et al., 2009; Kortenkamp et al., 2011). Organic molecules are also an environmental problem and may lead to global changes, such as the greenhouse effect or the ozone hole (Manisalidis et al., 2020; Petrescu et al., 2018; Varela et al.,2020).

2.7 Regulations and limits of Endocrine Disrupting Chemicals

The World Health Organization and (WHO) has established maximum tolerable daily intake of Endocrine Disrupting Chemicals as non- toxic (Bergman, United Nations Environment Programme., World Health Organization. 2013). These values vary, depending on the body mass index (BMI) of a person. The toxicity extent of each endocrine disruptor also varies from person to person, and is based on the age at exposure, the latency from exposure, non-traditional dose-response dynamics, and transgenerational effects (Lymperi and Giwercman, 2018; Schug et al., 2011). Table 2.2 shows the levels of six endocrine disruptors and their estimated mean daily intake.

Table 2.2 Maximum average tolerance limits per day for an adult consumer

Endocrine disrupting chemicals	Tolerable mean adult intake($\mu g\ kg^{-1} day^{-1}$)	References
1. Dimethyl Phthalate	13.5 in fruits and vegetables	(Serrano et al. 2014)
2. Diethyl Phthalate	13.8	(Koch *et al.*, 2003)

3. 4-Nonylphenol	1500	(Nielsen *et al.*, 2000)
4. 4,4'-DDT	3.5-10 in fruits and vegetables	(FAO/WHO, 1968)
5. 4,4-DDD	3.5 – 10 in fruits and vegetables	(FAO/WHO, 1968)
6. 4,4'-DDE	3.5 – 10 in fruits and vegetables	(FAO/WHO, 1968)

2.8 Gamma- Hexachlorocyclohexane as an Internal standard

$\gamma - Hexachlorocyclohexane, AKA\ \gamma - Lindane$ is used an insecticide on fruits and vegetable farms (Glover-Amengor and Tetteh, 2008). It behaves similar chromatographically, but not identical to 4,4'-DDD, 4,4'-DDT and 4,4'-DDE (Franken et al., 1976 ; Garcia Lara et al., 2021), which are being monitored in this study. It can be added to all the samples to be extracted in constant amount which can be used for calibration by plotting the ratio of the peak area of the analyte signal against the peak area of the internal standard signal as a function of the analyte standard calibration curve. This ratio is known as the response factor (Boysen et al., 2018; Čapoun & Krykorková, 2021). It is done to validate the QuEChERS method. The response factor equation is given as (Chandra et al., 2019; Wang et al., 2017):

$$RF = \frac{((A_X)(C_{IS}))}{((A_{IS})(C_X))}\ \ where:$$

$RF: Response\ factor$

C_X is the concentration of the target EDC

A_X is the area of the target EDC

A_{IS} is the area of the internal standard

C_{IS} is the concentration of the internal standard

The areas are deduced from the calibration curve of the analyte standard.

The structure of $\gamma - Hexachlorocyclohexane$ and its chromatogram is given in figure 2.5 and 2.6. It is essential to note the similarities of the structure in the hydrogen, carbon and chlorine empirical chains and substituent between the compound and the organochlorine pesticides being targeted in this present study. However, it is also essential that the gas chromatography detection is able to distinguish between the internal standard and the target compounds, to be able to quantify it individually. The disadvantage with the chosen internal standard ($\gamma - Hexachlorocyclohexane$) is that it must not be commonly found in the samples that are being extracted. This is to ensure that the internal standard is not affected by matrix interferences and coelutions, or target analytes interferences.

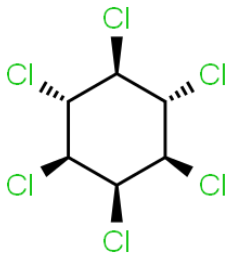


Figure 2.5 Chemical structure of γ-Hexachlorocyclohexane.

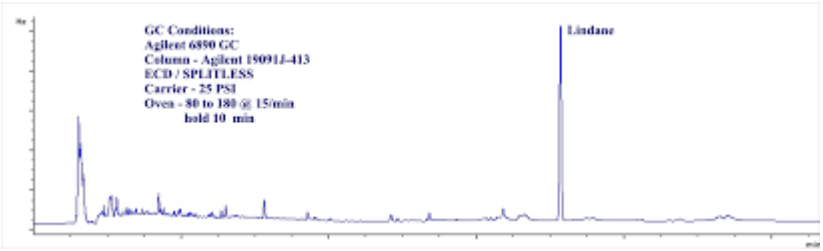


Figure 2.6 Certified chromatogram of γ-Hexachlorocyclohexane

As discussed for the other EDCs, workers in factories that manufacture the compound are also at risk of dermal contact and by inhalation during its formulation. To add, during its disposal, traces of the chemical may travel by wind from contaminated soil. $\gamma - Hexachlorocyclohexane$ has been detected in groundwater and surface water samples that

were tested near hazardous waste sites (Rodríguez et al., 2018; Seopela et al., 2021; Vasseghian et al., 2021). It has been listed as a pollutant of concern to EPA's Great Waters Program due to its persistence in the environment, potential to bioaccumulate, and toxicity to humans and the environment (Barnhoorn and Cobus Van Dyk, 2020; Luisa et al., 2020; Olisah et al., 2020).

2.9 Different techniques for the extraction of Endocrine Disrupting Chemicals

Several techniques have been developed over the years for the extraction of pesticide residues, such as EDCs. Environmental analytical chemists are continuously improving these techniques by using modern developments like automation and new clean-up sorbents which shortens analysis time and make the techniques greener and more sustainable, without compromising accuracy and sensitivity (Khezeli and Daneshfar, 2017; Maciel et al., 2019; Parrilla Vázquez et al., 2019).

Traditional sample preparation methods such as Soxhlet extraction requires considerable time and effort, they are expensive and uses up large volumes of organic solvents and the recovery is often compromised (Boyaci et al., 2015; Goh et al., 2019; Mwaurah et al., 2020; Pérez-Rodríguez et al., 2018; Shams et al., 2015; Xu et al., 2016). Therefore, modern sample preparation methods have been developed to overcome these drawbacks. Some modern extraction techniques include QuEChERS (quick, easy, cheap, effective, rugged, and safe), Supercritical Fluid Extraction, Pressurized Hot Water Extraction (PHWE), Soxhlet extraction, Ultrasound-Assisted Extraction (UAE) and Pressurized Fluid Extraction (PFE) among others. This chapter aspect describes several modern extraction techniques, looking at their advantages and drawbacks and their capacity to extract Endocrine Disrupting Chemicals.

2.9.1 QuEChERS extraction method

There has always been an interest to improve sample extraction techniques over the years, together with a call to limit the labor intensity and the amount of organic solvent used for extraction, to limit toxicity and environmental pollution (Armenta et al., 2019; Spietelun et al., 2014). This resulted in the development of many extraction techniques (Anastassiades et

al., 2003). For solid samples, microwave extraction and pressurized liquid extraction are the most popular techniques followed by analysis using the GC-MS or HPLC-MS (Itoh et al., 2008). However, these sample preparation techniques are expensive to buy. An extraction procedure must be efficient, safe, cheap, and easy to use. Thus, QuEChERS has emerged as a cheap alternative method for extracting these organic compounds in solid materials (Luo et al., 2018).

The QuEChERS method was introduced in 2003 (Anastassiades et al., 2003). The method reduces the complications of analytical processes by using less solvent meaning less solvent waste generation (Luo et al., 2018). Multiple extracts can be prepared in minimum time by a single analyst and it produces high recoveries with RSD generally < 5%. It requires minimum use of lab ware, less labor and has shown high versatility (Anastassiades et al., 2003; Kemmerich et al., 2015; Lehotay, 2011). The limitation however is the concentration of the final extract to achieve the necessary sensitivity required in the target samples(Barbieri et al., 2019). This, however, is also dependent on the analytical instrument used.

The QuEChERS method has been used in the extraction of several compounds such as pesticides and EDCs from solid samples (Kalachova et al., 2011). The procedure involves three stages: firstly, the extraction stage which involves the mixing of sample and extraction solvent. There after the partition of liquid phase containing analytes by using salts and lastly the clean-up stage (Pinto et al., 2010).

The QuEChERS method can be applied to different matrix types, with different types of sorbent requirements for the clean-up process as seen in table 2.3 below.

Table 2.3 QuEChERS method sorbent clean-up requirements for different matric types

Matrix type	Examples	Sorbent requirements for clean-up
General matrix	• Apples • Melon • Cucumber	$MgSO_4$ *and PSA*/C18
Fatty Matrix	• Fish	$MgSO_4$ *and PSA*/C18

Pigmented matrix	• Milk • Cereals • Lettuce • Carrot • Wine	$MgSO_4$ *and* PSA,C18, Activated carbon

Yang *et al.* (2015) did an analysis on the determination of twenty-six Endocrine Disrupting Chemicals in fish and water samples using modified QuEChERS method combined with solid extraction and HPLC-MS. Various experimental parameters that could affect the extraction efficiencies was investigated. For analysis, the sample was extracted by a modified QuEChERS method with 20 mL acetonitrile for fish samples. The clean-up was done using solid-phase extraction cartridge and the analytes were quantified using an isotope labelled internal standards and recoveries of 69.1% and 120.5% were obtained. The relative standards deviation was less than 20% and the detection limits ranged from 0.01 to 9.04 $ng\ g^{-1}$. On the other hand, Rudel et al. (2003) did an analysis of phthalates, pesticides, polybrominated diphenyls esters and other Endocrine Disrupting Chemicals in air and dust, which greatly accounts for the amount that end up in food items that are sold in markets for example. Fifty-two compounds were detected from air while and sixty-six were detected from dust. The most abundant compounds in air included phthalates, 4-nonylphenol and 4-tert-butylphenol with typical concentration range of 50-1500 $ng\ m^3$. The banned pesticides DDT and its metabolites were also detected, and the levels exceeded government health-based guidelines for fifteen compounds.

Applications of QuEChERS have been introduced commercially. Other applications are still being developed and require modification (Noril et al.,2011), such as analysis of veterinary drugs, mycotoxins, and other contaminants which cannot be neglected (González-Curbelo et al., 2015). So far, QuEChERS has been predominantly applied for the analysis of contaminants in foods of plant origin (Sadowska-Rociek, 2013). In addition to pesticides in various fruits and vegetables, acrylamide in potato chips, peanut butter, and chocolate and aflatoxins in noodles have been determined using QuEChERS, including acrylamide and veterinarian drugs (De Paola et al., 2017). It has already been applied after proper validation

to assess pesticides in non-aqueous samples such as olive oil and in a hydroethanolic matrix (e.g., wines).

2.9.2 Supercritical Fluid Extraction (SFE)

A supercritical fluid is a fluid that is compressed below their critical temperature while kept in the fluid state but below the pressure required to compress it into a solid state (Sharif et al., 2014; West, 2018). The density of a supercritical fluid is comparable to that of a liquid whereas its viscosity compares to that of a gas (Abhari and Khaneghah., 2016). As a result, these properties permit the fluid to penetrate faster and deeper into solid samples for efficient extraction. The restriction to using carbon dioxide as the supercritical fluid is that it does not extract polar compounds well enough and as a result, an organic solvent such as methanol is usually incorporated in combination to improve extraction efficiency of polar compounds (Cifuentes and Herrero,2017). The extraction conditions are mainly pressure and temperature, which are responsible for the selectivity of the compounds of interest in the supercritical fluid (Baldino et al., 2020; Gallego et al., 2019; da Silva et al., 2016).

Supercritical Fluid Extraction is a technique for separating an extractant from the matrix using supercritical fluids as the extraction solvent, such as carbon dioxide which is commonly used due to reasonable cost and safety purposes (Abhari and Khaneghah.,2016). Both the pressure and the temperature are kept higher above their critical point. Recently, Supercritical Fluid Extraction method has been utilized in food, pharmaceuticals and chemical fields (Lv et al.,2000). When using $SSCO_2$ as the fluid, the $SSCO_2$ is used as the solvent to extract and remove oils and other non-polar impurities to yield a highly pure phospholipid. The conditions for this are a pressure of approximately 30 MP and temperature of 40-60 ℃ which is not high enough to cause a deterioration of the resulting phospholipid. The extraction time using this technique is short and the $SSCO_2$ escapes into a gas state without any solvent with it. This is due to the excellent mass transfer performance of the $SSCO_2$ (Asiri and Isloor, 2009).

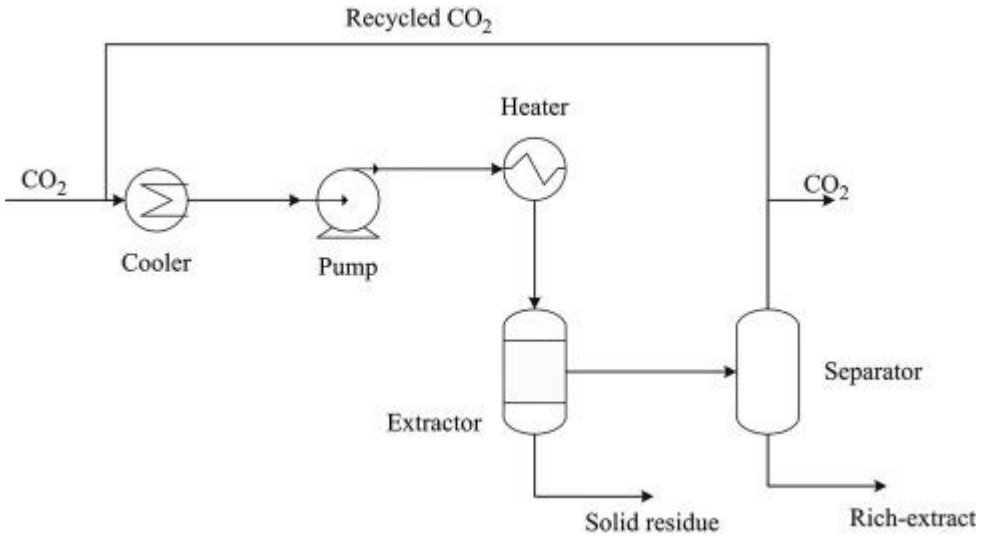


Figure 2.7 Simplified flow diagram of the supercritical fluid extraction with $SSCO_2$ (Dávila et al., 2014)

Figure 2.7 shows an overview of the Supercritical Fluid Extraction. In this process, the carbon dioxide is cooled to a temperature below its normal boiling point so that the carbon dioxide is a liquid when it enters the pump to avoid pressure changes, leading to cavitation. The pressurized carbon dioxide is then heated above its critical temperature and hot water added so that the extractor is kept at the operating temperature. The $SSCO_2$ flows into the extractor, which extract its soluble compounds. The solvent and extract leave the extractor, and goes to the separator, which is at atmospheric pressure (Alvarez et al., 2019; Rai et al., 2014; Sánchez-Camargo et al., 2017; Yao et al.,2018; Yousefi et al., 2019). Here the extract forms a precipitate due to depressurization (Abhari and Khaneghah., 2016).

Rissato *et al.* (2004) reported the Supercritical Fluid Extraction of pesticide multiresidue in honey by GC-ECD. The report shows that the recoveries for most of pesticides of honey at fortification levels of 0.01–0.10 $mg\ kg^{-1}$ ranged between 75–94%. The limits of detection found were less than 0.01 $mg\ kg^{-1}$ for ECD (Rissato et al., 2004).

2.9.3 Pressurised Hot Water Extraction (PHWE)

Pressurized Hot Water Extraction (PHWE) is an extraction technique that has become very popular as a green extraction method for different classes of compounds in environmental and food related fields (Plaza & Turner, 2015; Vazquez-Roig and Picó, 2015). PHWE is also used to extract contaminants in soils and sediments for environmental regulations and safety analysis (Sushkova et al., 2014). Parameters that affect the extraction efficiency in PHWE are temperature which is the most essential, extraction time and flow rates (Plaza and Turner, 2015). Addition of additives and modifiers are also reported to enhance the extraction efficiencies when this method is employed (Teo et al., 2010).

Compared to traditional methods of extraction such as Soxhlet extraction and sonication, PHWE uses less organic solvent, which can be less-environmentally-friendly. Instead, it utilizes pressurized water at high temperatures and controlled pressure for the extraction of less-polar compounds (Santos-Buelga and González-Paramás,2009). Water is readily available, non-toxic, low cost and environmentally friendly both for usage and disposal (Kronholm et al., 2007; Smith et al., 2002).

Water has some essential physicochemical properties which makes it suitable for pressurized hot water extraction of polar or inorganic compounds (Plaza and Turner, 2015; Tekin et al., 2014). At room temperature and atmospheric pressure, water has a notably high dielectric constant (ε) due to the hydrogen bonds within the structure. Raising the temperature of water above room temperature, its surface tension and permittivity decreases, which in turn increases its rate of diffusion (Fuentes-Azcatl and Alejandre, 2014; Mao et al., 2020). With enough pressure to maintain the water as a liquid at raised temperatures, the dielectric constant of water decreases to 27. This value is between that of ethanol and methanol so under these conditions, water behaves like an organic solvent, with the ability to dissolve low to medium polar analytes (Cvjetko Bubalo et al., 2015; Shitu et al.,2015; Yang et al., 2018). Figure 2.8 depicts the working principle and the extraction mechanism of a laboratory Pressurized Hot Water Extraction system.

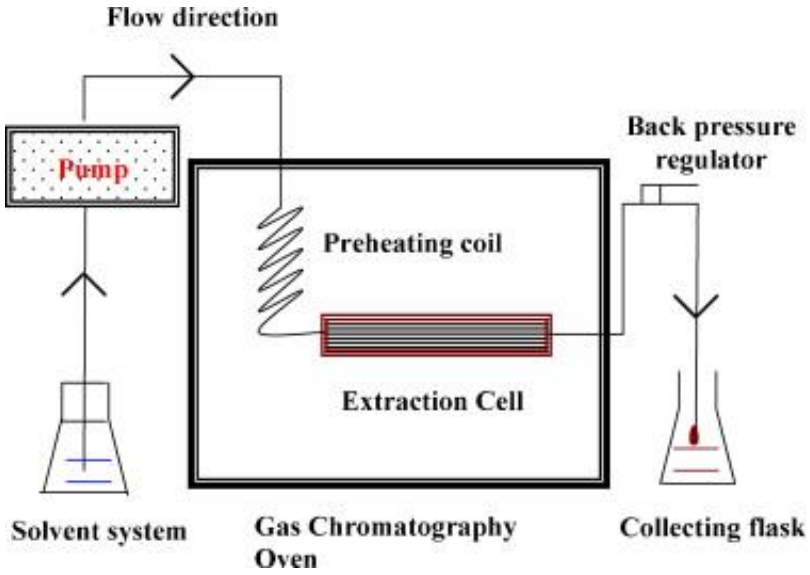


Figure 2.8 A schematic diagram of a laboratory PHWE system (Teo et al., 2010)

As seen in the figure, the extraction takes place in four sequential steps. The extraction takes place in an extraction cell that is filled with the sample and a high portion of sand material. The first step is called desorption. It involves the release of the solutes from the active surface of the sample matrix under the elevated temperature and pressure conditions. The extraction fluid then diffuses into the matrix and the solutes may partition themselves from the matrix into the extraction fluid to be eluted out of the extraction cell to the collection vial (Kronholm *et al.*, 2007; Ong *et al.*, 2006).

For the analysis of pesticides such as some Endocrine Disrupting chemicals, Concha-Graña et al. (2010) reported the Pressurized Hot Water Extraction of organochlorine pesticides in sediments. They further studied the parameters that affects the efficiency of extraction, such as organic modifier, percentage of organic modifier, temperature, and static extraction time. Results show that quantitative recoveries ranging from 80–115% and satisfactory precisions were obtained. The detection and quantification limits were between 0.11 and 16 $\mu g\ kg^{-1}$ and between 0.24 and 22 $\mu g\ kg^{-1}$, respectively with good linearity between LOQs.

2.9.4 Soxhlet extraction method

Soxhlet extraction is typically used when the sample has a limited solubility in the solvent while the impurities remain insoluble in the solvent (Kašpárková et al., 2016; Lim et al., 2020). The technique allows for an unmonitored and unmanaged operation while recycling a small amount of solvent in order to dissolve a large amount of sample (Russo et al., 2015; Supakot et al., 2015). The technique has been used for over a century as a new extraction technique for solid sample preparations.

The conventional Soxhlet type was originally implemented to determine fat in milk (Ellefson, 2017). Using this technique, the sample is placed in a thimble holder that is gradually filled with condensed fresh solvent from a distillation flask, as seen in figure 2.9.

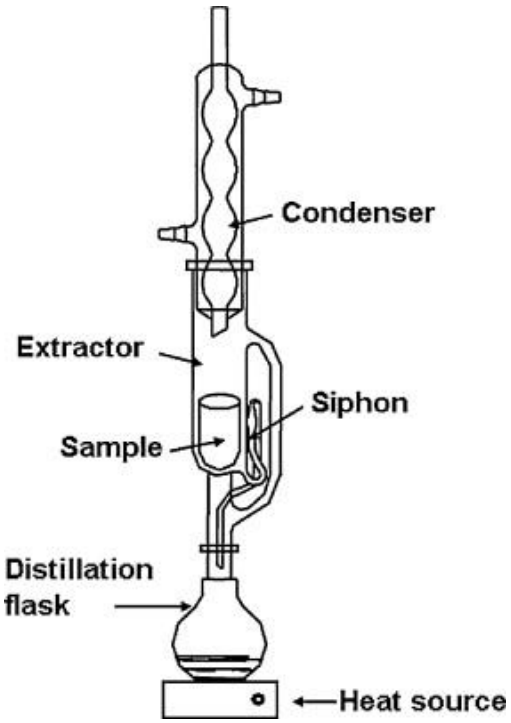


Figure 2.9 A conventional Soxhlet extraction set-up (de Castro and Priego-Capote., 2010).

When the solvent reaches the overflow level, a siphon repeatedly loads the solute from the thimble-holder and then offloads it back into the distillation flask thus carrying the extracted analytes into the bulk liquid until the extraction is complete (Heleno et al., 2016; Russo et al., 2015; Supakot et al., 2015). There are some advantages associated with the conventional

Soxhlet extraction. Since the sample is repeatedly bought into contact with fresh solvent, it enables the displacement of the transfer equilibrium. The distillation flask is heated and as a result, the high temperature enables the system to reach the extraction cavity. To add, no filtration is required, and multiple simultaneous extraction can be possible using one low-cost setup, which increases sample quantity and yield (Pandey and Tripathi, 2014a; Teixeira et al., 2018; Zhang et al., 2018b). There are also some disadvantages associated with Soxhlet extraction as compared to other techniques for solid sample preparation. The most serious is long extraction time and large amount of solvent wasted, which carries expensive disposal and negative environmental effects. The rate of extraction is not enhanced due to the technique providing no agitation, which would assist greatly to accelerate the extraction process. Lastly, no automation is possible as the Soxhlet technique is limited by solvent (Pandey and Tripathi, 2014; Subramanian et al., 2016).

For the application of organochlorine pesticides in plants, Barriada-Pereira et al. (2003) compared microwave-assisted extraction and Soxhlet extraction for twenty-one organochlorine pesticides. The pesticides were eluted with hexane–ethyl acetate (80:20, v / v) and determined by GC-ECD. Results show that the recoveries were obtained in the range 75.5–132.7% for Soxhlet extraction and 81.5–108.4% for MAE. This concludes that both methods are suitable for the determination of chlorinated pesticides in vegetation samples.

2.9.5 Ultrasound-Assisted Extraction method (UAE)

Ultrasonic extraction is a rapid yet effective technique that has been utilized for extraction of phytochemical compounds by various companies including food and pharmaceutical areas (Vieira da Silva et al., 2016; Zabot et al., 2021). An ultrasound is sound wave that has frequencies above the audible limit of human hearing and requires an elastic medium for its propagation (Dal Prá et al., 2017). The extraction is based on the difference between the physical and chemical properties of the sample matrix after their interactions with the ultrasound waves (Tomšik et al., 2016). Cavitation is the fast formation and possible collapse of millions of bubbles in a liquid and it is this cavitation effect that eases the extraction

process and thus increases the mass transfer between the sample and the solvent by disrupting the plant cell walls (Zhang et al., 2017).

To add, parameters that affect the rate of UAE process are frequency, temperature, solvent and the power of the ultrasound (Albuquerque et al., 2018). These factors affect the extraction yield. For instance, increasing the temperature to a controllable amount will speed up the diffusion process of the solvent to the sample and in turn increase its solubility. A further increase will likely degrade the sample (Giacometti et al., 2018). An increase in the power of the ultrasound increases the cavitation effect (Irakli et al.,2018).
Organochlorine pesticides, like Endocrine Disrupting Chemicals have been extracted before using UAE methods with promising results. Tor *et al.*, (2006) reported the ultrasonic solvent extraction of organochlorine pesticides such as $\alpha, \beta, \gamma \; and \; \Delta - BHC,$ aldrin, $4,4' - DDE, 4,4' - DDD \; and \; 4,4' - DDT$ extracted from soil. The determination of the presence and quantity of the pesticides was done using GC-ECD and recoveries of the pesticides were well over 88% for three different fortification levels between 15 and 200 $\mu g \; Kg^{-1}$ with standards deviation less than 6%.

2.9.6 Pressurised fluid extraction (PFE)

Also known as accelerated solvent extraction or pressurized liquid extraction, this technique has been used to extract extracts from solid samples for the past sixty years, with modifications throughout the years (Lefebvre et al., 2020; Oliveira et al., 2014). The technique uses an organic solvent and operates on high temperature and pressure (Dean, 2000). Together with extraction time, these are the four factors that affect the efficiency of the extraction using pressurized fluid extraction (Björklund et al.,2000). Temperature is an essential factor which account for the improved recoveries in several extraction techniques such as SFE and MAE. Richter *et al.*, (1996) reported an increased recovery of polychlorinated biphenyls and poly-aromatic hydrocarbons in sediments at higher temperatures. They further reported that at higher temperatures, the organic solvent has increased capacity to dissolve analytes and increase diffusion rates for extraction. Some

researchers also reported the elevated temperature to improve mass transfer of the analytes, which in turn increases its recovery. To add, the surface tension and viscosity are decreased with increased temperature, which leads to improved matric penetration (Richter et al., 1996).

Increased pressures used in pressurized liquid extraction is used to keep the solvent from boiling but plays little to no effect in recoveries of the target analytes (Andreu and Picó, 2019; Hoff and Pizzolato, 2018). Pressure mainly plays an essential role for sample with small particle size, as it increases the fill time of the solvent into the cells. To ensure that the maximum analyte is extracted, new solvent is poured into the extraction chamber to produce an uneven distribution of solute in the solution by increasing the rate of diffusion (Alara et al., 2021)

Figure 2.10 summarizes the process of pressurized liquid extraction. The sample is placed vertically in the extraction cell and the organic solvent filled into the cell through a solvent tube. The extraction cell is then heated to a temperature of $150℃\ to\ 200℃$ with a pressurized to a set value for 5 to 10 mins. This is called a pre-extraction step. The temperature and pressure set are kept at their specified values by electronical heaters and pumps. This is called a static extraction step. The solvent is the collected in a vial and then new solvent poured into the cell to remove any analyte left in the cell. Nitrogen gas is then purged onto the cell to remove solvent left in the extraction cell (Dierkes et al., 2019; Hirondart et al., 2020; Subedi et al., 2015; Xynos et al., 2014).

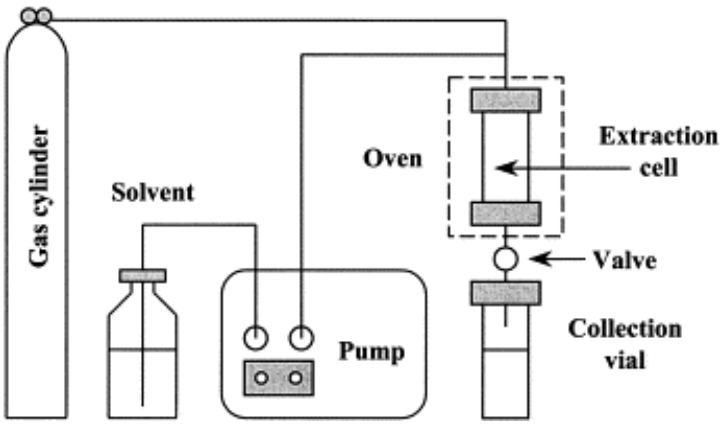


Figure 2.10 A schematic diagram of a basic PLE setup (Bjorklund and Nilsson, 2000).

Prior to loading the sample into the extraction cell, the sample is often dried either by plain drying, which takes about 24 to 48 hours, or freeze dried to remove moisture as water in the matrix may reduce extraction efficiency and sometimes sodium sulphate is added to the sample to compensate high amount of water (Laskin and Lanekoff, 2016). The drying step is often followed by sieving or grinding for small and uniform particle sizes.

2.10 Analysis of EDCs

2.10.1 HPLC-MS analysis of EDCs

High-Performance Liquid Chromatography (HPLC), also called liquid chromatography is the most applied separation method for complex matrices such as proteins and plant-based materials (Huang et al., 2004). Its instrumentation is usually made of a pump, an injector, a column, a detector, and a data acquisition system (Johnson et al., 2000) (As seen in Figure 2.12). In the HPLC, the mobile phase consists of a single solvent or a mixture of solvents in which the pH is regularly modified with acids, bases or buffer solutions depending on the analyte to be isolated (Motilva et al., 2013). The mobile phase moves through the column with the help of the pump.

The stationary phase is packed in the column and its physical and chemical properties are important to predict further separation (Rodríguez-Pérez et al., 2015). Normal phase HPLC separates analytes based on polarity and the interactions between the analyte and the stationary phase is purely adsorptive (West and Lemasson, 2019). This type uses polar stationary phase (amino, cyano or silica) and a non-polar mobile phase (e.g., dichloromethane). It is used when an analyte of interest is relatively polar. Reverse phase chromatography consists of a non-polar stationary phase (C_{18}, C_8 etc.) and a polar mobile phase (methanol – water mixture) (Olsson et al., 2014). It is used when an analyte of interest is non-polar. Reverse phase operates on the principle of hydrophobic interactions which results from the repulsive forces between a relatively polar solvent, the relatively nonpolar analyte, and the non-polar stationary phase (Huang et al., 2006).

There are many types of detectors that can be used in HPLC. Some of the more common detectors include refractive index, ultra-violet, fluorescent, radiochemical, electrochemical,

near-infra red, mass spectroscopy, nuclear magnetic resonance, and light scattering (Swartz, 2010). Mass spectrometry detectors ionize a sample and use a mass analyzer to detect the ion current (Seger and Stuppner, 2013). Figure 2.11 shoes a chematic diagram for HLPC-MS. Nuclear magnetic resonance detectors irradiate nuclei that are placed between the poles of a strong magnet. The radiation is absorbed, the parallel nuclei enter a higher energy state, and each atom produces a spectrum specific to its location and chemical composition (Lindon et al., 2000). The most utilized detector in HPLC work is UV-VIS (Herzallah, 2009). UV absorption detectors respond to those substances that absorb light in the range 180 to 900 nm. Three examples of UV detectors are fixed wavelength, which measures at one wavelength, usually 254 nm, variable wavelength which measures at one wavelength at a time but can detect over a wide range of wavelengths and diode array which measures a spectrum of wavelengths simultaneously. The diode array has an advantage in that it is used for further identification. The UV spectrum of any compound is unique though compounds of the same family tend to have similar spectrum (Bechtel et al., 2005; Gülfen et al., 2020). These can be differentiated based on retention time (Seeley, 2007).

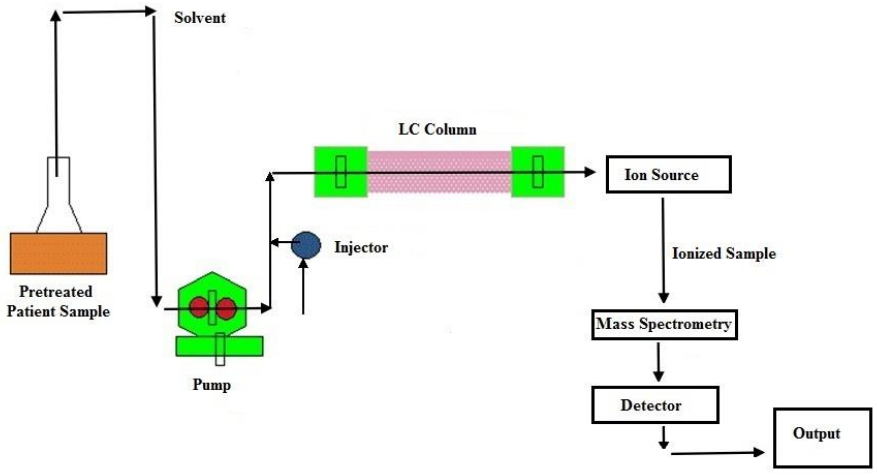


Figure 2.11 Schematic diagram of HPLS-MS working set-up.

Figure 2.12 Laboratory HPLC set-up

Work done by Villar-Navarro et al. (2013) reported the HPLC determination of two Endocrine Disrupting Chemicals (n-octylphenol and n-nonylphenol) in environmental samples using fluorescence detection. The extraction was carried out through a dihexyl ether liquid membrane and optimum pH and extraction time was determined. The method was successfully applied for the determination of these EDCs in environmental water samples. For fish samples, Jakimska et al. (2013) reported the development of HPLC-MS procedure for the determination of nineteen Endocrine Disrupting Chemicals in various classes of fish species from four impacted Mediterranean rivers. This was done using the QuEChERS approach and the matrix effect on the analytical performance was evaluated. Eleven out of nineteen target EDCs were found present at least once in each fish. Among the rest, bisphenol A and methyl and benzyl paraben which are potential EDCs were detected in high concentrations. The procedure provided satisfactory recoveries in range from 40% to 103% with accuracy <20%. HPLC-MS (Figure 2.11) is the best approach when looking at speed, efficiency and accuracy. It uses a pump rather than gravity to force a liquid solvent through a solid adsorbent material (Ganzera and Sturm, 2018; Timofeeva et al., 2017).

2.10.2 Gas Chromatography analysis of EDCs

This it suited for compounds that can be volatized such as most non-polar compounds and medium polar compounds. Polar compounds like most pharmaceuticals are not suited for GC analysis unless are derivatized to make them volatile (Schummer *et al.*, 2009).

In gas chromatography, gaseous analytes are transported through the column by a gaseous and inert mobile phase, called carrier gas (Al-Rubaye et al.,2017). The carrier gas is hydrogen, helium or nitrogen depending on the sensitivity of detection.

There are various detectors used in gas chromatography such as Flame ionization detector, electron capture detector, thermal conductivity detector, mass spectrometry detector. Mass spectrometry is the most popular because it is sensitive and identification is accurate (Miller et al., 2008).

The flame ionization detector (FID) is used to measure concentrations of hydrocarbons within a sampled gas. The presence of hydrocarbons is detectable by burning the sample gas in an air-hydrogen flame (Nwoko et al., 2019; Pan et al., 2017). The presence of hydrocarbons in the sample, when burnt with an air-hydrogen mix causes high levels of ionization. The ionization occurs because of the carbon atoms present in the sampled gas (Bertuzzi et al., 2018; Spanjers et al., 2017). The level of ionisation is proportional to the number of carbon atoms within the sample .This relationship is used for the quantification by running standard samples. Figure 2.13 shows a set up of a basic laboratory GC instrument with manual injection using ECD/FID detection.

Figure 2.13 Laboratory GC-ECD/FID

GC-MS had been applied to real samples. Yu et al. (2015) determined the occurrence of thirteen EDCs in fish muscles and bile in Californian lake. Seven of the thirteen EDCs were detected with total concentrations of up to 39 $ng.L^{-1}$ with recoveries ranging from 60% to 91%. He further found that the level of bisphenol A from fish diet is unlikely to pose a health risk as its mean concentration was 7.26 $ng.g^{-1}$. Schillirò et al. (2011) investigated the endocrine disrupting activity in forty-four fruits and vegetable samples of which ten did not contain any EDC residues. The other thirty-fur samples had EDC concentrations ranging from 0.03 to 1.91 ppm. The study further suggested that the calculated intake of dietary EDCs might represent the concentration comparable to that of the normal estrogen levels found in human blood. Another study by Jeannot et al. (2002) focused on the determination of EDCs in environmental samples using GC-MS. It is interesting to note that levels of bisphenol A, 4-nonylohenol and 4-octylphenol among other EDCs were determined with recoveries more than 60%, which are also target EDCs in this study. This was done using C_{18} sorbents. Quantification limits for the target EDCs were at ppb levels when using HPLC-MS and further reached ppt levels when using GC-MS. Figure 2.14 shows the main components of a GC-MS system.

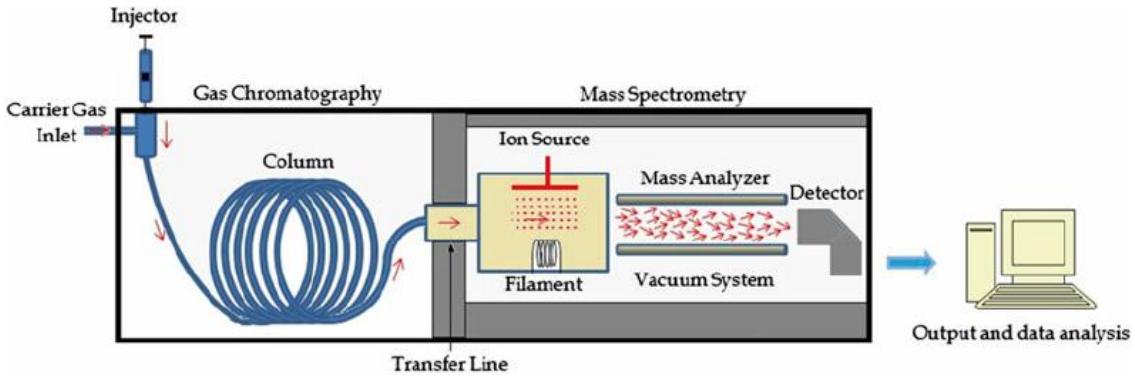


Figure 2.14 GC-MS schematic diagram (Emwas et al., 2015)

A recent development to GC-MS is a method where a sample mixture is first separated by gas chromatography then the sample ions with different masses are accelerated to the same (known) kinetic energy and the time taken for each ion to reach a detector at a known distance is measured. This is called GCxGC TOF-MS (Walters et al., 2018; X. Zeng et al., 2020). The time is dependent on the mass-to-charge ratio of the ion. GC × GC MSTOF (Figure 2.16) offers a solution to the co-elution problem and provides high sensitivity and selectivity. In principle, the method consists of two GC systems (GC × GC) equipped with columns of different polarity connected by an interface with an integrated cryogenic trap (Abdulhussain et al., 2021). The cryogenic trap repeatedly condenses compounds eluting from the primary column and releases them periodically as short pulses to the secondary column. Parameters like duration and frequency of both condensation and injection pulses are variable and allow precise tuning of the instrument according to the requirements of the analysis. Since the GC × GC produces very narrow peaks (down to 50 ms, depending on the frequency of cryogenic modulation) a TOFMS detector with a high acquisition rate (up to 500 spectra per second) is required (Christensen et al., 2018; Mahdavi, 2017). The pulsed nature of the TOFMS source of ionisation further enhances the system accuracy by avoiding spectral skewing common in a continuous ionisation mode. GC × GC with TOFMS detection thus operates with a high precision independent of concentration range (Cecchi et al., 2020; Kalinová et al., 2006, 2006; Lebedev et al., 2018; Stefanuto et al., 2021; L. Wang et al., 2019; X. Zeng et al., 2020b)

Figure 2.15 Laboratory GCxGC TOF-MS

2.11 Heavy trace metals: Inorganic Endocrine Disrupting Chemicals

2.11.1 Definitions and overview

Heavy metals like Hg are of interest due to their bioaccumulation tendencies in organisms (Ali et al., 2019; Hao et al., 2019). They have toxic effects even in trace amounts and can cause harmful effects on human health (Nuapia et al., 2018). Example of this is methyl mercury, which is an organo form of mercury (Ralston et al., 2010). Pb is a neuron toxin, acts on the brain and can delay intellectual developments in children, by causing an injury to the brain cells (Tong et al., 2000). Cd affects the kidneys and can damage some cells, potentially leading to cancer (Genchi et al., 2020). It is of necessity to assess the levels of these metals in raw food items like fruits, vegetables, beef, and fish in order to eventually protect human health and well-being.

Toxicity by heavy metals in food can be lethal to consumers. Majority of the metals are of a health concern due to their toxicity properties, but some other metals are essential to the health and well-being of humans and animals (Ore and Adeola, 2021). Heavy metals are defined generally, as metals that have relatively high densities and or atomic weights. However, the criteria used for defining heavy metals vary depending on the context of each author and whether or not metalloids are included. Examples of heavy metals include mercury (Hg),), cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb) and Copper (Cu).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed (Vijaya et al., 2020). To a small extent they enter our bodies via food, drinking water and air(Mishra et al., 2009). As trace elements, some heavy metals (e.g. copper, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g., lead pipes), high ambient air concentrations near emission sources, or intake via the food chain (Cannas et al., 2020; Huseen and Mohammed, 2019).

As said, heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment (Ali and Khan, 2019; Aniţaş et al., 2020). Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. The accumulation of heavy metals (e.g

(Mercury (Hg), Arsenic (As), Lead (Pb), Aluminium (Al), Copper (Cu), Iron (Fe), Cadmium (Cd), manganese (Mn) and Zinc (Fe)) in humans can cause adverse effects , both short and long term. This can happen by affecting the metabolic activities, thus causing homeostatic changes within the body (Chakraborty, 2021; González-Casanova et al., 2020; Sabir, et al., 2019; Sargis et al., 2019; Velmurugan et al., 2017; Vieira et al., 2021). They perform this action by mimicking the normal binding of different hormones in the body, thus complicating the different body signals, while causing an identical cellular response from the host as normal binding (Soto et al., 2010). Some are reported to discontinue the production of the bodily hormones and thus prevent any cellular response (Newbold et al., 2007; Heindel et al., 2015). Chronic exposure to metals increases the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-),which eventually leads to oxidative stress of the cell, finally cell death (Atli and Canli, 2010; Mittler, 2002). Metals like aluminum (Al), cadmium (Cd), copper (Cu), and lead (Pb), are classified as metalloestrogens due to their ability to interfere in the action of estrogenic hormones (Safe, 2003).

Heavy metals can make their way into water supply by acid rain which breaks down soils thus releasing series of heavy metals into streams and groundwater (Sikdar and Kundu, 2018; Wei et al., 2019). Figure 2.16 depicted shows the effect and toxicity mechanisms of heavy metals after they make their way into streams, thus affecting algal growth. Heavy metals exert toxic effect on algae at both cellular and molecular levels (Priyadarshini et al., 2019). An inverse relationship between concentration of metal and microbial growth rate is well reported (Priyadarshini et al., 2019; Wei et al., 2019). Ochari (1997) postulated that the toxic effect of metal ions on microorganism is exerted by three different mechanisms like (i) blocking the functional groups of biomolecules (enzymes and proteins), (ii) displacement of the essential metal ions by the toxic metal, and (iii) modification/alteration of the biomolecule conformation leading to alteration in its activity

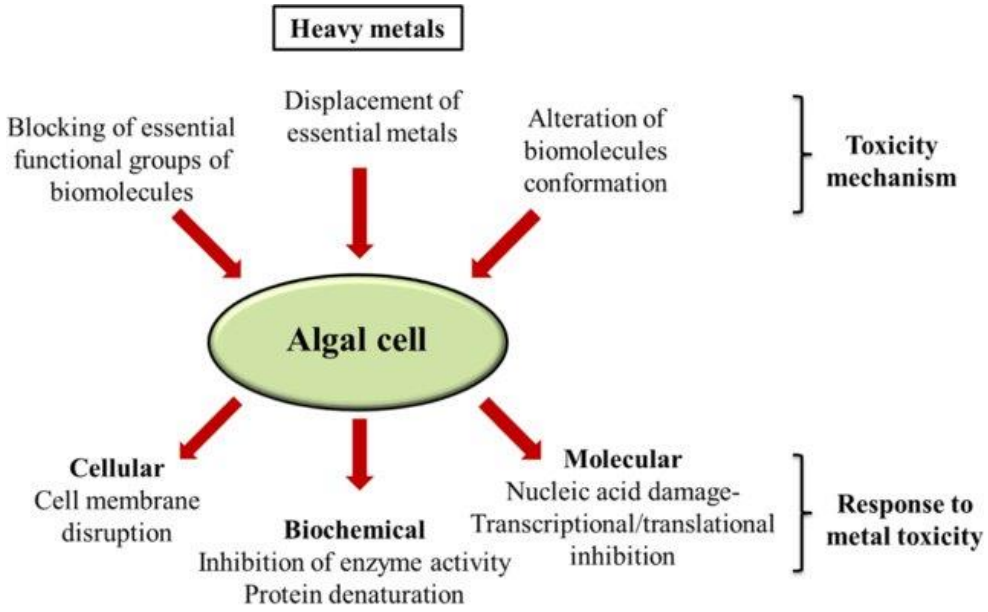


Figure 2.16 The toxic effect of heavy metals and consequent tolerance mechanism adapted by the algal system (Priyadarshini et al., 2019)

To add, the schematic diagram also shows that the algal system adapts strategies to survive in presence of metals. Some of the basic means include binding of metal ions to the high affinity functional groups, accumulation of metals in cell organelles, metal complication with different microbial biomolecules, and active efflux or exclusion of metals (P. Sharma et al. 2021). Table 2.4 shows different health efects by heavy metals exposure over the years.

Table 2.4 Heavy metals effects

Year		Effects
1932	Minamata	Sewage containing mercury is released by Chisso's chemicals works into Minamata Bay in Japan. The mercury accumulates in sea creatures, leading eventually to mercury poisoning in the population.
1952	Minamata Syndrome	In 1952, the first incidents of mercury poisoning appear in the population of Minamata Bay in Japan, caused by consumption of fish polluted with mercury, bringing over 500 fatalities. Since then,

		Japan has had the strictest environmental laws in the industrialised world.
1986-11-01	Sandoz	Water used to extinguish a major fire carries c. 30 fungicide containing mercury into the Upper Rhine. Fish are killed over a stretch of 100 km. The shock drives many FEA projects forwards. See also "Pollution of the Rhine at Basel / Sandoz".
1998-04	Spanish nature reserve contaminated after environmental disaster	Toxic chemicals in water from a burst dam belonging to a mine contaminate the Coto de Donana nature reserve in southern Spain. C. 5 million m_ of mud containing sulphur, lead, copper, zinc, and cadmium flow down the Rio Guadimar. Experts estimate that Europe's largest bird sanctuary, as well as Spain's agriculture and fisheries, will suffer permanent damage from the pollution.

2.11.2 Estimated daily intake (EDI) and target health quotients (THQ)

Estimated daily intake (EDI) of heavy metals is estimated based on the heavy metal concentration, the food consumption rate and body weight of an individual of various age groups. This can be done for fish, beef, and cabbage. Comparison is then possible with limits set by various legislatures. Table 2.5 below shows the food consumption rates of the samples under investigation(Cano-Sancho et al. 2010).

Table 2.5 Food consumption rates in South Africa

Sample	Consumption rate ($g\ day^{-1} person^{-1}$)
Fish	20.90
Beef	77.90
Cabbage	95.00

The carcinogenic risk (having the potential to cause cancer) and non-carcinogenic risks of the heavy metals in selected fruits and vegetables sold in South African open markets in adults and children age groups consumers can also be estimated. The non-carcinogenic risk is calculated using the Target hazard quotient (THQ) (Fathabad et al., 2018; Huang et al., 2016). The THQ is given by equation 1 below:

$$THQ = \frac{CDI}{RfD}$$

Where:

- CDI (chronic daily intake) is the exposure expressed as amount of heavy metal contacted per unit body weight per unit time, averaged over a long period of time
- RfD is the oral reference dose of the heavy metals ($mg\ kg^{-1}\ day^{-1}$) when exposed orally.

RfDs for target metals are given in table 2.6

Table 2.6 Oral reference dose for selected heavy metals

Metal	RfD – oral reference dose ($mg\ kg^{-1}\ day^{-1}$)	Author/s
Al	1.00E00	Risk assessment information system (accessed 13/05/21)
As	3.00E-04	(Department of Environmental Affairs (DEA) 2010; De Miguel et al. 2007)
Cd	5.00E-04	(Department of Environmental Affairs (DEA) 2010)d
Cr	3.00E-03	(USEPA 2007)
Cu	3.7.00E-02	(Department of Environmental Affairs (DEA) 2010; Ibrahim, Kana, and Abdullahiauta 2018)
Fe	7.00E-3	USEPA IRIS(2011)
Hg	3.00E-04	(Department of Environmental Affairs (DEA) 2010)
Mn	0.14	(Kamunda, Mathuthu, and Madhuku 2016)
Pb	3.60E-03	(Department of Environmental Affairs (DEA) 2010; Luo et al. 2012)
Se		
Zn	3.00E-01	(Department of Environmental Affairs (DEA) 2010; Ibrahim et al. 2018)

2.12 Metal analysis instrumentation

2.12.1 The Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

The Inductively Coupled Plasma Mass Spectrometer (ICP-MS)(Figure 2.17 shows a common laboratory ICP-MS setup) has been used for elemental analysis and metal speciation (Snyder et al., 2003). ICP-MS parameters such as ion optics voltages, mass scan, time scan, pump speed, argon flow need to be optimized for a better resolution and analyte-background intensity ratio (Satyanarayanan et al., 2018; Williams and Marcus, 2020)

Figure 2.17 Laboratory ICP-MS used in this study

Snyder et al. (2003) used ICP-MS to measure metalloid EDCs in water, with Hg and Cr being the primary target metals. It showed good sensitivity at low concentration levels and with high selectivity. According to Georgescu et al. (2011), heavy metals like Hg act as endocrine disruptors, with effects being the induction of alterations in male and female fertility and disrupt biosynthesis of steroid hormones.

2.12.2 Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES)

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) technique has been applied to the analysis of agricultural and food materials including soils, fertilizers, plant materials and food (Borges et al. 2020; Khan et al. 2021). This includes determination of major, minor and traces metals. A challenge associated with metal analysis by ICP-OES is the potential for spectral interferences since many metals have very complex emission spectra (Boss and Fredeen, 2004). Figure 2.18 shows the set up on ICP-OES in the laboratory.

Figure 2.18 Spectro Genesis ICP-OES laboratory set-up for the study

Table 2.7 shows concentrations of seventeen elements in plant leaves (*Almus glutinosa*), collected in 2002. The elements in **bold** were determined using ICP-OES and the rest with ICP-MS. The results suggested that only environmental pollution affected the elemental levels in the sample, with no history of exposure to pollution beyond the environment they were collected in. The study was done by Weltjie and Oehlmann (2002) from the Goethe University.

Table 2.7 Concentrations ($mg\ kg^{-1}$ dry wt) of seventeen elements in selected leaf samples (Weltjie and Oehlmann, 2002).

Element	Concentrations ($mg.kg^{-1}dry\ wt$)	Relative standard deviation (%)
Cr	7.49	0.59
Ni	5.69	0.56
Cu	10.3	0.19
Zn	85.8	0.89
As	2.46	2.56
Cd	0.129	0.42
Pb	5.04	1.04
Co	2.56	0.38
Tl	0.0506	1.77
Mn	1.466	0.85
Ba	64.0	1.38
Fe	***1.690***	***0.83***

Zn	***85.5***	***1.13***
Al	***2.320***	***2.00***
K	***4.070***	***1.18***
Mg	***1.830***	***1.87***
Ca	***18.700***	***0.35***
Ba	***61.4***	***1.40***
Ti	***93/5***	***3.06***

2.13 Moringa Oleifera seed protein bio sorbent

The use of *Moringa Oleifera* protein seeds (Figure 2.19) as a natural coagulant is of great interest for low-cost pollutant removal (Yamaguchi et al., 2021). Traditionally, aluminum and amine salts like the common primary secondary amine (PSA) are the commonly used coagulants in pollutant removal, especially water treatment (Boonchum et al., 2011). These plants are indigenous to North-western India, and they are largely valued for their tender pods, which are often consumed as vegetables (Pandey et al., 2011). The paste derived from the Moringa leaves can be used as an external application for wound healing, and the leaves can also be pressed to release a juice which has shown some antibacterial activity (Rathi et al., 2006). *Moringa Oleifera* comprises of many natural antioxidants, including Vitamin C, flavonoids and phenolic compounds (Makita et al., 2016; Pakade et al., 2012). The flavonoid and phenolic content is reported to be directly related to the antioxidant activity of these proteins, which has led to an increasing cultivation of these plants in their native communities (Pakadeet al., 2012). The *Moringa Oleifera* protein exert their antioxidant activities as they readily provide a source of hydrogen from the ortho-substituted hydroxyl group, which scavenges a free radical peroxide in the pollutant, and converts it to hydrogen peroxide and oxygen (Pakade et al.,2012). Testing this natural coagulant as a clean-up sorbent for the QuEChERS method in developing countries like South Africa could effectively elevate the economic status and as a long-term application, it can allow further extension of water supply to rural areas.

Figure 2.19 Moringa Oleifera (M.O) seeds

A study done by Latif et. al. (2011) evaluated five commercial enzyme mixtures for protein extraction recovery from Moringa seeds using enzyme-assisted aqueous extraction technique. Hexane was the chosen solvent for the oil extraction as it will be done in this study. Their results show very high recovery of the protein in the aqueous phase which can be employed in the application of food items.

A similar study carried out by Yarahmadi et al. (2009) compared the turbidity removal efficiency between Moringa Oleifera seed protein and polyaluminium chloride using a synthetic kaolinite. Results shows that the seed protein is more efficient in removing water turbidity. Furthermore, it shows that polyaluminium chloride required lower pH to remove the water turbidity efficiently.

(Iliyasu, 2020) studied the characterization and properties of *Moringa Oleifera* seed powder, including the recovery of the extracted protein. Results shows that the extracted protein is major component of the seed, with a recovery of 44.5%. The rest of the composition was oil, fats and ash. As a result, the protein was proved to be an effective clean up sorbent in water treatment as well as recovery of precious metals.

2.14 Freeze-dying as a sample drying technique for the analysis of EDC's

Freeze-drying refers to a dehydration process using low temperatures, which involves freezing the sample, lowering the pressure, and thus removing the ice in the sample by sublimation

process (Bhatta et al., 2020; Oyinloye and Yoon, 2020; Silva-Espinoza et al., 2021). Controlled freeze-drying process allows the sample to dry without changing the characteristics and structure of the dried sample (Nowak and Jakubczyk, 2020). Sublimation refers to a direct phase change of a solid (ice) to a vapor without first changing to a liquid state. This involves freezing, vacuum and drying. The sample is completely frozen in a suitable container or glass vial, flask or tray. The sample is then placed in deep vacuum, well below the triple point of water, and finally dried by applying heat energy to the sample causing the ice to sublime (Hriberšek et al., 2018; Lenaerts et al., 2019; Zhao et al., 2020). Figure 2.20 depicts the process of the freeze-drying process.

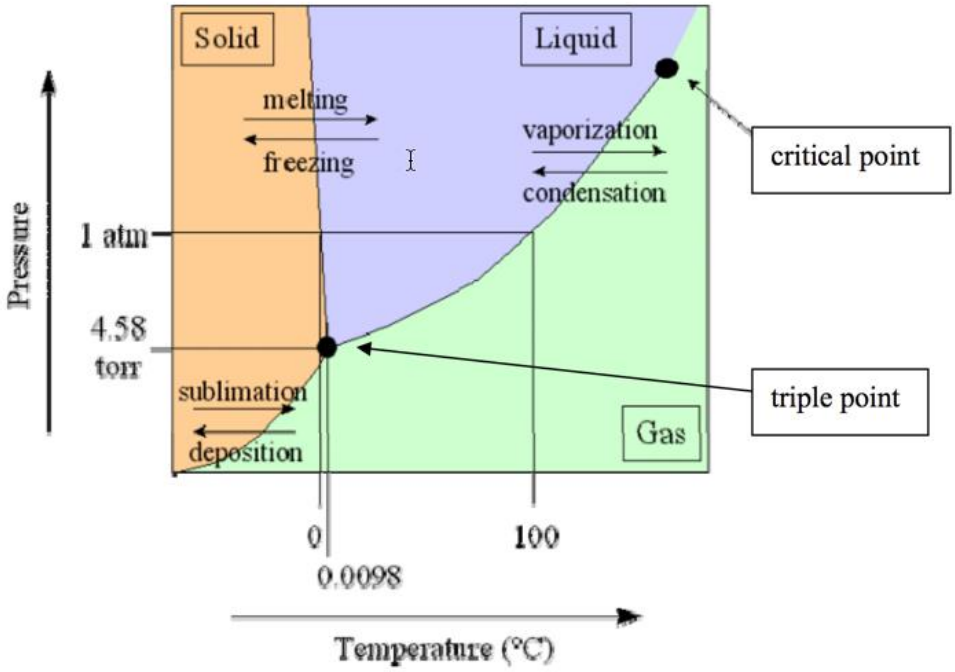


Figure 2.20 Basic principle of freeze-drying

The steps required to freeze-dry a sample (Figure 2.21) is summarized as follows:

i. Pre-treatment of the sample. Sample is pre-frozen before loading and the maximum cooling achieved with the doors closed. Sample temperature is monitored with probes.

ii. Loading of the sample into a suitable Container (Bulk, Flask, Vials).

iii. Primary Drying (Sublimation) under vacuum. The collector temperature is lower than -40 ℃

iv. Secondary Drying (Desorption) under vacuum

v. Backfill & Stoppering (for product in vials) under partial vacuum

vi. Removal of Dried Product from Freeze Dryer

Figure 2. 21 LABCONCO Freeze- drier (LABCONCO CORP. 811 Kansas City, Missouri)

CHAPTER 3: OBJECTIVES OF THE STUDY

Organic and inorganic Endocrine Disrupting Chemicals are receiving attention now as they are among the most detected pesticides in foodstuff. The chapter outlines the specific steps and objectives completed to achieve the main research aim of the study.

3.1 General objective

Endocrine Disrupting Chemicals like some organochlorine pesticides are still marketed and used today in South Africa in crops protection against insects and pests, despite their ban years ago (Plaza et al.,2019). South Africa also a developing country, thus the use of non-edible biomass for analytical processes can potentially offer a cheaper advantage over the current commercial ones.

The project aims to develop and optimize QuEChERS extraction technique to quantify the presence and concentration of EDCs in selected open market food sold in South Africa using RSM chemomemtric aproach. The secondary aim to compare PSA and M.O protein as a clean up sorbent for QuEChERS.

3.2 Specific objectives

The general objective (section 3.1) was achieved on completion of the specific objectives, which was to:

i. Optimize the various parameters that affect the QuEChERS method for the extraction of EDCs from fruits, vegetables, beef and fish. This was done with the use of a Response Surface Methodology chemometric.

ii. Test and compare the performance of various QuEChERS clean-up sorbents like *Moringa Oleifera* seed protein, PSA, Hemp cellulose, C18 and activated carbon.

iii. Apply the developed QuEChERS method to determine and quantify the organic Endocrine Disrupting Chemicals (Dimethyl phthalate, Diethyl phthalate, 4-n-Nonylphenol, 4,4'-DDD, 4,4'-DDE and 4,4'-DDT) in thirty-eight samples including vegetables (spinach, cabbage, carrots, potatoes, onion, tomato, and banana, red, yellow and green pepper, Scallion), fish and meat (Bovine beef muscle and chicken liver) sold in open markets, main markets and farms in Johannesburg and Pretoria.

iv. Apply a microwave digestion program to extract inorganic Endocrine Disrupting Chemicals (Metals) in the same thirty-eight foodstuffs sold on open markets, main markets and farms in Johannesburg and Pretoria (South Africa).

v. Compare the levels of the target endocrine disrupting chemicals in Johannesburg and Pretoria.

3.3 Hypothesis and Research Question

3.3.1 Hypothesis

i. The fruits and vegetables sold in Johannesburg and Pretoria local markets are contaminated with EDCs due to excessive operation on busy roads and unsanitary environments, which make it easier for the chemicals and pollutants make their way into the foodstuff sold there.

ii. The fish sold in the markets are hosts for 4,4'- DDT, 4,4'-DDE and 4,4'-DDD due to bioaccumulation and bioconcentration tendencies the EDCs in the aquatic environment.

iii. *Moringa Oleifera* seed protein bio sorbent will have a high cleaning potential due to its structural functional groups and chemistry.

3.3.2 Research questions

i. Does *Moringa Oleifera* bio sorbent really have better selectivity than PSA sorbent?

ii. Can the selectivity in QuEChERS method be improved by using different cleaning sorbents?

iii. Are South African consumers at risk following ingestion of fruits and vegetables from open markets?

iv. Is chemometric approach faster than manual method development and optimisation?

3.4 Novelty and impact

Recent developments like the use of chemometrics and new clean-up sorbents account for the novelty of this research. No identical analysis has been successfully reported to attempt to use *M.O* protein as a QuEChERS clean-up bio sorbent. Also, no comprehensive work was dedicated to analyzing the presence and quantity of Endocrine Disrupting Chemicals in South African open markets. A study published by Nuapia et al. (2016) assessed the presence of organochlorine pesticides and metals in raw food items in South African markets. However, they used a commercial PSA strictly as the clean-up sorbent. In another study by Ramalhosa *et al.* (2009) looked at QuEChERS-extracted Polycyclic Aromatic Hydrocarbons (PAHs) analyzed using gas chromatography in fish but with limited samples.

Therefore, it is sensible to modify and optimize the existing accepted QuEChERS method to minimize its cost and improve its effectiveness with a biobased sorbent.

CHAPTER 4: MATERIALS AND METHODS

This chapter reviews the methodologies involved to complete the research aims. Response Surface Methodology (RSM) that was used to understand the effect of the parameters for screening and optimization studies are also explained in detail.

The chapter is made up of two parts:

Part 1: Organic Endocrine Disrupting Chemicals extraction and quantification

Part 2: Inorganic Endocrine Disrupting Chemicals (metals) extraction and quantification

4.1 Study area and sampling

Moringa Oleifera seeds were collected from a Farm in Limpopo, South Africa (Figure 2.19).

Fresh fruits, vegetables, beef, and fish samples were purchased from two different cities in South Africa: Johannesburg and Pretoria. The areas were divided into: (i) Open markets, (ii) Main markets, (iii) Farm along Hennops river and irrigates using water from the river and (iv) Control sample bought from a supermarket. Targeted open market sites were markets where most people purchase the foodstuff, markets that operate in close proximity to taxi ranks and heavy vehicles emissions.

The first city is Johannesburg. It is in the Gauteng province and is the largest city in South Africa (Moosa *et al.*, 2017). The population is estimated to be 7, 2% of South African population (Nuapia et al., 2016; Nhamo et al., 2021). The air pollution is moderate (48.65 index) and drinking water pollution is low (29.64 index). However, dirty, and untidy index is high (65.22), and water pollution is moderate (56.88 index) (Lourens,2012). The particulates from pollution eventually make their way into foodstuff (Oladejo *et al.*, 2020), besides possible contamination from growing conditions.

i. Johannesburg Open markets

For Johannesburg open markets, *Site A* (Figure 4.1) is located at the Central Johannesburg District (CBD) where generally more Johannesburg people buy the foodstuff.

Figure 4.1 Study Area SITE A

Site B (Figure 4.2) is located at the exit of Park station shopping Centre. Other JHB residents prefer to buy the foodstuff there because it is the safest site. It is also the most unsanitary of the three sites.

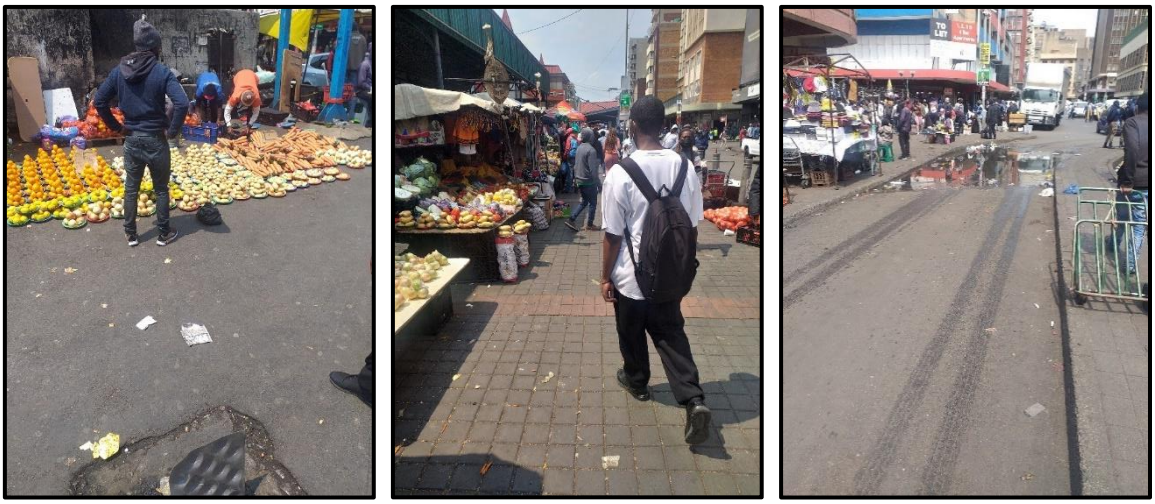


Figure 4.2 Study Area SITE B

Finally, *Site C* (Figure 4.3) was chosen because it is near a very busy taxi rank with heavy vehicle exhaust emissions.

Figure 4.3 Study Area SITE C

The sites were surveyed, and a random sampling method was chosen for each site. At the sites, the state of the environment was not sanitary (Site A, B), and the roads were very busy, with heavy vehicles exhaust emissions (Site C).

ii. Johannesburg main market – City Deep

Some samples were bought from City Deep main market (Figure 4.4) in Johannesburg for fresh produce and transported to the lab.

Figure 4.4 City Deep main market

iii. Farm - Hennops

Furthermore, some samples (Figure 4.5) were collected along Hennops river where they use the river water directly that is sewage contaminated.

Figure 4.5 Hennops river farm

(iv) Supermarket foodstuff : Control sample

Apple samples from Pick n Pay retailer (Figure 4.6) was bought with minimal pesticides as reported by Pick n Pay's code of ethics consumer safety documentation (Pick-n-Pay-Slr-Interactive-2019, 2019).

Figure 4.6 Pick n pay supermarket samples

The second city is Pretoria. It is the administrative capital of South Africa, and the population is estimated to be 5.6% of South African population (Cole and Broadhurst, 2021). It is one of the busiest cities in South Africa. Pretoria markets sell vegetables, fish, meat, and other foodstuffs from many areas around the city, many which are close to heavy gas emissions from cars on busy roads. The status of Endocrine Disrupting Chemicals (organic and inorganic) is currently unknown. The city sampling sites were divided into open markets and Tshwane main market.

The Pretoria open markets are similar to those of Johannesburg due to the unsanitary conditions and situated in environment close to heavy vehile emissions, some which end up in the foodstuff.

The vegetables from Tshwane main market are obtained from producers around South Africa and the market is also supported by buyers from South African cities, mainly Pretoria (Ferreira, 2014).

4.2 Sample collection and preparation

Moringa Oleifera seeds were collected and transported to the lab in a sack. At the lab, the seeds were manually deshelled and cleaned with distilled water to remove ground dirt. The seeds were then left to dry in the oven overnight at 40℃. After drying, the seeds were ground using a domestic blender and further powdered using a mortar and pestle and sieved using a 0.20 mm sieve to obtain uniform particle size. The powder was then stored in the refrigerator (4 ℃).

As stated, the foodstuffs were collected from JHB and PTA (Open markets, main markets, and farm) from November 2020 to August 2021.The samples were divided into fruits and

vegetables (Banana, spinach, cabbage, carrots, potatoes, onion, scallion, rape, tomato, green pepper, yellow pepper, red pepper), fish and meat samples (Beef muscle and chicken liver). A total of thirty-eight samples were collected. At the open markets, the samples were purchased from different vendors laid on the floor without any hygiene precautions, and most were sold very close to taxi ranks with exhaust emissions. The sources at the open markets were not clarified. Some rape and spinach were collected from a farm along Hennops river where they use river water for irrigation directly that is sewage contaminated. The samples from the open markets and main markets were collected in their original plastic packaging and the farm samples were also placed in a plastic bag. The different samples were treated as follows:
The samples were transported to the lab where they were unwrapped, rinsed with distilled water to clean any ground dirt and dust. Thereafter, only the edible vegetable and fruit parts were either shredded or cut into cubes and contained in a petri-dish.
The fish samples were bought from Johannesburg CBD. They were bought dead but not treated so were treated by washing with distilled water and manually filleted for muscles and stored in petri dishes and covered with foils. The mean length of the fish was 25 cm. The beef muscle was bought frozen and packaged in clear plastics. They were treated the same way as fish.

All the samples were stored at -20 ℃ . Thereafter, they were freeze dried at -37 ℃ for five to seven days. The freeze-dried samples were reduced using mortar and pestle to obtain uniform particle size and stored in PTFE tubes, lined with aluminum foil to prevent any growth of microorganisms. These were then stored at -20 ℃ until analysis. The target organic chemicals are dimethyl phthalate, diethyl phthalate 4-n-Nonylphenol, 4,4'-DDD, 4,4'-DDE and 4,4'-DDT.

The samples from the open markets were chosen because they are staple in the chosen cities. Most are bought because they are cost friendly, ready-to-serve and easily accessible on display. The main markets foodstuff was purchased to assess the extent to which the handing of foodstuff in open markets affects the levels of the EDCs.

4.3 Reagents

Petroleum ether and Hexane fraction (99.5%) was purchased from Associated Chemical Enterprise (Johannesburg, South Africa). The Ammonium sulphate, $(NH_4)_2SO_4$ used for precipitation was obtained from Analytic PLC (Johannesburg, South Africa). Dialysis

membrane tubing was from Sigma Aldrich (Johannesburg, South Africa). These reagents were used for *Moringa Oleifera* seed protein extraction.

Six organic EDCs standards used, namely Diethyl phthalate, Dimethyl phthalate , 4-n-Nonylphenol, 4, 4'-DDT; 4, 4,'-DDD, 4,4'-DDE were purchased from Sigma-Aldrich (Johannesburg, South Africa). All the standard EDCs were over 95% pure. Stock and standard solutions were prepared using HPLC-grade methanol from Merck Johannesburg, South Africa. Acetonitrile and Acetone were also obtained from Merck (Johannesburg, South Africa). Anhydrous magnesium sulphate was obtained from the analytical lab, purchased from Merck (Johannesburg, South Africa). For clean-up, primary secondary amine (PSA) bonded with silica will be purchased from Sigma-Aldrich (Johannesburg, South Africa). Nitrogen gas (99.999%) was used for blowing and evaporation of the solvent to the required volume. Further, Certified Reference Materials (Dimethyl phthalate, Diethyl phthalate, 4-Nonylphenol, 4,4'-DDT, 4,4'-DDD and 4,4'-DDE) were bought from Dr. Ehrenstorfer GmbH of LGC chemicals (Augsburg, Germany). An internal standard ($\gamma - hexachlorocyclohexane, \gamma - BHC, or\ \gamma - lindane,\ 100\ \mu g\ L^{-1}$) was purchased from Sigma-Aldrich (Johannesburg, South Africa). All the other used reagents were of analytical grade.

For metal analysis, all used chemicals and reagents were of analytical grade or higher for the analysis of inorganic EDCs at $mg\ kg^{-1}$ levels. 55% w/v Nitril acid, HNO_3 and 30% w/v hydrogen peroxide, $H_2O_2, supra\ pure\ grade$ were purchased from Merck (Johannesburg, South Africa). 5% HNO_3 used for instrument cleaning was prepared from 55 % HNO_3 using distilled water.

4.4 Instruments

The centrifuge used was a S-8 centrifuge from Boeco (Hamburg, Germany). FTIR spectra of the freeze dried *Moringa Oleifera (M.O)* protein were obtained using a Bruker Tensor 27 equipped with ATR diamond tip. The spectra were obtained in a transmittance mode in the spectra region of $4000 - 500\ cm^{-1}$.

The optimization of QuEChERS was carried out using a GC 7890A (Agilent Technologies, DE, USA) equipped with and Electron Capture Detector (ECD) with a WCOT fused silica capillary column (30 x 0.25 mm ID, 0.25 m film thickness) as seen depicted in figure 4.7. The software used for analysis was Chemstation version B.04.03. GC-ECD procedure was as follows: The gas chromatography method was programmed to best separate the Endocrine

Disrupting Chemicals of interest as follows: Oven 60℃ (5 min), increased to 150 ℃ at a rate of 10 ℃ min^{-1}(1 min), further increased to 200 ℃ at a rate of 30 ℃ min^{-1} (1 min) then lastly to 300 ℃ at 15 ℃ min^{-1} for 10 min. Nitrogen gas was used as carrier gas for GC-ECD with a flow rate of 1 $ml\ min^{-1}$. A capillary column coated with ZB-5 (30 m x 0.25 mm, 0.25 mm film thickness) was used. The temperature of the injector operating in split less mode was held a 300 ℃ and electron-capture detector temperature was 250 ℃. Volume injected was 10 μL.

Figure 4.7 GC-ECD instrument

A LECO GC-MS with a capacity for a GC x GC equipped with an TOF/MS detector (Figure 4.8) was used for application, quantification, and confirmation studies with the split less mode conditions employed. The software used is Pegasus ChromaTOF . The GC x GCMSTOF procedure was as follows: The mass spectrometer was programmed with a transfer line temperature of 320 ℃ ; ion source temperature 250 ℃ and multiplier voltage 1450 V. A programmed temperature vaporization injector operating in splitless mode was employed. The volume injected was 10 μL, split flow 50 $ml\ min^{-1}$ and injection time: 0.50 min, injection flow: 100 $ml\ min^{-1}$. Helium was used as carrier gas flow rate of 1 $ml\ min^{-1}$. Ion trap mass detection was operated in full scan mode from 50 to 500 amu. The temperature was programmed as follows: 50 ℃ increased to 150 ℃ at 10 ℃ ramp rate for 1 min followed by an increase to 300 ℃ at a rate of 5 ℃ for 1 min. The column used was BP5MS (30m x 0.25 mm, 0.25 mm film thickness). Figure 4.8 shows a LECO GC-MS with a capacity for a GC x GC equipped with an TOF/MS detector used for confirmation and application studies.

Figure 4.8 GC x GC MSTOF instrument

4.5 Organic endocrine disrupting chemicals analysis

4.5.1 *Moringa Oleifera* Protein Extraction

The bio sorbent used was extracted with distilled water and purified. The intermediate protein material and purified protein products of the extraction of *Moringa Oleifera* seed protein from the seed material is depicted in figure 4.9**.**

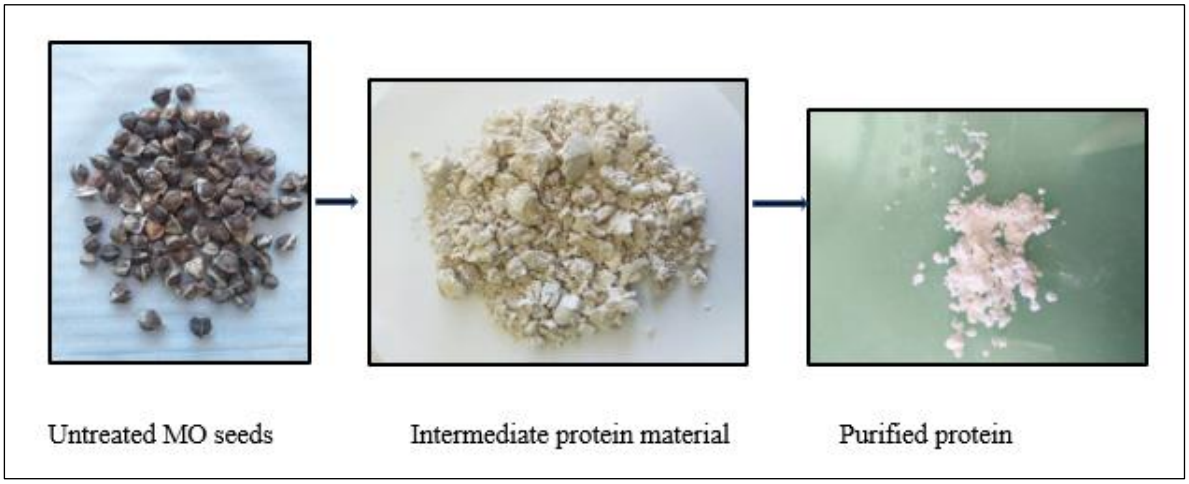


Figure 4.9 Starting material and products of *Moringa Oleifera* protein extraction

The extraction of the protein from the prepared seeds was done following the method described by Kebede *et al.* (2018), with modifications were necessary. The *Moringa Oleifera* (MO) powder was mixed with 37% w/v petroleum ether to extract the water insoluble oil and fats. The solid protein material was separated from the oil material by filtration using a Whatman filter paper no. 1. The solid protein material was then left to dry in the lab at room temperature overnight. After drying, the solid was dissolved in distilled water and stirred using a stirrer bar for 30 minutes to extract the water-soluble protein. The mixture was subject to 15 mins

centrifuge at 4000 rpm and filtered using a Whatman paper No.3 to remove the water-insoluble material. 200 mL of filtrate was treated with ammonium sulphate while stirring to precipitate the protein, with a continuous addition of the salt until saturation. After saturation, the precipitated protein solution was centrifuged for 10 mins at 4000 rpm to concentrate the protein. The precipitated concentrated protein was re-dissolved in distilled water and filtered using a Whatman No. 3 paper to remove the insoluble junk material. The water-soluble protein was subject to dialysis through a cellulose dialysis tube for reverse osmosis activity for purification. The dialysis tubes were placed in a 1 L beaker filled with distilled water and stored at room temperature during the day, with the distilled water changed twice daily. The dialysis was used for the removal of some unwanted small molecular weight solutes and "contaminants" from the protein solution which cleans the protein material, thus accounting for its purity and in turn concentrating the protein. This works by separating the protein and solutes in the protein solution by the difference in their rates of diffusion. Overnight, the beaker was stored at 4℃ to remove the ammonnium sulphate. After dialysis, the pure protein was freeze dried and a white powder was obtained, which is stored at room temperature until analysis using FTIR.

4.5.2 Preparation of the stock solution

A 1000 $mg\ L^{-1}$ stock solution of all EDCs used (Dimethyl phthalate, Diethyl phthalate, 4-Nonylphenol, 4,4'-DDD, 4,4'-DDE and 4,4'-DDT) was prepared in a 25 mL volumetric flask by weighing 25 mg of each standard chemical into the flask and filling it to the mark with methanol. A 10 $mg\ L^{-1}$ standard solution of the seven compounds was prepared from the 1000 $mg\ L^{-1}$ stock solution by withdrawing 100 μL of the prepared stock solution into a 10 mL volumetric flask and diluting to the mark with methanol. It is from this 10 $mg\ L^{-1}$ standard solution that a 1 $mg\ L^{-1}$ standard solution was made using dilutions equation. The prepared mixed solutions were then stored at 4℃ until analysis.

4.5.3 Preparation of GC-ECD calibration curve

A 10 $mg\ L^{-1}$ standards solution was prepared from the 1000 $mg\ L^{-1}$ stock solution prepared earlier. A standard solution of EDCs of different concentration ranging from 0.2 $mg\ L^{-1}$ to 1.0 $mg\ L^{-1}$ was prepared and used for determination of calibration curve. Five concentrations of standards were prepared in a 10 ml volumetric flask using dilution calculations. The calibration

curves were linearly fitted, and the data is shown is Table 4.1. The limit of detection is for manual injections.

Table 4.1 GC-ECD calibration curve data and retention times

Endocrine disrupting chemical	r^2	Retention time	Detection limits ($\mu g\ kg^{-1}$)		Precision (RSD %)
			LOD	LOQ	$n = 3$
Diethyl phthalate	0.9318	10.871	1.03	10.3	8.55
Dimethyl Phthalate	0.9252	11.643	0.61	6.1	4.39
$\gamma - BHC$	0.9479	12.829	1.18	11.8	4.88
$4 - n - Nonylohenol$	0.9887	13.214	0.89	8.9	9.86
$4,4' - DDD$	0.9599	17.347	0.16	1.6	13.32
$4,4' - DDE$	0.9567	18.214	1.12	11.2	4.44
$4,4' - DDT$	0.9638	20.422	1.77	17.7	2.25

4.5.4 Preparation of GC x GC MSTOF calibration curve

Furthermore, a calibration curve was also done for GCxGC MSTOF. The results are displayed in the Table 4.2. The preparation of standards is the same as seen for GC-ECD.

Table 4.2 GCxGC MSTOF calibration curve data and retention times.

Endocrine disrupting compound	r^2	Slope	RSD %	Detection limits ($\mu g\ kg^{-1}$)	
				LOD	LOQ
Dimethyl Phthalate	0.9252	0.0117	1.23	0.61	6.1
Diethyl phthalate	0.9479	0.0145	3.33	1.18	11.8
$4-n-Nonylohenol$	0.9887	0.0087	2.25	0.89	0.89
$4,4'-DDE$	0.9567	0.021	1.95	1.12	11.2
$4,4'-DDT$	0.9638	0.1874	3.63	1.77	17.7

4.5.5 Preparation of the internal standard

The $\gamma-lindane$ ($C_{14}H_9Cl_{15}$) was chosen as an internal standard because it is structurally similar to 4,4'-DDT and its metabolites, which are target chemicals being monitored in this study and its retention time does not interfere with that of the target endocrine disrupting chemicals. A 1 $mg\ L^{-1}$ internal standard was prepared from a 10 $mg\ L^{-1}$ stock of $\gamma-lindane$. This was done using dilution calculations, where C_1 is 10 $mg\ L^{-1}$ and C_2 is 1 $mg\ L^{-1}$. The preparation was done in 10 mL volumetric flask. Thereafter, equal amounts (10 μL) of the internal standard and EDC standards were combined and analysed concurrent for any interferences.

4.6 Optimisation of QuEChERS method

4.6.1 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques in the development of a series of experiments for the adequate prediction of a response (denoted by y) from several associated control variables (denoted by $x_1, x_2, \ldots\ldots, x_k$) (Edwards, 2002; Yolmeh and Jafari, 2017). Such a relationship can be denoted by the low degree polynomial equation:

$y = f'(x)\beta + \in$ where:

- $f'(x)$ denotes the mean response which is the expected value of y.

- $x = x_1, x_2, \ldots\ldots, x_k$
- $f(x)$ is a vector function of p elements consisting of powers and cross-product powers of $x_1, x_2, \ldots\ldots, x_k$
- β is vector of p unknown constant coefficients (parameters)
- $\in$ is an experimental error with a zero mean (Ba and Boyaci, 2007; Trinh and Kang, 2010).

A design of experiment was performed for the optimisation methodology, made up of screening and optimisation experiments.

4.6.2 Design of experiments: Screening and Optimisation

To get the best recovery in the application of real food samples, the sample mass, speed and time of centrifuge, amount of sodium chloride (NaCl) and magnessiun sulphate ($MgSO_4$), solvent type and solvent extraction volume which affects the extraction efficiency of QuEChERS method was screened using L8 (3 levels with 7 factors, 6 responses and 21 runs) linear model. The selected parameters were optimized by a Central Composite Orthogonal (CCO) design which composed of a full or fractional factorial design (Quadratic model, 17 runs). The statistical tool used for the design of experiment is MODDE Pro 13.0.1 (Sartorius Stedim Biotech, Malmö, Sweden).
Three Centre points replicates were employed for the design and for each experimental run. The screened factors are displayed in Table 4.3.

Table 4.3 Parameters screened using L8 linear model

QuEChERS parameter	**Minimum**	**Maximum**	**Factor Type**
Sample mass	0.5 grams	3 grams	Quantitative
Centrifuge time	5 mins	20 mins	Quantitative
Centrifuge speed	3400 rpm	4000 rpm	Quantitative
Amount of NaCl	1 g	3 g	Quantitative
Amount of $MgSO_4$	1 g	3 g	Quantitative
Solvent volume	5 mL	10 mL	Quantitative

Solvent type	Methanol, Acetone and Acetonitrile	Qualitative

4.6.3 QuEChERS extraction method

As explained, a Response Surface Methodology was used for the screening and optimization of the QuEChERS method. The QuEChERS method was done using the procedure by (Ramalhosa et al., 2009) ,with modifications where necessary. The method was first performed following different screening conditions as displayed in Table 4.4 (a full factorial design was used as screening tools). Each run was done following the combination shown in Table 4.5. Varying amounts of sample (Table 4.5) was weighed in a 50 mL centrifuge tube and spiked with 500 μL of 200 $\mu g\ L^{-1}$ standard mixture to give varying concentrations in $\mu g\ kg^{-1}$. The spiked samples were allowed to stand for 30 min to allow the standards to integrate into the samples. Varying volumes of solvent was added according to Table 4.4, and the sample was vortex-shaken for 1 min. This was followed by an addition of sodium chloride and anhydrous magnesium sulphate and the mixture was vortex-shaken vigorously for 1 min and then centrifuged for varying minutes under different speeds (Table 4.5). After centrifuge, the supernatant was transferred into a second centrifuge tube for the clean-up process with anhydrous magnesium sulphate and PSA. PSA was used for screening and will be replaced with the *Moringa Oleifera* seed protein bio sorbent at optimal conditions. The solution was further vortexed for 30 seconds, and then centrifuged for 5 min under the same conditions and filtered using first a 0.45 μm PTFE syringe filters followed by 0.22 μm PTFE syringe filters and injected in the GC-ECD for analysis.

Table 4.4 Experimental responses of independent factors for screening

Run Order	Sample mass	Centrifuge speed	Centrifuge time	NaCl mass	$MgSO_4$ mass	Solvent volume	Solvent type	DMP	DEP	4-Nonylphenol	4,4'-DDT	4,4'-DDE	4,4'-DDD
5	0,5	3400	5	1	1	5	methanol	44,99	123,3	68,86	23,24	123,5	132,0
8	0,5	3700	12,5	2	2	5	acetone	76,85	132,5	88,65	37,04	96,65	96,37
18	0,5	4000	20	3	3	5	acetonitrile	44,24	72,63	88,62	76,85	87,64	130,7
2	1,75	3400	5	2	2	5	acetonitrile	28,88	68,85	66,96	55,67	147,9	66,61
12	1,75	3700	12,5	3	3	5	methanol	32,65	42,25	84,56	42,55	76,68	101,9
15	1,75	4000	20	1	1	5	acetone	67,65	142,6	136,69	71,04	119,7	145,6
10	3	3400	12,5	1	3	5	acetone	98,89	108,1	123,25	13,66	176,64	65,55
21	3	3700	20	2	1	5	acetonitrile	11,92	64,56	105,98	65,95	67,32	127,62
16	3	4000	5	3	2	5	methanol	12,6	58,46	75,55	59,67	46,95	101,05
9	0,5	3400	20	3	2	10	acetone	45,68	56,69	45,98	88,37	48,15	77,79
20	0,5	3700	5	1	3	10	acetonitrile	47,06	64,56	32,97	74,91	119,9	68,55
1	0,5	4000	12,5	2	1	10	methanol	16,95	45,56	45,5	75,68	38,69	140,23
3	1,75	3400	12,5	3	1	10	acetonitrile	14,65	26,65	38,66	89,51	52,23	90,58
19	1,75	3700	20	1	2	10	methanol	22,89	43,56	105,45	79,98	71,23	73,09
14	1,75	4000	5	2	3	10	acetone	105,26	65,59	89,65	65,87	54,44	61,22
4	3	3400	20	2	3	10	methanol	26,68	20,56	102,23	65,66	69,99	46,96
7	3	3700	5	3	1	10	acetone	56,66	65,64	58,69	88,96	36,96	55,56
11	3	4000	12,5	1	2	10	acetonitrile	42,63	32,22	71,25	108,66	56,68	75,66
6	1,75	3700	12,5	2	2	7,5	methanol	23,36	49,68	66,69	59,69	39,64	46,96
17	1,75	3700	12,5	2	2	7,5	methanol	21,99	33,65	65,58	55,26	29,38	86,66
13	1,75	3700	12,5	2	2	7,5	methanol	33,1	39,65	87,88	67,85	36,66	88,89

Once all the parameters were screened and the important ones determined, the optimization process was done with reduced runs using most important QuEChERS parameters. The data is displayed in Table 4.5. The optimization tool employed has an advantage to determine the interaction between the independent quantitative variables, namely sample mass, speed, and time of centrifuge. It then models the system mathematically. This saved time and cost by reducing the number of trials and experiments that could have been performed when using the traditional way of optimization. The traditional way entails dealing with one parameter at a time while keeping the rest of the parameters constant. At optimal PSA amounts, the PSA was then replaced with the same amount of *Moringa Oleifera* protein. The QuEChERS methodology schematic diagram is displayed in Figure 4.10.

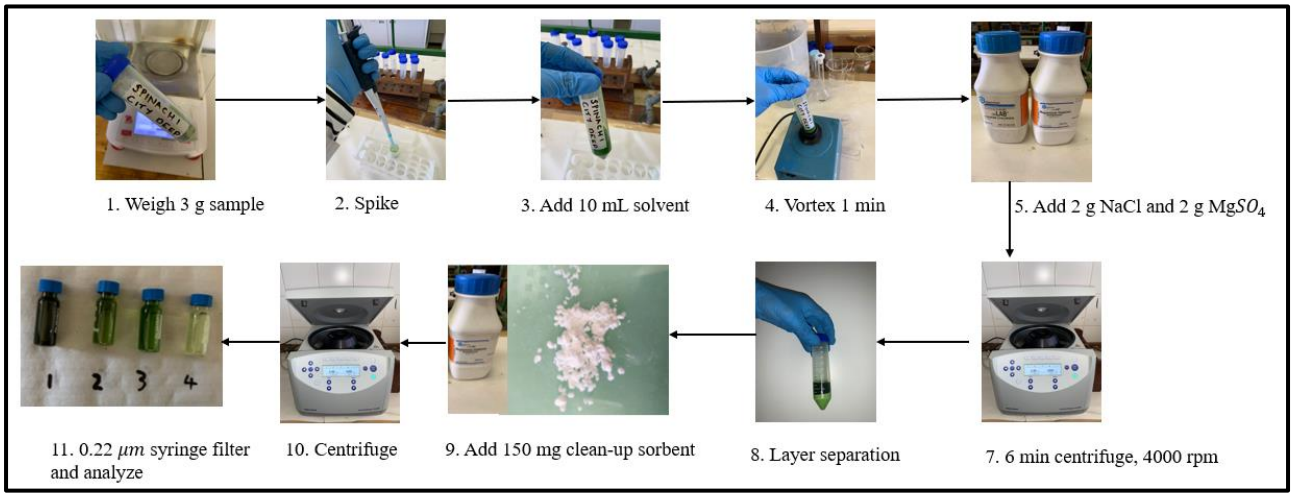


Figure 4.10 Schematic QuEChERS experimental procedure.

Table 4.5 Experimental design for the optimization model

Exp No	Run Order	Sample mass	centrifuge time	centrifuge speed	Dimethyl phthalate	Diethyl phthalate	4-Nonylphenol	4,4'-DDT	4,4'-DDD	4.4'-DDE
1	4	0,5	5	3400	42,11	82,36	60,41	20,41	71,38	69,66
2	6	3	5	3400	49,81	54,11	41,91	80,41	57,85	74,21
3	12	0,5	20	3400	16,96	21,22	98,41	66,21	64,24	41,22
4	8	3	20	3400	24,58	71,11	45,27	88,22	72,64	77,71
5	3	0,5	5	4000	58,11	55,21	72,29	61,66	48,65	96,34
6	11	3	5	4000	28,96	77,41	85,69	72,77	85,21	71,02
7	15	0,5	20	4000	41,21	33,24	114,22	69,94	74,21	49,21
8	17	3	20	4000	35,91	86,96	76,96	36,66	100,05	48,76
9	16	0,1	13	3700	55,54	81,21	79,68	12,1	66,21	71,28
10	9	3,4	13	3700	58,64	101,23	71,26	39,65	88,57	56,65
11	2	1,75	2	3700	35,65	30,21	71,21	48,65	61,24	88,85
12	14	1,75	23	3700	12,71	31,24	80,21	67,41	61,21	63,33
13	1	1,75	12,5	3300	19,89	22,21	31,28	86,21	42,88	58,87
14	10	1,75	12,5	4100	32,88	54,21	88,27	52,47	64,52	71,26
15	5	1,75	12,5	3700	34,56	38,27	71,41	51,48	37,58	72,24
16	13	1,75	12,5	3700	33,24	41,27	63,41	55,21	39,21	69,71
17	7	1,75	12,5	3700	35,59	50,44	65,22	50,28	42,81	72,36

4.7 Application to real samples

4.7.1 Optimal QuEChERS extraction method

Fresh fruits, vegetables, meat and fish samples ($n = 38$) were bought from open markets, Main markets and a farm along Hennops river in Johannesburg and Pretoria and prepared as described in section 4.2. 3 g of each sample was weighed in a 50 ml centrifuge tube. 10 ml of methanol was added, and the sample was vortex-shaken for 1 min. This was followed by an addition of 2 g sodium chloride and 2 g of anhydrous magnesium sulphate and the mixture was vortex-shaken vigorously for 1 min. Rapid vortex at this stage prevents the formation of magnesium sulphate aggregates at a later stage. The solution was centrifuged for 6 mins under 4000 rpm. After centrifuge, the supernatant was transferred into a second centrifuge tube for the clean-up process with 75 mg anhydrous magnesium sulphate and 150 mg of *Moringa Oleifera* protein bio sorbent and some with PSA sorbent. The solution was further vortexed for 30 seconds. The solution was then centrifuged for 3 min under 3000 rpm and filtered using a 0.45 μm and 0.22 μm PTFE syringe filter and injected in the $GC \times GCMSTOF$ for analysis. To concentrate the sample, nitrogen blowing was used to reduce the solvent to required volume. For each sample, there were three rounds of extractions and for each extraction there were also three rounds of analysis. The reported values are $mean \pm RSD$.

4.7.2 Quality assurance and Certified Reference material (CRM) preparation

Appropriate quality assurance procedures were carried out to ensure reliability and reproducibility of the results such as spiking and replicate extraction of samples. To add, a correct cleaning protocol was followed to minimize contamination by using blanks and washing of experiments apparatus using 10% (v/v) HNO_3 for a minimum of three days and rinsed with distilled water with an electrical resistivity of 18.2 MΩ cm (Millipore, USA). Some apparatuses were further dried using acetone. Limit of detection was also calculated as signal to noise ratio of three times.

The Certified Reference Materials (CRMs) were used for the QuEChERS method validation. The material certificate of analysis was designed in accordance with ISO 17034 and ISO Guide 31. 0.7 grams of fish, cabbage and beef sample was fortified with $100\mu L$ of $1000\ \mu g/L$ of each reference materials to give a concentration of 142.86 $\mu g\ kg^{-1}$ of each EDC in the sample.

They were extracted with 5 ml of methanol. After extraction, the organic liquid was filtered and reduced to 1 ml. This was then analyzed on GCxGC MSTOF along with standards.
To ensure the reliability and reproducibility of the digestion and the results thereof, it was very essential for proper lab quality assurance to be carried out. The glassware used was soaked in 10% Nitric acid and then washed with detergent and tap water and dried using acetone. Thereafter they were rinsed again with deionized water. All the reagents used are of analytical grade. The Standards were prepared for each metal from their stock solution for calibration of the instrument.

4.8 Metal analysis

Metals (Inorganic Endocrine Disrupting Chemicals) analysis in foodstuff is described in this section.

4.8.1 Microwave digestion of foodstuff for metal analysis.

Anton Paar microwave Go digestion system (Switzerland)(Figure 4.11) was used to digest the samples. The metal concentration analysis was done using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Spectro, Kleve, Germany) with Coupled Charge Detection (CCD) system.

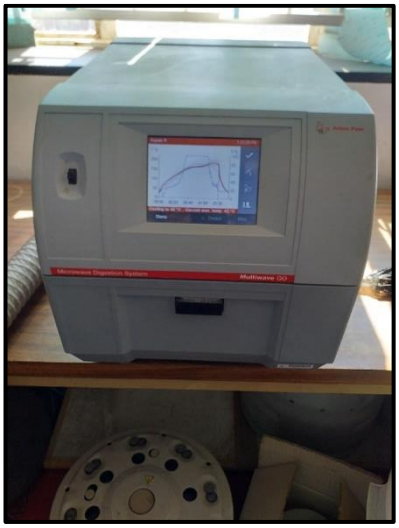


Figure 4.11 Anton Paar Go microwave

The foodstuff was digested using the program (Table 4.6).

Table 4.6 Microwave Digestion Program

Ramp (mm:ss)	**Temperature (℃)**	**Hold (mm:ss)**
35 :00	180	30: 00

Application type: Digestion; Vessel mode: multi-vessel; Temperature program mode : Average; Temperature limit (℃) : 190.

The foodstuffs were digested in the microwave system as follows:

A mass of 0.15 ± 0.008 g of sample powder was weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TFM liners). 10 mL $HNO_3$and 2 mL H_2O_2 was added to the tubes. The tubes were sealed and placed into a motor, which was subject to Anton Paar microwave Go digestion. The digestion program conditions are displayed in table 4.6. After digestion was complete, the digested solution was transferred into 50 mL PTFE tubes onto which distilled water was added and diluted to the 50 mL mark. 10 mL of the diluted solution was then filtered using 0.22 μm PVDF syringe filters into an acid washed PTFE tubes for ICP-OES analysis for concentration determination. The concentrations of ten trace elements ((Mercury (Hg), Arsenic (As), Lead (Pb), Aluminum (Al), Copper (Cu), Iron (Fe), Cadmium (Cd), manganese (Mn) and Zinc (Fe)) were analyzed in all samples.

4.8.2 Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP - OES) analysis

The ICP-OES (Figure 4.12) was first calibrated to get the best sensitivity when analyzing the metals. Each EDC was analyzed at different metal wavelengths to accommodate the relative concentrations of the metals as sensitivity increases. The instrument was calibrated with a blank and by using metal standards ranging from 0.1 $mg\,L^{-1}$ to 10 $mg\,L^{-1}$. The ICP-OES parameters on the day of analysis was provided by the instrument as follows (Table 4.7).

Table 4.7 Parameters for ICP-OES for day of analysis

Parameter used	Optimal condition
Coolant flow	$13\ ml\ min^{-1}$
Auxiliary flow	$1\ ml\ min^{-1}$

Plasma power	$1400\ W$
Type of Nebulizer	$Cross\ flow$
Nebulizer flow	$1\ ml\ min^{-1}$
Injector tube diameter	0.890 m

Figure 4.12 Inductively coupled plasma-optical emission spectroscopy (ICP - OES)

4.8.3 Method limit of detection

The ICP-OES method limit of detection is the minimum measured concentration of each EDC that was reported with 99% confidence that is distinguishable from method blank results. This was done using the formula (Barros et al., 2019; Virgilio et al., 2020):

$$LOD = 3\ x\ standard\ deviation\ of\ the\ blank\ samples \quad \text{.....(2)}$$

4.9 Statistical data analysis

Statistical analysis for mean, standard deviation and P values for comparison studies was done using Microsoft excel with ANOVA plugin and Origin Pro software.

CHAPTER 5: RESULTS AND DISCUSSION

This chapter presents the empirical findings of the research methods used.

The chapter is made up of two parts:

Part 1: Organic Endocrine Disrupting Chemicals analysis

Part 2: Metals as Inorganic Disrupting Chemicals analysis

5.1 Verification of QuEChERS extraction method with the use of Certified Reference Materials

Certified Reference Materials (DRE-GA09011090ME for Dimethyl phthalate, MM 0006.28 for Diethyl phthalate, DRE - C15630000 for 4-Nonylphenol, DRA-C12031000 for 4,4'-DDD and DRE - C12041000 for 4,4'-DDE) were fortified for beef, fish and vegetables and used for verification of the applied QuEChERS method. The material certificate of analysis was designed in accordance with ISO 17034 and ISO Guide 31. The chromatographic representation of the individual Certified Reference Materials analyzed with GC x GC MSTOF is presented in Appendix 1 (figure 1a).

The obtained CRM recovery data was used to judge the QuEChERS performance at optimised conditions in terms of accuracy of the analysis. The highest recovery was detected for 4-Nonylphenol (114.69%) in Cabbage Reference Material and the lowest was detected for Diethyl phthalate (52.34%) in Fish Reference Material (Table 5.1). For the analysis of EDCs at $\mu g\ kg^{-1}$ levels, recoveries between 70-120% are considered acceptable (Valles *et al.*,2012). Analysis of beef meat, cabbage and fish reference materials generally revealed similar values of acceptable accuracy and precision of the method for most EDCs (Table 5.1).

Table 5.1 Measures of Certified Reference Materials concentration ($\mu g\ kg^{-1}$) and their respective recoveries in vegetable, fish, and beef sample (n=3, RSD% < 21.66).

	Cabbage			Fish			Beef		
EDC	Amount in extract (μg)	Amount in sample (μg)	Recovery %	Amount in extract (μg)	Amount in sample (μg)	Recovery %	Amount in extract (μg)	Amount in sample (μg)	Recovery %
DMP	0.146	0.1	69%	0.148	0.1	67%	0.182	0.1	55%
DEP	0.149	0.1	67%	0.191	0.1	52%	0.168	0.1	59%
4-nonylphenol	0.087	0.1	114%	0.092	0.1	108%	0.090	0.1	111%
4,4-DDE	0.141	0.1	71%	0.183	0.1	54%	0.156	0.1	64%

% Recovery = $(100 * amount\ in\ extract/amount\ in\ sample)$

The data obtained for internal standards is presented in Figures 1b-1d, Appendix I. The chromatograms shows no peak interferences between the internal stand and target EDCs.

The identities of the EDCs were further confirmed with mass spec detection. Figure 1e, appendix I shows representations of mass spectra of the EDCs under study which clearly shows true peaks matching the references from the NIST library. Further, the standard representations of EDCs under investigations theroughout the project is shown in figure 1f-1h of Appendix I.

5.2 Choice of QuEChERS clean-up sorbent

This section compares the performance of *Moringa Oleifera* seed protein to commercial PSA as QuEChERS clean-up sorbent. Other clean-up sorbents are also compared including Hemp cellulose (extracted in the lab using 4 wt% sodium hydroxide (NaOH)), commercial Hemp cellulose, C_{18} and Activated carbon. The optimization of the amount of clean-up sorbent was done using PSA.

5.2.1 Optimal amount of PSA sorbent

The optimization of the amount of PSA was investigated using single factor experiments. PSA amount was varied from 25 to 75 mg (25 mg increments) while keeping the other parameters constant at 1.5 g of sample, 4000 rpm for 6 mins centrifuge, 1 g of each salt ($NaCl\ and\ MgSO_4$) , and 5 mL of methanol. With the increase of PSA mass from 25 to 75 mg, the extraction efficiency gradually increased and then remained the same above 75 mg for most analytes (Figures 2a and 2b, Appendix I). Therefore, 75 mg of PSA was selected as the optimal clean-up amount. After the PSA optimization, each parameter was scaled up by a factor of 2, giving optimal PSA amount of 150 mg. As a result, 150 mg PSA was substituted with 150 mg *Moringa Oleifera* protein and applied as the optimum cleaning sorbent mass to obtain an acceptable recovery. This correlates to 3 g samples mass, 2 grams NaCl mass, 2 grams $MgSO_4$ mass and 10 mL methanol volume.

5.2.2 Comparison of PSA and *Moringa Oleifera* seed protein

Figure 5.1 shows the comparison of selectivity between 150 mg PSA and 150 mg *Moringa Oleifera* seed protein as clean up sorbents in QuEChERS in the extraction of beef sample. Similarly, Figure 5.2 shows comparison of the same but for extraction of fish sample. In both cases, chromatograms using *Moringa Oleifera* seed protein as clean-up shows much selectivity compared to PSA.

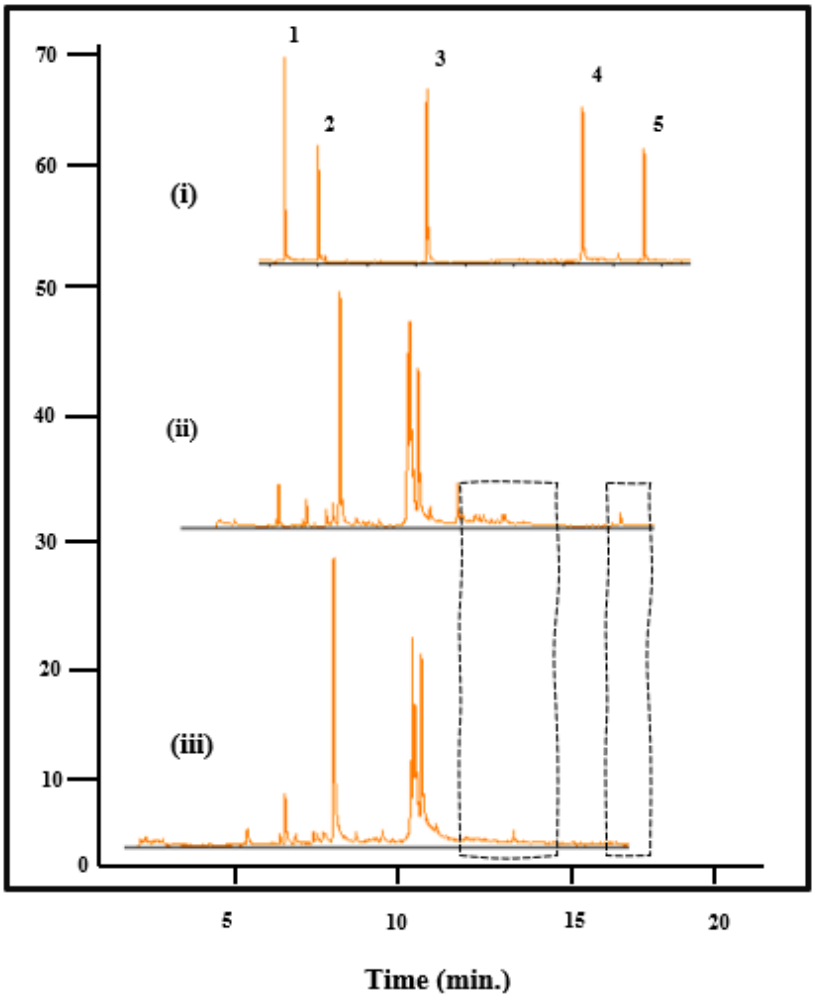


Figure 5.1 GC x GC MSTOF chromatograms of (i) 1 $mg\ L^{-1}$ standard mixture of five endocrine disrupting compounds standards, (ii) PSA-Cleaned beef sample spiked with 142.86 $\mu g\ kg^{-1}$ standard mixture, (iii) Moringa Oleifera protein cleaned beef sample spiked with 142.86 $\mu g\ kg^{-1}$ standard mixture (Peak markers: 1. Dimethyl phthalate. 2: Diethyl phthalate 3: 4-Nonylphenol 4: 4,4'-DDE, 5: 4,4'-DDT).

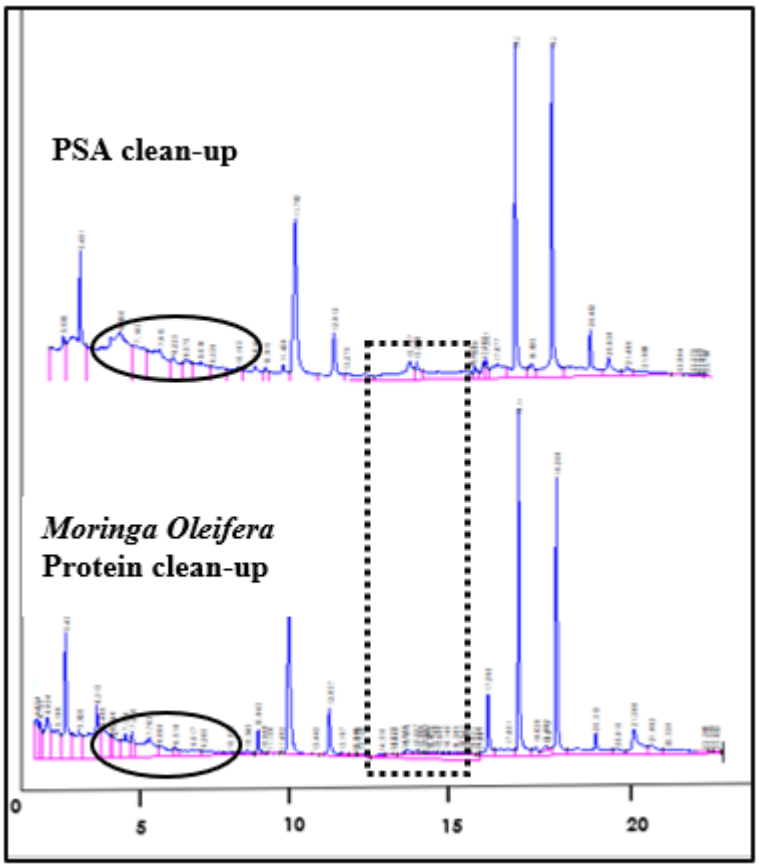


Figure 5.2 GC-ECD chromatogram of fish samples extracted using QuEChERS and (i) PSA and (ii) *Moringa Oleifera* Protein clean-up sorbent.

5.2.3 Comparison of the Recovery between PSA and *Moringa Oleifera* seed protein

Recovery comparison between PSA and *Moringa Oleifera* seed protein in QuEChERS as clean-up sorbents was also compared. The results are given in Table 1 (a and b, Appendix I). The mean recoveries of the EDCs using PSA as a clean-up sorbent ranged from 74.1± 1.26 to 104.1± 0.96% for fruits and vegetables, 79.9± 0.96 to 107.4±0.88% for fish, 80.7±0.52 to 104.5±0.21% for beef, respectively. Moreover, then mean recoveries of the EDCs when using *Moringa Oleifera* protein as clean-up sorbent ranged from 86.5± 0.56 to 103.2± 0.68% for fish, and 76.2 ± 0.85 to 105.1± 2.24% for beef. When using *Moringa Oleifera* protein, the detection limits ranged between 0.16 to 1.77 $\mu g\ kg^{-1}$ with RSD values $\leq$ 13.32%, respectively. Overall, recoveries using *Moring Oleifera* seed protein as clean-up sorbent were slightly higher than those from PSA. These recoveries in QuEChERS are comparable to those reported by (Hakme

and Poulsen, 2021). The high recovery values and good RSD indicate accuracy of the QuEChERS procedure used in this investigation. The R^2 values were over 0.9252.

5.2.4 *Moringa Oleifera* seed protein characterisation

i. FTIR

FTIR was used to characterize the Moringa Oleifera protein in order to understand the interaction between the protein and the food samples. The FTIR spectra obtained for this study is presented in Figure 1, Appendix I. The water-soluble seed protein shows characteristic bands which are due to OH and NH amide stretches in the secondary structure. These are the bands at $3200.87\ cm^{-1}$ to $3280.10\ cm^{-1}$ (1). Furthermore, there is a $C=O$ stretch at $1643.72\ cm^{-1}$ (2), two N-H amides bands at $1538.14\ cm^{-1}$ and $1415.37\ cm^{-1}$ (3). There is a C-N stretch at $1238.02\ cm^{-1}$ from the amide (4). Lastly, at $1078.95\ cm^{-1}$, there is a C-C-N amide bend (5) and N-C=O bend at $611.98\ cm^{-1}$(6).

In a recent work by Kebede et al. (2018), it was observed that *Moringa Oleifera* seed protein has amide, amine, carboxylic acid, and other functional groups. This agreed to what was observed in this study.

ii. Protein recovery studies

Moringa Oleifera seeds are a protein source, which accounts for the second most constituent of the seeds, after oils and lipids which may account for over 60% of the total seed contents.

Table 5.2 shows the protein recovery of *Moringa Oleifera* seeds, which was extracted with distilled water. Various reports have reported *Moringa Oleifera* protein recoveries with a mean value of 4 - 19% (Saa et al.,2019 and the references thereof) in which most were not manually extracted.

Table 5.2 Recovery of MO seed protein per 37 g of seed powder (n=2)

Mass of MO seed powder/ g	Mass of water-soluble protein extracted/ g	Percentage recovery (%)
37	0.8365	2.26 %

5.2.5 Comparison of *Moringa Oleifera* protein bio sorbent with other sorbent types

The sorbent used for extraction is very essential as it can have various effect on recovery and selectivity (Oshita and Jardim,2015). In this study, different types of cleaning sorbents were applied in the extraction of fish sample. The effects of sorbent type were evaluated by comparing selectivity of unwanted matrix in the presence of the target EDCs. Figure 5.3 shows the selectivity of 150 mg PSA, 150 mg *Moringa Oleifera* protein and 150 mg Hemp cellulose sorbent for the extraction of target EDCs in fish. The chromatogram is much cleaner when Hemp cellulose is used. This may be due to the chemistry and affinity of hemp cellulose. However, more studies in its chemistry need to be investigated to fully understand its cleaning capabilities. The disadvantage with Hemp cellulose in this study is that it also has an affinity for 4,4'-DDE, which is a target EDC. 4,4'-DDE has a LogP value of 5.80, making it more lipophilic and non-polar than most analytes. It has an affinity for organic plant-based adsorbents like hemp. However, 4,4'-DDT also has a similar LogP value of 5.80 and was not taken away. The reason for this may be in the structure of 4,4'-DDT as it has more substituents (and molecular mass) than 4,4'-DDE which plays a role in its polarity.

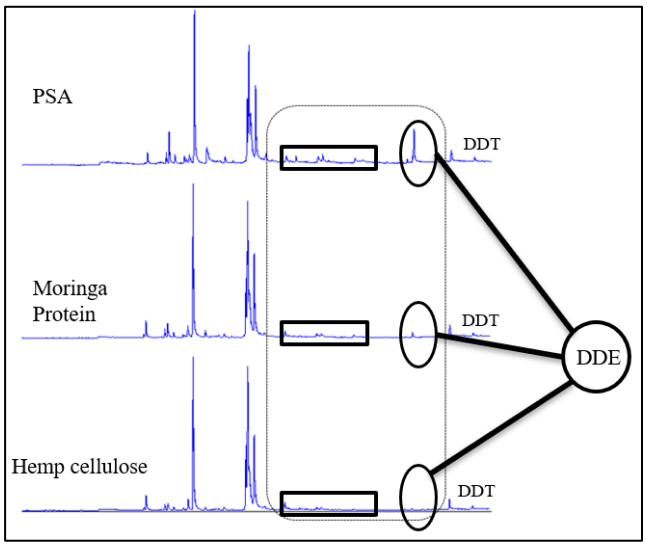


Figure 5.3 Chromatograms of QuEChERS fish samples extracted using different clean-up sorbents (150 mg each).

When 150 mg Activated carbon is used as a clean-up sorbent (figure 5.4), there was no improvement in the selectivity and cleanliness of the chromatogram. Thus the protein was closen as the better and optimal sorbent type over activated carbon and PSA.

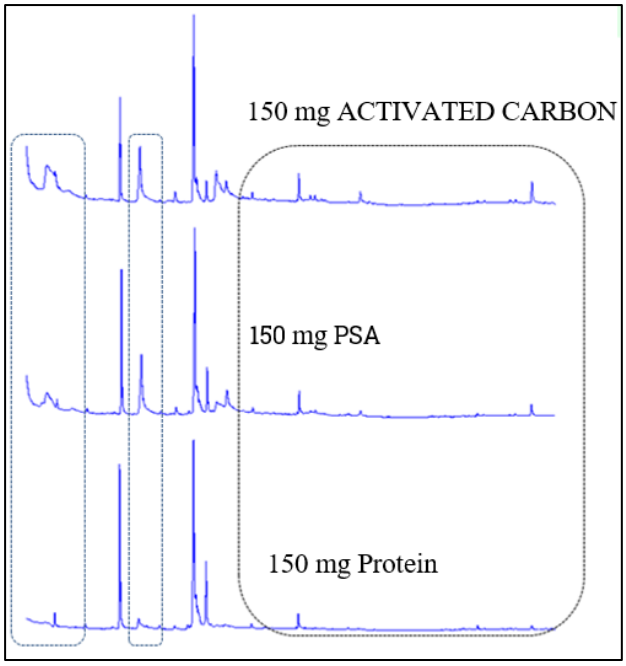


Figure 5.4 Chromatograms of QuEChERS fish sample extracted using QuEChERS and different clean-up sorbents

Further, a combination of 75 mg PSA and 75 mg C_{18} sorbent was used to clean the fish extract (figure 5.5). The clean-up activity was good but the selectivity was not better than *Moringa Oleifera* protein. Even though C_{18} is the most hydrophobic bonded silica sorbent, earlier eluted EDCs with low LogP values were not cleaned-up properly. A combination of commercial hemp and lab extracted hemp cellulose only gave better results for the earlier eluted EDCs in terms of selectivity. However in later retention times, even the target EDCs were taken away. Thus, *Moringa Oleifera* protein was still chosen overall as the best clean-up sorbent as it gave superior results.

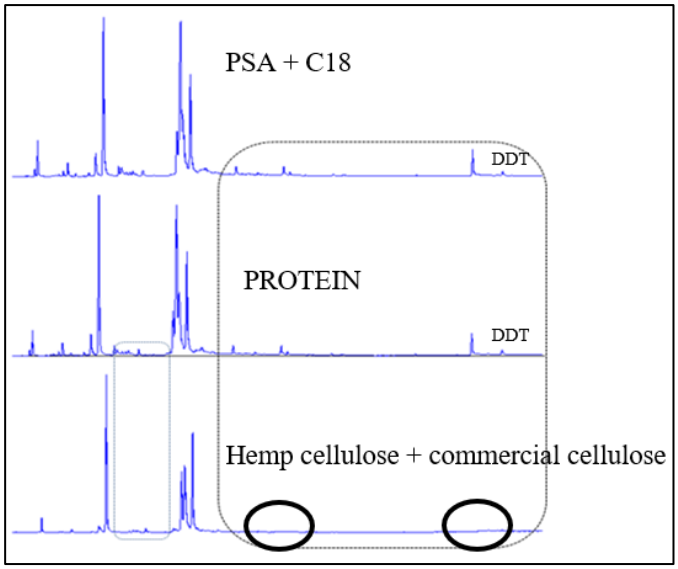


Figure 5.5 Chromatograms of QuEChERS fish sample extracted using QuEChERS and C18 and mixture of commercial and lab extracted cellulose

5.3 RSM optimisation of QuEChERS conditions

5.3.1 Screening experiments

The parameters which affect the efficiency of QuEChERS method (sample mass, speed and time of centrifuge, amount of $NaCl$ and $MgSO_4$, solvent type and solvent extraction volume) were screened using a linear L8 model. The L8 model was then fitted using MLS (Multiple linear Regression).

R^2 represents the percent of the variation of the response showing how well the model fits the data. The findings are shown in Table 5.3. A large R^2 (close to 1) is a necessary condition for a good model. For this model, R^2 values are in the range 0.78 - 0.94 (Table 5.3)

Table 5.3 Multiple linear regression (MLR) statistical analysis (n=21)

Chemical	R^2	Q^2	RSD (%)	Model Validity	Reproducibility
Dimethyl phthalate	0,92	0,64	9,80	0,68	0,95
Diethyl phthalate	0,94	0,77	11,27	0,75	0,94

4-Nonylphenol	0,88	0,58	12,18	0,88	0,78
4,4'-DDT	0,89	0,60	9,65	0,71	0,91
4,4'-DDE	0,84	0,56	20,76	0,26	0,98
4,4'-DDD	0,78	0,49	18,33	0,94	0,41

Some factors were deemed “non-essential” from the screening. They are the type and volume of solvent and amount of salt. However, the type of solvent used for extraction needs to satisfy several requirements for maximum recovery of the EDCs. The chosen solvent needs to have high affinity and high selectivity for the endocrine disrupting chemicals of interest. It needs to have a good chromatographic behavior and its interaction with water is also essential. The solvent needs to be immiscible with water and must be less dense than water (Bazregar et al., 2016).

The solvent polarity, cost, toxicity, and selectivity as well as the structure of the target analyte was taken into consideration since the chosen solvent should be able to extract all the target compounds. Figure 5.6 shows different peak concentration when three solvent types are varied for the extraction of endocrine disrupting chemicals of interest. All fish samples were spiked with $106.67\ \mu g\ kg^{-1}$ of the EDC’s. From the Figure 5.6, methanol is more selective towards unwanted matrix in the presence of the target endocrine disrupting chemicals in the extraction of fish sample.

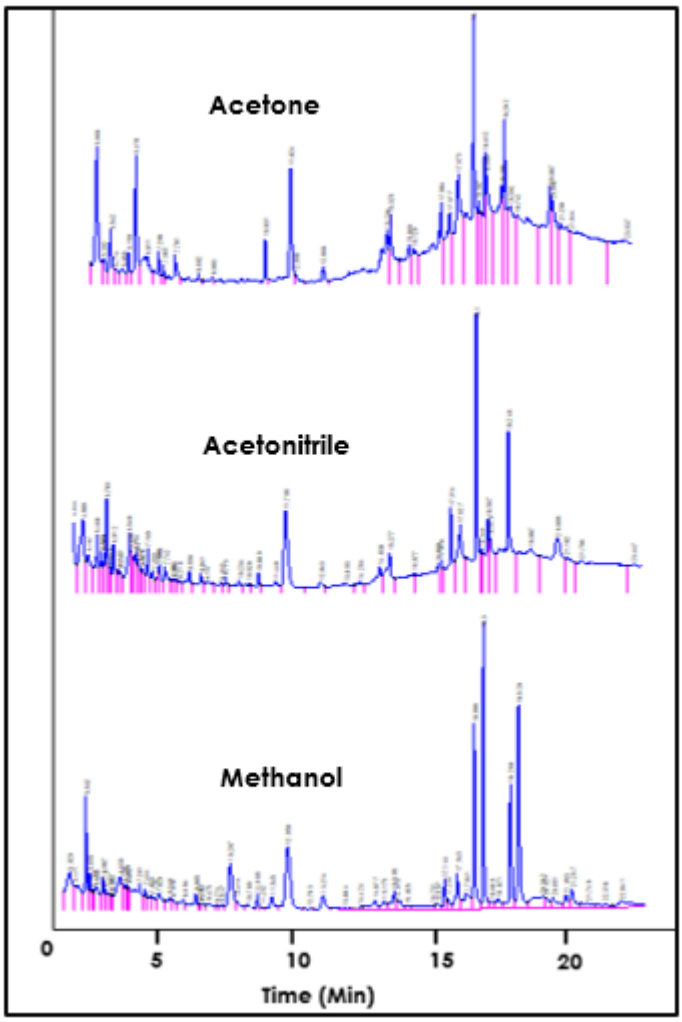


Figure 5.6 Effect of solvent type on QuEChERS extraction of a fish sample. (i) Acetone (ii) Acetonitrile (iii) Methanol.

The volume of the chosen solvent was also optimized. This is because extraction is based on the affinity differences between the analytes of the organic and aqueous phase. Generally, low solvent volumes are favored when taking enrichment factors into consideration. Low solvent volumes give higher enrichment factors. For this work, the final volume was reduced by nitrogen blowing to a required volume. For the extraction, 10 mL of solvent was optimized to give the best extraction of the analytes while ensuring that the final volume is enough to be collected for analysis. The final volume was $\pm$ 1 mL.

The amount of NaCl and $MgSO_4$ salts were also investigated as this is the step in QuEChERS where target EDCs are extracted. The amount added may have salting out effects, salting in effects or no effect at all on the extraction of endocrine disrupting chemicals (Zhang and Lee,

2013). The optimal amount of salt added was determined to be 2 g of NaCl and 2 g $MgSO_4$. Increasing this amount further decreased the concentration of all seven EDCs as the solution viscosity was also increased, consequently.

The fitted model shows a cross-validated predictability of $0.49 \leq Q^2 \leq 0.77$ and total explained variance of $0.78 \leq R^2 \leq 0.94$ where Q^2 shows an estimation of the future prediction and R^2 shows the model total variance. Figure 5.7 shows the linearity of the predicted versus observed values plot highlighted the validity of the model and its ability to predict the most important parameters to be optimized.

Ceylan et al. (2008) conducted a similar RSM study and found that the predicted data of the response from the empirical model agrees with the observed ones in the range of the operating variables. The high value of their adjusted R^2 (0.89) indicated that the model fit the observed data well. Other similar studies (Nuapia *et al.*, 2021) showed a cross-validated predictability of 65% (Q^2 = 0.65) and total explained variance of 95% (R^2=0.95). Makadi and Nanavati (2013) found that verification experiments carried out for predicted and observed results show that the empirical models developed were within 6% error.

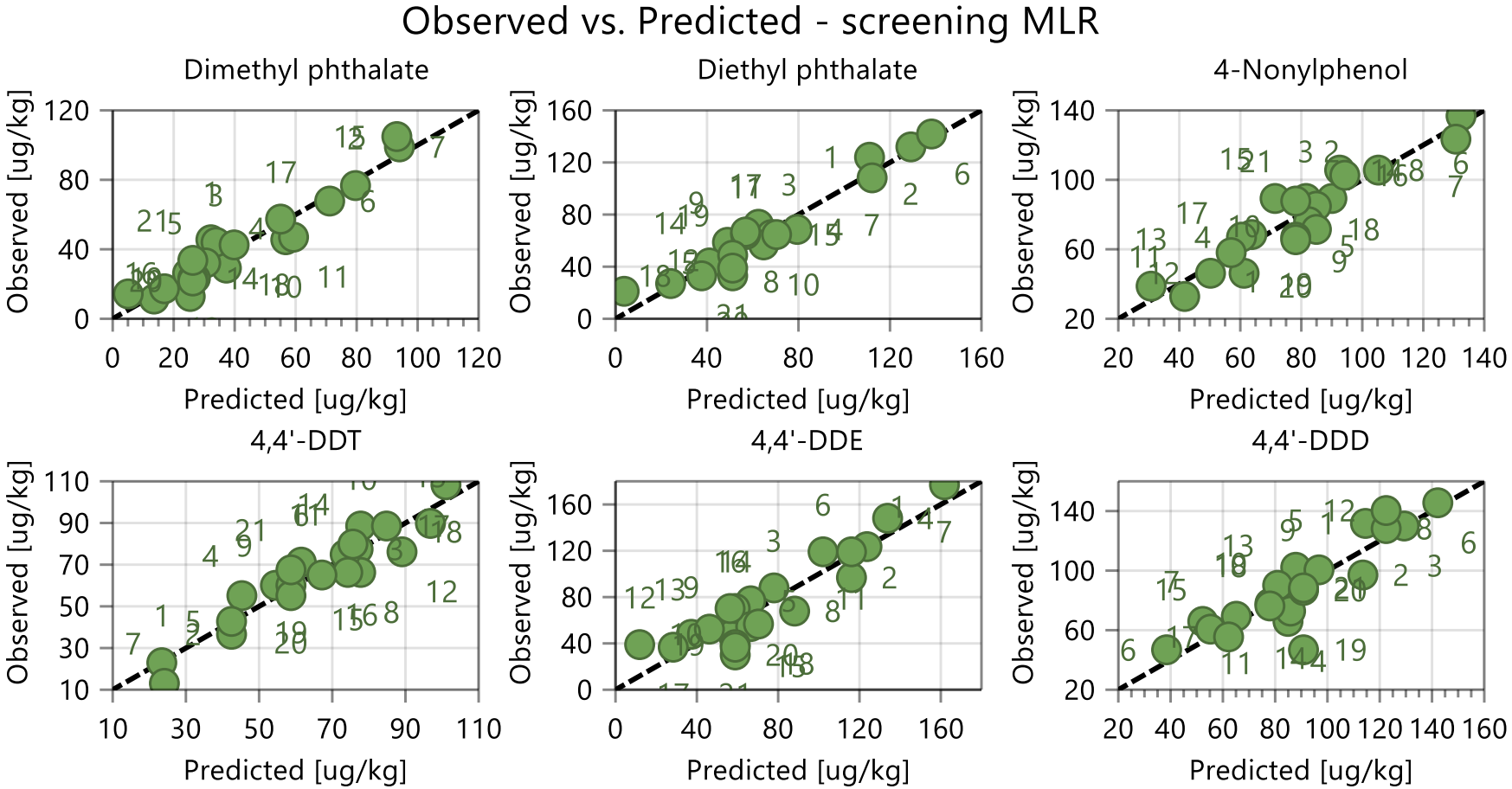


Figure 5.7 Observed vs Predicted concentrations of endocrine disrupting chemicals

The coefficient plots show the parameters that have significant influence on the endocrine disrupting chemical extraction when QuEChERS method is employed. It shows that centrifuge conditions and sample mass have significant influence on the extraction of 4,4'-DDT, 4,4'-DDD, 4,4'-DDE and 4-nonylphenol. Acetone as the solvent is not essential as it is not the optimum solvent even though it influences phthalates group significantly (Figure 5.8). The reasoning behind the significances is given in section 3.2.2. Figure 5.8 further shows that acetonitrile as a solvent is very relevant to the extraction of 4-Nonylphenol. Oscar Galarce-Bustos (2018) found that acetonitrile solvent greatly impacts polyphenols extraction in their study. No other study has reported the effect of partition salts (NaCl and M_gSO_4) on QuEChERS extraction using chemometrics.

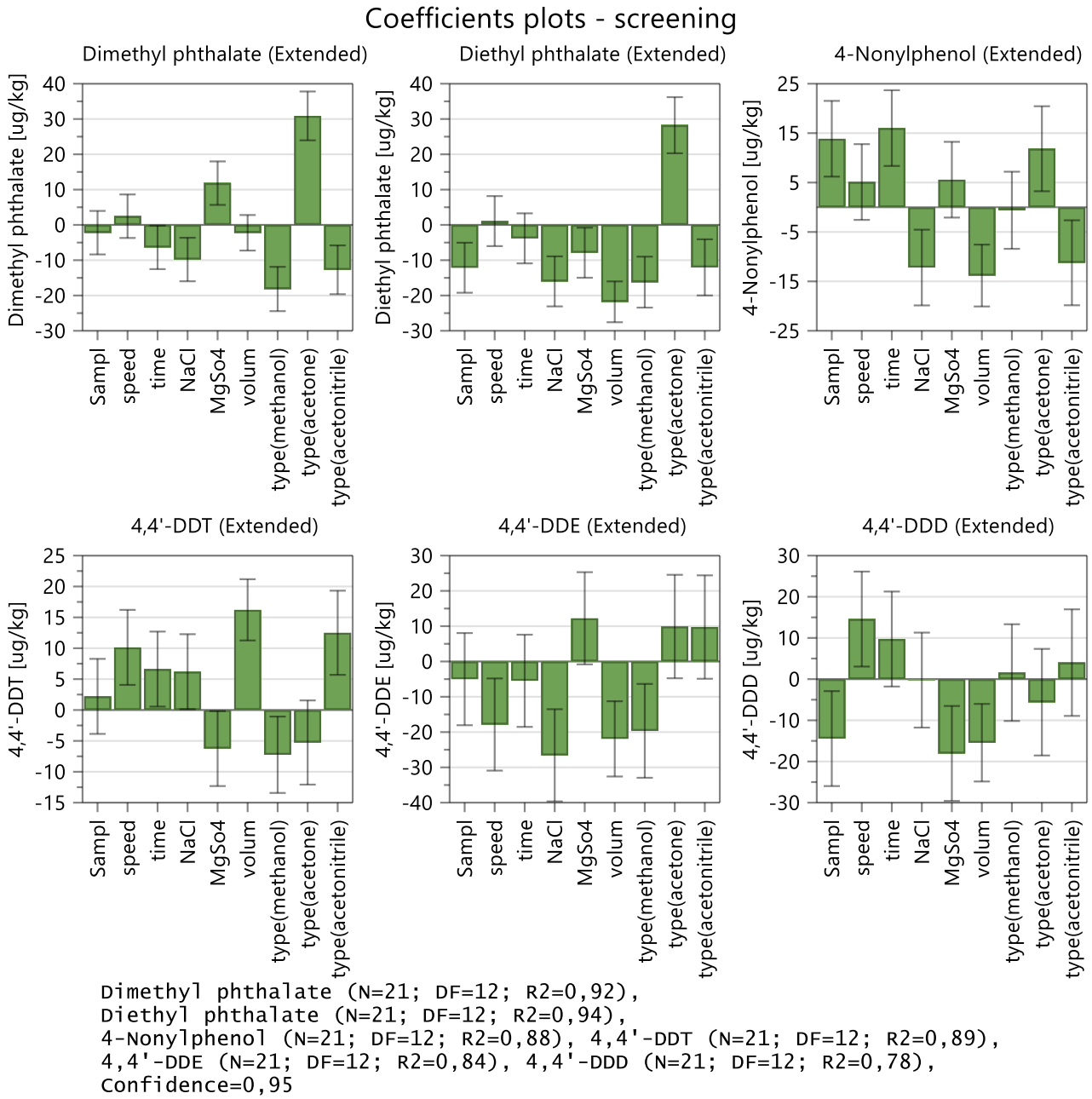


Figure 5.8 Coefficient plots: Effect of different screening factors on the concentration of endocrine disrupting chemicals.

5.3.2 Optimisation (RSM) using CCO quadratic design

The optimum values from the optimization are given on Table 5.4 with their factor contributions. From the design employed, it is essential to note that the sample mass, centrifuge time and centrifuge speed are the factors with the highest contributions. This is due to their

large influence on the efficiency of QuEChERS extraction of endocrine disrupting chemicals over the other screened parameters.

Table 5.4 RSM- Optimised values

Factor	Role	Value	Factor contribution
Sample mass	Free	3	52,8265
centrifuge time	Free	6	24,3663
centrifuge speed	Free	4000	22,8072

Centrifuge speed and time are essential parameters in QuEChERS extraction to get the target compound in the final extract from the solid. The centrifuge is used in two phases, firstly to separate the supernatant that contains the extracted target analytes from the solid sample after addition of partition salts (NaCl and M_gSO_4) and secondly to separate the final extract from the sample matrix in the extract. In this work, Figure 5.9 shows the optimum extraction conditions obtained by using graphical and numerical analysis based on the response surface plots. The contour plot shows that at optimal centrifuge speed of 4000 rpm, the optimal centrifuge time is at 6 mins. E.g., For 4,4'-DDE, increasing the centrifuge time from 6 to 17 mins decreases the 4,4'-DDE concentration from 90 to 60 $\mu g\ kg^{-1}$, corresponding to the coefficient plot at 95% confidence interval (Figure 5.8).

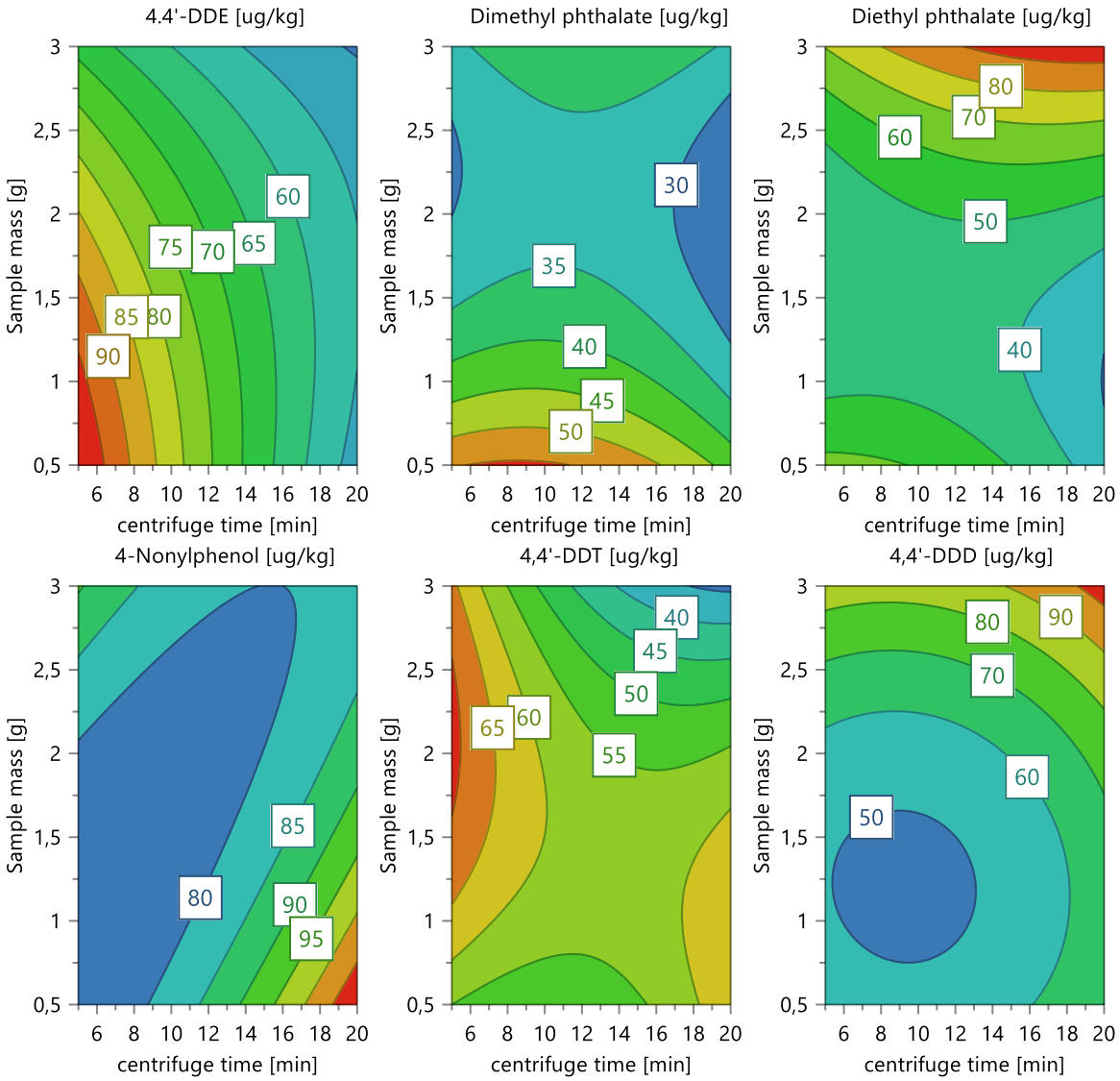


Figure 5.9 Contour plots of the interaction of the selected parameters on the concentrations of target endocrine disrupting chemicals

5.4 Concentration of Organic Endocrine Disrupting Chemicals (EDCs) from Johannesburg

In total, twenty one samples from Johannesburg were analysed for organic EDCs. The chromatograms are presented in the Appendix.

5.4.1 Concentration of organic EDCs in raw foodstuff sold in JHB open markets

Fourteen different samples from Johannesburg open markets were analyzed for organic EDCs concentration. The following EDCs were identified in Johannesburg food items in the order: DMP > 4-Nonylphenol > DEP > DDT >DDE (Table 5.5). The lowest detected mean concentration in Johannesburg samples was 4,4'-DDT in cabbage samples (5.2 ± 1.827 μg kg^{-1}), and the highest detected concentration was also 4,4'-DDT in (Tilapia fish - 188.9 ± 8.89 μg kg^{-1} and Beef muscle- 143.7 ± 11.87 μg kg^{-1}) followed by DMP in Banana (169.5 ± 12.28 μg kg^{-1}). The high concentration of 4,4'-DDT in fish can be attributed to the extent of bioaccumulation of the pesticides in fish' fat/lipid membranes. This is due to extensive pesticide use in farms, which eventually leaches into water bodies and aquatic environment in rainy seasons. Furthermore, when the herding cattle drink the water having DDT residue, the DDT bioconcentrates in the lipid tissue of the cattle. These levels of EDCs then accumulate in the bodies of human consumers in the process of biomagnification to potentially cause endocrine disrupting effects. 4,4'-DDT is quite persistent in the environment. Half the 4,4'-DDT in soil will break down in 2–15 years (Chopra et al., 2011). The 4,4'-DDT detected in this study in fish and beef samples was generally comparable to the results reported by (Nuapia et al., 2016) who reported 4,4'-DDT in beef as 167.89 $\pm$ 1.50 μg kg^{-1} and 134.57 $\pm$ 5.20 μg kg^{-1} in fish respectively in Johannesburg open markets. The levels of 4,4'-DDT detected in this study were higher than the limits recommended by the FAO/WHO (2012). No target EDC's was detected in tomato samples. This can be attributed to tomatoes being sold in boxes at the open market other than plastic packaging as we would anticipate DMP or DEP to be detected in plastic wrapped samples.

The mean concentration of 4-Nonylphenol varied from 34.7 ± 6.39 μg kg^{-1} to 141.5 ± 7.68 μg kg^{-1}, the highest being in the chicken liver. Similar work on Nonylphenols in South Africa was reported by (Günther and Sumpter, 2002) who analysed a chicken liver sausage sample,

targeting 4-Nonylphenol. The concentration was 13 μg kg^{-1}. Their highest concentration of 4-Nonylphenol is 18.5 μg kg^{-1} in potato samples. For this present study, 4-Nonylphenol was not detected in any potato samples. Nonylphenols accumulate in fat environments since they are hydrophilic, which correlates to the highest levels in this study being detected in beef and liver samples. However, some 4-Nonylphenol was detected in onion and cabbage with minimal fat. This can be attributed to pesticides. Nonylphenol ethoxylates (NPEs), which eventually degrade to NPs are commonly used in pesticide formulations. When it degrades, the nonylphenols accumulate in the wax coats of the cabbage and onion samples (Fang et al., 2015).

Table 5.5 Concentration of endocrine disrupting chemicals in fruits and vegetables ($\mu g\ kg^{-1}$) in Johannesburg open market.

	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Banana	169.5±12.28	88.3±9.96	nd	nd	nd
Potato	155.9±4.56	nd	nd	nd	nd
Garlic	nd	nd	nd	nd	nd
Red onion	nd	nd	nd	nd	nd
Green pepper	nd	nd	nd	nd	nd
Red pepper	nd	nd	nd	nd	nd
Yellow pepper	nd	nd	nd	nd	nd
Carrot	45.6±2.28	nd	nd	nd	nd
Onion	37.2±8.86	nd	77.2±7.23	nd	nd
Cabbage	8.1±5.69	nd	34.7±6.39	nd	5.2±1.87
Tomato	nd	nd	Nd	nd	nd

nd : Not detected

Table 5.6 Concentration of endocrine disrupting chemicals in beef, fish, and chicken samples ($\mu g\ kg^{-1}$) in Johannesburg open Market

	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT

Sample					
Fish	17.2±10.11	72.6±5.52	nd	86.3±8.56	188.9±8.89
Beef muscle	21.2±5.56	nd	93.8±4.56	62.8±8.86	143.7±11.87
Chicken Liver	23.7±6.69	76.1±6.63	141.5±7.68		nd

nd: Not detected

5.4.2 Concentration of organic EDCs in fruits and vegetables sold in JHB City deep main market

Seven different samples from Johannesburg City Deep main market were analyzed for organic EDCs concentration. The following EDCs were identified in the order: DEP > DMP >4-Nonylphenol. No 4,4'-DDE and 4,4'-DDT was detected. High residue levels (By mean concentration) were detected in Potato, followed by cabbage, Onion, Spinach and Peppers (Table 5.7).

Table 5.7 Concentration of endocrine disrupting compounds in food items ($\mu g\ kg^{-1}$) from Johannesburg deep city main market

	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Cabbage	10.2 ± 4.88	2.8 ± 1.55	12.8 ± 5.02	nd	nd
Potato	112.9±4.44	nd	nd	nd	nd
Yellow pepper	nd	2.3 ± 8.22	nd	nd	nd
Green pepper	nd	nd	nd	nd	nd
Red pepper	nd	4.8 ± 2.34	nd	nd	nd
Onion	nd	12.8 ± 4.12	8.8 ± 9.12	nd	nd
Spinach	8.8±4.11	nd	nd	nd	nd

nd : Not detected

5.4.3 Levels of organic EDCs in vegetables sold along JHB Hennops river farm

4,4'-DDE and 4,4'-DDT was not detected in the samples, which contradicts the hypothesis since the contaminated river water is mainly used to grow the samples. The occurrence of 4-Nonylphenol residues in the rape and vegetables are probably derived from the applied pesticides in the

agricultural soil. The concentration of 4-Nonylphenol in the leafy vegetables was higher than the commercial samples in the main market.

Table 5.8 Concentration of endocrine disrupting compounds in food items ($\mu g\ kg^{-1}$) from Johannesburg Hennops River farm

	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Rape 1	nd	nd	15.5±5.44	nd	nd
Rape 2	nd	nd	17.2±7.23	nd	nd
Spinach	nd	nd	nd	nd	nd

nd: not detected

5.5 Concentration of Organic EDCs from Pretoria foodstuff

In total, eighteen samples from Pretoria were analysed for organic EDCs. This was made of samples from open markets and Tshwane main market. The chromatograms are given in Appendix.

5.5.1 Concentration of organic EDCs in foodstuff sold in Pretoria open markets

The mean concentration of EDCs in Pretoria open market foodstuff is summarized in Table 5.9. The following EDCs were identified in the order: DEP >DMP 4-Nonylphenol > DDT . High residue levels were detected in carrot, rape followed by cabbage, onion, and tomato.

The highest concentration was recorded for Diethyl phthalate (69.2 ±2.44 µg kg^{-1}) in carrot. The lowest concentration was detected for 4-Nonylphenol (2.45 ±0.87 µg kg^{-1}) in rape. 4,4'-DDT was only detected in the leafy vegetables (cabbage and spinach). The presence of 4,4'-DDT pesticides could be attributed to its usage in killing pests or other plants that also grow with these vegetables. Also, the water regularly used to rinse the vegetables in open markets to keep them fresh might be a source of 4,4'-DDT contamination.

Similar study was conducted by Olatunji (2019) who evaluated DDT levels in fresh root and leafy vegetables collected from different farm areas in Cape Town. Analysis was done using GC-MS. They found the DDT levels to be in the range 38.9 to 66.1 µg kg^{-1} . Further, Nuapia et al., 2016

found the levels of DDT in cassava to be in the range of 57.56 ± 9.34 - 86.67 ± 6.23 µg kg^{-1}. The concentrations of DDT detected in this work were lower (range of 9.2 ± 7.44 to 17.3 ±8.22 µg kg^{-1}) compared to those of similar works done in Johannesburg.

Table 5.9 Concentration of endocrine disrupting compounds in food items ($\mu g\ kg^{-1}$) in Pretoria Open Markets

	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Carrot	61.4 ± 9.92	69.2±2.44	nd	nd	nd
Lettuce	nd	23.62 ± 8.62	nd	nd	nd
Onion	44.15 ± 4.23	12.5 ± 5.45	nd	nd	nd
Rape	34.33 ± 6.63	22.34 ± 3.34	2.45 ± 0.87	nd	nd
Tomato 1	nd	nd	67.2±2.36	nd	nd
Tomato 2	Nd	12.33 ± 5.33	nd	nd	nd
Cabbage 1	24.6±8.85	51.22 ±7.21	nd	nd	17.3±8.22
Cabbage 2	44.12 ± 4.41	nd	nd	nd	nd
Spinach	nd	nd	nd	nd	9.2 ± 7.44

5.5.2 Concentration of organic EDCs in foodstuff sold in Pretoria Tshwane market

Further, nine more samples from Tshwane market were analyzed for EDCs concentration. The following EDCs were identified in Johannesburg food items in the order: DMP > 4-Nonylphenol > DEP > DDT >DDE (Table 5.10). The levels of EDCs were detected in the range of 6.53 ± 2.77 µg kg^{-1} for 4-Nonylphenol in butternut to 95.8 ± 6.69 µg kg^{-1} for Diethyl phthalate in Red pepper. In terms on mean concentration, beetroot samples presented high EDC residue levels over all samples. 4,4'-DDT in beetroot was detected as 44.2±3.73 µg kg^{-1}. Again, this can be speculated to come from the use of DDT as insecticides for insect control in the farms and also irrigation with contaminated water. The residues can accumulate in the tubers or root growth of the beetroot. The EDCs detected from Tshwane main market foodstuff do not impose endocrine disrupting danger to consumers as they are within the permissible limits set by the relevant

legislation agencies. Tshwane main market stock their produce from eleven commissioned agents that have a good reputation in the fresh produce industry.

Table 5.10 Concentration of endocrine disrupting compounds in food items ($\mu g\ kg^{-1}$) in Pretoria Tshwane Main Market

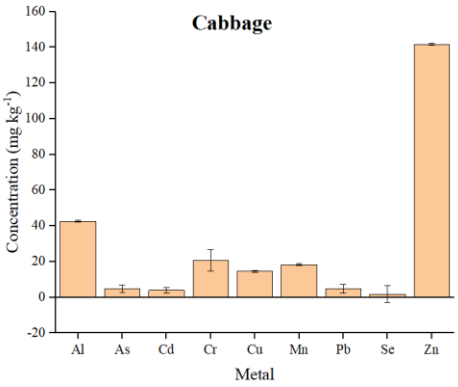
	DMP	DEP	4-nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Lettuce	nd	nd	nd	nd	nd
Butternut	nd	nd	16.53 ± 7.77	nd	nd
Yellow pepper	nd	22.4 ± 4.52	nd	nd	nd
Red pepper	46.7±8.96	95.8±6.69	nd	nd	nd
Green pepper	74.1±1.10	nd	61.2±8.36	nd	nd
Sweet potato	22.4±6.69	nd	27.8±4.69	nd	nd
Beetroot	34.6±8.85	75.8±7.63	nd	nd	44.2±3.73
Eggplant	nd	nd	16.78 ± 9.22	nd	nd
Carrot	52.24 ± 7.77	45.33 ± 6.38	nd	nd	nd

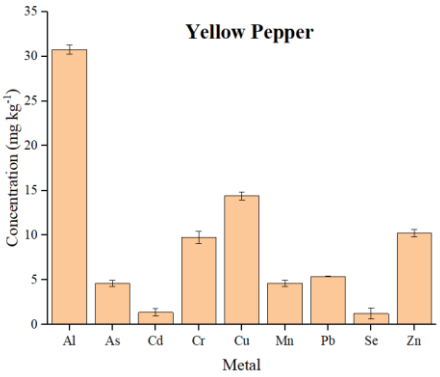
5.6 Concentration of Metals in Johannesburg Foodstuff

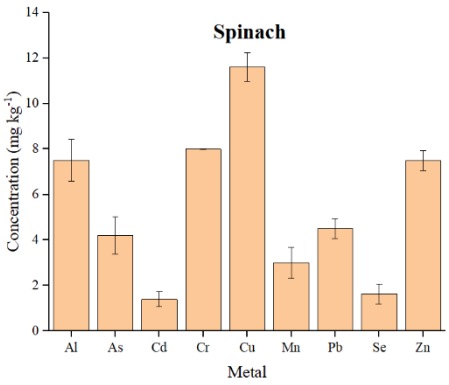
The same Twenty samples from Johannesburg analysed for organic EDCs were also analysed for inorganic EDCs (metals) using Inductively Coupled Plasma - Optical Emission Spectroscopy, ICP-OES (section 4.9.2).

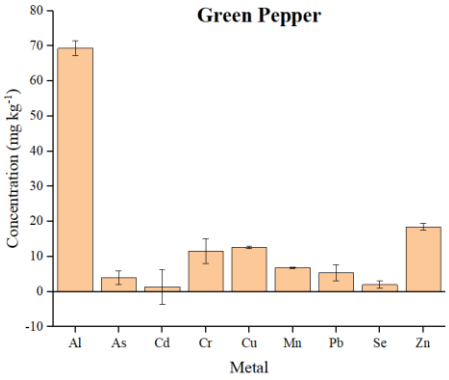
5.6.1 Concentration of Metals in Johannesburg raw vegetables sold in Open Markets

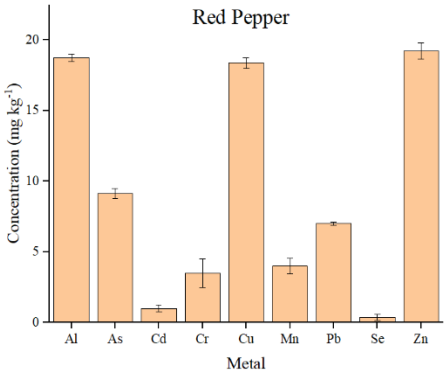
Selected vegetable samples (n= 9) were analyzed for metals concentration. Nine metals (Al, Zn, Cu, Cd, Pb, Mn, As, Se and Cr) were investigated. The analyzed metals (in terms of mean concentration) were detected as follows: Zn > Cu > Al > As > Cr > Cd > Mn > Pb > Se. (Figure 5.10).

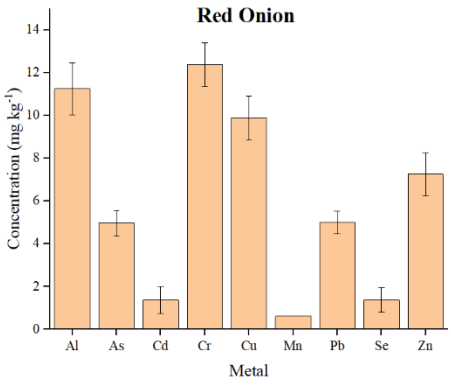












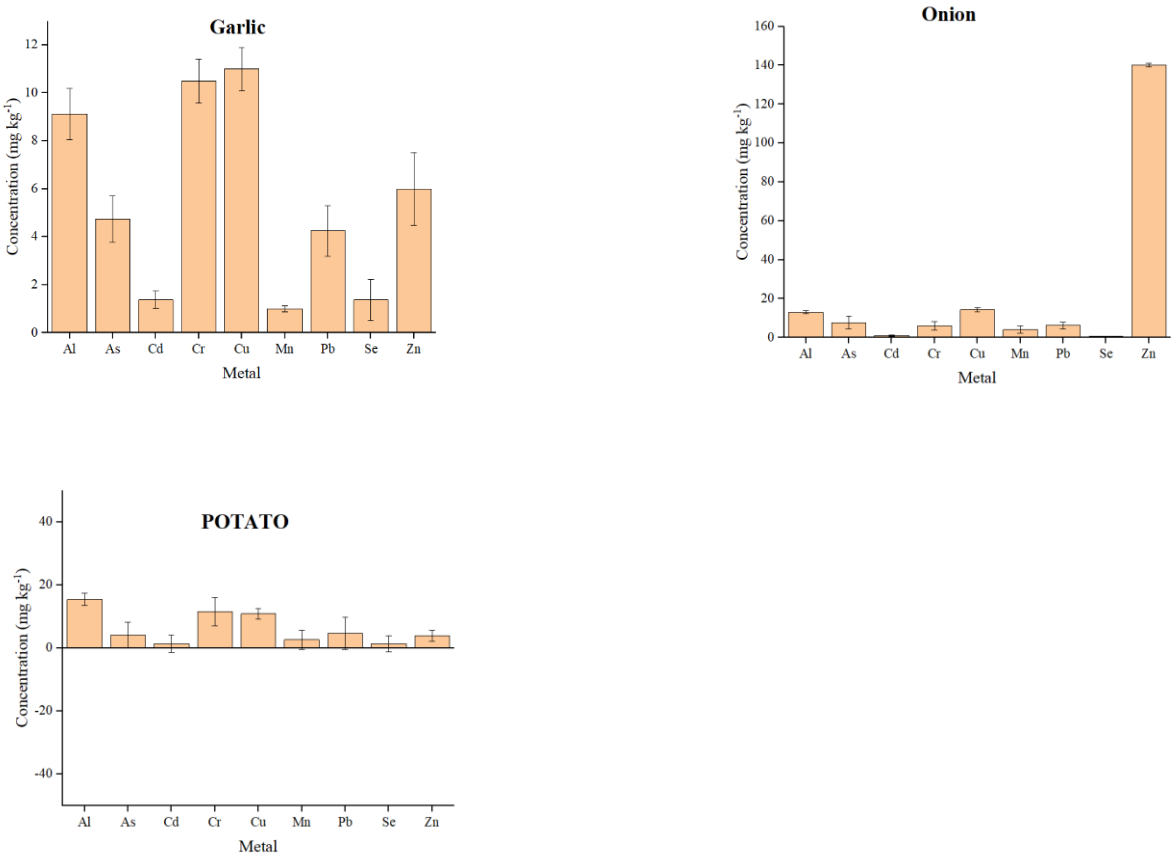


Figure 5.10 Concentration ($mg\ kg^{-1}$) of Metals detected in vegetable foodstuff from Johannesburg Open Markets

The maximum permitted FAO/WHO (2000) concentrations are presented in Table 1 of Appendix II. In all the studied foodstuff, Zn was the highest detected metal in terms of mean concentration. The highest detected Zn concentration was for Cabbage ($141.63 \pm 0.540\ \text{mg kg}^{-1}$) and the lowest detected Zn concentration was for Potato ($3.88 \pm 1.688\ \text{mg kg}^{-1}$). The mean concentration of Zn detected in the present study was similar to that reported by (Nuapia et al., 2018) in Cabbage. Zn is an essential element for human health, but it can cause adverse health effects if intake is either too low (Zn deficiency) or too high (Zn toxicity). The FAO/WHO maximum permitted concentration of Zn is between 8 and 11 mg kg^{-1} (WHO/FAO, 2000). 78% of the investigated foodstuff in JHB open markets in this study were within the permissible levels and 22% exceeded the permissible limit for Zn set by the FAO/WHO (2000).

The highest detected Cu concentration was 18.38 ± 1.600 mg kg^{-1} in red pepper samples and the lowest detected was 9.88 ± 0.719 mg kg^{-1} in red onion. Cu is an essential metal that works together with Fe to enable the body to form red blood cells (Collins et al., 2010). It helps maintain healthy bones thus preventing osteoporosis and is also required for healthy immune function (Alagawany et al., 2020). At the same time, it can be toxic when present in excess, the most noticeable chronic effect being liver damage (de Jong et al., 2019). According to FAO/WHO (2000), the permissible limit for Cu in food is set between 2 to 10 mg kg^{-1}. About 10 % of the samples investigated in this study were lower than the maximum FAO/WHO permitted levels.

Al is a known endocrine disruptor and a neurotoxic agent because this metal tends to accumulate in the brain (Briffa et al., 2020; Gade et al., 2021). Several studies have correlated certain Aluminum concentration and different diseases such Alzheimer's disease (Inan-Eroglu and Ayaz, 2018; Hardisson et al., 2017). In addition, aluminum can interfere with some essential metals. The Al concentration ranged from 7.50 ± 0.917 mg kg^{-1} in spinach samples which was the lowest detected to 69.33 ± 2.064 mg kg^{-1} in green pepper which was the highest detected in this study. The concentration detected in the present investigation was similar to that reported by Hardisson et al. (2017) who detected mean Al in South African vegetables as 16.8 mg kg^{-1}. According to FAO/WHO (2000), the permissible limits for Al content was set for 12 to 71 mg kg^{-1} in foodstuffs. 100 % of the investigated vegetables samples in this study were within the permissible limit. The highest Al content in green pepper may be due to high Al content in the soil where the peppers were harvested.

As was detected in all studied foodstuff in the range 3.96 ± 1.911 mg kg^{-1} in green pepper to 9.13 ± 0.365 mg kg^{-1} in red pepper. According to FAO/WHO (2000), the permissible concentration limits for As in food is 0.1 to 0.2 mg kg^{-1} . All the studied food samples in this study were above the permissible limit . The results found in this study for Arsenic were above those reported by Nuapia et al. (2018) (1.01 -3.09 mg kg^{-1}). The reason for this may be due to the As in the environment that end up in the open market vegetables as they are exposed to the environment when on display and also from the rinsing water that is contaminated with the metal.

There have been no reported cases of Cr toxicity due to food intake. Cr (III) helps to transport blood glucose from the bloodstream into the cells to be used as energy (Chang et al., 2020). Cr (VI) has been shown to be potent occupational carcinogens (Nikfar et al., 2011). In the current

study, Cr was detected in all food samples with varying concentration ranging from 3.5 ± 1.012 mg kg^{-1} in red pepper to 20.8 ± 1.547 mg kg^{-1} in cabbage. Regarding to WHO/FAO (2000) permissible limit, the level of Cr in all the samples were above the permissible limit in vegetables food.

Cd was found in all the JHB open market foodstuff in the range 1.0 ± 0.244 mg kg^{-1} in red pepper to 4.0 ± 1.569mg kg^{-1} in cabbage. According to the FAO limits of 0.30 mg kg^{-1}, the level of Cd in the studied samples are above the FAO permissible limits but not too concentrated to cause endocrine disruption related complications. Thus, the impacts of sample handling and sampling treatment from the farms to the open market may play a role in the increased Cd concentration.

Pb occurs in foodstuff because of its presence in the environment that end up in foodstuff. E.g., Pb in the farm soil can settle on or be absorbed by vegetables grown there. Pb that gets into the vegetables roots cannot be completely removed merely by washing or rinsing. Pb was found in all studied food samples in the range 4.25 ± 1.062 mg kg^{-1} in garlic to 7.000 ± 0.096 mg kg^{-1} in red pepper. The means value of Pb found in the vegetables from Johannesburg open markets were similar with that reported by (Manzoor et al., 2018) who reported a mean value of 5.5 mg kg^{-1} in spinach.

Se was detected in all vegetable food samples ranging from 0.38 ± 0.021 mg kg^{-1} in red pepper to 2.0 ± 0.107 mg kg^{-1} in green pepper. The level of selenium found in this current study was lower to that reported by Bañuelos wt al. (2020) who reported the Se concentration in vegetables as follows: broccoli 10.0 ± 3.7 mg kg^{-1}, green cabbage 13.1 ± 2.9 mg kg^{-1}, red cabbage 17.2 ± 6.4 mg kg^{-1}, savoy cabbage 11.0 ± 3.0 mg $kg^{-1}$and Swiss chard 4.8 ± 1.4 mg kg^{-1}. The maximum permitted levels for Selenium in food is not specified by the World Health Organization or other legislation agencies. However, for soil, it is set at 6 mg kg^{-1}. If soil is the major source of selenium concentration in the studied vegetables, then the detected mean concentrations are below the maximum permitted levels.

5.6.2 Concentration of Metals in raw beef and chicken sold in Open Markets

Samples of beef meat and chicken liver were purchased from an open market in Johannesburg CBD. The metals detected in the beef muscles were identified in the following sequence: Al > Cu > Cr > Pb > As > Mn > Zn > Cd > Se. The concentration of Al in the sample is 4.17 ± 0.315 mg kg^{-1} which is the highest detected metal in beef. In chicken liver, the highest detected metal is Zn (89.63 ± 0.168 mg kg^{-1}) . The metals detected in the liver were identified in the following sequence: Zn > Al > Cu > Cr > As > Pb > Se > Mn > Cd This is represented in figure 5.11.
Al is common through water, plants, air, and food chain (Nuapia et al., 2018 ; Oniawa et al., 2000). The presence of Al in beef and chicken liver samples may be due to the high Al content in plants that are eaten by the animals and passed via bioaccumulation and biomagnification.

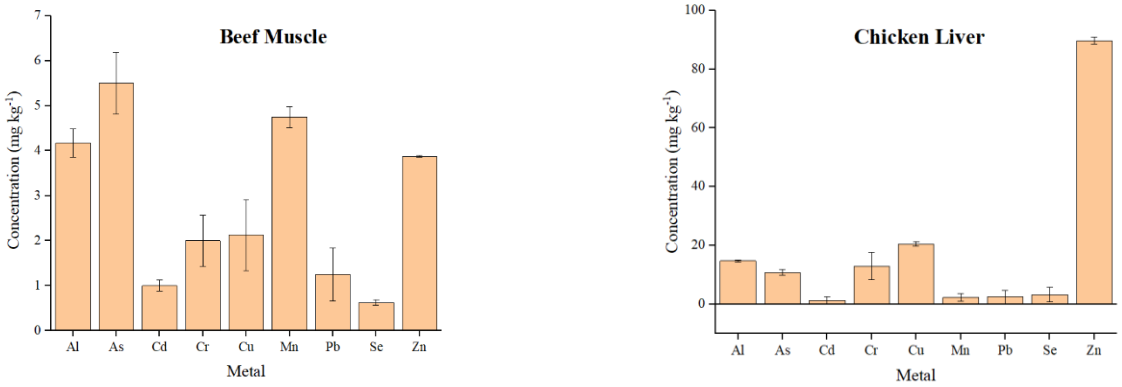


Figure 5.11 Concentration ($mg\ kg^{-1}$) of Metals detected in beef and chicken meat from Johannesburg Open Markets.

Table 5.11 shows the comparison between detected concentrations in this study compared to similar studies in South African open markets by (Nuapia et al., 2018) for metals concentrations in beef meat. Most metals detected in this study are higher (but in same magnitude) to those of the referenced report. The beef meat was bought from an open market operating very close to a taxi station and industrial manufacturing businesses in JHB CBD. Some level of metals is likely to originate from there. In close proximity to the markets, there was also untreated sewage sludge (Figure 4.3).

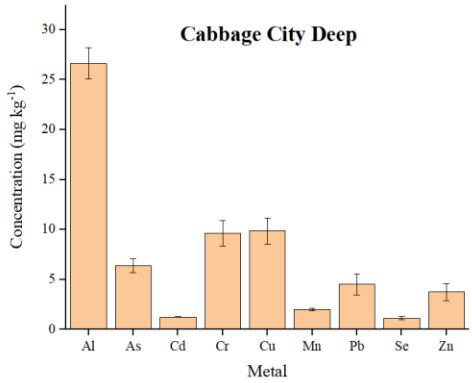
Table 5.11 Comparison of metal concentration in beef with similar South African studies

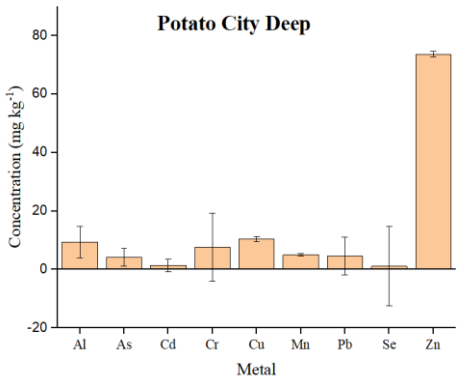
Metal	Concentration in this study in $mg\ kg^{-1}$	Ref. Concentration ($mg\ kg^{-1}$)
Al	4.17 ± 0.315	3.57 – 4.19
Cu	2.13 ± 0.788	0.32 – 0.35
Cr	2.00 ± 0.566	0.45 – 0.78
Pb	1.25 ± 0.588	0.28 – 0.37
As	5.5 ± 0.685	0.35 – 0.86
Mn	4.75 ± 0.233	0.24 – 0.39
Zn	3.88 ± 0.025	2.98 – 6.38
Cd	1.00 ± 0.125	0.29 – 0.32
Se	3.875 ± 0.056	0.28 – 0.30

5.6.3 Concentration of Metals in Johannesburg City Deep main market fruits and vegetables

Figure 5.12 shows mean concentrations of the selected metals for Johannesburg City Deep market samples.

Seven samples vegetables were collected from JHB City Deep main market. Nine metals were determined the foodstuff. The mean concentrations from highest to lowest were detected in the order : Zn > Al > Cu > Cr > Pb > As > Mn > Cd > Se .





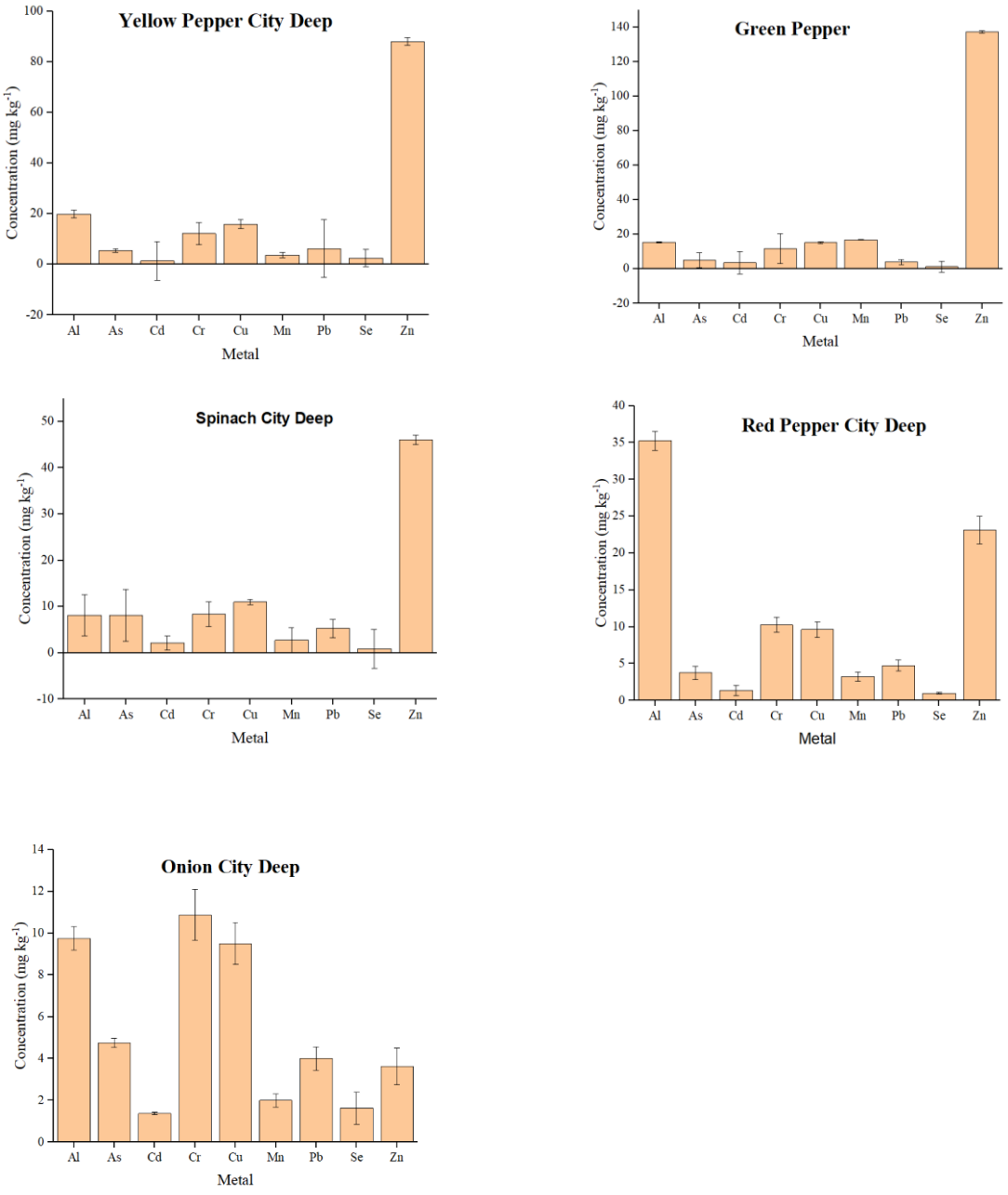


Figure 5.12 Concentration ($mg\ kg^{-1}$) of Metals detected in vegetables from City Deep main market

5.6.4 Concentration of metals in Hennops river farm vegetables

Rape and Spinach samples collected from a farm along Hennops river were analyzed for metals. The highest detected metal in rape was 15.25 ± 0.499 mg kg^{-1} for Cu and the lowest detected

was 1.38 ± 0.107 mg kg^{-1} for Cd. In Spinach, the highest detected metal was 42.4 ± 1.211 mg kg^{-1} for Al and the lowest detected was 0.75 ± 0.058 mg kg^{-1} for Pb. Spinach presented the highest concentrations for Al, Cd, Cr, Cu, and Mn when compared to levels in open markets and main markets. This is likely due to the metals accumulated in the sewage contaminated water used for irrigation along the Hennops river farm. Figure 5.13 shows mean concentrations of the selected metals for Hennops river farm samples.

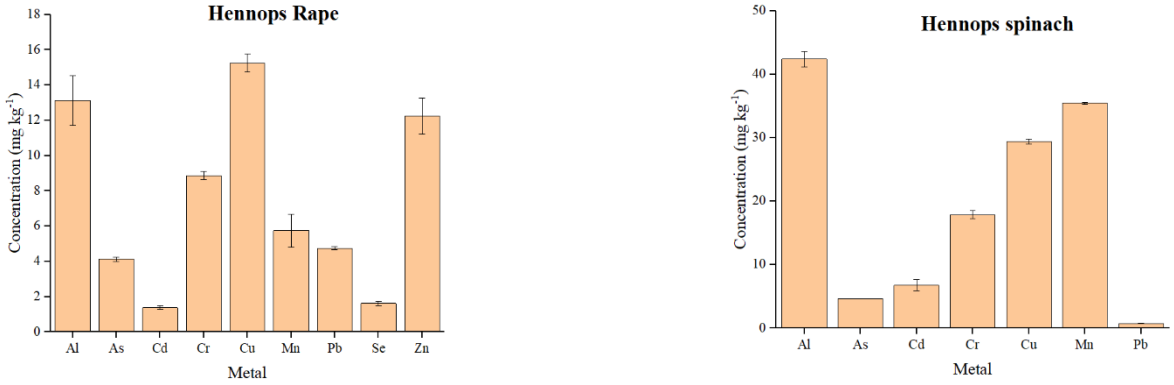


Figure 5.12 Concentration ($mg\ kg^{-1}$) of Metals detected in vegetables from Hennops river farm.

5.6.5 Statistical Analysis and comparison on the concentration of metals in Johannesburg open market and City Deep.

Statistical hypothesis testing of the data was done using student's t-test in Origin Pro software. This allowed comparison of vegetable samples from Johannesburg open markets with vegetable samples from the main market. The results are presented in table 5.15.

Table 5.12 Comparative mean concentrations ($mg\ kg^{-1}$) of metals in vegetables collected in Pretoria and Johannesburg

Sample	P-Value
Cabbage	0.098
Green Pepper	0.289
Yellow Pepper	0.193

Since P > 0.05, the mean concentration of metals in Johannesburg open markets were not significantly higher than those in main markets.

5.7 Concentration of Metals in Pretoria Foodstuff

Eighteen samples from Pretoria analysed for organic EDCs were also analysed for inorganic EDCs (metals) using Inductively Coupled Plasma - Optical Emission Spectroscopy, ICP-OES (section 4.9.2).

5.7.1 Concentration of Metals in Pretoria raw vegetables sold in Open Markets

Eight samples of fruits and vegetables were collected from an open market in Pretoria. The summary of the mean concentrations of the metals detected in Pretoria is given in Table 5.13 . Nine metals were investigated, and the mean concentrations are detected in the sequence : Al > Zn > Mn > As > Cu > Cr > Pb > Cd > Se. When comparing with Johannesburg Open markets (Zn > Cu > Al > As Cr > Cd > Mn > Pb > Se), Selenium is the lowest detected metal in both cities. Cd was also detected in lower concentration in Pretoria.

Table 5.13 Concentration $(mg\ kg^{-1})$ of Metals detected in vegetables from Pretoria open markets.

Vegetable	Metals								
	Al	As	Cd	Cr	Cu	Mn	Pb	Se	Zn
Carrot	141.25	5.2	1.25	12.125	16.63	177.25	3.125	1.25	4.75
	±0.811	±0.244	±0.214	±1.052	±2.036	±0.889	±0.662	±0.028	±0.215
Tomato	8.375	4.625	1.375	11.0	10.75	6.75	4.0	1.25	118.87
	±0.455	±0.588	±0.058	±1.024	±1.085	±0.522	±0.422	±0.211	±1.529
Lettuce	12.25	5.0	1.375	10.875	16.375	6.375	5.0	0.50	59.375
	±1.211	±0.276	±0.251	±1.009	±1.531	±1.100	±0.642	±0.017	±1.118
Onion	64.5	4.375	1.375	10.875	11.875	51.375	4.875	1.125	14.50
	±1.811	±0.625	±0.124	±1.296	±1.522	±2.366	±0.346	±0.106	±1.011
Rape	17.0	6.5	1.25	9.375	10.375	4.575	5.625	1.125	6.50
	±0.822	±0.566	±0.068	±1.038	±1.085	±0.622	±0.211	±0.063	±0.127
Tomato 2	19.0	4.25	1.25	9.875	10.625	1.375	5.375	1.75	6.125
	±1.077	±0.277	±0.106	±1.155	±1.285	±0.201	±0.311	±0.102	±0.625

Cabbage	9.0	8.05	1.125	9.125	10.015	2.625	5.250	1.00	2.050
	± 0.522	± 0.622	± 0.067	± 1.066	± 1.055	± 0.112	± 0.216	± 0.024	± 0.104
Spinach	774.250	6.125	3.625	11.875	2.505	54.00	5.250	2.005	14.625
	± 2.227	± 0.658	± 0.021	± 1.061	± 1.339	± 2.015	± 0.452	± 0.137	± 1.182

Se in certain amounts is essential for better health. The low mean concentration of Selenium detected in vegetables in this study can be linked to the Se concentration in soil used in farming and agriculture where the vegetables originate. When Se concentration is lower than the limits set by legislation bodies (6 mg kg^{-1} in soil), it can lead to an increased risk of several diseases such as cancer, heart disease, viral diseases and other conditions that involve increased levels of oxidative stress (Huag et al., 2009).

Regarding the maximum permissible limits stipulated by FAO/WHO (2000) for metals in vegetables food, 25% of the vegetables exceeded maximum Al limits, 100 % of the vegetables exceeded maximum As limits, 100% of the vegetables exceeded maximum Cr limits, 0% of the vegetables exceeded maximum Cu limits, 0% of the vegetables exceeded maximum Mn limits, 100% of the vegetables exceeded maximum Pb limits, and 0% of the vegetables exceeded maximum Al limits.

CHAPTER SIX : CONCLUSIONS AND RECOMMENDATIONS

Finally, the concludes the results discussed above.

6.1 Conclusions

Chemometric optimization was found to be an accurate and easy way of optimizing different parameters that affects extraction efficiency. The QuEChERS technique was optimized for the maximum extraction of some Endocrine Disrupting Chemicals in food. The optimized QuEChERS technique has been found to be very effective in extracting the EDCs from vegetables, beef and fish. The clean-up using *Moringa Oleifera* was very easy because the step was very fast and no intensive labor and instrumentation was required. The performance of the *Moringa Oleifera* and PSA as clean-up sorbents was compared and the protein gave superior results. This is seen by cleaner chromatograms when the protein is used as sorbent, most likely due to the chemistry of the protein functional groups.

The results obtained from the FTIR show that the water-extracted *Moringa Oleifera* protein powder contains the necessary functional groups which are responsible to clean-up sorbent activity and removal of coagulants from Pesticide-determination methods, primarily the amine, amide and alcohol groups.

The concentration of EDCs such as Dimethyl phthalate ,diethyl phthalate, 4-Nonylphenol, DDT, DDE and DDD were high in Johannesburg raw food as compared to Pretoria. The difference in concentration was not significant since $P > 0.05$. The highest levels of some EDCs in the raw food from Johannesburg can be attributed to the higher agricultural and mining activities carried out around Johannesburg compared to Pretoria. The concentrations in open markets were greater than in main markets, suggesting that the environment and handling of food in open markets is likely a large contributor to the contaminants levels in the foodstuff. Samples collected from a farm near Hennops river presented very high EDC concentrations due to the use of sewage treated water for irrigation.

The metals levels were also generally higher in Johannesburg open markets over Pretoria for the same reasons stated above. Some metals concentrations exceed the maximum levels set by WHO/FAO which alerts food safety in open markets.

6.2 Recommendations and Future Work

- Future work for thorough carcinogenic risk assessments must be established in terms of Daily limit Intake of Metals (DIM) and Hazard index (HI).
- The target Endocrine Disrupting Chemicals should be detected directly from the human urine and blood to get the true representation of the chemicals that affects the human health
- Other QuEChERS clean-up sorbents should be compared for performance
- **2-D** analysis of EDCS using GC x GC MSTOF

CHAPTER 7 : REFERENCES

Abbassy, Moustafa A., Moustafa A. Khalifa, Atef M. K. Nassar, Eman E. Nour El-Deen, and Yehia M. Salim. 2021. "Analysis of Organochlorine Pesticides Residues in Fish from Edko Lake (North of Egypt) Using Eco-Friendly Method and Their Health Implications for Humans." *Toxicological Research* 37(4):495–503. doi: 10.1007/s43188-020-00085-8.

AbdElgawad, Hamada, Gaurav Zinta, Badreldin A. Hamed, Samy Selim, Gerrit Beemster, Wael N. Hozzein, Mohammed A. M. Wadaan, Han Asard, and Walid Abuelsoud. 2020. "Maize Roots and Shoots Show Distinct Profiles of Oxidative Stress and Antioxidant Defense under Heavy Metal Toxicity." *Environmental Pollution* 258. doi: 10.1016/j.envpol.2019.113705.

Abdulhussain, Noor, Suhas Nawada, and Peter Schoenmakers. 2021. "Latest Trends on the Future of Three-Dimensional Separations in Chromatography." *Chemical Reviews* 121(19):12016–34.

Adzitey, Frederick, David Mireku, and Nurul Huda. 2018. "Assessment of Selected Heavy Metal Concentration in Fresh and Grilled Beef - A Case Study in East Legon, Ghana." *International Journal of One Health* 4:40–44. doi: 10.14202/IJOH.2018.40-44.

Afonne, Onyenmechi Johnson, and Emeka Chinedu Ifediba. 2020. "Heavy Metals Risks in Plant Foods – Need to Step up Precautionary Measures." *Current Opinion in Toxicology* 22:1–6.

Agency for Toxic Substances and Disease Registry. 1995. "Chapter 4 . Chemical and Physical Information - Silica." (14808):198–207.

Ahmed, Sarah Mustafa, Mohd Raihan Taha, and Omer Muhie Eldeen Taha. 2018. "Kinetics and Isotherms of Dichlorodiphenyltrichloroethane (DDT) Adsorption Using Soil–Zeolite Mixture." *Nanotechnology for Environmental Engineering* 3(1). doi: 10.1007/s41204-017-0033-8.

Alagawany, Mahmoud, Shaaban S. Elnesr, Mayada R. Farag, Ruchi Tiwari, Mohd Iqbal Yatoo, Kumaragurubaran Karthik, Izabela Michalak, and Kuldeep Dhama. 2020. "Nutritional Significance of Amino Acids, Vitamins and Minerals as Nutraceuticals in Poultry Production and Health–a Comprehensive Review." *Veterinary Quarterly* 41(1):1–29.

Alara, Oluwaseun Ruth, Nour Hamid Abdurahman, and Chinonso Ishamel Ukaegbu. 2021. "Extraction of Phenolic Compounds: A Review." *Current Research in Food Science* 4:200–214.

Ali, Hazrat, and Ezzat Khan. 2019. "Trophic Transfer, Bioaccumulation, and Biomagnification of Non-Essential Hazardous Heavy Metals and Metalloids in Food Chains/Webs—Concepts and Implications for Wildlife and Human Health." *Human and Ecological Risk Assessment* 25(6):1353–76.

Alon, Uri. 2021. *Hormone Circuits Lecture Notes The Insulin-Glucose Circuit*. Vol. 3.

Al-Rubaye, Abeer Fauzi, Imad Hadi Hameed, and Mohanad Jawad Kadhim. 2017. "A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants." *INTERNATIONAL JOURNAL OF TOXICOLOGICAL AND PHARMACOLOGICAL RESEARCH* 9(01). doi: 10.25258/ijtpr.v9i01.9042.

Altarawneh, Rakan M. 2021. "Levels of Selected Heavy Metals (Pb, Ni, Cd, and Cr) in Various Widely Consumed Fruits and Vegetables in Jordan." *International Journal of Environmental Analytical Chemistry* 101(7):1026–33. doi: 10.1080/03067319.2019.1675653.

Alvarez, María v., Santiago Cabred, Cristina L. Ramirez, and María A. Fanovich. 2019. "Valorization of an Agroindustrial Soybean Residue by Supercritical Fluid Extraction of Phytochemical Compounds." *Journal of Supercritical Fluids* 143:90–96. doi: 10.1016/j.supflu.2018.07.012.

Ambwani, Sonu, Tanuj Kumar Ambwani, and Ramswaroop Singh Chauhan. 2018. "Immunotoxic Effects of Cypermethrin in Mitogen Stimulated Chicken Lymphocytes Due to Oxidative Stress and Apoptosis." *~ 37 ~ Journal of Entomology and Zoology Studies* 6(2).

Amuanyena, Marta O. N., Martha Kandawa-Schulz, and Habauka M. Kwaambwa. 2019. "Magnetic Iron Oxide Nanoparticles Modified with Moringa Seed Proteins for Recovery of

Precious Metal Ions." *Journal of Biomaterials and Nanobiotechnology* 10(02):142–58. doi: 10.4236/jbnb.2019.102008.

Anastassiades, Michelangelo, Ellen Scherbaum, and Dorothea Bertsch. 2003. "Validation of a Simple and Rapid Multiresidue Method and Its Implementation in Routine Pesticide Analysis." *Filtration* (May):7.

Andreu, Vicente, and Yolanda Picó. 2019. "Pressurized Liquid Extraction of Organic Contaminants in Environmental and Food Samples." *TrAC - Trends in Analytical Chemistry* 118:709–21.

Anh, Hoang Quoc, Keidai Tomioka, Nguyen Minh Tue, Go Suzuki, Tu Binh Minh, Pham Hung Viet, and Shin Takahashi. 2019. "Comprehensive Analysis of 942 Organic Micro-Pollutants in Settled Dusts from Northern Vietnam: Pollution Status and Implications for Human Exposure." *Journal of Material Cycles and Waste Management* 21(1):57–66. doi: 10.1007/s10163-018-0745-2.

Aniţaş, Simona, Mirela Coman, and Bogdan-Vasile Cioruța. 2020. "Some Considerations Regarding the Presence of Heavy Metals in Soil and the Human Body." *Asian Soil Research Journal* 39–46. doi: 10.9734/asrj/2020/v3i430081.

Anon. n.d. "Pick-n-Pay-Slr-Interactive-2019."

Armenta, Sergio, Salvador Garrigues, Francesc A. Esteve-Turrillas, and Miguel de la Guardia. 2019. "Green Extraction Techniques in Green Analytical Chemistry." *TrAC - Trends in Analytical Chemistry* 116:248–53.

Autrup, Herman, Frank A. Barile, Sir Colin Berry, Bas J. Blaauboer, Alan Boobis, Herrmann Bolt, Christopher J. Borgert, Wolfgang Dekant, Daniel Dietrich, Jose L. Domingo, Gio Batta Gori, Helmut Greim, Jan Hengstler, Sam Kacew, Hans Marquardt, Olavi Pelkonen, Kai Savolainen, Pat Heslop-Harrison, and Nico P. Vermeulen. 2020. "Human Exposure to Synthetic Endocrine Disrupting Chemicals (S-EDCs) Is Generally Negligible as Compared to Natural Compounds with Higher or Comparable Endocrine Activity: How to Evaluate the Risk of the S-EDCs?" *Archives of Toxicology* 94(7):2549–57.

Aworh, O. Charles. 2021. "Food Safety Issues in Fresh Produce Supply Chain with Particular Reference to Sub-Saharan Africa." *Food Control* 123.

Azzouz, Abdelmonaim, Laura Palacios Colón, Badredine Souhail, and Evaristo Ballesteros. 2019. "A Multi-Residue Method for GC-MS Determination of Selected Endocrine Disrupting

Chemicals in Fish and Seafood from European and North African Markets." *Environmental Research* 178. doi: 10.1016/j.envres.2019.108727.

Ba, Deniz, and Ismail H. Boyaci. 2007. "Modeling and Optimization i: Usability of Response Surface Methodology." *Journal of Food Engineering* 78(3):836–45. doi: 10.1016/j.jfoodeng.2005.11.024.

Bai, Xuelian, and Kumud Acharya. 2019. "Removal of Seven Endocrine Disrupting Chemicals (EDCs) from Municipal Wastewater Effluents by a Freshwater Green Alga." *Environmental Pollution* 247:534–40. doi: 10.1016/j.envpol.2019.01.075.

Baldino, Lucia, Mariarosa Scognamiglio, and Ernesto Reverchon. 2020. "Supercritical Fluid Technologies Applied to the Extraction of Compounds of Industrial Interest from Cannabis Sativa L. and to Their Pharmaceutical Formulations: A Review." *Journal of Supercritical Fluids* 165.

Bañuelos, Gary S., John Freeman, and Irvin Arroyo. 2020. "Accumulation and Speciation of Selenium in Biofortified Vegetables Grown under High Boron and Saline Field Conditions." *Food Chemistry: X* 5. doi: 10.1016/j.fochx.2019.100073.

Barbieri, Maria Vittoria, Cristina Postigo, Nuria Guillem-Argiles, Luis Simon Monllor-Alcaraz, Julliana Izabelle Simionato, Elisa Stella, Damià Barceló, and Miren López de Alda. 2019. "Analysis of 52 Pesticides in Fresh Fish Muscle by QuEChERS Extraction Followed by LC-MS/MS Determination." *Science of the Total Environment* 653:958–67. doi: 10.1016/j.scitotenv.2018.10.289.

Barnhoorn, Irene, and &. Cobus Van Dyk. n.d. "The First Report of Selected Herbicides and Fungicides in Water and Fish from a Highly Utilized and Polluted Freshwater Urban Impoundment." doi: 10.1007/s11356-020-09930-7/Published.

Barros, Ariane I., Fernanda C. Pinheiro, and Joaquim A. Nóbrega. 2019. "Calibration Strategies to Correct for Matrix Effects in Direct Analysis of Urine by ICP OES: Internal Standardization and Multi-Energy Calibration." *Analytical Methods* 11(27):3401–9. doi: 10.1039/c9ay00907h.

Ben-Iwo, Juliet, Vasilije Manovic, and Philip Longhurst. 2016. "Biomass Resources and Biofuels Potential for the Production of Transportation Fuels in Nigeria." *Renewable and Sustainable Energy Reviews* 63:172–92.

Bergman, Åke., United Nations Environment Programme., and World Health Organization. 2013. *State of the Science of Endocrine Disrupting Chemicals - 2012 an Assessment of the State of the Science of Endocrine Disruptors*. WHO.

Bertuzzi, Andrea S., Paul L. H. McSweeney, Mary C. Rea, and Kieran N. Kilcawley. 2018. "Detection of Volatile Compounds of Cheese and Their Contribution to the Flavor Profile of Surface-Ripened Cheese." *Comprehensive Reviews in Food Science and Food Safety* 17(2):371–90. doi: 10.1111/1541-4337.12332.

Bhatta, Sagar, Tatjana Stevanovic Janezic, and Cristina Ratti. 2020. "Freeze-Drying of Plant-Based Foods." *Foods* 9(1).

Bhuyan, Md Simul, Muhammad Abu Bakar, Aysha Akhtar, M. Belal Hossain, Mir Mohammad Ali, and Md Shafiqul Islam. 2017. "Heavy Metal Contamination in Surface Water and Sediment of the Meghna River, Bangladesh." *Environmental Nanotechnology, Monitoring and Management* 8:273–79. doi: 10.1016/j.enmm.2017.10.003.

Bilal, Muhammad, Hafiz M. N. Iqbal, and Damiá Barceló. 2019. "Persistence of Pesticides-Based Contaminants in the Environment and Their Effective Degradation Using Laccase-Assisted Biocatalytic Systems." *Science of the Total Environment* 695.

Billings, Alex, Kevin C. Jones, M. Glória Pereira, and David J. Spurgeon. 2021. "Plasticisers in the Terrestrial Environment: Sources, Occurrence and Fate." *Environmental Chemistry* 18(3):111–30.

Borges, Camila S., David C. Weindorf, Geila S. Carvalho, Luiz R. G. Guilherme, Thalita Takayama, Nilton Curi, Geraldo J. E. O. Lima, and Bruno T. Ribeiro. 2020. "Foliar Elemental Analysis of Brazilian Crops via Portable X-Ray Fluorescence Spectrometry." *Sensors (Switzerland)* 20(9). doi: 10.3390/s20092509.

Borghesi, Fabrizio, Francesca Migani, and Enrico Dinelli. 2016. "Geochemical Characterization of Surface Sediments from the Northern Adriatic Wetlands around the Po River Delta. Part II: Aqua Regia Results." *Journal of Geochemical Exploration* 169:13–29. doi: 10.1016/j.gexplo.2016.06.012.

Borsellino, Valeria, Emanuele Schimmenti, and Hamid el Bilali. 2020. "Agri-Food Markets towards Sustainable Patterns." *Sustainability (Switzerland)* 12(6).

Boulo, Sébastien, Julia Kuhlmann, Ulf Andreasson, Britta Brix, Iswariya Venkataraman, Victor Herbst, Sandra Rutz, Ekaterina Manuilova, Manu Vandijck, Filip Dekeyser, Maria Bjerke,

Josef Pannee, Jean Charoud-Got, Guy Auclair, Stéphane Mazoua, Gregor Pinski, Stefanie Trapmann, Heinz Schimmel, Hendrik Emons, Milena Quaglia, Erik Portelius, Magdalena Korecka, Leslie M. Shaw, Mary Lame, Erin Chambers, Hugo Vanderstichele, Erik Stoops, Andreas Leinenbach, Tobias Bittner, Rand G. Jenkins, Vesna Kostanjevecki, Piotr Lewczuk, Johan Gobom, Henrik Zetterberg, Ingrid Zegers, and Kaj Blennow. 2020. "First Amyloid B1-42 Certified Reference Material for Re-Calibrating Commercial Immunoassays." *Alzheimer's and Dementia* 16(11):1493–1503. doi: 10.1002/alz.12145.

Boyaci, Ezel, Ángel Rodríguez-Lafuente, Krzysztof Gorynski, Fatemeh Mirnaghi, Érica A. Souza-Silva, Dietmar Hein, and Janusz Pawliszyn. 2015. "Sample Preparation with Solid Phase Microextraction and Exhaustive Extraction Approaches: Comparison for Challenging Cases." *Analytica Chimica Acta* 873:14–30.

Boysen, Angela K., Katherine R. Heal, Laura T. Carlson, and Anitra E. Ingalls. 2018. "Best-Matched Internal Standard Normalization in Liquid Chromatography-Mass Spectrometry Metabolomics Applied to Environmental Samples." *Analytical Chemistry* 90(2):1363–69. doi: 10.1021/acs.analchem.7b04400.

Braun, Joseph M. 2017. "Early-Life Exposure to EDCs: Role in Childhood Obesity and Neurodevelopment." *Nature Reviews Endocrinology* 13(3):161–73.

Briffa, Jessica, Emmanuel Sinagra, and Renald Blundell. 2020. "Heavy Metal Pollution in the Environment and Their Toxicological Effects on Humans." *Heliyon* 6(9).

Burgos-Aceves, Mario Alberto, Vincenzo Migliaccio, Ilaria di Gregorio, Gaetana Paolella, Marilena Lepretti, Caterina Faggio, and Lillà Lionetti. 2021. "1,1,1-Trichloro-2,2-Bis (p-Chlorophenyl)-Ethane (DDT) and 1,1-Dichloro-2,2-Bis (p, p'-Chlorophenyl) Ethylene (DDE) as Endocrine Disruptors in Human and Wildlife: A Possible Implication of Mitochondria." *Environmental Toxicology and Pharmacology* 87.

Campêlo, Jacqueline de M., Taís B. Rodrigues, Jose L. Costa, and Jandyson M. Santos. 2021. "Optimization of QuEChERS Extraction for Detection and Quantification of 20 Antidepressants in Postmortem Blood Samples by LC-MS/MS." *Forensic Science International* 319. doi: 10.1016/j.forsciint.2020.110660.

Cannas, Daniela, Eleonora Loi, Matteo Serra, Davide Firinu, Paolo Valera, and Patrizia Zavattari. 2020. "Relevance of Essential Trace Elements in Nutrition and Drinking Water for Human Health and Autoimmune Disease Risk." *Nutrients* 12(7):1–22.

Cano-Sancho, German, Sonia Marin, Antonio J. Ramos, Juan Peris-Vicente, and Vicente Sanchis. 2010. “Incidencia de Aflatoxina M1 y Evaluación de La Exposición En Cataluña (España).” *Revista Iberoamericana de Micologia* 27(3):130–35. doi: 10.1016/j.riam.2010.05.003.

Čapoun, Tomáš, and Jana Krykorková. n.d. *INTERNAL STANDARDS FOR QUANTITATIVE ANALYSIS OF CHEMICAL WARFARE AGENTS BY THE GC/MS METHOD: BLISTER AGENTS*.

Carnevali, Oliana, Stefania Santangeli, Isabel Forner-Piquer, Danilo Basili, and Francesca Maradonna. 2018. “Endocrine-Disrupting Chemicals in Aquatic Environment: What Are the Risks for Fish Gametes?” *Fish Physiology and Biochemistry* 44(6):1561–76. doi: 10.1007/s10695-018-0507-z.

Casella, Patrizia, Tiziana Marino, Angela Iovine, Vincenzo Larocca, Roberto Balducchi, Dino Musmarra, and Antonio Molino. 2021. “Optimization of Lutein Extraction from Scenedesmus Almeriensis Using Pressurized Liquid Extraction.” *Chemical Engineering Transactions* 87(February):475–80. doi: 10.3303/CET2187080.

van Cauwenbergh, Olivia, Alessandra di Serafino, Jan Tytgat, and Adelheid Soubry. 2020. “Transgenerational Epigenetic Effects from Male Exposure to Endocrine-Disrupting Compounds: A Systematic Review on Research in Mammals.” *Clinical Epigenetics* 12(1).

Cecchi, Lorenzo, Francesca Ieri, Pamela Vignolini, Nadia Mulinacci, and Annalisa Romani. 2020. “Characterization of Volatile and Flavonoid Composition of Different Cuts of Dried Onion (Allium Cepa L.) by HS-SPME-GC-MS, HS-SPME-GC×GC-TOF and HPLC-DAD.” *Molecules* 25(2). doi: 10.3390/molecules25020408.

Chakraborty, Suman Bhusan. 2021. “Non-Essential Heavy Metals as Endocrine Disruptors: Evaluating Impact on Reproduction in Teleosts.” *Proceedings of the Zoological Society*. doi: 10.1007/s12595-021-00399-x.

Chandra, Rachna, N. Sharpanabharathi, B. Anjan Kumar Prusty, P. A. Azeez, and Rama Mohan Kurakalva. 2021. “Organochlorine Pesticide Residues in Plants and Their Possible Ecotoxicological and Agri Food Impacts.” *Scientific Reports* 11(1). doi: 10.1038/s41598-021-97286-4.

Chandra, Suman, Mohamed M. Radwan, Chandrani G. Majumdar, James C. Church, Tom P. Freeman, and Mahmoud A. ElSohly. 2019. “New Trends in Cannabis Potency in USA and

Europe during the Last Decade (2008–2017)." *European Archives of Psychiatry and Clinical Neuroscience* 269(1):5–15. doi: 10.1007/s00406-019-00983-5.

Chang, Geng Ruei, Po Hsun Hou, Wen Kai Chen, Chien Teng Lin, Hsiao Pei Tsai, and Frank Chiahung Mao. 2020. "Exercise Affects Blood Glucose Levels and Tissue Chromium Distribution in High-Fat Diet-Fed C57BL6 Mice." *Molecules* 25(7). doi: 10.3390/molecules25071658.

Chaturvedi, Aaditya, Santanu Bhattacharjee, Abhay Kumar Singh, and Vipin Kumar. 2018. "A New Approach for Indexing Groundwater Heavy Metal Pollution." *Ecological Indicators* 87:323–31. doi: 10.1016/j.ecolind.2017.12.052.

Chen, Haibo, Chen Wang, Hui Li, Ruixue Ma, Ziling Yu, Liangzhong Li, Mingdeng Xiang, Xichao Chen, Xin Hua, and Yunjiang Yu. 2019. "A Review of Toxicity Induced by Persistent Organic Pollutants (POPs) and Endocrine-Disrupting Chemicals (EDCs) in the Nematode Caenorhabditis Elegans." *Journal of Environmental Management* 237(September 2018):519–25. doi: 10.1016/j.jenvman.2019.02.102.

Chen, Tse Lun, Hyunook Kim, Shu Yuan Pan, Po Chih Tseng, Yi Pin Lin, and Pen Chi Chiang. 2020. "Implementation of Green Chemistry Principles in Circular Economy System towards Sustainable Development Goals: Challenges and Perspectives." *Science of the Total Environment* 716. doi: 10.1016/j.scitotenv.2020.136998.

Cheng, Min, Guangming Zeng, Danlian Huang, Cui Lai, Piao Xu, Chen Zhang, and Yang Liu. 2016. "Hydroxyl Radicals Based Advanced Oxidation Processes (AOPs) for Remediation of Soils Contaminated with Organic Compounds: A Review." *Chemical Engineering Journal* 284:582–98.

Cheng, Zhang, Jia Rui Chen, Chao Zheng, Zhan Biao Yang, Xiao Xun Xu, and Ming Hung Wong. 2021. "Bioaccumulation and Health Risk Assessment of Phthalate Esters in Cultured Low Trophic Level Fish Feded with Food Waste-Based Diets." *Chemosphere* 276. doi: 10.1016/j.chemosphere.2021.130189.

Chmielewski, Jarosław, Jarogniew Łuszczki, Małgorzata Czarny-Działak, Ewa Dutkiewicz, Halina Król, Barbara Gworek, and Grażyna Nowak-Starz. 2021. "Environmental Exposition to Xenoestrogens and Related Health Effects." *Journal of Elementology* 26(3):717–30. doi: 10.5601/jelem.2021.26.2.2157.

Choi, Seul Min, Sun Dong Yoo, and Byung Mu Lee. 2004. "Toxicological Characteristics of Endocrine-Disrupting Chemicals: Developmental Toxicity, Carcinogenicity, and Mutagenicity." *Journal of Toxicology and Environmental Health - Part B: Critical Reviews* 7(1):1–23. doi: 10.1080/10937400490253229.

Chopra, A. K., Mukesh Kumar Sharma, and Shikha Chamoli. 2011. "Bioaccumulation of Organochlorine Pesticides in Aquatic System-an Overview." *Environmental Monitoring and Assessment* 173(1–4):905–16. doi: 10.1007/s10661-010-1433-4.

Christensen, Earl, Gina M. Fioroni, Seonah Kim, Lisa Fouts, Erica Gjersing, Robert S. Paton, and Robert L. McCormick. 2018. "Experimental and Theoretical Study of Oxidative Stability of Alkylated Furans Used as Gasoline Blend Components." *Fuel* 212:576–85. doi: 10.1016/j.fuel.2017.10.066.

Chung, Stephen W. C. 2021. "The Development of Isomer-Specific Analysis of Branched 4-Nonylphenol in Food for Dietary Exposure - a Critical Review of Analytical Methods and Occurrence in Foodstuffs." *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* 38(5):842–55.

Cloutier, Pierre Luc, Frédérik Fortin, Paule Emilie Groleau, Pauline Brousseau, Michel Fournier, and Mélanie Desrosiers. 2017. "QuEChERS Extraction for Multi-Residue Analysis of PCBs, PAHs, PBDEs and PCDD/Fs in Biological Samples." *Talanta* 165(October 2016):332–38. doi: 10.1016/j.talanta.2016.12.080.

Cohn, Barbara A., Piera M. Cirillo, and Mary Beth Terry. 2019. "DDT and Breast Cancer: Prospective Study of Induction Time and Susceptibility Windows." *Journal of the National Cancer Institute* 111(8):803–10. doi: 10.1093/jnci/djy198.

Cole, Megan J., and Jennifer L. Broadhurst. 2021. "Measuring the Sustainable Development Goals (SDGs) in Mining Host Communities: A South African Case Study." *Extractive Industries and Society* 8(1):233–43. doi: 10.1016/j.exis.2020.11.012.

Collins, James F., Joseph R. Prohaska, and Mitchell D. Knutson. 2010. "Metabolic Crossroads of Iron and Copper." *Nutrition Reviews* 68(3):133–47.

Cooper, Andrew M., Didra Felix, Fatima Alcantara, Ilya Zaslavsky, Amy Work, Paul L. Watson, Keith Pezzoli, Qi Yu, Dan Zhu, Alexander J. Scavo, Yasman Zarabi, and Julian I. Schroeder. 2020. "Monitoring and Mitigation of Toxic Heavy Metals and Arsenic Accumulation in Food

Crops: A Case Study of an Urban Community Garden." *Plant Direct* 4(1). doi: 10.1002/pld3.198.

de Coster, Sam, and Nicolas van Larebeke. 2012. "Endocrine-Disrupting Chemicals: Associated Disorders and Mechanisms of Action." *Journal of Environmental and Public Health* 2012.

Cvjetko Bubalo, Marina, Senka Vidović, Ivana Radojčić Redovniković, and Stela Jokić. 2015. "Green Solvents for Green Technologies." *Journal of Chemical Technology and Biotechnology* 90(9):1631–39. doi: 10.1002/jctb.4668.

Darbre, Philippa D. 2018. "Overview of Air Pollution and Endocrine Disorders." *International Journal of General Medicine* 11:191–207.

Das, Mihir Tanay, Smita S. Kumar, Pooja Ghosh, Goldy Shah, Sandeep K. Malyan, Somvir Bajar, Indu Shekhar Thakur, and Lakhveer Singh. 2021. "Remediation Strategies for Mitigation of Phthalate Pollution: Challenges and Future Perspectives." *Journal of Hazardous Materials* 409.

Davis, Frederick Rowe. 2019. "Pesticides and the Perils of Synecdoche in the History of Science and Environmental History." *History of Science* 57(4):469–92. doi: 10.1177/0073275319848964.

Demirtaş, Güray, Kültiğin Çavuşoğlu, and Emine Yalçin. 2020. "Aneugenic, Clastogenic, and Multi-Toxic Effects of Diethyl Phthalate Exposure." *Environmental Science and Pollution Research* 27(5):5503–10. doi: 10.1007/s11356-019-07339-5.

Deng, Man, Xu Han, Jiali Ge, Xinxin Liang, Bibai Du, Juan Li, and Lixi Zeng. 2021. "Prevalence of Phthalate Alternatives and Monoesters alongside Traditional Phthalates in Indoor Dust from a Typical E-Waste Recycling Area: Source Elucidation and Co-Exposure Risk." *Journal of Hazardous Materials* 413. doi: 10.1016/j.jhazmat.2021.125322.

Department of Environmental Affairs (DEA). 2010. "Framework for the Management of Contaminated Land, South Africa. Republic of South Africa. 79 p." *Framework* (May):326.

Dierkes, Georg, Tim Lauschke, Susanne Becher, Heike Schumacher, Corinna Földi, and Thomas Ternes. 2066. "Quantification of Microplastics in Environmental Samples via Pressurized Liquid Extraction and Pyrolysis-Gas Chromatography." doi: 10.1007/s00216-019-02066-9/Published.

Ding, Yang, Li Li, Frank Wania, Huanfang Huang, Yuan Zhang, Bo Peng, Yingjie Chen, and Shihua Qi. 2021. "Do Dissipation and Transformation of γ-HCH and p,p'-DDT in Soil

Respond to a Proxy for Climate Change? Insights from a Field Study on the Eastern Tibetan Plateau." *Environmental Pollution* 278. doi: 10.1016/j.envpol.2021.116824.

D'Souza, Lucas, and Deepta Bhattacharya. 2019. "Plasma Cells: You Are What You Eat." *Immunological Reviews* 288(1):161–77.

DUMAN, Erman, Mustafa Mete Özcan, Mehmet Hamurcu, and Mehmet Musa Özcan. 2019. "Bazı Hayvan Karaciğerlerinin Mineral ve Ağır Metal Içerkleri." *European Journal of Science and Technology* 302–7. doi: 10.31590/ejosat.506340.

Edwards, Jeffrey R. 2002. "Alternatives to Difference Scores: Polynomial Regression Analysis and Response Surface Methodology." *Measuring and Analyzing Behavior in Organizations: Advances in Measurement and Data Analysis* 350–400.

El-Moselhy, Kh. M., A. I. Othman, H. Abd El-Azem, and M. E. A. El-Metwally. 2014. "Bioaccumulation of Heavy Metals in Some Tissues of Fish in the Red Sea, Egypt." *Egyptian Journal of Basic and Applied Sciences* 1(2):97–105. doi: 10.1016/j.ejbas.2014.06.001.

El-Nahhal, Ibrahim, and Yasser El-Nahhal. 2021. "Pesticide Residues in Drinking Water, Their Potential Risk to Human Health and Removal Options." *Journal of Environmental Management* 299.

Encarnação, Telma, Alberto A. C. C. Pais, Maria G. Campos, and Hugh D. Burrows. 2019. "Endocrine Disrupting Chemicals: Impact on Human Health, Wildlife and the Environment." *Science Progress* 102(1):3–42. doi: 10.1177/0036850419826802.

Evans, Richard M., Martin Scholze, and Andreas Kortenkamp. 2015. "Examining the Feasibility of Mixture Risk Assessment: A Case Study Using a Tiered Approach with Data of 67 Pesticides from the Joint FAO/WHO Meeting on Pesticide Residues (JMPR)." *Food and Chemical Toxicology* 84:260–69. doi: 10.1016/j.fct.2015.08.015.

Farounbi, Adebayo I., and Nosiphiwe P. Ngqwala. 2020. "Occurrence of Selected Endocrine Disrupting Compounds in the Eastern Cape Province of South Africa." *Environmental Science and Pollution Research* 27(14):17268–79. doi: 10.1007/s11356-020-08082-y.

Fathabad, Ayub Ebadi, Nabi Shariatifar, Mojtaba Moazzen, Shahrokh Nazmara, Yadolah Fakhri, Mahmood Alimohammadi, Ali Azari, and Amin Mousavi Khaneghah. 2018. "Determination of Heavy Metal Content of Processed Fruit Products from Tehran's Market Using ICP- OES: A Risk Assessment Study." *Food and Chemical Toxicology* 115(April):436–46. doi: 10.1016/j.fct.2018.03.044.

Ferreira, Dominique. 2014. *An Exploratory Investigation into Tshwane Postmodern Consumers' Consciousness and Practices That Relate to Sustainable Food Procurement*.

Fuentes-Azcatl, Raúl, and José Alejandre. 2014. "Non-Polarizable Force Field of Water Based on the Dielectric Constant: TIP4P/ε." *Journal of Physical Chemistry B* 118(5):1263–72. doi: 10.1021/jp410865y.

Gade, Meethila, Nicole Comfort, and Diane B. Re. 2021. "Sex-Specific Neurotoxic Effects of Heavy Metal Pollutants: Epidemiological, Experimental Evidence and Candidate Mechanisms." *Environmental Research* 201.

Gallego, Rocío, Mónica Bueno, and Miguel Herrero. 2019. "Sub- and Supercritical Fluid Extraction of Bioactive Compounds from Plants, Food-by-Products, Seaweeds and Microalgae – An Update." *TrAC - Trends in Analytical Chemistry* 116:198–213.

Gallo, Frederic, Cristina Fossi, Roland Weber, David Santillo, Joao Sousa, Imogen Ingram, Angel Nadal, and Dolores Romano. 2018. "Marine Litter Plastics and Microplastics and Their Toxic Chemicals Components: The Need for Urgent Preventive Measures." *Environmental Sciences Europe* 30(1).

Ganzera, Markus, and Sonja Sturm. 2018. "Recent Advances on HPLC/MS in Medicinal Plant Analysis—An Update Covering 2011–2016." *Journal of Pharmaceutical and Biomedical Analysis* 147:211–33.

Garcia Lara, Bianey, Katarzyna Wrobel, Alma Rosa Corrales Escobosa, Oracio Serrano Torres, Israel Enciso Donis, and Kazimierz Wrobel. 2021. "Mass Spectrometry-Based Identification of Bacteria Isolated from Industrially Contaminated Site in Salamanca (Mexico) and Evaluation of Their Potential for DDT Degradation." *Folia Microbiologica* 66(3):355–69. doi: 10.1007/s12223-020-00848-8.

Gaudriault, Pierre, Séverine Mazaud-Guittot, Vincent Lavoué, Isabelle Coiffec, Laurianne Lesné, Nathalie Dejucq-Rainsford, Martin Scholze, Andreas Kortenkamp, and Bernard Jégou. 2017. "Endocrine Disruption in Human Fetal Testis Explants by Individual and Combined Exposures to Selected Pharmaceuticals, Pesticides, and Environmental Pollutants." *Environmental Health Perspectives* 125(8). doi: 10.1289/EHP1014.

Gaur, Nisha, Korrapati Narasimhulu, and Y. PydiSetty. 2018. "Recent Advances in the Bio-Remediation of Persistent Organic Pollutants and Its Effect on Environment." *Journal of Cleaner Production* 198:1602–31.

Genchi, Giuseppe, Maria Stefania Sinicropi, Graziantonio Lauria, Alessia Carocci, and Alessia Catalano. 2020. “The Effects of Cadmium Toxicity.” *International Journal of Environmental Research and Public Health* 17(11).

Ghahvechi Khaligh, Fereshteh, Navid Dinparast Djadid, Mostafa Farmani, Zahra Asadi Saatlou, Samira Frooziyan, Fatemeh Abedi Astaneh, Fariba Farnoosh, Aioub Sofizadeh, Firoozeh Naseri, Delnia Adib, Mohammad Taheri, Emran Lalehzari, Shabnam Pashaei, Shadiyeh Soltanbeiglou, Aboozar Soltani, Hassan Khooban, Ebrahim Abbasi, Ali-Ashraf Aivazi, Samrand Saeedi, Behrooz Taghilou, Abbas Jafari, Kamal Dashti Khavidak, Ariel C. Toloza, and Saber Gholizadeh. 2021. “Molecular Monitoring of Knockdown Resistance in Head Louse (Phthiraptera: Pediculidae) Populations in Iran.” *Journal of Medical Entomology* 58(6):2321–29. doi: 10.1093/jme/tjab101.

Ghani, Z. A., A. N. Anuar, Z. A. Majid, and M. Yoneda. 2017. “Multimedia Environmental Fate and Transport Model of Dichlorodiphenyltrichloroethane (DDT): Case Study Sayong River Watershed, Johor, Malaysia.” *Sains Malaysiana* 46(12):2383–92. doi: 10.17576/jsm-2017-4612-15.

Glover-Amengor, M., and F. M. Tetteh. 2008. *Effect of Pesticide Application Rate on Yield of Vegetables and Soil Microbial Communities*. Vol. 12.

Goh, Brandon Han Hoe, Hwai Chyuan Ong, Mei Yee Cheah, Wei Hsin Chen, Kai Ling Yu, and Teuku Meurah Indra Mahlia. 2019. “Sustainability of Direct Biodiesel Synthesis from Microalgae Biomass: A Critical Review.” *Renewable and Sustainable Energy Reviews* 107:59–74.

Golge, Ozgur, Ahmet Koluman, and Bulent Kabak. 2018. “Validation of a Modified QuEChERS Method for the Determination of 167 Pesticides in Milk and Milk Products by LC-MS/MS.” *Food Analytical Methods* 11(4):1122–48. doi: 10.1007/s12161-017-1066-0.

González-Casanova, Jorge Enrique, Sonia Liliana Pertuz-Cruz, Nelson Hernando Caicedo-Ortega, and Diana Marcela Rojas-Gomez. 2020. “Adipogenesis Regulation and Endocrine Disruptors: Emerging Insights in Obesity.” *BioMed Research International* 2020.

Graca, Bożena, Agata Rychter, Marta Staniszewska, Katarzyna Smolarz, Adam Sokołowski, and Karina Bodziach. 2021. “Bioaccumulation of Phenolic Endocrine Disruptors in the Clam Rangia Cuneata: Storage in Shells and Influence of Size and Sex.” *Environmental Research* 197. doi: 10.1016/j.envres.2021.111181.

Greenberg, Stephen. 2017. “Corporate Power in the Agro-Food System and the Consumer Food Environment in South Africa.” *Journal of Peasant Studies* 44(2):467–96. doi: 10.1080/03066150.2016.1259223.

Guarino, Annalise, Antonio Aruta, Pooria Ebrahimi, Salvatore Dominech, Annamaria Lima, Benedetto de Vivo, Shihua Qi, and Stefano Albanese. 2021. “Organochlorine Pesticides in the Soils of the Acerra Plain : Concentration and Distribution of DDT Isomers and Metabolites.” (2015):4–5.

Gülfen, Mustafa, Yazgı Canbaz, and Abdil Özdemir. 2020. “Simultaneous Determination of Amoxicillin, Lansoprazole, and Levofloxacin in Pharmaceuticals by HPLC with UV–Vis Detector.” *Journal of Analysis and Testing* 4(1):45–53. doi: 10.1007/s41664-020-00121-4.

Günther, Klaus, and John Sumpter. 2002. “Environmental M Ne.”

Guo, Wenjing, Bohu Pan, Sugunadevi Sakkiah, Gokhan Yavas, Weigong Ge, Wen Zou, Weida Tong, and Huixiao Hong. 2019. “Persistent Organic Pollutants in Food: Contamination Sources, Health Effects and Detection Methods.” *International Journal of Environmental Research and Public Health* 16(22).

Gupta, Sanjay Kumar, Faiz Ahmed Ansari, Mahmoud Nasr, Mayuri Chabukdhara, and Faizal Bux. 2018. “Multivariate Analysis and Health Risk Assessment of Heavy Metal Contents in Foodstuffs of Durban, South Africa.” *Environmental Monitoring and Assessment* 190(3). doi: 10.1007/s10661-018-6546-1.

Gusso-Choueri, Paloma Kachel, Giuliana Seraphim de Araújo, Ana Carolina Feitosa Cruz, Tatiana Roselena de Oliveira Stremel, Sandro Xavier de Campos, Denis Moledo de Souza Abessa, Ciro Alberto de Oliveira Ribeiro, and Rodrigo Brasil Choueri. 2018. “Metals and Arsenic in Fish from a Ramsar Site under Past and Present Human Pressures: Consumption Risk Factors to the Local Population.” *Science of the Total Environment* 628–629:621–30. doi: 10.1016/j.scitotenv.2018.02.005.

Hao, Zhe, Lihong Chen, Chenglong Wang, Xinqing Zou, Fangqin Zheng, Weihua Feng, Dongrong Zhang, and Ling Peng. 2019. “Heavy Metal Distribution and Bioaccumulation Ability in Marine Organisms from Coastal Regions of Hainan and Zhoushan, China.” *Chemosphere* 226:340–50. doi: 10.1016/j.chemosphere.2019.03.132.

Harding, Anna K., George P. Daston, Glen R. Boyd, George W. Lucier, Stephen H. Safe, Juarine Stewart, Donald E. Tillitt, and Glen van der Kraak. 2006. “Endocrine Disrupting Chemicals

Research Program of the U.S. Environmental Protection Agency: Summary of Peer-Review Report." *Environmental Health Perspectives* 114(8):1276–82.

Hassan, Mostafa, Ali Ahmed, Nabil Marzouk, and Mohamed Maky. 2020. "Detection of Organochlorine Pesticides Residues in Nile Fish and Its Risks in Qena City." *SVU-International Journal of Veterinary Sciences* 3(1):51–65. doi: 10.21608/svu.2020.19713.1036.

Heleno, Sandrina A., Patrícia Diz, M. A. Prieto, Lillian Barros, Alírio Rodrigues, Maria Filomena Barreiro, and Isabel C. F. R. Ferreira. 2016. "Optimization of Ultrasound-Assisted Extraction to Obtain Mycosterols from Agaricus Bisporus L. by Response Surface Methodology and Comparison with Conventional Soxhlet Extraction." *Food Chemistry* 197:1054–63. doi: 10.1016/j.foodchem.2015.11.108.

Hirondart, Mathilde, Natacha Rombaut, Anne Sylvie Fabiano-Tixier, Antoine Bily, and Farid Chemat. 2020a. "Comparison between Pressurized Liquid Extraction and Conventional Soxhlet Extraction for Rosemary Antioxidants, Yield, Composition, and Environmental Footprint." *Foods* 9(5). doi: 10.3390/foods9050584.

Hirondart, Mathilde, Natacha Rombaut, Anne Sylvie Fabiano-Tixier, Antoine Bily, and Farid Chemat. 2020b. "Comparison between Pressurized Liquid Extraction and Conventional Soxhlet Extraction for Rosemary Antioxidants, Yield, Composition, and Environmental Footprint." *Foods* 9(5). doi: 10.3390/foods9050584.

Hochella, Michael F., David W. Mogk, James Ranville, Irving C. Allen, George W. Luther, Linsey C. Marr, B. Peter McGrail, Mitsu Murayama, Nikolla P. Qafoku, Kevin M. Rosso, Nita Sahai, Paul A. Schroeder, Peter Vikesland, Paul Westerhoff, and Yi Yang. 2019. "Natural, Incidental, and Engineered Nanomaterials and Their Impacts on the Earth System." *Science* 363(6434).

Hoff, Rodrigo Barcellos, and Tânia Mara Pizzolato. 2018. "Combining Extraction and Purification Steps in Sample Preparation for Environmental Matrices: A Review of Matrix Solid Phase Dispersion (MSPD) and Pressurized Liquid Extraction (PLE) Applications." *TrAC - Trends in Analytical Chemistry* 109:83–96.

Horak, Ilzé, Suranie Horn, and Rialet Pieters. 2021a. "Agrochemicals in Freshwater Systems and Their Potential as Endocrine Disrupting Chemicals: A South African Context." *Environmental Pollution* 268.

Horak, Ilzé, Suranie Horn, and Rialet Pieters. 2021b. “Agrochemicals in Freshwater Systems and Their Potential as Endocrine Disrupting Chemicals: A South African Context.” *Environmental Pollution* 268.

Hou, Haonan, Yihao Min, Xueke Liu, Peng Wang, Zhiqiang Zhou, and Donghui Liu. 2021. “Occurrence and Migration of Phthalates in Adhesive Materials to Fruits and Vegetables.” *Journal of Hazardous Materials* 418. doi: 10.1016/j.jhazmat.2021.126277.

Hriberšek, Matjaž, Matej Zadravec, Žiga Časar, and Jure Ravnik. 2018. “The Influence of a Vial Stopper on Flow and Mass Transfer Conditions inside a Vial.” Pp. 193–200 in *WIT Transactions on Engineering Sciences*. Vol. 120. WITPress.

Huang, Chun Li, Lian Jun Bao, Pei Luo, Zhao Yi Wang, Shao Meng Li, and Eddy Y. Zeng. 2016. “Potential Health Risk for Residents around a Typical E-Waste Recycling Zone via Inhalation of Size-Fractionated Particle-Bound Heavy Metals.” *Journal of Hazardous Materials* 317:449–56. doi: 10.1016/j.jhazmat.2016.05.081.

Huseen, Hadeel M., and Ahmed J. Mohammed. 2019. “Heavy Metals Causing Toxicity in Fishes.” in *Journal of Physics: Conference Series*. Vol. 1294. Institute of Physics Publishing.

Ibrahim, Umar, Aisha Abubakar Kana, and Abbas Abdullahiauta. 2018. “Health Risk Assessment of Heavy Metals in Swampy Agricultural Soils in Nasarawa West , Nigeria.” (December).

Iliyasu, Dauda. n.d. “Evaluation of Moringa Oleifera (L) Aqueous Seed Extracts on Aphrodisiac, Gonadal and Epididymal Sperm Reserves of Wistar Rats Infectious Courses of Abortion in Livestock View Project EFFECT OF AQUEOUS SEEDS EXTRACT OF Moringa Oleifera (L) ON SOME REPRODUCTIVE AND HAEMATOLOGICAL PARAMETERS OF YANKASA RAMS. View Project.” doi: 10.5455/ijlr.20200316.

Inan-Eroglu, Elif, and Aylin Ayaz. 2018. “Is Aluminum Exposure a Risk Factor for Neurological Disorders?” *Journal of Research in Medical Sciences* 23(1). doi: 10.4103/jrms.JRMS_921_17.

Ingerslev, Flemming, Elvira Vaclavik, and Bent Halling-Sørensen. 2003. *Topic 2.4 Pharmaceuticals and Personal Care Products: A Source of Endocrine Disruption in the Environment?**. Vol. 75.

Inobeme, Abel, Alexander Ikechukwu Ajai, Abdullahi Mann, and Yahaya Ahmed Iyaka. n.d. *DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METAL CONTENTS OF BARBECUE BEEF, FISH AND CHICKEN*. Vol. XVII.

Islam, Rafiquel, Sazal Kumar, Joyanto Karmoker, Md Kamruzzaman, Md Aminur Rahman, Nirupam Biswas, Thi Kim Anh Tran, and Mohammad Mahmudur Rahman. 2018. "Bioaccumulation and Adverse Effects of Persistent Organic Pollutants (POPs) on Ecosystems and Human Exposure: A Review Study on Bangladesh Perspectives." *Environmental Technology and Innovation* 12:115–31.

Jaacks, Lindsay M., Sudesh Yadav, Parinya Panuwet, Sushil Kumar, Girish H. Rajacharya, Cierra Johnson, Ishita Rawal, Deepa Mohan, Viswanathan Mohan, Nikhil Tandon, Dana Boyd Barr, K. M. Venkat Narayan, and Dorairaj Prabhakaran. 2019. "Metabolite of the Pesticide DDT and Incident Type 2 Diabetes in Urban India." *Environment International* 133. doi: 10.1016/j.envint.2019.105089.

Jackson, Erin, Robin Shoemaker, Nika Larian, and Lisa Cassis. 2017. "Adipose Tissue as a Site of Toxin Accumulation." *Comprehensive Physiology* 7(4):1085–1135. doi: 10.1002/cphy.c160038.

Javed, Mehjbeen, and Nazura Usmani. 2019. "An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health." *Proceedings of the National Academy of Sciences India Section B - Biological Sciences* 89(2):389–403.

Jeong, Seung Hyun, Ji Hun Jang, Hea Young Cho, and Yong Bok Lee. 2019. "Simultaneous Determination of Diethyl Phthalate and Its Major Metabolite, Monoethyl Phthalate, in Rat Plasma, Urine, and Various Tissues Collected from a Toxicokinetic Study by Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectrometry." *Journal of Pharmaceutical and Biomedical Analysis* 173:108–19. doi: 10.1016/j.jpba.2019.05.027.

Jeong, Seung Hyun, Ji Hun Jang, Hea Young Cho, and Yong Bok Lee. 2020. "Risk Assessment for Humans Using Physiologically Based Pharmacokinetic Model of Diethyl Phthalate and Its Major Metabolite, Monoethyl Phthalate." *Archives of Toxicology* 94(7):2377–2400. doi: 10.1007/s00204-020-02748-9.

Jin, Xiaoling, Yan Liu, Xiaocui Qiao, Rui Guo, Chengyou Liu, Xing Wang, and Xingru Zhao. 2019. "Risk Assessment of Organochlorine Pesticides in Drinking Water Source of the Yangtze River." *Ecotoxicology and Environmental Safety* 182. doi: 10.1016/j.ecoenv.2019.109390.

de Jong, Wim H., Eveline de Rijk, Alessandro Bonetto, Wendel Wohlleben, Vicki Stone, Andrea Brunelli, Elena Badetti, Antonio Marcomini, Ilse Gosens, and Flemming R. Cassee. 2019.

"Toxicity of Copper Oxide and Basic Copper Carbonate Nanoparticles after Short-Term Oral Exposure in Rats." *Nanotoxicology* 13(1):50–72. doi: 10.1080/17435390.2018.1530390.

al Juhaimi, Fahad, Mehmet Musa Özcan, Kashif Ghafoor, Elfadıl E. Babiker, and Shahzad Hussain. 2018. "Comparison of Cold-Pressing and Soxhlet Extraction Systems for Bioactive Compounds, Antioxidant Properties, Polyphenols, Fatty Acids and Tocopherols in Eight Nut Oils." *Journal of Food Science and Technology* 55(8):3163–73. doi: 10.1007/s13197-018-3244-5.

Kabir, Eva Rahman, Monica Sharfin Rahman, and Imon Rahman. 2015. "A Review on Endocrine Disruptors and Their Possible Impacts on Human Health." *Environmental Toxicology and Pharmacology* 40(1):241–58.

Kalinová, Blanka, Pavel Jiroš, Jan Žd'árek, Xiujun Wen, and Michal Hoskovec. 2006a. "GC × GC/TOF MS Technique-A New Tool in Identification of Insect Pheromones: Analysis of the Persimmon Bark Borer Sex Pheromone Gland." *Talanta* 69(3):542–47. doi: 10.1016/j.talanta.2005.10.045.

Kalinová, Blanka, Pavel Jiroš, Jan Žd'árek, Xiujun Wen, and Michal Hoskovec. 2006b. "GC × GC/TOF MS Technique-A New Tool in Identification of Insect Pheromones: Analysis of the Persimmon Bark Borer Sex Pheromone Gland." *Talanta* 69(3):542–47. doi: 10.1016/j.talanta.2005.10.045.

Kamunda, Caspah, Manny Mathuthu, and Morgan Madhuku. 2016. "Health Risk Assessment of Heavy Metals in Soils from Witwatersrand Gold Mining Basin, South Africa." *International Journal of Environmental Research and Public Health* 13(7). doi: 10.3390/ijerph13070663.

Kanduč, Tjaša, Zdenka Šlejkovec, Nataša Mori, Mirijam Vrabec, Timotej Verbovšek, Sergej Jamnikar, and Marko Vrabec. 2019. "Multielemental Composition and Arsenic Speciation in Low Rank Coal from the Velenje Basin, Slovenia." *Journal of Geochemical Exploration* 200:284–300. doi: 10.1016/j.gexplo.2018.08.001.

Kapeleka, Jones A., Elingarami Sauli, Omowunmi Sadik, and Patrick A. Ndakidemi. 2020. "Co-Exposure Risks of Pesticides Residues and Bacterial Contamination in Fresh Fruits and Vegetables under Smallholder Horticultural Production Systems in Tanzania." *PLoS ONE* 15(7 July). doi: 10.1371/journal.pone.0235345.

Kasozi, Keneth Iceland, Phyllis Candy Natabo, Sarah Namubiru, Dickson Stuart Tayebwa, Andrew Tamale, and Pwaveno H. Bamaiyi. 2018. "Food Safety Analysis of Milk and Beef

in Southwestern Uganda." *Journal of Environmental and Public Health* 2018. doi: 10.1155/2018/1627180.

Kašpárková, Věra, Petr Humpolíček, Jaroslav Stejskal, Jitka Kopecká, Zdenka Kuceková, and Robert Moučka. 2016. "Conductivity, Impurity Profile, and Cytotoxicity of Solvent-Extracted Polyaniline." *Polymers for Advanced Technologies* 27(2):156–61. doi: 10.1002/pat.3611.

Kaur, R., A. Kumari, G. Sharma, D. Singh, and R. Kaur. 2021. "Biodegradation of Endocrine Disrupting Chemicals Benzyl Butyl Phthalate and Dimethyl Phthalate by Bacillus Marisflavi RR014." *Journal of Applied Microbiology* 131(3):1274–88. doi: 10.1111/jam.15045.

Kaushik, Ankita, and Deepa Bhartiya. 2018. "Pluripotent Very Small Embryonic-Like Stem Cells in Adult Testes – An Alternate Premise to Explain Testicular Germ Cell Tumors." *Stem Cell Reviews and Reports* 14(6):793–800. doi: 10.1007/s12015-018-9848-3.

Keijer, Tom, Vincent Bakker, and J. Chris Slootweg. 2019. "Circular Chemistry to Enable a Circular Economy." *Nature Chemistry* 11(3):190–95.

Keshavarzi, Behnam, Mina Hassanaghaei, Farid Moore, Meisam Rastegari Mehr, Siyavash Soltanian, Ahmad Reza Lahijanzadeh, and Armin Sorooshian. 2018. "Heavy Metal Contamination and Health Risk Assessment in Three Commercial Fish Species in the Persian Gulf." *Marine Pollution Bulletin* 129(1):245–52. doi: 10.1016/j.marpolbul.2018.02.032.

Khadka, Sujan, Jean Bosco Nshimiyimana, Piao Zou, Niranjan Koirala, and Li Xiong. 2020. "Biodegradation Kinetics of Diethyl Phthalate by Three Newly Isolated Strains of Pseudomonas." *Scientific African* 8. doi: 10.1016/j.sciaf.2020.e00380.

Khalid, Madiha, and Mohammad Abdollahi. 2021. "Environmental Distribution of Personal Care Products and Their Effects on Human Health." *Iranian Journal of Pharmaceutical Research* 20(1):216–53.

Khan, Sharib Raza, Babita Sharma, Pooja A. Chawla, and Rohit Bhatia. 2021. "Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES): A Powerful Analytical Technique for Elemental Analysis." *Food Analytical Methods*. doi: 10.1007/s12161-021-02148-4.

Khezeli, Tahere, and Ali Daneshfar. 2017. "Development of Dispersive Micro-Solid Phase Extraction Based on Micro and Nano Sorbents." *TrAC - Trends in Analytical Chemistry* 89:99–118.

Kopittke, Peter M., Neal W. Menzies, Peng Wang, Brigid A. McKenna, and Enzo Lombi. 2019. "Soil and the Intensification of Agriculture for Global Food Security." *Environment International* 132.

Korkmaz, Cengiz, Özcan Ay, Yakup Ersoysal, Mehmet Ali Köroğlu, and Cahit Erdem. 2019. "Heavy Metal Levels in Muscle Tissues of Some Fish Species Caught from North-East Mediterranean: Evaluation of Their Effects on Human Health." *Journal of Food Composition and Analysis* 81:1–9. doi: 10.1016/j.jfca.2019.04.005.

Kubincová, Petra, Eliška Sychrová, Jan Raška, Amrita Basu, Affiefa Yawer, Aneta Dydowiczová, Pavel Babica, and Iva Sovadinová. 2019. "Polycyclic Aromatic Hydrocarbons and Endocrine Disruption: Role of Testicular Gap Junctional Intercellular Communication and Connexins." *Toxicological Sciences* 169(1):70–83. doi: 10.1093/toxsci/kfz023.

Kumar, Reeti, and Suparna Mukherji. 2017. "Threat Posed by Persistent Organochlorine Pesticides and Their Mobility in the Environment." *Current Organic Chemistry* 22(10):954–72. doi: 10.2174/1385272822666171227150106.

Kuzukiran, Ozgur, Begum Yurdakok-Dikmen, Sedat Sevin, Ufuk Tansel Sireli, Guzin Iplikcioglu-Cil, and Ayhan Filazi. 2018. "Determination of Selected Endocrine Disruptors in Organic, Free-Range, and Battery-Produced Hen Eggs and Risk Assessment." *Environmental Science and Pollution Research* 25(35):35376–86. doi: 10.1007/s11356-018-3400-5.

Lalonde, Benoit, and Christine Garron. 2021. "Nonylphenol, Octylphenol, and Nonylphenol Ethoxylates Dissemination in the Canadian Freshwater Environment." *Archives of Environmental Contamination and Toxicology* 80(2):319–30. doi: 10.1007/s00244-020-00807-x.

Langston, W. J. 2020. "Endocrine Disruption and Altered Sexual Development in Aquatic Organisms: An Invertebrate Perspective." *Journal of the Marine Biological Association of the United Kingdom* 100(4):495–515.

Laskin, Julia, and Ingela Lanekoff. 2016. "Ambient Mass Spectrometry Imaging Using Direct Liquid Extraction Techniques." *Analytical Chemistry* 88(1):52–73.

Lauretta, Rosa, Andrea Sansone, Massimiliano Sansone, Francesco Romanelli, and Marialuisa Appetecchia. 2019a. "Endocrine Disrupting Chemicals: Effects on Endocrine Glands." *Frontiers in Endocrinology* 10(March):1–7. doi: 10.3389/fendo.2019.00178.

Lauretta, Rosa, Andrea Sansone, Massimiliano Sansone, Francesco Romanelli, and Marialuisa Appetecchia. 2019b. “Endocrine Disrupting Chemicals: Effects on Endocrine Glands.” *Frontiers in Endocrinology* 10.

Lazareva, E. v., I. N. Myagkaya, I. S. Kirichenko, M. A. Gustaytis, and S. M. Zhmodik. 2019. “Interaction of Natural Organic Matter with Acid Mine Drainage: In-Situ Accumulation of Elements.” *Science of the Total Environment* 660:468–83. doi: 10.1016/j.scitotenv.2018.12.467.

Lebedev, A. T., O. v. Polyakova, D. M. Mazur, V. B. Artaev, I. Canet, A. Lallement, M. Vaïtilingom, L. Deguillaume, and A. M. Delort. 2018. “Detection of Semi-Volatile Compounds in Cloud Waters by GC×GC-TOF-MS. Evidence of Phenols and Phthalates as Priority Pollutants.” *Environmental Pollution* 241:616–25. doi: 10.1016/j.envpol.2018.05.089.

Lefebvre, Thibault, Emilie Destandau, Caroline West, and Eric Lesellier. 2020. “Supercritical Fluid Chromatography Development of a Predictive Analytical Tool to Selectively Extract Bioactive Compounds by Supercritical Fluid Extraction and Pressurised Liquid Extraction.” *Journal of Chromatography A* 1632. doi: 10.1016/j.chroma.2020.461582.

Lehotay, Steven J., Kyung Ae Son, Hyeyoung Kwon, Urairat Koesukwiwat, Wusheng Fu, Katerina Mastovska, Eunha Hoh, and Natchanun Leepipatpiboon. 2010. “Comparison of QuEChERS Sample Preparation Methods for the Analysis of Pesticide Residues in Fruits and Vegetables.” *Journal of Chromatography A* 1217(16):2548–60. doi: 10.1016/j.chroma.2010.01.044.

Lenaerts, Jan T. M., Brooke Medley, Michiel R. van den Broeke, and Bert Wouters. 2019. “Observing and Modeling Ice Sheet Surface Mass Balance.” *Reviews of Geophysics* 57(2):376–420. doi: 10.1029/2018RG000622.

Lestido-Cardama, Antía, Ana Rodríguez Bernaldo de Quirós, Juana Bustos, M. Luisa Lomo, Perfecto Paseiro Losada, and Raquel Sendón. 2020. “Estimation of Dietary Exposure to Contaminants Transferred from the Packaging in Fatty Dry Foods Based on Cereals.” *Foods* 9(8). doi: 10.3390/foods9081038.

Li, Helian, Zongquan Sun, Yanhua Qiu, Xiuyue Yu, Xuemei Han, and Yibing Ma. 2018. “Integrating Bioavailability and Soil Aging in the Derivation of DDT Criteria for Agricultural

Soils Using Crop Species Sensitivity Distributions." *Ecotoxicology and Environmental Safety* 165:527–32. doi: 10.1016/j.ecoenv.2018.09.035.

Li, Ning, Di Wu, Jichao Liu, Na Hu, Xuexiang Shi, Chunji Dai, Zhiwei Sun, Yourui Suo, Guoliang Li, and Yongning Wu. 2018. "Magnetic Covalent Organic Frameworks Based on Magnetic Solid Phase Extraction for Determination of Six Steroidal and Phenolic Endocrine Disrupting Chemicals in Food Samples." *Microchemical Journal* 143:350–58. doi: 10.1016/j.microc.2018.08.036.

Lim, Jihyun, Na Yeong Kim, Woongsik Jang, Un Su An, Aung Ko Ko Kyaw, Yun Hi Kim, and Dong Hwan Wang. 2020. "Selective Soxhlets Extraction to Enhance Solubility of Newly-Synthesized Poly(Indoloindole-Selenophene Vinylene Selenophene) Donor for Photovoltaic Applications." *Nano Convergence* 7(1). doi: 10.1186/s40580-020-0219-9.

Liu, Peng, Xin Zhan, Xiaowei Wu, Jinli Li, Hanyu Wang, and Shixiang Gao. 2020. "Effect of Weathering on Environmental Behavior of Microplastics: Properties, Sorption and Potential Risks." *Chemosphere* 242.

Lourens, ASM. 2012. "Air Quality in the Johannesburg-Pretoria Megacity: Its Regional Influence and Identification of Parameters That Could Mitigate Pollution/ASM Lourens." (November).

Lu, Jian, Jun Wu, Cui Zhang, and Yuxuan Zhang. 2020. "Possible Effect of Submarine Groundwater Discharge on the Pollution of Coastal Water: Occurrence, Source, and Risks of Endocrine Disrupting Chemicals in Coastal Groundwater and Adjacent Seawater Influenced by Reclaimed Water Irrigation." *Chemosphere* 250. doi: 10.1016/j.chemosphere.2020.126323.

Lu, Jian, Cui Zhang, Jun Wu, Yuxuan Zhang, and Yichen Lin. 2020. "Seasonal Distribution, Risks, and Sources of Endocrine Disrupting Chemicals in Coastal Waters: Will These Emerging Contaminants Pose Potential Risks in Marine Environment at Continental-Scale?" *Chemosphere* 247. doi: 10.1016/j.chemosphere.2020.125907.

Lu, Meiyu, Wankui Jiang, Qinqin Gao, Mingliang Zhang, and Qing Hong. 2020. "Degradation of Dibutyl Phthalate (DBP) by a Bacterial Consortium and Characterization of Two Novel Esterases Capable of Hydrolyzing PAEs Sequentially." *Ecotoxicology and Environmental Safety* 195. doi: 10.1016/j.ecoenv.2020.110517.

Luisa, Maria, Baiño Salingay, Daniel Giesen, and Chris Zevenbergen. 2020. "Pesticide Assessment Using Passive Samplers in Two River System of Cagayan De Oro River Basin,

Philippines Pesticide Assessment Using Passive Samplers in Two River Systems of Cagayan de Oro River Basin, Philippines 2." doi: 10.21203/rs.3.rs-98094/v1.

Luo, Xiao San, Jing Ding, Bo Xu, Yi Jie Wang, Hong Bo Li, and Shen Yu. 2012. "Incorporating Bioaccessibility into Human Health Risk Assessments of Heavy Metals in Urban Park Soils." *Science of the Total Environment* 424:88–96. doi: 10.1016/j.scitotenv.2012.02.053.

Lymperi, Stefania, and Aleksander Giwercman. 2018. "Endocrine Disruptors and Testicular Function." *Metabolism: Clinical and Experimental* 86:79–90. doi: 10.1016/j.metabol.2018.03.022.

Maciel, Edvaldo Vasconcelos Soares, Ana Lúcia de Toffoli, Eduardo Sobieski Neto, Carlos Eduardo Domingues Nazario, and Fernando Mauro Lanças. 2019. "New Materials in Sample Preparation: Recent Advances and Future Trends." *TrAC - Trends in Analytical Chemistry* 119.

Mahalakshmi, R., Arivalagan Pugazhendhi, Kathirvel Brindhadevi, and Nachimuthu Ramesh. 2020. "Analysis of Alkylphenol Ethoxylates (APEOs) from Tannery Sediments Using LC–MS and Their Environmental Risks." *Process Biochemistry* 97:37–42. doi: 10.1016/j.procbio.2020.06.015.

Mahdavi, Behnam. 2017. *Chemical Compositions of Essential Oils from Etlingera Brevilabrum: A Comparative Analysis Using GC×GC/TOFMS*. Vol. 1.

Mahurpawar, Manju. 2015. "EFFECTS OF HEAVY METALS ON HUMAN HEALTHEFFECTS OF HEAVY METALS ON HUMAN HEALTH." *International Journal of Research - GRANTHAALAYAH* 3(9SE):1–7. doi: 10.29121/granthaalayah.v3.i9se.2015.3282.

Maia, Maria Luz, Sara Sousa, Maria Luísa Correia-Sá, Cristina Delerue-Matos, Conceição Calhau, and Valentina Fernandes Domingues. 2020. "Organochlorine Pesticides, Brominated Flame Retardants, Synthetic Musks and Polycyclic Aromatic Hydrocarbons in Shrimps. An Overview of Occurrence and Its Implication on Human Exposure." *Heliyon* 6(9).

Malaisé, Yann, Hélène le Mentec, Lydie Sparfel, and Laurence Guzylack-Piriou. 2020. "Differential Influences of the BPA, BPS and BPF on in Vitro IL-17 Secretion by Mouse and Human T Cells." *Toxicology in Vitro* 69. doi: 10.1016/j.tiv.2020.104993.

Malusá, Eligio, Małgorzata Tartanus, Witold Danelski, Artur Miszczak, Ewelina Szustakowska, Joanna Kicińska, and Ewa M. Furmanczyk. 2020. "Monitoring of DDT in Agricultural Soils

under Organic Farming in Poland and the Risk of Crop Contamination." *Environmental Management* 66(5):916–29. doi: 10.1007/s00267-020-01347-9.

Manisalidis, Ioannis, Elisavet Stavropoulou, Agathangelos Stavropoulos, and Eugenia Bezirtzoglou. 2020. "Environmental and Health Impacts of Air Pollution: A Review." *Frontiers in Public Health* 8.

Manzoor, Javid, Manoj Sharma, and Khursheed Ahmad Wani. 2018a. "Heavy Metals in Vegetables and Their Impact on the Nutrient Quality of Vegetables: A Review." *Journal of Plant Nutrition* 41(13):1744–63.

Manzoor, Javid, Manoj Sharma, and Khursheed Ahmad Wani. 2018b. "Heavy Metals in Vegetables and Their Impact on the Nutrient Quality of Vegetables: A Review." *Journal of Plant Nutrition* 41(13):1744–63.

Mao, Hui, Yan Yang, Hao Zhang, Jiang Zhang, and Yan Huang. 2020. "A Critical Review of the Possible Effects of Physical and Chemical Properties of Subcritical Water on the Performance of Water-Based Drilling Fluids Designed for Ultra-High Temperature and Ultra-High Pressure Drilling Applications." *Journal of Petroleum Science and Engineering* 187.

Marcon, Naiane Sabedot, Rosicler Colet, Denise Bibilio, Adriana Marcia Graboski, Clarice Steffens, and Clarissa Dalla Rosa. 2019. "Production of Ethyl Esters by Direct Transesterification of Microalga Biomass Using Propane as Pressurized Fluid." *Applied Biochemistry and Biotechnology* 187(4):1285–99. doi: 10.1007/s12010-018-2882-4.

Marziali, L., F. Rosignoli, A. Drago, S. Pascariello, L. Valsecchi, B. Rossaro, and L. Guzzella. 2017. "Toxicity Risk Assessment of Mercury, DDT and Arsenic Legacy Pollution in SedimentsA Triad Approach under Low Concentration Conditions." *Science of the Total Environment* 593–594:809–21. doi: 10.1016/j.scitotenv.2017.03.219.

Maurya, Pradip Kumar, D. S. Malik, Krishna Kumar Yadav, Amit Kumar, Sandeep Kumar, and Hesam Kamyab. 2019. "Bioaccumulation and Potential Sources of Heavy Metal Contamination in Fish Species in River Ganga Basin: Possible Human Health Risks Evaluation." *Toxicology Reports* 6:472–81. doi: 10.1016/j.toxrep.2019.05.012.

Mazlan, Norida, Mohammed Ahmed, Farrah Melissa Muharam, and Amirul Alam. 2017. "Status of Persistent Organic Pesticide Residues in Water and Food and Their Effects on Environment and Farmers: A Comprehensive Review in Nigeria." *Semina:Ciencias Agrarias* 38(4):2221–36.

la Merrill, Michele A., Laura N. Vandenberg, Martyn T. Smith, William Goodson, Patience Browne, Heather B. Patisaul, Kathryn Z. Guyton, Andreas Kortenkamp, Vincent J. Cogliano, Tracey J. Woodruff, Linda Rieswijk, Hideko Sone, Kenneth S. Korach, Andrea C. Gore, Lauren Zeise, and R. Thomas Zoeller. 2020. "Consensus on the Key Characteristics of Endocrine-Disrupting Chemicals as a Basis for Hazard Identification." *Nature Reviews Endocrinology* 16(1):45–57. doi: 10.1038/s41574-019-0273-8.

Mezcua, M., M. A. Martínez-Uroz, M. M. Gómez-Ramos, M. J. Gómez, J. M. Navas, and A. R. Fernández-Alba. 2012. "Analysis of Synthetic Endocrine-Disrupting Chemicals in Food: A Review." *Talanta* 100:90–106. doi: 10.1016/j.talanta.2012.07.078.

De Miguel, E., I. Iribarren, E. Chacón, A. Ordoñez, and S. Charlesworth. 2007. "Risk-Based Evaluation of the Exposure of Children to Trace Elements in Playgrounds in Madrid (Spain)." *Chemosphere* 66(3):505–13. doi: 10.1016/j.chemosphere.2006.05.065.

Miller, W. Greg, Jeffrey Budd, Neil Greenberg, Cas Weykamp, Harald Althaus, Heinz Schimmel, Mauro Panteghini, Vincent Delatour, Ferruccio Ceriotti, Thomas Keller, Douglas Hawkins, Chris Burns, Robert Rej, Johanna E. Camara, Finlay MacKenzie, Eline van der Hagen, and Hubert Vesper. 2020. "IFCC Working Group Recommendations for Correction of Bias Caused by Noncommutability of a Certified Reference Material Used in the Calibration Hierarchy of an End-User Measurement Procedure." *Clinical Chemistry* 66(6):769–78. doi: 10.1093/clinchem/hvaa048.

Min, Ning, Jun Yao, Langping Wu, Meseret Amde, Hans Hermann Richnow, Yafei Chen, Chaochang Wu, and Hao Li. 2021. "Isotope Fractionation of Diethyl Phthalate during Oxidation Degradation by Persulfate Activated with Zero-Valent Iron." *Chemical Engineering Journal* 132132. doi: 10.1016/j.cej.2021.132132.

Mirzabeygi, Majid, Abbas Abbasnia, Masoud Yunesian, Ramin Nabizadeh Nodehi, Nader Yousefi, Mahdi Hadi, and Amir Hossein Mahvi. 2017. "Heavy Metal Contamination and Health Risk Assessment in Drinking Water of Sistan and Baluchistan, Southeastern Iran." *Human and Ecological Risk Assessment* 23(8):1893–1905. doi: 10.1080/10807039.2017.1322895.

Mishra, Virendra K., Alka R. Upadhyay, and B. D. Tripathi. 2009. "Bioaccumulation of Heavy Metals and Two Organochlorine Pesticides (DDT and BHC) in Crops Irrigated with

Secondary Treated Waste Water." *Environmental Monitoring and Assessment* 156(1–4):99–107. doi: 10.1007/s10661-008-0466-4.

Mohamed, Wafaa A. M., Walaa El-Houseiny, Rowida E. Ibrahim, and Yasmina M. Abd-Elhakim. 2019. "Palliative Effects of Zinc Sulfate against the Immunosuppressive, Hepato- and Nephrotoxic Impacts of Nonylphenol in Nile Tilapia (Oreochromis Niloticus)." *Aquaculture* 504:227–38. doi: 10.1016/j.aquaculture.2019.02.004.

Mohammed Golam Rasul. 2018. "Conventional Extraction Methods Use in Medicinal Plants, Their Advantages and Disadvantages." *International Journal of Basic Sciences and Applied Computing* (6):10–14.

Montemurro, Nicola, Joel Joedicke, and Sandra Pérez. 2021. "Development and Application of a QuEChERS Method with Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry for the Determination of 50 Wastewater-Borne Pollutants in Earthworms Exposed through Treated Wastewater." *Chemosphere* 263. doi: 10.1016/j.chemosphere.2020.128222.

Moreira, Patricia V. L., Larissa Galastri Baraldi, Jean Claude Moubarac, Carlos Augusto Monteiro, Alex Newton, Simon Capewell, and Martin O'Flaherty. 2015. "Comparing Different Policy Scenarios to Reduce the Consumption of Ultra-Processed Foods in UK: Impact on Cardiovascular Disease Mortality Using a Modelling Approach." *PLoS ONE* 10(2). doi: 10.1371/journal.pone.0118353.

Mostafa, Mohamed K., and Robert W. Peters. 2017. "Reuse Paint Wastewater in the Manufacture of Cement Bricks and Tiles." *Journal of Material Cycles and Waste Management* 19(2):840–50. doi: 10.1007/s10163-016-0485-0.

Moyo, Babra, Vhahangwele Matodzi, Malebogo A. Legodi, Vusumzi E. Pakade, and Nikita T. Tavengwa. 2020. "Determination of Cd, Mn and Ni Accumulated in Fruits, Vegetables and Soil in the Thohoyandou Town Area, South Africa." *Water SA* 46(2):285–90. doi: 10.17159/wsa/2020.v46.i2.8244.

Mwaurah, Peter Waboi, Sunil Kumar, Nitin Kumar, Arun Kumar Attkan, Anil Panghal, Vijay Kumar Singh, and Mukesh Kumar Garg. 2020. "Novel Oil Extraction Technologies: Process Conditions, Quality Parameters, and Optimization." *Comprehensive Reviews in Food Science and Food Safety* 19(1):3–20. doi: 10.1111/1541-4337.12507.

Nascimento, Sabrina, Gabriela Göethel, Bruna Gauer, Elisa Sauer, Jessica Nardi, Larissa Cestonaro, Douglas Correia, Caroline Peruzzi, Luciano Mota, Rafael v. Machry, Tania W. Furlanetto, Tatiana Saint' Pierre, Adriana Gioda, Marcelo D. Arbo, and Solange C. Garcia. 2018. "Exposure to Environment Chemicals and Its Possible Role in Endocrine Disruption of Children from a Rural Area." *Environmental Research* 167:488–98. doi: 10.1016/j.envres.2018.07.039.

Nesan, Dinushan, Laronna C. Sewell, and Deborah M. Kurrasch. 2018. "Opening the Black Box of Endocrine Disruption of Brain Development: Lessons from the Characterization of Bisphenol A." *Hormones and Behavior* 101:50–58.

Nhamo, Luxon, Lameck Rwizi, Sylvester Mpandeli, Joel Botai, James Magidi, Henerica Tazvinga, Nafiisa Sobratee, Stanley Liphadzi, Dhesigen Naidoo, Albert T. Modi, Rob Slotow, and Tafadzwanashe Mabhaudhi. 2021. "Urban Nexus and Transformative Pathways towards a Resilient Gauteng City-Region, South Africa." *Cities* 116. doi: 10.1016/j.cities.2021.103266.

Nowak, Dorota, and Ewa Jakubczyk. 2020. "The Freeze-Drying of Foods⇔the Characteristic of the Process Course and the Effect of Its Parameters on the Physical Properties of Food Materials." *Foods* 9(10).

Nuapia, Yannick, Luke Chimuka, and Ewa Cukrowska. 2016a. "Assessment of Organochlorine Pesticide Residues in Raw Food Samples from Open Markets in Two African Cities." *Chemosphere* 164:480–87. doi: 10.1016/j.chemosphere.2016.08.055.

Nuapia, Yannick, Luke Chimuka, and Ewa Cukrowska. 2016b. "Assessment of Organochlorine Pesticide Residues in Raw Food Samples from Open Markets in Two African Cities." *Chemosphere* 164:480–87. doi: 10.1016/j.chemosphere.2016.08.055.

Nuapia, Yannick, Luke Chimuka, and Ewa Cukrowska. 2016c. "Assessment of Organochlorine Pesticide Residues in Raw Food Samples from Open Markets in Two African Cities." *Chemosphere* 164:480–87. doi: 10.1016/j.chemosphere.2016.08.055.

Nuapia, Yannick, Luke Chimuka, and Ewa Cukrowska. 2018. "Assessment of Heavy Metals in Raw Food Samples from Open Markets in Two African Cities." *Chemosphere* 196:339–46. doi: 10.1016/j.chemosphere.2017.12.134.

Nuerxiati, Rehebati, Aytursun Abuduwaili, Paiheerding Mutailifu, Atikan Wubulikasimu, Nigora Rustamova, Cui Jingxue, Haji Akber Aisa, and Abulimiti Yili. 2019. "Optimization of Ultrasonic-Assisted Extraction, Characterization and Biological Activities of Polysaccharides

from Orchis Chusua D. Don (Salep)." *International Journal of Biological Macromolecules* 141:431–43. doi: 10.1016/j.ijbiomac.2019.08.112.

Nwoko, Chris Obioma, Uwakmfon L. Bassey, Tochi Ebe, Peter N. Okeke, Mbuka-Nwosu E. Iheoma, Uloma O. Njionye, and Chris O Nwoko. 2019. "Assessment of the Contamination Level of Polycyclic Aromatic Hydrocarbons in the Soil around Ekeatai River, Eket, Akwa Ibom State, Nigeria Assessment of Contaminated Soils in Owerri Municipality View Project Environmental Monitoring View Project Mbuka-Nwosu Iheoma Ezichi 2 PUBLICATIONS 2 CITATIONS SEE PROFILE Assessment of the Contamination Level of Polycyclic Aromatic Hydrocarbons in the Soil around Ekeatai River, Eket, Akwa Ibom State, Nigeria." *Applied Ecology and Environmental Sciences* 7(6):270–77. doi: 10.12691/aees-7-6-9.

Nyaba, Luthando, and Philiswa N. Nomngongo. 2020. "Determination of Trace Metals in Vegetables and Water Samples Using Dispersive Ultrasound-Assisted Cloud Point-Dispersive μ-Solid Phase Extraction Coupled with Inductively Coupled Plasma Optical Emission Spectrometry." *Food Chemistry* 322. doi: 10.1016/j.foodchem.2020.126749.

Okpashi, Victor Eshu, Agnes Edet Asuquo Offiong, Etim Nkanu Efut, and Ogre Gabriel Ogre. 2019. "EQA-International Journal of Environmental Quality Evaluation of Endocrine Disruptors in the Environment of Eket Metropolis in Nigeria: An Insight into Public Health and Risk Assessment." doi: 10.6092/issn.2281-4485/9364.

Olaniyan, Lamidi W. B., and Anthony I. Okoh. 2020. "Determination and Ecological Risk Assessment of Two Endocrine Disruptors from River Buffalo, South Africa." *Environmental Monitoring and Assessment* 192(12). doi: 10.1007/s10661-020-08717-0.

Olatunji, Olatunde S. 2019. "Evaluation of Selected Polychlorinated Biphenyls (PCBs) Congeners and Dichlorodiphenyltrichloroethane (DDT) in Fresh Root and Leafy Vegetables Using GC-MS." *Scientific Reports* 9(1). doi: 10.1038/s41598-018-36996-8.

Olisah, Chijioke, Omobola O. Okoh, and Anthony I. Okoh. 2020. "Occurrence of Organochlorine Pesticide Residues in Biological and Environmental Matrices in Africa: A Two-Decade Review." *Heliyon* 6(3).

Oliveira, Alessandra L., Emilie Destandau, Laëtitia Fougère, and Michel Lafosse. 2014. "Isolation by Pressurised Fluid Extraction (PFE) and Identification Using CPC and HPLC/ESI/MS of Phenolic Compounds from Brazilian Cherry Seeds (Eugenia Uniflora L.)." *Food Chemistry* 145:522–29. doi: 10.1016/j.foodchem.2013.08.065.

Oloruntoba, Adefarati, and Isaiah Adukwu Nathaniel. 2019. “Assessment of Heavy Metal Levels in Offal Meats (Kidney and Liver) of Beef Sold at Gwagwalada Market, Abuja, Nigeria.” *Asian Journal of Physical and Chemical Sciences* 1–8. doi: 10.9734/ajopacs/2019/v7i230090.

Olsson, Petter, Jan Holmbäck, and Bengt Herslöf. 2014. “A Single Step Reversed-Phase High Performance Liquid Chromatography Separation of Polar and Non-Polar Lipids.” *Journal of Chromatography A* 1369:105–15. doi: 10.1016/j.chroma.2014.10.010.

Onal, Gizem, Ozlem Kutlu, Devrim Gozuacik, and Serap Dokmeci Emre. 2017. “Lipid Droplets in Health and Disease.” *Lipids in Health and Disease* 16(1).

Ore, Odunayo T., and Adedapo O. Adeola. 2021. “Toxic Metals in Oil Sands: Review of Human Health Implications, Environmental Impact, and Potential Remediation Using Membrane-Based Approach.” *Energy, Ecology and Environment* 6(2):81–91.

Osman, K. A., A. M. Al-Humaid, S. M. Al-Rehiayani, and K. N. Al-Redhaiman. 2010. “Monitoring of Pesticide Residues in Vegetables Marketed in Al-Qassim Region, Saudi Arabia.” *Ecotoxicology and Environmental Safety* 73(6):1433–39. doi: 10.1016/j.ecoenv.2010.05.020.

Oyinloye, Timilehin Martins, and Won Byong Yoon. 2020. “Effect of Freeze-Drying on Quality and Grinding Process of Food Produce: A Review.” *Processes* 8(3).

Ozaki, Hitomi, Kazumi Sugihara, Yoko Watanabe, Kyoko Moriguchi, Naoto Uramaru, Tomomichi Sone, Shigeru Ohta, and Shigeyuki Kitamura. 2017. “Comparative Study of Hydrolytic Metabolism of Dimethyl Phthalate, Dibutyl Phthalate and Di(2-Ethylhexyl) Phthalate by Microsomes of Various Rat Tissues.” *Food and Chemical Toxicology* 100:217–24. doi: 10.1016/j.fct.2016.12.019.

Pais, Gillian, Kartik Jayaram, and Arend van Wamelen. 2020. *Safeguarding Africa’s Food Systems through and beyond the Crisis Understanding the Impact of COVID-19 on African Agriculture-and How Governments and Private-Sector Actors Can Respond*.

Pan, Yi, Zhi Ang Li, Xin Zhou, Wei Kang Wang, Xing Wang, Zheng Fang, and Cheng bin Zheng. 2017. “A System Consisted of Flame Ionization Detector and Sulfur Chemiluminescence Detector for Interference Free Determination of Total Sulfur in Natural Gas.” *Chinese Chemical Letters* 28(8):1670–74. doi: 10.1016/j.cclet.2017.04.014.

Pandey, Amita, and Shalini Tripathi. 2014a. *Concept of Standardization, Extraction and Pre Phytochemical Screening Strategies for Herbal Drug*. Vol. 2.

Pandey, Amita, and Shalini Tripathi. 2014b. *Concept of Standardization, Extraction and Pre Phytochemical Screening Strategies for Herbal Drug*. Vol. 2.

Paolella, Gaetana, Antonio M. Romanelli, Stefania Martucciello, Silvia Sposito, Marilena Lepretti, Carla Esposito, Anna Capaldo, and Ivana Caputo. 2021. "The Mechanism of Cytotoxicity of 4-Nonylphenol in a Human Hepatic Cell Line Involves ER-Stress, Apoptosis, and Mitochondrial Dysfunction." *Journal of Biochemical and Molecular Toxicology* 35(7). doi: 10.1002/jbt.22780.

Parkkinen, Veli-Pekka, Christian Wallmann, Michael Wilde, Brendan Clarke, Phyllis Illari, Michael P. Kelly, Charles Norell, Federica Russo, Beth Shaw, and Jon Williamson. n.d. *SPRINGER BRIEFS IN PHILOSOPHY Evaluating Evidence of Mechanisms in Medicine Principles and Procedures*.

Parrilla Vázquez, P., C. Ferrer, M. J. Martínez Bueno, and A. R. Fernández-Alba. 2019. "Pesticide Residues in Spices and Herbs: Sample Preparation Methods and Determination by Chromatographic Techniques." *TrAC - Trends in Analytical Chemistry* 115:13–22.

Paschoalini, A. L., L. A. Savassi, F. P. Arantes, E. Rizzo, and N. Bazzoli. 2019. "Heavy Metals Accumulation and Endocrine Disruption in Prochilodus Argenteus from a Polluted Neotropical River." *Ecotoxicology and Environmental Safety* 169:539–50. doi: 10.1016/j.ecoenv.2018.11.047.

Pavlikova, Nela, Jan Sramek, Michael Jelinek, Petr Halada, and Jan Kovar. 2020. "Markers of Acute Toxicity of DDT Exposure in Pancreatic Beta-Cells Determined by a Proteomic Approach." *PLoS ONE* 15(10 October). doi: 10.1371/journal.pone.0229430.

Pérez-Rodríguez, Michael, Roberto Gerardo Pellerano, Leonardo Pezza, and Helena Redigolo Pezza. 2018. "An Overview of the Main Foodstuff Sample Preparation Technologies for Tetracycline Residue Determination." *Talanta* 182:1–21.

Perrot-Applanat, Martine, Martine Kolf-Clauw, Cécile Michel, and Claire Beausoleil. 2018. "Alteration of Mammary Gland Development by Bisphenol a and Evidence of a Mode of Action Mediated through Endocrine Disruption." *Molecular and Cellular Endocrinology* 475:29–53.

Petrescu, Relly Victoria, Raffaella Aversa, Antonio Apicella, and Florian Ion Tiberiu Petrescu. 2018. "NASA Sees First in 2018 the Direct Proof of Ozone Hole Recovery." *Journal of Aircraft and Spacecraft Technology* 2(1):53–64. doi: 10.3844/jastsp.2018.53.64.

Petta, Julia C., Oliver N. Shipley, Sabine P. Wintner, Geremy Cliff, Matt L. Dicken, and Nigel E. Hussey. 2020. “Are You Really What You Eat? Stomach Content Analysis and Stable Isotope Ratios Do Not Uniformly Estimate Dietary Niche Characteristics in Three Marine Predators.” *Oecologia* 192(4):1111–26. doi: 10.1007/s00442-020-04628-6.

Plaza, Merichel, and Charlotta Turner. 2015a. “Pressurized Hot Water Extraction of Bioactives.” *TrAC - Trends in Analytical Chemistry* 71:39–54.

Plaza, Merichel, and Charlotta Turner. 2015b. “Pressurized Hot Water Extraction of Bioactives.” *TrAC - Trends in Analytical Chemistry* 71:39–54.

Plaza, Merichel, and Charlotta Turner. 2015c. “Pressurized Hot Water Extraction of Bioactives.” *TrAC - Trends in Analytical Chemistry* 71:39–54.

Plaza, Pablo I., Emma Martínez-López, and Sergio A. Lambertucci. 2019. “The Perfect Threat: Pesticides and Vultures.” *Science of the Total Environment* 687:1207–18.

Popescu, Maria, Talia B. Feldman, and Tanuja Chitnis. 2021. “Interplay Between Endocrine Disruptors and Immunity: Implications for Diseases of Autoreactive Etiology.” *Frontiers in Pharmacology* 12.

Prasad, Bablu. 2021. “Phthalate Pollution: Environmental Fate and Cumulative Human Exposure Index Using the Multivariate Analysis Approach.” *Environmental Science: Processes and Impacts* 23(3):389–99.

Prathyusha, K., Ysyv Jagan Mohan, S. Sridevi, and B. v Sandeep. 2016. “ISOLATION AND CHARACTERIZATION OF PETROLEUM HYDROCARBON DEGRADING INDIGENOUS BACTERIA FROM CONTAMINATED SITES OF ISOLATION AND CHARACTERIZATION OF PETROLEUM HYDROCARBON DEGRADING INDIGENOUS BACTERIA FROM CONTAMINATED SITES OF VISAKHAPATNAM.” *International Journal of Advanced Research* 4(3):357–62.

Priyadarshini, Eepsita, Sushree Sangita Priyadarshini, and Nilotpala Pradhan. 2019. “Heavy Metal Resistance in Algae and Its Application for Metal Nanoparticle Synthesis.” *Applied Microbiology and Biotechnology* 103(8):3297–3316.

Rai, Amit, Kumargaurao D. Punase, Bikash Mohanty, and Ravindra Bhargava. 2014. “Evaluation of Models for Supercritical Fluid Extraction.” *International Journal of Heat and Mass Transfer* 72:274–87. doi: 10.1016/j.ijheatmasstransfer.2014.01.011.

Rajmohan, Kunju Vaikarar Soundararajan, Chandrasekaran Ramya, Manakkal Raja Viswanathan, and Sunita Varjani. 2019. “Plastic Pollutants: Effective Waste Management for Pollution Control and Abatement.” *Current Opinion in Environmental Science and Health* 12:72–84.

Ramalhosa, Maria João, Paula Paíga, Simone Morais, Cristina Delerue-Matos, and Maria Beatriz Prior Pinto Oliveira. 2009. “Analysis of Polycyclic Aromatic Hydrocarbons in Fish: Evaluation of a Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction Method.” *Journal of Separation Science* 32(20):3529–38. doi: 10.1002/jssc.200900351.

Rehman, Saba, Zeenat Usman, Sabeen Rehman, Moneera AlDraihem, Noor Rehman, Ibraheem Rehman, and Gulfam Ahmad. 2018. “Endocrine Disrupting Chemicals and Impact on Male Reproductive Health.” *Translational Andrology and Urology* 7(3):490–503.

Ripamonti, Emiliano, Elena Allifranchini, Stefano Todeschi, and Elena Bocchietto. 2018. “Endocrine Disruption by Mixtures in Topical Consumer Products.” *Cosmetics* 5(4).

Rodgers, Kathryn M., Julia O. Udesky, Ruthann A. Rudel, and Julia Green Brody. 2018. “Environmental Chemicals and Breast Cancer: An Updated Review of Epidemiological Literature Informed by Biological Mechanisms.” *Environmental Research* 160:152–82.

Rodrigues, M. O., N. Abrantes, F. J. M. Gonçalves, H. Nogueira, J. C. Marques, and A. M. M. Gonçalves. 2019. “Impacts of Plastic Products Used in Daily Life on the Environment and Human Health: What Is Known?” *Environmental Toxicology and Pharmacology* 72.

Rodríguez, Angel Gabriel Polanco, María Inmaculada Riba López, Ángel Del Valls Casillas, Jesús Alfredo Araujo León, and Sudip Datta Banik. 2018. “Impact of Pesticides in Karst Groundwater. Review of Recent Trends in Yucatan, Mexico.” *Groundwater for Sustainable Development* 7:20–29.

Rosenfeld, Cheryl S., and Paul S. Cooke. 2019. “Endocrine Disruption through Membrane Estrogen Receptors and Novel Pathways Leading to Rapid Toxicological and Epigenetic Effects.” *Journal of Steroid Biochemistry and Molecular Biology* 187:106–17. doi: 10.1016/j.jsbmb.2018.11.007.

Rotondo, Eleonora, and Francesco Chiarelli. 2020. “Endocrine-Disrupting Chemicals and Insulin Resistance in Children.” *Biomedicines* 8(6).

Ruiz, Daniel, Marisol Becerra, Jyotsna S. Jagai, Kerry Ard, and Robert M. Sargis. 2018. “Disparities in Environmental Exposures to Endocrine-Disrupting Chemicals and Diabetes Risk in Vulnerable Populations.” *Diabetes Care* 41(1):193–205. doi: 10.2337/dc16-2765.

Rushing, Blake R., and Mustafa I. Selim. 2019. “Aflatoxin B1: A Review on Metabolism, Toxicity, Occurrence in Food, Occupational Exposure, and Detoxification Methods.” *Food and Chemical Toxicology* 124:81–100.

Russo, Mario Vincenzo, Pasquale Avino, Luisa Perugini, and Ivan Notardonato. 2015. “Extraction and GC-MS Analysis of Phthalate Esters in Food Matrices: A Review.” *RSC Advances* 5(46):37023–43.

Sabir, Shakila, Muhammad Sajid Hamid Akash, Fareeha Fiayyaz, Uzma Saleem, Malik Hassan Mehmood, and Kanwal Rehman. 2019. “Role of Cadmium and Arsenic as Endocrine Disruptors in the Metabolism of Carbohydrates: Inserting the Association into Perspectives.” *Biomedicine and Pharmacotherapy* 114.

Sabir, Shakila, Muhammad Furqan Akhtar, and Ammara Saleem. 2019. “Endocrine Disruption as an Adverse Effect of Non-Endocrine Targeting Pharmaceuticals.” *Environmental Science and Pollution Research* 26(2):1277–86.

Sánchez-Camargo, Andrea del Pilar, Fabián Parada-Alfonso, Elena Ibáñez, and Alejandro Cifuentes. 2017. “On-Line Coupling of Supercritical Fluid Extraction and Chromatographic Techniques.” *Journal of Separation Science* 40(1):213–27.

Sanford, Elissa. n.d. *THE DEFICIENCIES OF THE EUROPEAN UNION’S REGULATORY SYSTEM GOVERNING THE CLASSIFICATION OF ENDOCRINE DISRUPTING CHEMICALS*.

Santana-Mayor, Álvaro, Bárbara Socas-Rodríguez, Antonio v. Herrera-Herrera, and Miguel Ángel Rodríguez-Delgado. 2019. “Current Trends in QuEChERS Method. A Versatile Procedure for Food, Environmental and Biological Analysis.” *TrAC - Trends in Analytical Chemistry* 116:214–35. doi: 10.1016/j.trac.2019.04.018.

Sargis, Robert M., Jerrold J. Heindel, and Vasantha Padmanabhan. 2019. “Interventions to Address Environmental Metabolism-Disrupting Chemicals: Changing the Narrative to Empower Action to Restore Metabolic Health.” *Frontiers in Endocrinology* 10(FEB). doi: 10.3389/fendo.2019.00033.

Satyanarayanan, M., V. Balaram, S. S. Sawant, K. S. V. Subramanyam, G. Vamsi Krishna, B. Dasaram, and C. Manikyamba. 2018. “Rapid Determination of REEs, PGEs, and Other Trace Elements in Geological and Environmental Materials by High Resolution Inductively

Coupled Plasma Mass Spectrometry." *Atomic Spectroscopy* 39(1):1–15. doi: 10.46770/as.2018.01.001.

Schug, Thaddeus T., Amanda Janesick, Bruce Blumberg, and Jerrold J. Heindel. 2011. "Endocrine Disrupting Chemicals and Disease Susceptibility." *Journal of Steroid Biochemistry and Molecular Biology* 127(3–5):204–15.

Seltenrich, Nate. 2015. "New Link in the Food Chain? Marine Plastic Pollution and Seafood Safety." *Environmental Health Perspectives* 123(2):A34–41.

Seopela, Mathapelo P., Robert I. McCrindle, Sandra Combrinck, and Wilma Augustyn. 2021. "Temporal and Spatial Variations of Organochlorine Pesticides (Ocps) and Phthalates Affecting the Quality of Water and Sediment from Loskop Dam, South Africa." *Water SA* 47(2):221–34. doi: 10.17159/wsa/2021.v47.i2.10918.

Serrano, Samantha E., Joseph Braun, Leonardo Trasande, Russell Dills, and Sheela Sathyanarayana. 2014. "Phthalates and Diet: A Review of the Food Monitoring and Epidemiology Data." *Environmental Health: A Global Access Science Source* 13(1). doi: 10.1186/1476-069X-13-43.

Shaheen, Nazma, Nafis Md Irfan, Ishrat Nourin Khan, Saiful Islam, Md Saiful Islam, and Md Kawser Ahmed. 2016. "Presence of Heavy Metals in Fruits and Vegetables: Health Risk Implications in Bangladesh." *Chemosphere* 152:431–38. doi: 10.1016/j.chemosphere.2016.02.060.

Shams, Khaled A., Nahla S. Abdel-Azim, Ibrahim A. Saleh, Mohamed-Elamir F. Hegazy, Mostafa M. El-Missiry, and Faiza M. Hammouda. 2015. "Green Technology: Economically and Environmentally Innovative Methods for Extraction of Medicinal & Aromatic Plants (MAP) in Egypt." *Journal of Chemical and Pharmaceutical Research* 7(5):1050–74.

Shariati, S., C. Ebenau-Jehle, A. A. Pourbabaee, H. A. Alikhani, M. Rodriguez-Franco, M. Agne, M. Jacoby, R. Geiger, F. Shariati, and M. Boll. 2021. "Degradation of Dibutyl Phthalate by Paenarthrobacter Sp. Shss Isolated from Saravan Landfill, Hyrcanian Forests, Iran." *Biodegradation*. doi: 10.1007/s10532-021-09966-7.

Sharif, K. M., M. M. Rahman, J. Azmir, A. Mohamed, M. H. A. Jahurul, F. Sahena, and I. S. M. Zaidul. 2014. "Experimental Design of Supercritical Fluid Extraction - A Review." *Journal of Food Engineering* 124:105–16.

Sharma, Brij Mohan, Girija K. Bharat, Paromita Chakraborty, Jakub Martiník, Ondřej Audy, Petr Kukučka, Petra Přibylová, Praveen Kumar Kukreti, Anežka Sharma, Jiří Kalina, Eirik Hovland Steindal, and Luca Nizzetto. 2021a. "A Comprehensive Assessment of Endocrine-Disrupting Chemicals in an Indian Food Basket: Levels, Dietary Intakes, and Comparison with European Data." *Environmental Pollution* 288. doi: 10.1016/j.envpol.2021.117750.

Sharma, Brij Mohan, Girija K. Bharat, Paromita Chakraborty, Jakub Martiník, Ondřej Audy, Petr Kukučka, Petra Přibylová, Praveen Kumar Kukreti, Anežka Sharma, Jiří Kalina, Eirik Hovland Steindal, and Luca Nizzetto. 2021b. "A Comprehensive Assessment of Endocrine-Disrupting Chemicals in an Indian Food Basket: Levels, Dietary Intakes, and Comparison with European Data." *Environmental Pollution* 288. doi: 10.1016/j.envpol.2021.117750.

Sharma, Pooja, Ranjna Sirohi, Yen Wah Tong, Sang Hyoun Kim, and Ashok Pandey. 2021. "Metal and Metal(Loids) Removal Efficiency Using Genetically Engineered Microbes: Applications and Challenges." *Journal of Hazardous Materials* 416. doi: 10.1016/j.jhazmat.2021.125855.

Sheikh, Ishfaq A. 2020. "Molecular Interactions of Thyroxine Binding Globulin and Thyroid Hormone Receptor with Estrogenic Compounds 4-Nonylphenol, 4-Tert-Octylphenol and Bisphenol A Metabolite (MBP)." *Life Sciences* 253. doi: 10.1016/j.lfs.2020.117738.

Shitu, A., S. Izhar, and T. M. Tahir. 2015. *A r c h i v e o f S I D Sub-Critical Water as a Green Solvent for Production of Valuable Materials from Agricultural Waste Biomass: A Review of Recent Work*. Vol. 1.

Shoaff, Jessica R., Brent Coull, Jennifer Weuve, David C. Bellinger, Antonia M. Calafat, Susan L. Schantz, and Susan A. Korrick. 2020. "Association of Exposure to Endocrine-Disrupting Chemicals during Adolescence with Attention-Deficit/Hyperactivity Disorder-Related Behaviors." *JAMA Network Open* 3(8). doi: 10.1001/jamanetworkopen.2020.15041.

Sikdar, Sayantani, and Madhusree Kundu. 2018. "A Review on Detection and Abatement of Heavy Metals." *ChemBioEng Reviews* 5(1):18–29.

da Silva, Rui P. F. F., Teresa A. P. Rocha-Santos, and Armando C. Duarte. 2016. "Supercritical Fluid Extraction of Bioactive Compounds." *TrAC - Trends in Analytical Chemistry* 76:40–51.

Silva-Espinoza, Marilú Andrea, María del Mar Camacho, Javier Martínez-Monzó, and Nuria Martínez-Navarrete. 2021. "Impact of the Freeze-Drying Conditions Applied to Obtain an Orange Snack on Energy Consumption." *Foods* 10(11):2756. doi: 10.3390/foods10112756.

Sobota, Daniel J., Jana E. Compton, Michelle L. McCrackin, and Shweta Singh. 2015. “Cost of Reactive Nitrogen Release from Human Activities to the Environment in the United States.” *Environmental Research Letters* 10(2). doi: 10.1088/1748-9326/10/2/025006.

Şolpan, Dilek, and Masoomeh Mehrnia. 2018. “Dimethyl Phthalate (DMP) Degradation in Aqueous Solution by Gamma-Irradiation/H2O2.” *Journal of Radioanalytical and Nuclear Chemistry* 317(2):841–51. doi: 10.1007/s10967-018-5944-8.

Spanjers, Charles S., Connor A. Beach, Andrew J. Jones, and Paul J. Dauenhauer. 2017. “Increasing Flame Ionization Detector (FID) Sensitivity Using Post-Column Oxidation–Methanation.” *Analytical Methods* 9(12):1928–34. doi: 10.1039/c6ay03363f.

Spietelun, Agata, Łukasz Marcinkowski, Miguel de La Guardia, and Jacek Namieśnik. 2014. “Green Aspects, Developments and Perspectives of Liquid Phase Microextraction Techniques.” *Talanta* 119:34–45.

Stefanuto, Pierre Hugues, Agnieszka Smolinska, and Jean François Focant. 2021. “Advanced Chemometric and Data Handling Tools for GC×GC-TOF-MS: Application of Chemometrics and Related Advanced Data Handling in Chemical Separations.” *TrAC - Trends in Analytical Chemistry* 139.

Steinmetz, Rosemary, Nancy G. Brown, Donald L. Allen, Robert M. Bigsby, and Nira Ben-Jonathan. 1997. *The Environmental Estrogen Bisphenol A Stimulates Prolactin Release in Vitro and in Vivo**. Vol. 138.

Street, Maria Elisabeth, Sabrina Angelini, Sergio Bernasconi, Ernesto Burgio, Alessandra Cassio, Cecilia Catellani, Francesca Cirillo, Annalisa Deodati, Enrica Fabbrizi, Vassilios Fanos, Giancarlo Gargano, Enzo Grossi, Lorenzo Iughetti, Pietro Lazzeroni, Alberto Mantovani, Lucia Migliore, Paola Palanza, Giancarlo Panzica, Anna Maria Papini, Stefano Parmigiani, Barbara Predieri, Chiara Sartori, Gabriele Tridenti, and Sergio Amarri. 2018. “Current Knowledge on Endocrine Disrupting Chemicals (EDCs) from Animal Biology to Humans, from Pregnancy to Adulthood: Highlights from a National Italian Meeting.” *International Journal of Molecular Sciences* 19(6).

Streets, David G., Hannah M. Horowitz, Daniel J. Jacob, Zifeng Lu, Leonard Levin, Arnout F. H. ter Schure, and Elsie M. Sunderland. 2017. “Total Mercury Released to the Environment by Human Activities.” *Environmental Science and Technology* 51(11):5969–77. doi: 10.1021/acs.est.7b00451.

Study, Research, and Dustin S. Ferguson. 2021a. *Preliminary Findings of Microplastic Particles and 4-Nonlyphenol in California and Wyoming Glaciers*.

Study, Research, and Dustin S. Ferguson. 2021b. *Preliminary Findings of Microplastic Particles and 4-Nonlyphenol in California and Wyoming Glaciers*.

Subedi, Bikram, Lissette Aguilar, Eleanor M. Robinson, Kimberly J. Hageman, Erland Björklund, Rebecca J. Sheesley, and Sascha Usenko. 2015. "Selective Pressurized Liquid Extraction as a Sample-Preparation Technique for Persistent Organic Pollutants and Contaminants of Emerging Concern." *TrAC - Trends in Analytical Chemistry* 68:119–32.

Subramanian, R., P. Subbramaniyan, J. Noorul Ameen, and V. Raj. 2016. "Double Bypasses Soxhlet Apparatus for Extraction of Piperine from Piper Nigrum." *Arabian Journal of Chemistry* 9:S537–40. doi: 10.1016/j.arabjc.2011.06.022.

Sukuroglu, Ayca Aktas, Dilek Battal, Kumsal Kocadal, Mehmet Ali Sungur, İsmet Cok, and Irfan Unlusayin. 2021. "Biomonitoring of Bisphenol A, 4-Nonylphenol, and 4-t-Octylphenol in Turkish Population: Exposure and Risk Assessment." *Environmental Science and Pollution Research*. doi: 10.1007/s11356-021-17796-6.

Sun, Hong Jie, Bala Rathinasabapathi, Bing Wu, Jun Luo, Li Ping Pu, and Lena Q. Ma. 2014. "Arsenic and Selenium Toxicity and Their Interactive Effects in Humans." *Environment International* 69:148–58.

Supakot, Pianpan, Jittawan Kubola, and Chuleeporn Bungthong. n.d. *Effects of Extraction Factors on Total Phenolic Compounds and Antioxidant Activity in Mulberry Leaves*. Vol. 1.

Sushkova, Svetlana N., Galina K. Vasilyeva, Tatjana M. Minkina, Saglara S. Mandzhieva, Irina G. Tjurina, Sergei I. Kolesnikov, Ridvan Kizilkaya, and Tayfun Askin. 2014. "New Method for Benzo[a]Pyrene Analysis in Plant Material Using Subcritical Water Extraction." *Journal of Geochemical Exploration* 144(PB):267–72. doi: 10.1016/j.gexplo.2014.02.018.

Szewczyńska, M., M. Pośniak, and E. Dobrzyńska. 2020. "Determination of Phthalates in Particulate Matter and Gaseous Phase Emitted into the Air of the Working Environment." *International Journal of Environmental Science and Technology* 17(1):175–86. doi: 10.1007/s13762-019-02435-y.

Talley, Theresa Sinicrope, Nina Venuti, and Rachel Whelan. 2020a. "Natural History Matters: Plastics in Estuarine Fish and Sediments at the Mouth of an Urban Watershed." *PLoS ONE* 15(3). doi: 10.1371/journal.pone.0229777.

Talley, Theresa Sinicrope, Nina Venuti, and Rachel Whelan. 2020b. “Natural History Matters: Plastics in Estuarine Fish and Sediments at the Mouth of an Urban Watershed.” *PLoS ONE* 15(3). doi: 10.1371/journal.pone.0229777.

Tato, Tania, Noelia Salgueiro-González, Víctor M. León, Sergio González, and Ricardo Beiras. 2018. “Ecotoxicological Evaluation of the Risk Posed by Bisphenol A, Triclosan, and 4-Nonylphenol in Coastal Waters Using Early Life Stages of Marine Organisms (Isochrysis Galbana, Mytilus Galloprovincialis, Paracentrotus Lividus, and Acartia Clausi).” *Environmental Pollution* 232:173–82. doi: 10.1016/j.envpol.2017.09.031.

Tawar, Neha, Basu Banerjee, Brijesh Mishra, Tusha Sharma, Shipra Tyagi, Sri Madhu, Vivek Agarwal, and Sanjay Gupta. 2021. “Adipose Tissue Levels of DDT as Risk Factor for Obesity and Type 2 Diabetes Mellitus.” *Indian Journal of Endocrinology and Metabolism* 25(2):160–65. doi: 10.4103/ijem.ijem_198_21.

Teixeira, Gerson Lopes, Saeed M. Ghazani, Marcos Lúcio Corazza, Alejandro G. Marangoni, and Rosemary Hoffmann Ribani. 2018. “Assessment of Subcritical Propane, Supercritical CO2 and Soxhlet Extraction of Oil from Sapucaia (Lecythis Pisonis) Nuts.” *Journal of Supercritical Fluids* 133:122–32. doi: 10.1016/j.supflu.2017.10.003.

Tekin, Kubilay, Selhan Karagöz, and Sema Bektaş. 2014. “A Review of Hydrothermal Biomass Processing.” *Renewable and Sustainable Energy Reviews* 40:673–87.

Timofeeva, Irina, Andrey Shishov, Daria Kanashina, Daria Dzema, and Andrey Bulatov. 2017. “On-Line in-Syringe Sugaring-out Liquid-Liquid Extraction Coupled with HPLC-MS/MS for the Determination of Pesticides in Fruit and Berry Juices.” *Talanta* 167:761–67. doi: 10.1016/j.talanta.2017.01.008.

Topal, Tufan, and Canan Onac. 2020. “Determination of Heavy Metals and Pesticides in Different Types of Fish Samples Collected from Four Different Locations of Aegean and Marmara Sea.” *Journal of Food Quality* 2020. doi: 10.1155/2020/8101532.

Torretta, Vincenzo, Ioannis A. Katsoyiannis, Paolo Viotti, and Elena Cristina Rada. 2018. “Critical Review of the Effects of Glyphosate Exposure to the Environment and Humans through the Food Supply Chain.” *Sustainability (Switzerland)* 10(4).

Trinh, Thuy Khanh, and Lim-Seok Kang. 2010. “Application of Response Surface Method as an Experimental Design to Optimize Coagulation Tests.” *Environmental Engineering Research* 15(2):63–70. doi: 10.4491/eer.2010.15.2.063.

Tse, Timothy J., Sarah K. Purdy, Jianheng Shen, Fina B. Nelson, Rana Mustafa, Daniel J. Wiens, and Martin J. T. Reaney. 2021. “Toxicology of Alcohol-Based Hand Rubs Formulated with Technical-Grade Ethanol.” *Toxicology Reports* 8:785–92.

Tsygankov, Vasiliy Yu. 2019. “Organochlorine Pesticides in Marine Ecosystems of the Far Eastern Seas of Russia (2000–2017).” *Water Research* 161:43–53.

Ubaid ur Rahman, Hafiz, Waqas Asghar, Wahab Nazir, Mansur Abdullah Sandhu, Anwaar Ahmed, and Nauman Khalid. 2021. “A Comprehensive Review on Chlorpyrifos Toxicity with Special Reference to Endocrine Disruption: Evidence of Mechanisms, Exposures and Mitigation Strategies.” *Science of the Total Environment* 755.

Ueda Yamaguchi, Natália, Luís Fernando Cusioli, Heloise Beatriz Quesada, Maria Eliana Camargo Ferreira, Márcia Regina Fagundes-Klen, Angélica Marquetotti Salcedo Vieira, Raquel Guttierres Gomes, Marcelo Fernandes Vieira, and Rosângela Bergamasco. 2021. “A Review of Moringa Oleifera Seeds in Water Treatment: Trends and Future Challenges.” *Process Safety and Environmental Protection* 147:405–20.

USEPA. 2007. “Framework for Determining a Mutagenic Mode of Action for Carcinogenicity.” *Cancer* 1–50.

Varela, Begoña, Vanessa Sesto, and Isabel García-Rodeja. 2020. “An Investigation of Secondary Students’ Mental Models of Climate Change and the Greenhouse Effect.” *Research in Science Education* 50(2):599–624. doi: 10.1007/s11165-018-9703-1.

Vasseghian, Yasser, Sevda Hosseinzadeh, Alireza Khataee, and Elena Niculina Dragoi. 2021. “The Concentration of Persistent Organic Pollutants in Water Resources: A Global Systematic Review, Meta-Analysis and Probabilistic Risk Assessment.” *Science of the Total Environment* 796. doi: 10.1016/j.scitotenv.2021.149000.

Vazquez-Duhalt, R., − F Marquez-Rocha, − E Ponce, − A F Licea, and − M T Viana. n.d. *Vazquez-Duhalt et al.: Nonyphenol, an Integrated Vision of a Pollutant-1-APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 4(1): 1-25. Http://Www NONYLPHENOL, AN INTEGRATED VISION OF A POLLUTANT. SCIENTIFIC REVIEW*.

Vazquez-Roig, Pablo, and Yolanda Picó. 2015. “Pressurized Liquid Extraction of Organic Contaminants in Environmental and Food Samples.” *TrAC - Trends in Analytical Chemistry* 71:55–64.

Velis, Costas A., and Ed Cook. 2021. “Mismanagement of Plastic Waste through Open Burning with Emphasis on the Global South: A Systematic Review of Risks to Occupational and Public Health.” *Environmental Science and Technology* 55(11):7186–7207.

Velmurugan, Ganesan, Tharmarajan Ramprasath, Mithieux Gilles, Krishnan Swaminathan, and Subbiah Ramasamy. 2017. “Gut Microbiota, Endocrine-Disrupting Chemicals, and the Diabetes Epidemic.” *Trends in Endocrinology and Metabolism* 28(8):612–25.

Ventura, C., C. D. Zappia, M. Lasagna, W. Pavicic, S. Richard, A. D. Bolzan, F. Monczor, M. Núñez, and C. Cocca. 2019. “Effects of the Pesticide Chlorpyrifos on Breast Cancer Disease. Implication of Epigenetic Mechanisms.” *Journal of Steroid Biochemistry and Molecular Biology* 186:96–104. doi: 10.1016/j.jsbmb.2018.09.021.

Vieira da Silva, Beatriz, João C. M. Barreira, and M. Beatriz P. P. Oliveira. 2016. “Natural Phytochemicals and Probiotics as Bioactive Ingredients for Functional Foods: Extraction, Biochemistry and Protected-Delivery Technologies.” *Trends in Food Science and Technology* 50:144–58.

Vieira, Wedja Timóteo, Marina Barbosa de Farias, Marcela Pires Spaolonzi, Meuris Gurgel Carlos da Silva, and Melissa Gurgel Adeodato Vieira. 2021. “Endocrine-Disrupting Compounds: Occurrence, Detection Methods, Effects and Promising Treatment Pathways - A Critical Review.” *Journal of Environmental Chemical Engineering* 9(1).

Vijaya, P., Harsimran Kaur, Nancy Garg, and Suman Sharma. 2020. “Protective and Therapeutic Effects of Garlic and Tomato on Cadmium-Induced Neuropathology in Mice.” *The Journal of Basic and Applied Zoology* 81(1). doi: 10.1186/s41936-020-00160-4.

Vikrant, Kumar, Daniel C. W. Tsang, Nadeem Raza, Balendu Shekher Giri, Deepak Kukkar, and Ki Hyun Kim. 2018. “Potential Utility of Metal-Organic Framework-Based Platform for Sensing Pesticides.” *ACS Applied Materials and Interfaces* 10(10):8797–8817.

Virgilio, Alex, Ana Beatriz S. Silva, Ana Rita A. Nogueira, Joaquim A. Nóbrega, and George L. Donati. 2020. “Calculating Limits of Detection and Defining Working Ranges for Multi-Signal Calibration Methods.” *Journal of Analytical Atomic Spectrometry* 35(8):1614–20. doi: 10.1039/d0ja00212g.

Vlassi, Eleni, Eleftheria Bempelou, Konstantinos Liapis, and Gerassimos Arapis. 2020. “Consumer Safety Evaluation after Monitoring of Endocrine Disruptor Pesticide Residues: A

Case Study of Thessaly, Central Greece." *Toxicological and Environmental Chemistry* 102(1–4):105–23. doi: 10.1080/02772248.2020.1770256.

Walters, Clifford C., Frank C. Wang, Meytal B. Higgins, and Marlene E. Madincea. 2018. "Universal Biomarker Analysis Using GC×GC with Dual FID and ToF-MS (EI/FI) Detection." *Organic Geochemistry* 115:57–66. doi: 10.1016/j.orggeochem.2017.10.003.

Wan, Murphy Lam Yim, Vanessa Anna Co, and Hani El-Nezami. 2021. "Endocrine Disrupting Chemicals and Breast Cancer: A Systematic Review of Epidemiological Studies." *Critical Reviews in Food Science and Nutrition*.

Wang, Lei, Jian Li, Yingquan Chen, Haiping Yang, Jingai Shao, Xiaoyu Zhang, Hongbo Yu, and Hanping Chen. 2019. "Investigation of the Pyrolysis Characteristics of Guaiacol Lignin Using Combined Py-GC × GC/TOF-MS and in-Situ FTIR." *Fuel* 251:496–505. doi: 10.1016/j.fuel.2019.04.061.

Wang, Miao, Chunyan Wang, and Xianlin Han. 2017. "Selection of Internal Standards for Accurate Quantification of Complex Lipid Species in Biological Extracts by Electrospray Ionization Mass Spectrometry—What, How and Why?" *Mass Spectrometry Reviews* 36(6):693–714.

Wang, Wei, Anna Oi Wah Leung, Lam Hang Chu, and Ming Hung Wong. 2018. "Phthalates Contamination in China: Status, Trends and Human Exposure-with an Emphasis on Oral Intake." *Environmental Pollution* 238:771–82.

Wang, Yan, Hui Liu, Yue'e Peng, Lei Tong, Liang Feng, and Kesen Ma. 2018. "New Pathways for the Biodegradation of Diethyl Phthalate by Sphingobium Yanoikuyae SHJ." *Process Biochemistry* 71:152–58. doi: 10.1016/j.procbio.2018.05.010.

Wanwimolruk, Sompon, Onnicha Kanchanamayoon, Kamonrat Phopin, and Virapong Prachayasittikul. 2015. "Food Safety in Thailand 2: Pesticide Residues Found in Chinese Kale (Brassica Oleracea), a Commonly Consumed Vegetable in Asian Countries." *Science of the Total Environment* 532:447–55. doi: 10.1016/j.scitotenv.2015.04.114.

Weaver, James A., Brandiese E. J. Beverly, Nagalakshmi Keshava, Anuradha Mudipalli, Xabier Arzuaga, Christine Cai, Andrew K. Hotchkiss, Susan L. Makris, and Erin E. Yost. 2020. "Hazards of Diethyl Phthalate (DEP) Exposure: A Systematic Review of Animal Toxicology Studies." *Environment International* 145.

Weber, R., L. Bell, A. Watson, J. Petrlik, M. C. Paun, and J. Vijgen. 2019. “Assessment of Pops Contaminated Sites and the Need for Stringent Soil Standards for Food Safety for the Protection of Human Health.” *Environmental Pollution* 249:703–15.

Wei, Lezhang, Yu Liu, Joyanto Routh, Jinfeng Tang, Guowei Liu, Lirong Liu, Dinggui Luo, Huosheng Li, and Hongguo Zhang. 2019. “Release of Heavy Metals and Metalloids from Two Contaminated Soils to Surface Runoff in Southern China: A Simulated-Rainfall Experiment.” *Water (Switzerland)* 11(7). doi: 10.3390/w11071339.

West, Caroline. 2018. “Current Trends in Supercritical Fluid Chromatography.” *Analytical and Bioanalytical Chemistry* 410(25):6441–57.

West, Caroline, and Elise Lemasson. 2019. “Unravelling the Effects of Mobile Phase Additives in Supercritical Fluid Chromatography—Part II: Adsorption on the Stationary Phase.” *Journal of Chromatography A* 1593:135–46. doi: 10.1016/j.chroma.2019.02.002.

White, J. Wilson, Bryan J. Cole, Gary N. Cherr, Richard E. Connon, and Susanne M. Brander. 2017. “Scaling Up Endocrine Disruption Effects from Individuals to Populations: Outcomes Depend on How Many Males a Population Needs.” *Environmental Science and Technology* 51(3):1802–10. doi: 10.1021/acs.est.6b05276.

Wilkinson, John, Peter S. Hooda, James Barker, Stephen Barton, and Julian Swinden. 2017. “Occurrence, Fate and Transformation of Emerging Contaminants in Water: An Overarching Review of the Field.” *Environmental Pollution* 231:954–70.

Williams, Tyler J., and R. Kenneth Marcus. 2020. “Coupling the Liquid Sampling-Atmospheric Pressure Glow Discharge, a Combined Atomic and Molecular (CAM) Ionization Source, to a Reduced-Format Mass Spectrometer for the Analysis of Diverse Species.” *Journal of Analytical Atomic Spectrometry* 35(9):1910–21. doi: 10.1039/d0ja00094a.

Wolmarans, Nico J., Lieven Bervoets, Ruan Gerber, Yared Beyene Yohannes, Shouta MM Nakayama, Yoshinori Ikenaka, Mayumi Ishizuka, Patrick Meire, Nico J. Smit, and Victor Wepener. 2021. “Bioaccumulation of DDT and Other Organochlorine Pesticides in Amphibians from Two Conservation Areas within Malaria Risk Regions of South Africa.” *Chemosphere* 274. doi: 10.1016/j.chemosphere.2021.129956.

Wooding, Madelien, Egmont R. Rohwer, and Yvette Naudé. 2017. “Determination of Endocrine Disrupting Chemicals and Antiretroviral Compounds in Surface Water: A Disposable Sorptive Sampler with Comprehensive Gas Chromatography – Time-of-Flight Mass

Spectrometry and Large Volume Injection with Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry." *Journal of Chromatography A* 1496:122–32. doi: 10.1016/j.chroma.2017.03.057.

Wu, Lingfeng, Lei Li, Shengjie Chen, Lu Wang, and Xue Lin. 2020. "Deep Eutectic Solvent-Based Ultrasonic-Assisted Extraction of Phenolic Compounds from Moringa Oleifera L. Leaves: Optimization, Comparison and Antioxidant Activity." *Separation and Purification Technology* 247(May):117014. doi: 10.1016/j.seppur.2020.117014.

Xiao, Douxin, Wenlong Liang, Zhengang Xie, Jingli Cheng, Yongjun Du, and Jinhao Zhao. 2021. "A Temperature-Responsive Release Cellulose-Based Microcapsule Loaded with Chlorpyrifos for Sustainable Pest Control." *Journal of Hazardous Materials* 403. doi: 10.1016/j.jhazmat.2020.123654.

Xu, Jing Jing, Rui Yang, Li Hong Ye, Jun Cao, Wan Cao, Shuai Shuai Hu, and Li Qing Peng. 2016. "Application of Ionic Liquids for Elution of Bioactive Flavonoid Glycosides from Lime Fruit by Miniaturized Matrix Solid-Phase Dispersion." *Food Chemistry* 204:167–75. doi: 10.1016/j.foodchem.2016.02.012.

Xynos, Nikos, Georgios Papaefstathiou, Evangelos Gikas, Aikaterini Argyropoulou, Nektarios Aligiannis, and Alexios Leandros Skaltsounis. 2014. "Design Optimization Study of the Extraction of Olive Leaves Performed with Pressurized Liquid Extraction Using Response Surface Methodology." *Separation and Purification Technology* 122:323–30. doi: 10.1016/j.seppur.2013.10.040.

YAMASHITA, Rei, Nagako HIKI, Fumika KASHIWADA, Hideshige TAKADA, Kaoruko MIZUKAWA, Britta Denise HARDESTY, Lauren ROMAN, David HYRENBACH, Peter G. RYAN, Ben J. DILLEY, Juan Pablo MUÑOZ-PÉREZ, Carlos A. VALLE, Christopher K. PHAM, João FRIAS, Bungo NISHIZAWA, Akinori TAKAHASHI, Jean-Baptiste THIEBOT, Alexis WILL, Nobuo KOKUBUN, Yuuki Y. WATANABE, Takashi YAMAMOTO, Kozue SHIOMI, Ui SHIMABUKURO, and Yutaka WATANUKI. 2021. "Plastic Additives and Legacy Persistent Organic Pollutants in the Preen Gland Oil of Seabirds Sampled across the Globe." *Environmental Monitoring and Contaminants Research* 1(0):97–112. doi: 10.5985/emcr.20210009.

Yang, Bin, Ling Tao, and Charles E. Wyman. 2018. “Strengths, Challenges, and Opportunities for Hydrothermal Pretreatment in Lignocellulosic Biorefineries.” *Biofuels, Bioproducts and Biorefining* 12(1):125–38.

Yang, Bing, Qunjun Wu, Yuxin Luo, Qiong Yang, Xunyu Wei, and Jianquan Kan. 2019. “High-Pressure Ultrasonic-Assisted Extraction of Polysaccharides from Hovenia Dulcis: Extraction, Structure, Antioxidant Activity and Hypoglycemic.” *International Journal of Biological Macromolecules* 137:676–87. doi: 10.1016/j.ijbiomac.2019.07.034.

Yao, Yuxiang, Nina F. Farac, and Gisele Azimi. 2018. “Supercritical Fluid Extraction of Rare Earth Elements from Nickel Metal Hydride Battery.” *ACS Sustainable Chemistry and Engineering* 6(1):1417–26. doi: 10.1021/acssuschemeng.7b03803.

Yilmaz, Bayram, Hakan Terekeci, Suleyman Sandal, and Fahrettin Kelestimur. 2020. “Endocrine Disrupting Chemicals: Exposure, Effects on Human Health, Mechanism of Action, Models for Testing and Strategies for Prevention.” *Reviews in Endocrine and Metabolic Disorders* 21(1):127–47.

Yolmeh, Mahmoud, and Seid Mahdi Jafari. 2017. “Applications of Response Surface Methodology in the Food Industry Processes.” *Food and Bioprocess Technology* 10(3):413–33. doi: 10.1007/s11947-016-1855-2.

Yousefi, Mohammad, Mehdi Rahimi-Nasrabadi, Seied Mahdi Pourmortazavi, Marcin Wysokowski, Teofil Jesionowski, Hermann Ehrlich, and Somayeh Mirsadeghi. 2019. “Supercritical Fluid Extraction of Essential Oils.” *TrAC - Trends in Analytical Chemistry* 118:182–93.

Zabot, Giovani Leone, Juliane Viganó, and Eric Keven Silva. 2021. “Low-Frequency Ultrasound Coupled with High-Pressure Technologies: Impact of Hybridized Techniques on the Recovery of Phytochemical Compounds.” *Molecules* 26(17).

Zeng, Hua, Fei Lyu, Wei Sun, Hai Zhang, Li Wang, and Yanxiu Wang. 2020. “Progress on the Industrial Applications of Red Mud with a Focus on China.” *Minerals* 10(9):1–28.

Zeng, Xiaofang, Jialing Liu, Hao Dong, Weidong Bai, Limei Yu, and Xiaomin Li. 2020a. “Variations of Volatile Flavour Compounds in Cordyceps Militaris Chicken Soup after Enzymolysis Pretreatment by SPME Combined with GC-MS, GC × GC-TOF MS and GC-IMS.” *International Journal of Food Science and Technology* 55(2):509–16. doi: 10.1111/ijfs.14294.

Zeng, Xiaofang, Jialing Liu, Hao Dong, Weidong Bai, Limei Yu, and Xiaomin Li. 2020b. "Variations of Volatile Flavour Compounds in Cordyceps Militaris Chicken Soup after Enzymolysis Pretreatment by SPME Combined with GC-MS, GC × GC-TOF MS and GC-IMS." *International Journal of Food Science and Technology* 55(2):509–16. doi: 10.1111/ijfs.14294.

Zhang, Chong, Li Liu, Yan Ma, and Fasheng Li. 2018. "Using Isomeric and Metabolic Ratios of DDT to Identify the Sources and Fate of DDT in Chinese Agricultural Topsoil." *Environmental Science and Technology* 52(4):1990–96. doi: 10.1021/acs.est.7b05877.

Zhang, Dan, Langping Wu, Jun Yao, Carsten Vogt, and Hans Hermann Richnow. 2019. "Carbon and Hydrogen Isotopic Fractionation during Abiotic Hydrolysis and Aerobic Biodegradation of Phthalate Esters." *Science of the Total Environment* 660:559–66. doi: 10.1016/j.scitotenv.2019.01.003.

Zhang, Jingfan, Chengnan Zhang, Yunping Zhu, Jinlong Li, and Xiuting Li. 2018. "Biodegradation of Seven Phthalate Esters by Bacillus Mojavensis B1811." *International Biodeterioration and Biodegradation* 132:200–207. doi: 10.1016/j.ibiod.2018.04.006.

Zhang, Lei, Cunshan Zhou, Bei Wang, Abu El Gasim A. Yagoub, Haile Ma, Xiao Zhang, and Mian Wu. 2017. "Study of Ultrasonic Cavitation during Extraction of the Peanut Oil at Varying Frequencies." *Ultrasonics Sonochemistry* 37:106–13. doi: 10.1016/j.ultsonch.2016.12.034.

Zhang, Qing Wen, Li Gen Lin, and Wen Cai Ye. 2018a. "Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review." *Chinese Medicine (United Kingdom)* 13(1):1–26. doi: 10.1186/s13020-018-0177-x.

Zhang, Qing Wen, Li Gen Lin, and Wen Cai Ye. 2018b. "Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review." *Chinese Medicine (United Kingdom)* 13(1).

Zhao, Y., Q. Guo, T. Lin, and P. Cheng. 2020. "A Review of Recent Literature on Icing Phenomena: Transport Mechanisms, Their Modulations and Controls." *International Journal of Heat and Mass Transfer* 159.

Zheng, Shuilin, Yue Yang, Cong Wen, Wenya Liu, Linghui Cao, Xiangling Feng, Jihua Chen, Hui Wang, Yan Tang, Li Tian, Xiaoyan Wang, and Fei Yang. 2021. "Effects of Environmental Contaminants in Water Resources on Nonalcoholic Fatty Liver Disease." *Environment International* 154.

Zhou, Xinyi, Fangyuan Peng, Zhoufei Luo, Yue Li, Haipu Li, and Zhaoguang Yang. 2020. "Assessment of Water Contamination and Health Risk of Endocrine Disrupting Chemicals in Outdoor and Indoor Swimming Pools." *Science of the Total Environment* 704. doi: 10.1016/j.scitotenv.2019.135277.

Zwolak, Aneta, Magdalena Sarzyńska, Ewa Szpyrka, and Kinga Stawarczyk. 2019. "Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: A Review." *Water, Air, and Soil Pollution* 230(7).

Appendix I

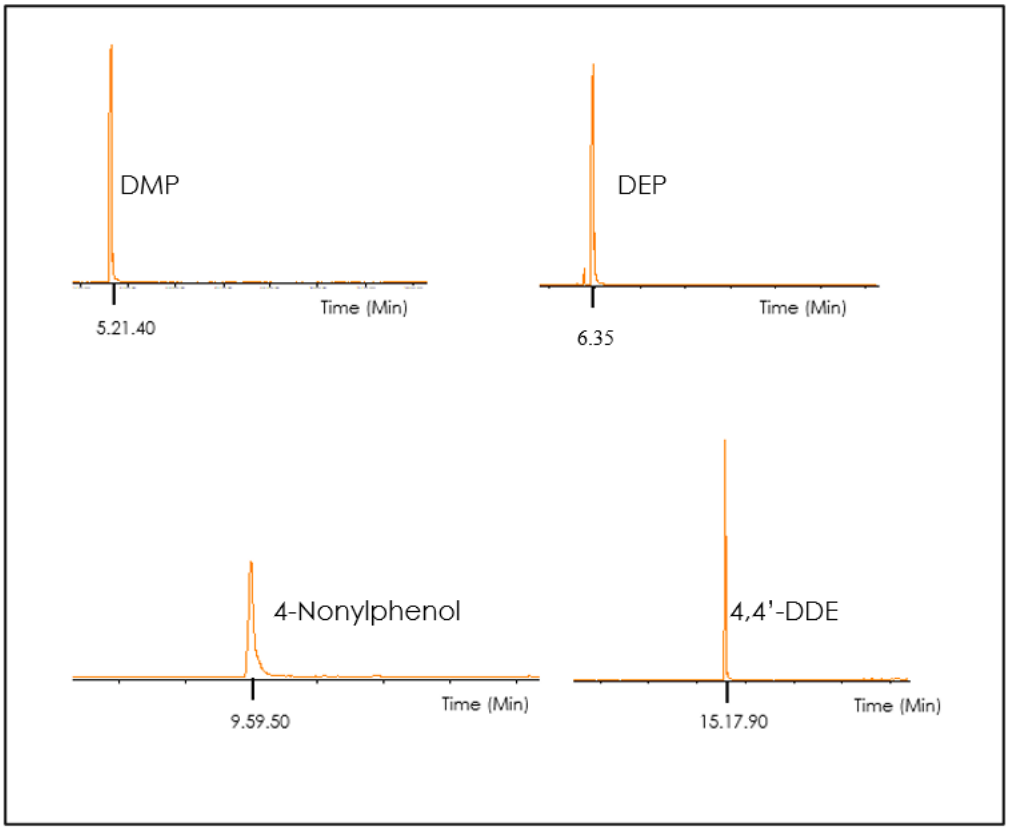


Figure. 1a : $GC \times GC\ TOFMS$ chromatogram of 1 $mg.L^{-1}$ Certified Reference Materials.

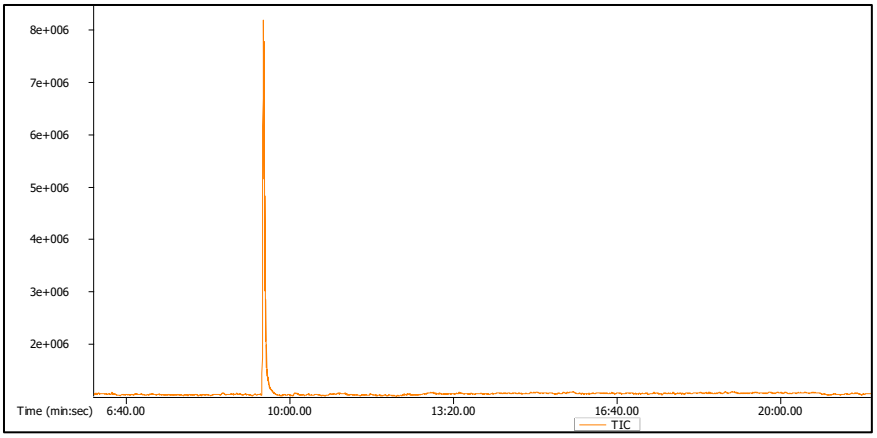


Figure 1b : 1 $mg\ L^{-1}$ representation of Internal Standard ($\gamma - BHC$)

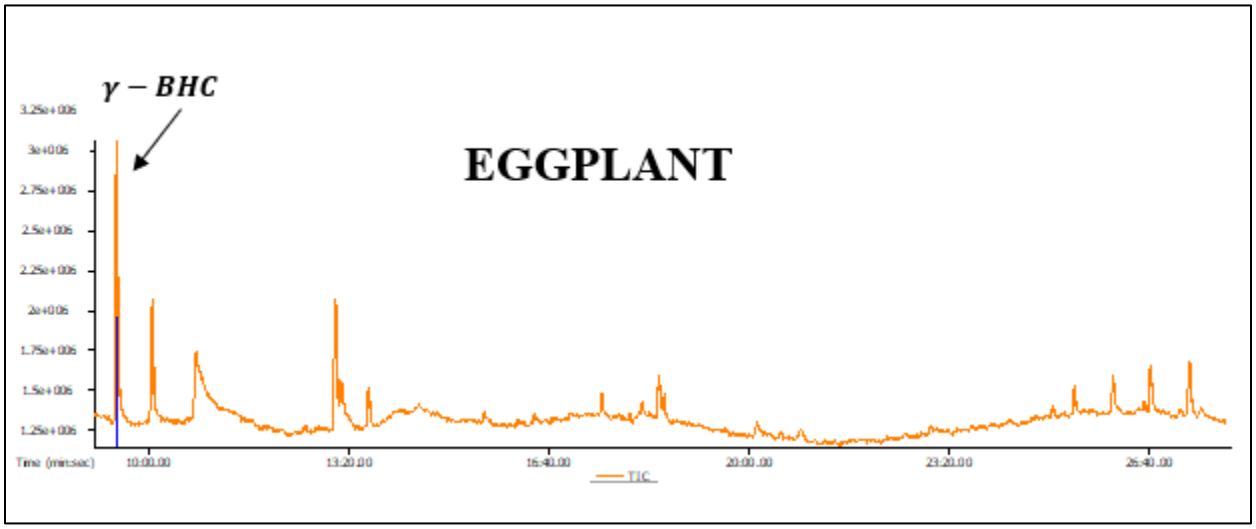


Figure 1c Eggplant sample spiked with $\mu g\ kg^{-1}$ of internal standard ($\gamma - BHC$)

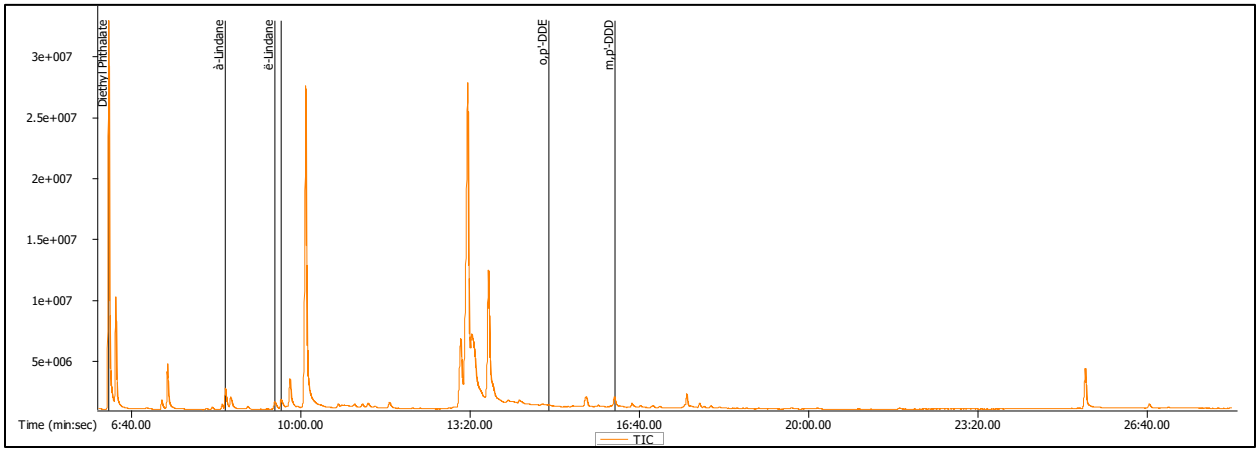
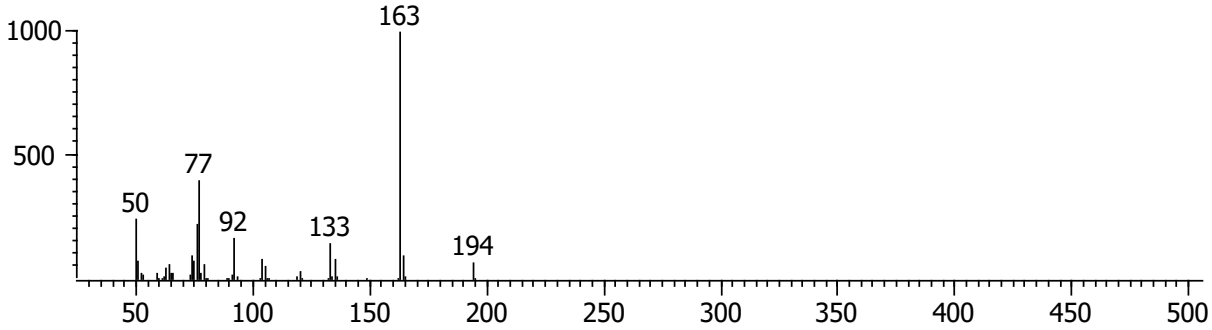
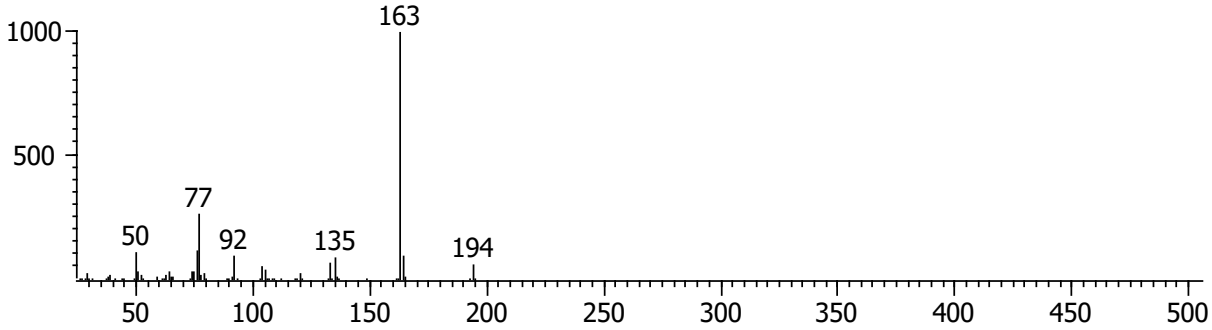


Figure 1d Meat sample spiked with 142.86 $\mu g\ kg^{-1}$ of internal standard and pure ECD standards .

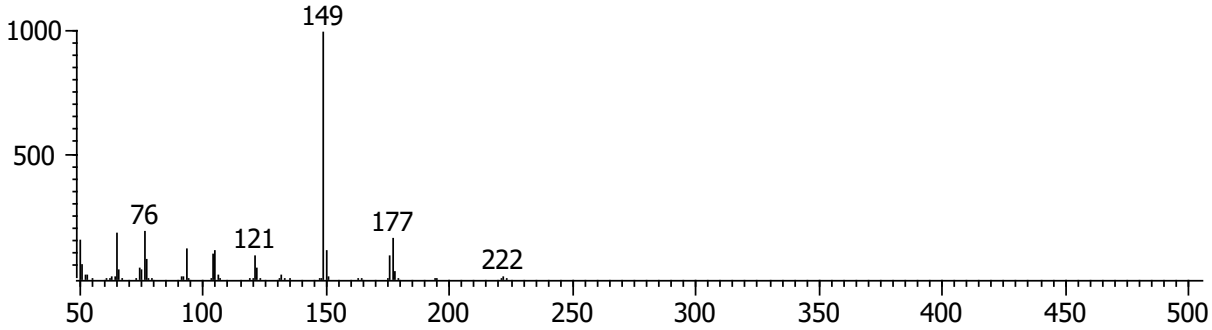
Peak True - sample "1 ppm:2", peak 41, at 5:31.50 min:sec



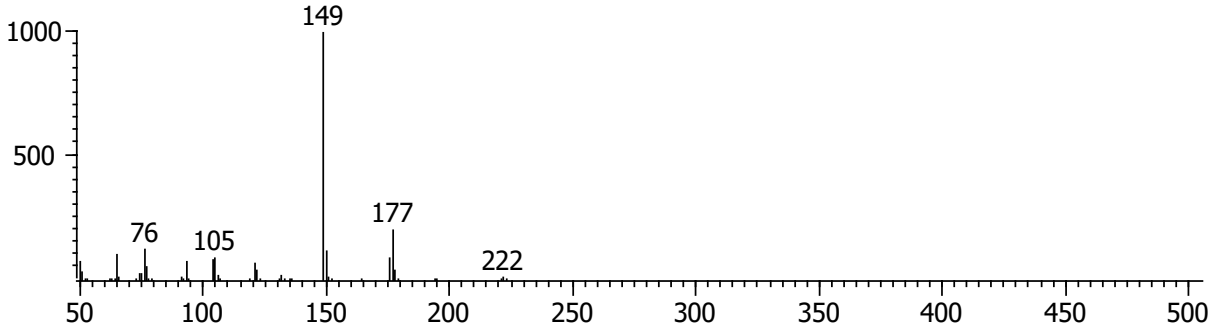
Library Hit - similarity 939, "Dimethyl phthalate"



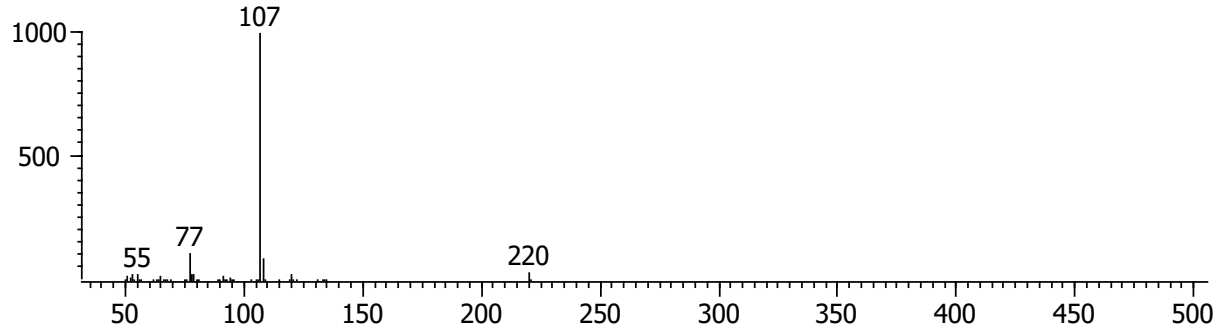
Peak True - sample "1 ppm:2", peak 45, at 6:40.30 min:sec



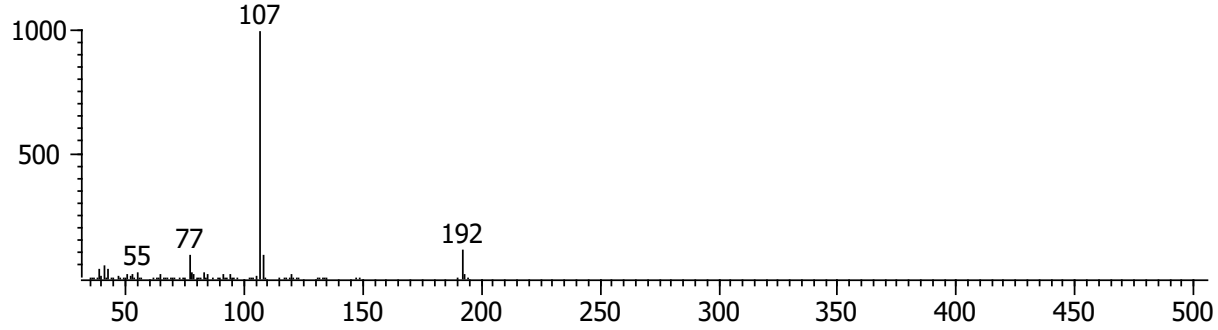
Library Hit - similarity 958, "Diethyl Phthalate"



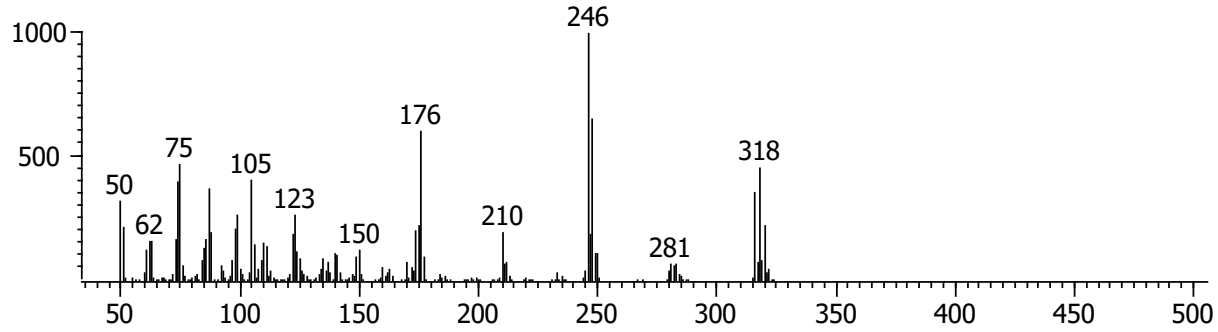
Peak True - sample "1 ppm:2", peak 47, at 10:21.50 min:sec



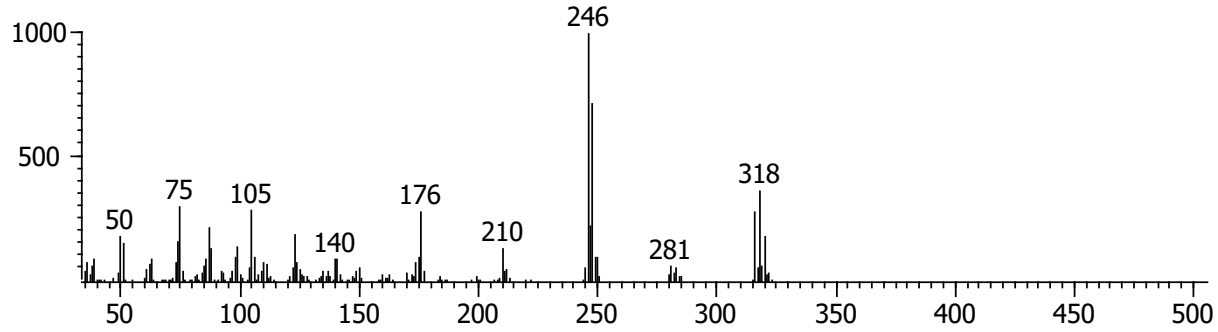
Library Hit - similarity 855, "Phenol, 4-heptyl-"



Peak True - sample "1 ppm:2", peak 49, at 15:38.20 min:sec



Library Hit - similarity 939, "o,p'-DDE"



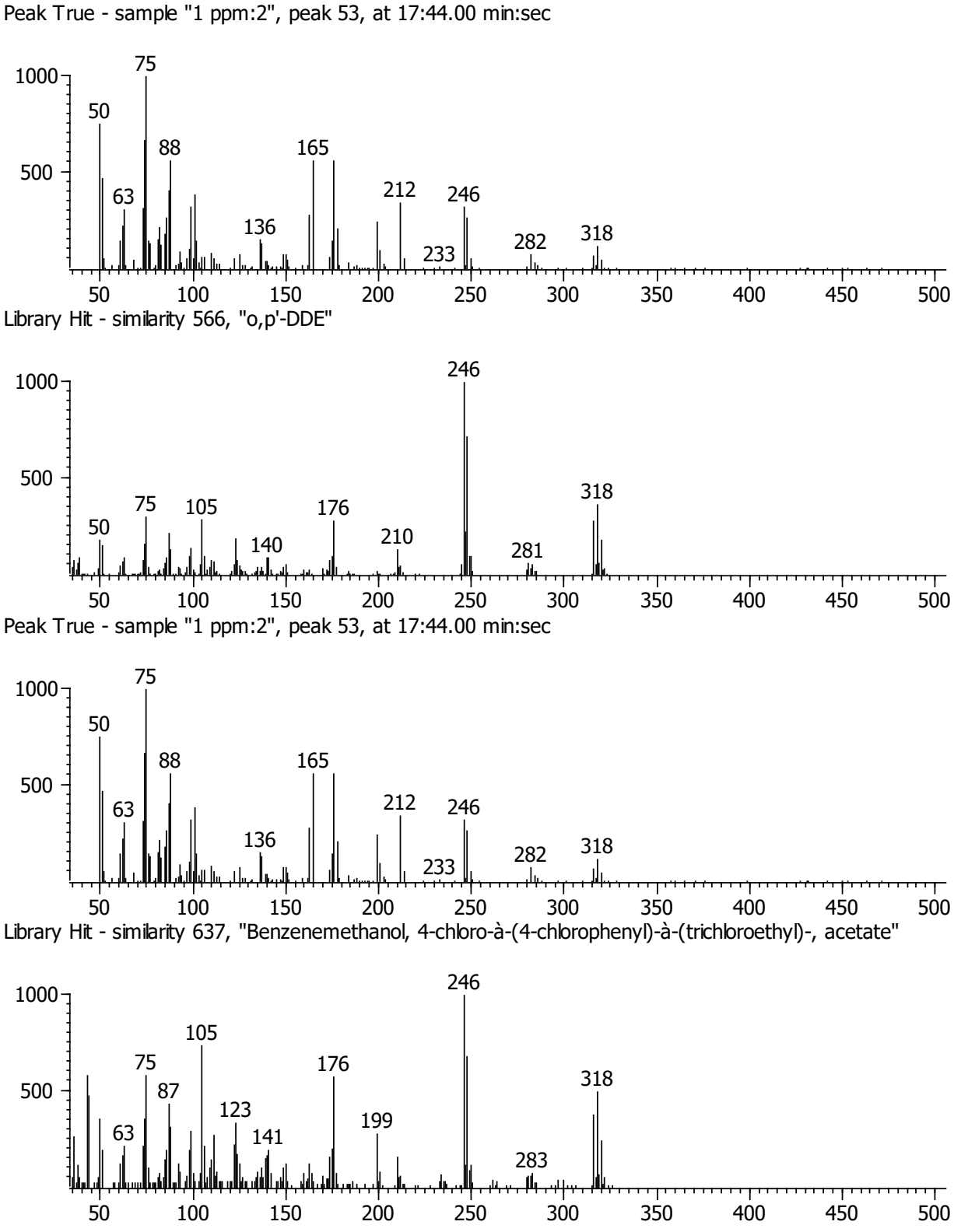


Figure 1e: Mass spectra representation of all EDCs under investigation

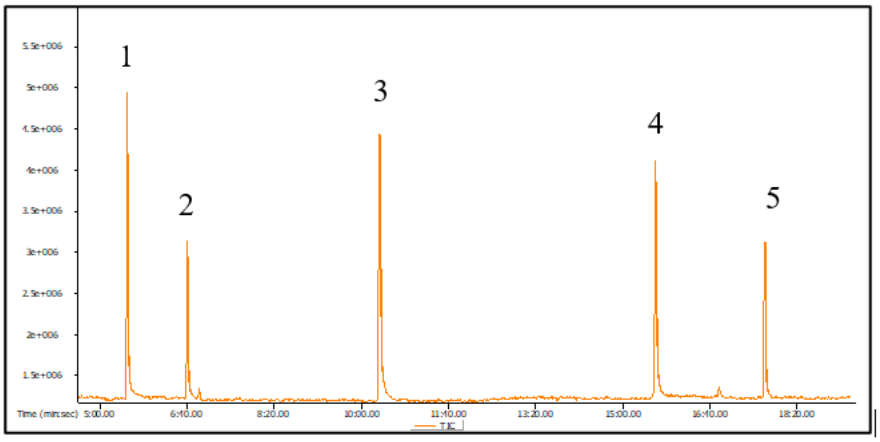


Figure 1f Chromatogram obtained for 1.0 $mg\ L^{-1}$ standard EDCs solution. 1: Dimethyl phthalate. 2: Diethyl phthalate 3: 4-Nonylphenol 4: 4,4'-DDE, 5: 4,4'-DDT.

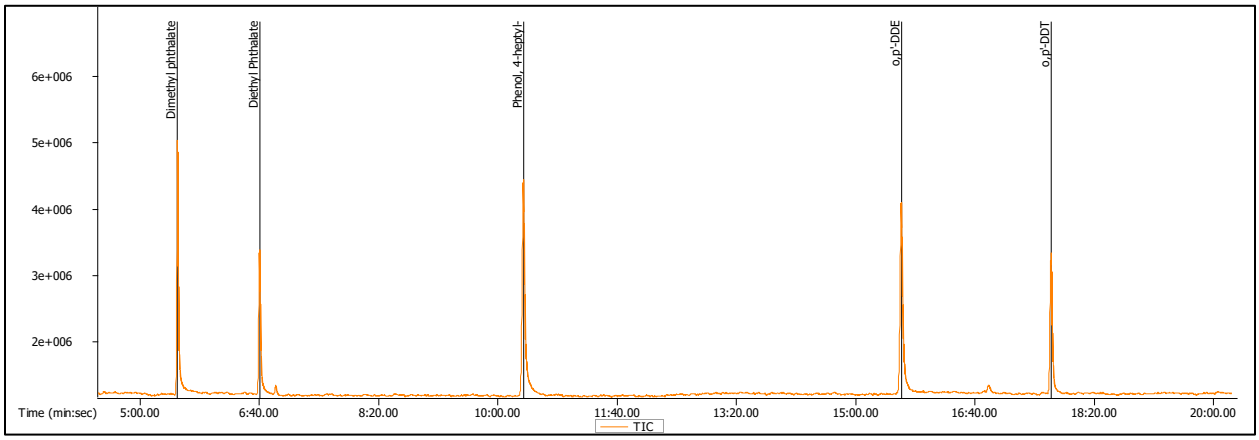


Figure 1g : 1 $mg\ L^{-1}$ representation of standard EDCs, n = 2

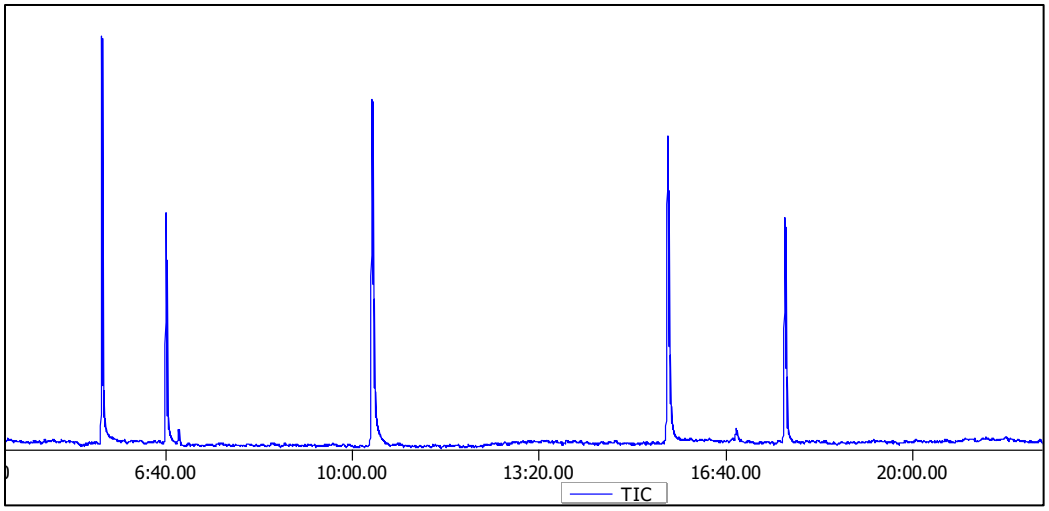


Figure 1h : 1 mg L^{-1} representation of standard EDCs, third analysis

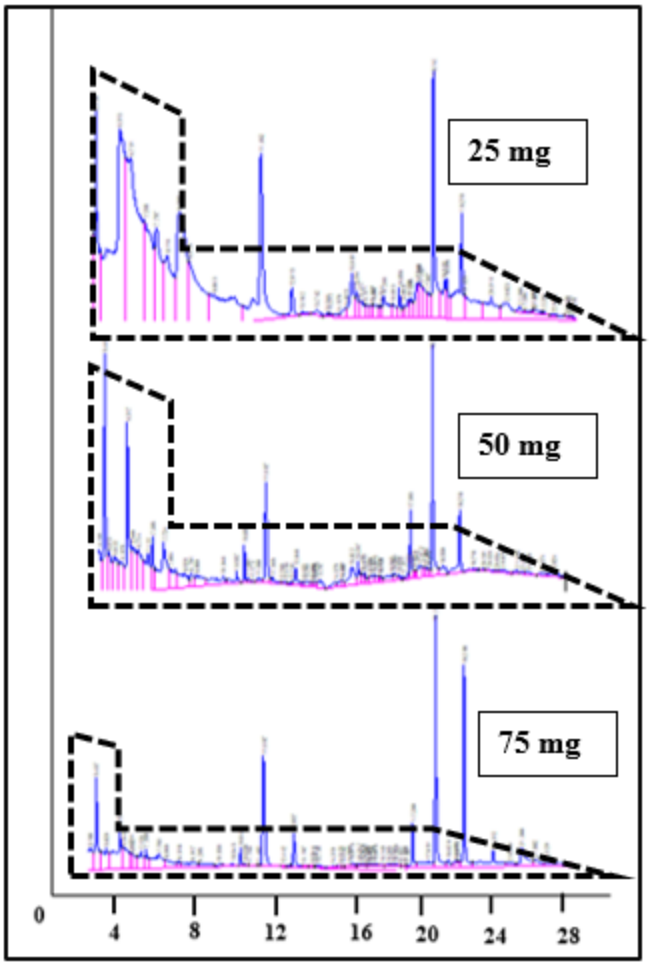


Figure 2a: Chromatograms of QuEChERS fish sample extracted using QuEChERS and PSA as a clean-up sorbent at quantities: (i) 25 mg, (ii) 50 mg, (iii) 75 mg

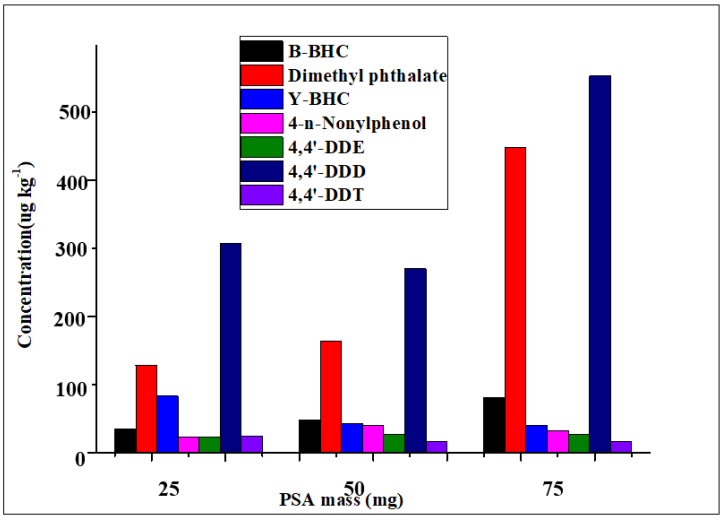


Figure 2b: One factor optimisation of PSA cleaning sorbent. 1.5 grams of hake fish was spiked with 106.67 $\mu g\ kg^{-1}$ standard mixture.

Table 1a: Analytical spike recovery and standard deviation (SD) of five endocrine disrupting chemicals for different food items sold in Johannesburg open markets at 142.86 $\mu g\ kg^{-1}$ and 714.29 $\mu g\ kg^{-1}$ fortification levels.
DMP: Dimethyl phthalate, DEP: Diethyl phthalate, SD: standard deviation.

EDC	**Banana**		**Potato**		**Onion**		**Cabbage**	
	142.86 $\mu g\ kg^{-1}$	714.29 $\mu g\ kg^{-1}$	142.86 $\mu g\ kg^{-1}$	714.29 $\mu g\ kg^{-1}$	142.86 $\mu g\ kg^{-1}$	714.29 $\mu g\ kg^{-1}$	142.86 $\mu g\ kg^{-1}$	714.29 $\mu g\ kg^{-1}$
DMP	87.8 ± 0.41	89.0±0.52	85.9±1.36	81.3±1.69	93.9±2.20	99.3±1.39	76,3±2.23	74,2±1.12
DEP	88.9±0.33	86.3±0.38	101,2±1.21	104,1±0.96	80,6±1.02	78,2±2.89	86,3±2.56	86,3±2.23
4-n-OH	83.9±0.17	85.9±0.23	94,3± 1.23	86,6±0.96	87,4±1.27	84,4±0.78	87,4±1.04	86,8±1.01
4,4'-DDE	89.2±0.88	86.9±1.20	74,3 ±1.08	74,1±1.26	78.0±0.85	80,1±1.05	86,1±0.76	87,2±0.87
4,4'-DDT	102.3±0.29	94.3±0.09	100,1±0.26	77,5±0.25	78.8±1.55	75.1±0.96	98,9±1.23	100,5±0.22

Table 1b: Choice of clean-up sorbent: Recovery comparison between PSA and *Moringa Oleifera*-extracted Protein on selected food from Johannesburg open markets at 142.86 $\mu g\ kg^{-1}$ and 714.29 $\mu g\ kg^{-1}$ fortification levels.

EDC	**Fish: Tilapia**				**Beef**			
	142.86 $\mu g\ kg^{-1}$		714.29 $\mu g\ kg^{-1}$		142.86 $\mu g\ kg^{-1}$		714.29 $\mu g\ kg^{-1}$	
	PSA	**Protein**	**PSA**	**Protein**	**PSA**	**Protein**	**PSA**	**Protein**
DMP	89.7±1.04	88,2± 0.85	81.0±1.20	89,3±1.26	90.7±0.53	93,6±0.21	104.5±0.21	105,1±2.24
DEP	93.3±1.36	86,5±0.56	107.4±0.88	98,5±0.85	79.8±1.35	76,2±0.85	80.8±2.32	98,6±0.45
4-Nonylphenol	86.0±0.89	91,1±1.11	88.1±1.03	91,2±0.63	82.7±1.22	90,9±2.23	85.2±1.01	97,7±1.24
4,4'-DDE	90.8±1.01	103,2±0.68	79.9±0.96	101,6±0.52	88.4±0.53	88,8±0.52	80.7±0.52	92,1±0.28
4,4'-DDT	103.1±0.96	94,3 ±0.89	96.5±1.01	92,6±0.75	86.7±0.21	96,9±1.18	82.2±0.32	96,0±0.96

Appendix II

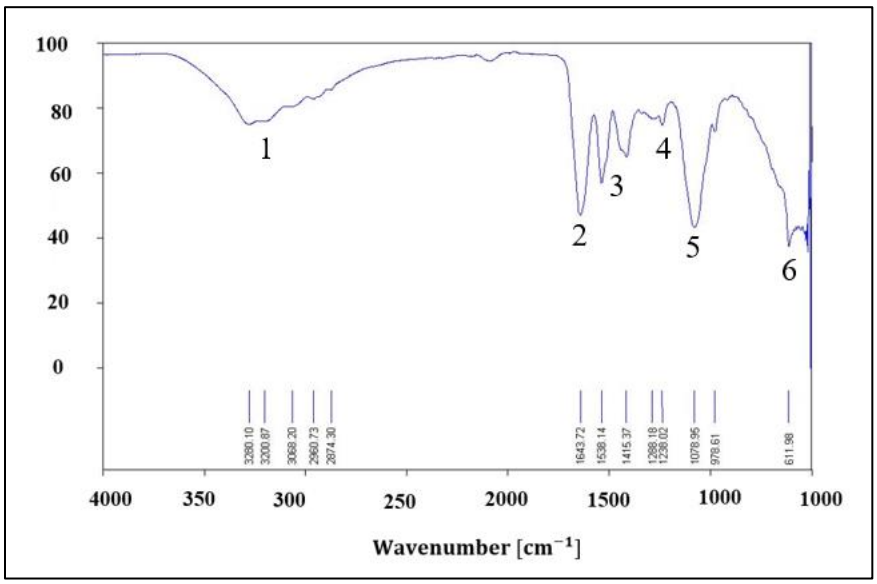
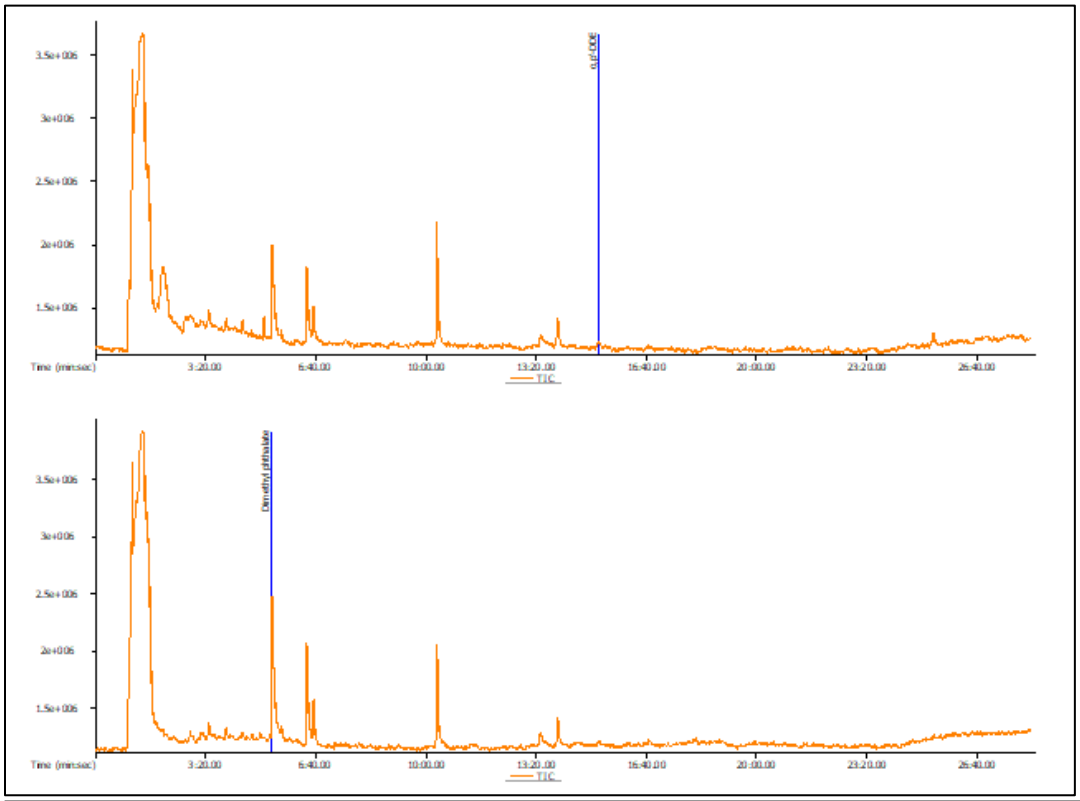


Figure 1: FTIR spectra of *Moringa Oleifera* seed protein

Table 1: Maximum Permitted concentrations of selected metals in food ($mg\ kg^{-1}$)

	Max. Permitted concentration in $mg\ kg^{-1}$
Al	71
As	0.2
Cd	0.3
Cr	1.3
Cu	10
Mn	5
Pb	0.3
Se	n/a in food. (6 in soil)
Zn	11


Dimethyl phthalate
TIC

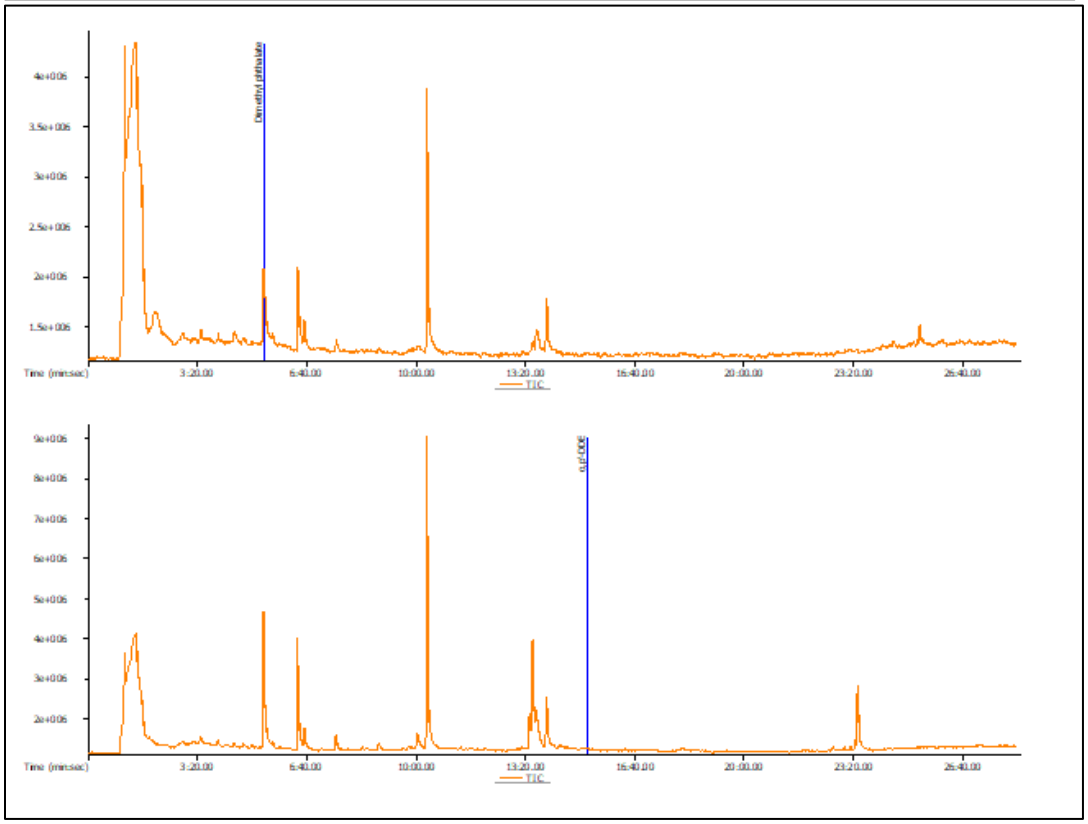

Dimethyl phthalate
TIC

Figure 2 : Cabbage sample spiked with 142.86 $\mu g\ kg^{-1}$ of Certified Reference Materials mixture

Chromatograms for application studies are available as supplementary data in a separate file (available when needed).