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# EVALUATION OF SPATIAL DISTRIBUTION AND HEALTH RISK OF PESTICIDE RESIDUES IN THE ENVIRONMENTAL MATRICES OF BUFFALO RIVER ESTUARY AND THEIR REMOVAL USING NANOSPONGES AND POLYMER MODIFIED CYCLODEXTRINS

**NONKULULEKO LANDY MDENI**

**DEPARTMENT OF CHEMISTRY**

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**ALICE 5700, SOUTH AFRICA**

**MARCH 2023**

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# CERTIFICATION

This dissertation entitled "Evaluation of Spatial Distribution and Health Risk of Pesticide Residues in the Environmental Matrices of Buffalo River Estuary and their Removal using Nanosponges and Polymer Modified Cyclodextrins" meets the regulation governing the award of degree of Master of Science of the University of Fort Hare and is approved for its contribution to scientific knowledge and literary presentation.

**Prof O. O. Okoh**
Supervisor

Date

**Dr. A. O. Adeniji**
Co-Supervisor

Date

# DECLARATION 1

I, the undersigned, declare that this dissertation entitled "Evaluation of Spatial Distribution and Health Risk of Pesticide Residues in the Environmental Matrices of Buffalo River Estuary and their Removal using Nanosponges and Polymer Modified Cyclodextrins" submitted to the University of Fort Hare for the degree of Master of Science in Chemistry in the Faculty of Science and Agriculture, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted to any other University in partial or entirely for the award of any degree.

Name: Nonkululeko Landy Mdeni

Signature: ………………………...

Date: ……………………….

# DECLARATION 2 – PLAGIARISM

I, **Nonkululeko Landy Mdeni**, student number: 201506727 hereby declare that I am fully aware of the University of Fort Hare’s policy on plagiarism, and I have taken every precaution to comply with the regulations.

Signature ____________________________ Date_______________________________

# DECLARATION 3 – PUBLICATIONS

Contribution to publication is given in details, which is part of chapters given in this dissertation.

**Publication**

Mdeni, N.L., Adeniji, A.O., Okoh, A.I. and Okoh, O.O., 2022. Analytical Evaluation of Carbamate and Organophosphate Pesticides in Human and Environmental Matrices: A Review. *Molecules*, *27*(3), p.618.

I conceived and designed the review under the guidance of Dr. A. O. Adeniji, Prof O. O. Okoh and Prof A. I. Okoh. Proof reading of the manuscript through the publication process was done by Prof A. I. Okoh, my supervisor, Prof O. O. Okoh and co-supervisor, Dr. A. O. Adeniji. This study is presented in this dissertation as Chapter 2.

Signature__________________________ Date___________________________________

# ETHICAL APPROVAL FOR THE STUDY

Ethical clearance certificate was obtained from the Faculty Research Ethics Committee. The supporting letter is attached (Appendix 1).

## DEDICATION

This dissertation is dedicated to the Most High God who has been with me throughout the period of the study.

## ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to the most-high God through whom anything is possible because His grace is sufficient for me, for His power is perfected in my weakness. My sincere appreciation also goes to Professor Omobola Okoh and Dr. Abiodun Olagoke Adeniji, for supervising this work and for their patience, guidance and valuable contribution to the work. I highly appreciate your great assistance and advice all through the period. My sincere appreciation goes to Prof A.I. Okoh for funding this research and providing all the resources needed to complete this work. I am also grateful to Dr. Mike for his valuable contribution to this project, also for reading through and correcting my synthesis chapter of this dissertation.

My deep gratitude also goes to the Department of Chemistry, University of Fort Hare for providing me an enabling environment to do this research. National Research Foundation (NRF) and SASOL are greatly appreciated for their financial supports in the form of student's bursary; every member of the Applied and Environmental Microbiology Research Group (AEMREG), as well as the Synthetic, Environmental and Applied Chemistry Research Group (SEACREG) were all there for me throughout this journey. May God bless you all.

My heartfelt appreciation also goes to Dr. Chinemerem, Mr. Olaniyan, Ms. Bika and Mr. Samuel, and other colleagues who were always willing to lend a hand of assistance, taking time off their busy schedules to help me in the laboratory, especially on how to use and operate different equipment, you guys were my family in the absence of my biological one, I wouldn't have the strength to keep going if it weren't for you, and for that, I thank you.

Last, but not the least, my profound gratitude goes to my family; my mother for always believing in me, consoling and encouraging me during tough times, my grandfather and siblings for being my support system and always cheering me, my daughter Lubabalwe and my nieces and nephews for their unconditional love towards me and being a breath of fresh air in my life, I love and appreciate you guys, you are my greatest gifts from God.

# LIST OF ACRONYMS

| | |
|---|---|
| AEMREG | Applied and Environmental Microbiology Research Group |
| SEACREG | Synthetic, Environmental and Applied Chemistry Research Group |
| NRF | National Research Foundation |
| SASOL | South Africa Synthetic Oil Liquid |
| OPs | Organophosphates |
| CDs | Cyclodextrins |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| GC-µECD | Gas chromatography micro electron capture detector |
| OM | Organic matter |
| OC | Organic carbon |
| TOC | Total Oil and Grease |
| HEM | n-Hexane extractable material |
| MC | Moisture Content |
| BNB | 1-Bromo-2-nitrobenzene |
| RQ | Risk Quotient |
| RSD | Relative standard deviation |
| SA | South Africa |
| SD | Standard Deviation |
| IARC | International Agency for Research on Cancer |
| AChE | Acetylcholinesterase |
| EL | East London |

| | |
|---|---|
| PNEC | Predicted no effect concentration |
| MEC | Measured environmental concentration |
| IC | Inclusion Complex |

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# ABSTRACT

The contamination of lotic ecosystems caused by agricultural, industrial and anthropogenic activities has attracted the attention of researchers, authorities and scientists because these water-bodies are of social and economic importance to human livelihoods. Pesticides and insecticides are a group of compounds that are toxic to pests and insects, respectively. Organophosphate pesticides (OPs) can be released into different environmental matrices through several sources including agricultural and urban runoff. They are one of the three main classes of synthetic organic pesticides that are used in agricultural sector and very often end up in other destinations than their target sites. In this study, four OPs, namely, ronnel, malathion, parathion and dursban were investigated. The Buffalo River Estuary, located in East London, is one of most popular areas and the river is a major source of raw water abstraction for three municipalities in the Eastern Cape Province, South Africa. Sediment samples were collected from six points in the estuary and soil samples were taken from a nearby dumpsite in winter and spring seasons of 2021. The physicochemical parameters of the samples including particle size, moisture content, oil and grease and organic carbon were determined. The samples were then extracted by ultra-sonication, the final extracts were analyzed using GC-μECD and the risk quotient (RQ) method was used to evaluate the risk of OP exposure to aquatic organisms. Polymer modified cyclodextrin (β-CD) for the removal of parathion in sediment and soil was synthesized and the morphology of the modified β-CD and vibrations of the synthesized material were determined using scanning electron microscope (SEM) and Fourier transform infrared (FTIR) spectroscopy, respectively.

The limit of detection (LOD) and the limit of quantification (LOQ) of the OPs investigated were in the range of 0.09 to 2.99 and 0.274 to 9.06 μg/L, respectively. OPs gave a good recovery except for malathion, which had a recovery percentage lower than 70%. Calibration curves were plotted for the four OPs and were linear with relative standard deviations (RSD) ranging from 1.02 to 3.50, and correlation coefficients ($R^2$) ranging from 0.9935 to 0.9972. The mean concentrations for sediments and soil in both seasons varied from 0.00231 to 63.8 μg/g in winter and from 0.0017 to 36.6 μg/g in spring, with malathion being the predominant congener and

dursban being the least dominant in both seasons. In winter, the particle size percentages of the sediment and soil samples ranged from 34.4 to 79.4%, 15.4 to 23.9% and from 7.77 to 38.3% for coarse, medium and fine fractions, respectively. While in spring, the particle size for the coarse, medium and fine mesh fractions ranged from 37.4 to 74.6%, 14.1 to 25.1% and 12.4 to 35.0% respectively; the percentage moisture content varied from 43.0 to 73.1 in winter, and from 57.1 to 63.4 in spring. In the soil samples, the physicochemical properties were lower in winter except for the n- hexane extractable material (HEM), which significantly decreased in spring from 2.6 to 1.63 mg/kg; while for sediments, the oil and grease ranged from 1.77 to 10.3 mg/kg in winter and from 1.63 to 4.28 mg/kg in spring. The risk quotient method was used to determine the risk of OP exposure to sediment-dwelling organisms and the results obtained indicated that the levels of OPs in this study, especially dursban, can pose a high risk to the organisms in sediments, with RQs higher than ten (>10) in both winter and summer. The Pearson product-moment correlation showed that parathion had a weak correlation with every other variable except for malathion in winter, whereas in spring, parathion correlated very weakly with malathion; suggesting that products containing parathion are mostly used to control pests found in crops, such as corn and in South Africa, the season for this crop is June to August, while malathion can be found in products used for the control of mosquitos, which are most active in the spring season. There was a very strong correlation between dursban and ronnel in spring and winter, implying that an increase in dursban concentration resulted in an increase in the concentration of ronnel. To determine the efficiency of the synthesized adsorbent CD-chitosan-alginate for the removal of Parathion from soil and sediment, different parameters such as concentration, time, pH and dose were varied. The adsorption capacity was found to be highest at pH 2 (99.19%), when 3 mL of the adsorbent was used for 30 minutes.

# CHAPTER ONE

## 1.0 INTRODUCTION

For many years, chemicals have been used to control pests and insects affecting humans, animals and crops (Freed, 2013). Many kinds of possibly harmful chemicals with different structures and properties have been found in different environments (Khan, 2013). Some chemicals, such as pesticides are synthetic compounds that may become environmental contaminants through their use and application while other materials, such as selenium and salt, are natural components of some environments, but contaminants of others. Such natural and synthetic materials may in some cases cause direct poisoning and death, but may also have several adverse effects on wildlife, causing damage to certain biological systems, including the reproductive and immune systems (Friend, 1999).

Pesticides and insecticides are a group of compounds that are toxic to pests and insects (Vlcˇek and Pohanka, 2012). These compounds work by attracting and terminating the insect or pest where prevalent (Mahmood *et al*., 2016). Although, pesticides may help in the production and quality improvement of crops, they can also cause environmental problems (Derbalah *et al*., 2019). Pesticides and insecticides can be classified as organophosphates, carbamates, organochlorines, pyrethroids and neonicitinoids (Mahmood *et al*., 2016). Organophosphate pesticides (OPs) are products of the reactions between alcohols and phosphoric acid, they have been used excessively all over the globe for over 60 years to protect vegetation, crops, human health and livestock. Poisoning by these compounds poses a serious problem especially in developing countries, poisoning by OPs differs with the chemical structure, route and extent of exposure (Kwong, 2002, Ghorab and Khalil, 2015).

Exposure to organophosphate pesticides can be through inhalation, ingestion or absorption through the skin and could lead to acute intoxication, which can later manifest as gastrointestinal upset, miosis, urination, sweating, lacrimation, muscle weakness, hypertension, and central nervous system depression or coma (Jaga and

Dharmani, 2003). Organophosphates have the ability to inhibit acetylcholinesterase (AChE), an enzyme that is responsible for the functioning of the nervous system. This irreversible process is the reason why OPs are used in many pharmaceutical products and unfortunately also in warfare agents (Chambers and Levi, 2013). The classification and nomenclature of OPs follow various schemes that may be complicated, the commonly used scheme considers the nature of the leaving group, which is the most reactive substituent thus, OPs can be classified into aliphatics, heterocyclics, aromatics and anhydrides (Chambers and Levi, 2013). The degradation of OPs differs as a function of microbial composition, pH, availability of light and temperature. These pesticides are persistent in the environment as they can be detected in the soil after many years of application and because they are fairly soluble, they enter surface and ground waters (Ragnarsdottir, 2000). There are many sources of organophosphate exposure, these include domestic use of pesticides, close proximity to farms that use OPs as pesticides, industries where pesticides are manufactured, office workers that get exposed to OPs applied by exterminators, florists that use pesticides to protect their flowers and so on (Jaga and Dharmani, 2003).

Organophosphate pesticides enter the environment through agricultural application and because they are water soluble, their adsorption to soil is high, this leads to the buildup of these pesticides in agricultural runoff water (Kaushal *et al.*, 2021). The fate of pesticides and their bioavailability depends on their physicochemical properties such as volatility, solubility, adsorption and persistency.

There is a high demand for the development of new methods for pesticide remediation in various environmental matrices due to their negative effects on the environment and human health. One of the prominent remediation methods is the use of cyclodextrins (CDs) (Waris *et al.*, 2021), which are polysaccharides with stable hydrophobic cavities and external surfaces that are hydrophilic. The primary hydroxyl groups on the external surface of the CDs have the ability to form complexes by covalently bonding with metal ions, thereby removing pollutants from a solution (Tian *et al.*, 2021). There are three types of CDs referred to as the native or parent CDs (α-, β- and γ-CD), which are made of six, seven and eight α-(1,4)-linked glycosyl units, respectively, with β-CD being the cheapest and most useful (Del Valle, 2004). CDs are cyclic oligosaccharides that are torus shaped due to the chair conformation of the glycosyl units, the cavity of CDs consists of skeletal carbons and ethereal oxygens which are responsible for the lipophilic character of the CDs, as the hydroxyl groups at the exterior give the hydrophobic character (Brewster and Loftsson, 2007).

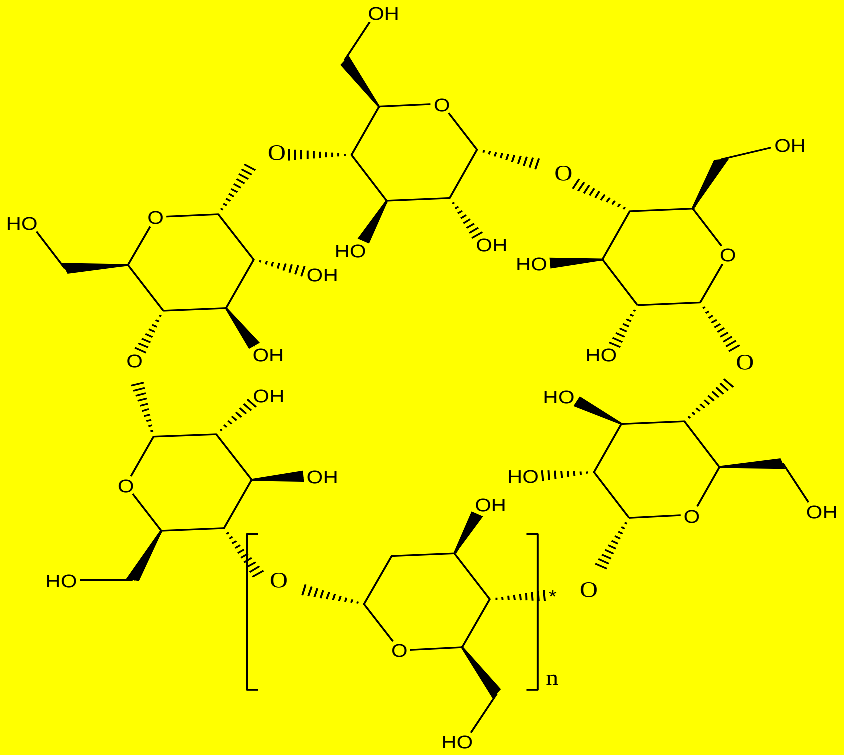

Figure 1.1: General structure of cyclodextrins

## 1.1 PROBLEM STATEMENT

Surface waters are very important for agricultural, industrial and recreational purposes, these waterbodies, such as dams, rivers and lakes are exposed to high levels of pollution, which has become a serious problem globally (Chigor *et al*., 2013). Persistent pesticides and other toxins have been accumulating in the environment over the years, and in recent times, this has sparked the interest of the public in environmental issues. A need for effective methods for both detection and remediation of these pollutants in areas that may possibly be contaminated has increased over the years because of public awareness and concern (Pletschke *et al*., 2010). Some OPs have been used as weapons of destruction in warfare and terrorist attacks, whereas some are used as therapeutic agents in human medicine. OPs are toxic and not selective, hence, they end up being a threat to marine life, wildlife, animals and human health, because this anticholinesterase agent has different toxicological effects in both target and non-target organs (Gupta, 2011).

Fatalities due to pesticide poisoning and information on them are not well reported or documented globally, especially in South Africa (Quinn *et al*., 2011). The water quality all around the world is deteriorating due to pollution caused by inorganic and organic compounds as a result of human activities (Salazar *et al*., 2018). Organophosphate pesticides are one of the three main classes of synthetic organic pesticides that are used in agricultural activities and very often end up in other destinations than their target sites. In the environment, the chemical structure of pesticides can undergo changes by biotic and abiotic processes that could either result in harmless or even more toxic substances (Cembranel *et al*., 2017).

## 1.2 JUSTIFICATION OF THE STUDY

The Buffalo River is a major source for raw water abstraction in the Eastern Cape Province of South Africa. It is used for irrigation and entertainment purposes, and has become populated with inadequate water resources, sewerage systems that are overloaded or not functional and sewerage treatment works, insufficient treatment capacity and poor management that result in the discharge of untreated or partly treated sewage into the river (Chigor *et al*., 2013). The Buffalo river estuary is located in the Buffalo City Metropolitan Municipality in East London (EL). The Buffalo river catchment has three municipalities that fall under it; dams such as the Maden, Bridle Drift and Rooikrantz, Laing are all dependent on the river. These dams were built as impoundments along the Buffalo River to provide water to communities such as Izele Town, Zwelitsha, King Williams Town, Mdantsane, Bhisho, Berlin and EL, which are all within the river's catchment. While the river flows through these areas, it is exposed to pollution via human activities, including dumping of agricultural, industrial and domestic wastes, especially those coming from an old tannery mill and a dumpsite near Zwelitsha Township (Yahaya *et al*., 2019).

With about 642 000 residents, the Buffalo river catchment has one of the highest population densities in the Eastern Cape Province, with the middle and lower reaches being the highest. The estuary mouth of the Buffalo River is South Africa's (SA) only river port and that is where most of the economic value of the estuary is derived (Carter *et al.*, 2016). Municipal and industrial wastewater, storm water and agricultural runoff containing pesticides, herbicides and suspended solids are the four key sources of pollution in estuaries that were listed by South Africa's National Programme of Action for Protection of the Marine Environment from

Land-based Activities (James *et al*., 2013). The Buffalo River passes through some urban and rural areas that are polluted from industrial and domestic sources, the pollution status of the river increases with increase in the anthropogenic activities in the area, thus, leading to elevated levels of bacteria and several organic and inorganic pollutants (Yahaya et al., 2017). In light of this, research on pollutants such as organophosphate pesticides is, therefore, a necessity.

## 1.3 NULL HYPOTHESIS

The hypothesis of this study is that the environmental matrices of the Buffalo river estuary and soil from the nearby dumpsite are not contaminated with organophosphate pesticides.

## 1.4 AIM AND OBJECTIVES

The aim of this study is to investigate the levels and distribution of organophosphate pesticides in the environmental matrices of the Buffalo River Estuary, and soil samples from a nearby dumpsite and to develop a method for their removal using modified cyclodextrins.

The objectives of the study are as follows:

1 To collect sediment samples from six points along the estuary; and soil samples from a nearby dumpsite for a period of four months (covering 2 seasons).

2 To determine the physicochemical characteristics of soil and sediment samples including pH, percentage moisture, organic matter, carbon contents particle size and oil and grease.

3 To validate the existing methods for the measurements analysis of the contaminants by determining their limit of detection (LOD), limit of quantification (LOQ) and by carrying out recovery studies.

4 To evaluate levels of organophosphate pesticides in soil and sediment samples using suitable extraction methods and analyse the extracts with gas chromatography coupled with mass spectrometer (GC-MS) and electron capture detector.

5 To identify the possible sources of the contaminants in the environment and assess their level of health risk to humans and animals.

6. To synthesize and characterize some modified cyclodextrins for the remediation of the contaminated soil and sediment samples.
7. To develop a method for the removal of the modified cyclodextrins from the sample matrices, and conduct regeneration studies as appropriate.

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# CHAPTER FOUR

## Validation of methods for the evaluation of pesticides in the environmental matrices of Buffalo River Estuary

### ABSTRACT

OPs are most commonly absorbed through diet; although they are considered safe for agricultural use, as they tend to reduce soil fertility and microbial community. Hence, there is a need to develop new and effective methods for their bioremediation. Sediment and soil samples were collected in this study, and certain physicochemical parameters such as particle size, oil and grease and organic carbon contents were determined. Different ratios of solvent mixtures of dichloromethane, acetone, ethyl acetate, and cyclohexane (2:1:1:1 and 3:2:1:1 v/v/v/v) were used to validate the determination of OPs in sediment and soil samples in the Buffalo river estuary and a dumpsite near the river. When comparing the ratios, the 3:2:1:1 ratio gave better recoveries of the OP pesticides, hence this ratio was used throughout the study. The samples were extracted by ultrasonication, concentrated with a rotary evapour ator and analyzed using GC-µECD. The recovery of the OPs ranged from 57.8 to 102%; the limits of detection and limits of quantification ranged from 0.09 to 2.99 µg/L and 0.274 to 9.06 µg/L, respectively. The relative standard deviation was generally less than 11%, and the instrument's response was linear. Based on the results obtained, it could be inferred that the method used in this study is suitable for the measurement of organophosphate levels in solid environmental media.

**Keywords**: Particle size, Oil and Grease, Total Organic Carbon, FTIR, GC-µECD

## 4.1 INTRODUCTION

Organophosphate pesticides were first introduced as chemical warfare agents and nerve poisons during World War II when they were discovered, now they are famously used to destroy pests. OPs are preferred over other pesticides such as organochlorines because they are less persistent (Jaga and Dharmani, 2003). OPs are absorbed in all routes but the most common route of exposure is through diet, as traces of OP pesticides are often detected in foods. Exposure to high doses of these pesticides can be fatal, it can also lead to paralysis and respiratory arrest for sublethal doses (Oates *et al*., 2014). The primary toxic effect of OPs is the inhibition of the enzyme AChE that catalyzes the hydrolysis of the neurotransmitter acetylcholine at nerve synapses and neuro-muscular junctions (Sams *et al*., 2000).

Although organophosphates are considered safe for agricultural use, they tend to reduce soil fertility and subside the microbial community. There is therefore a need to develop new and effective methods for their bioremediation in the environment (Sidhu *et al*., 2019). The pesticidal properties of some OPs such as Parathion are derived from AChE inhibition, which is also its toxic property. Toxic effects of Parathion poisoning include sweating, dizziness, vomiting, diarrhea, convulsions, cardiac arrest, respiratory arrest, and death in severe cases (Garcia *et al*., 2003). Parathion together with tetrachlorvinphos were classified as possibly carcinogenic to humans by eleven countries at the International Agency for Research on Cancer (IARC), where through occupational studies, Parathion was associated with some cancers (Fritschi *et al*., 2015). OPs like many other pesticides end up being transported to other environmental matrices. Sorption of pesticides affects other processes, such as transport, degradation, volatilization, and adsorption, which determine their final fate in the soil. Studies have shown that the adsorption of insecticides by soils and sediment varies based on the type and concentration of the insecticide, pH, temperature, clay, and organic matter. Desorption is also important to pesticide release, as it determines how mobile pesticides will be in the soil (Krishna and Philip, 2008). In this chapter, the methods for the determination of OP pesticides in the environmental matrices of the Buffalo river estuary, South Africa, were validated.

## 4.2 MATERIALS AND METHOD

### 4.2.1 Reagents and chemicals

β-cyclodextrin, chitosan, sodium alginate, and calcium chloride were all purchased from Sigma-Aldrich. Solvents such as dichloromethane, acetone, ethyl acetate and cyclohexane of HPLC and analytical grades were all purchased from Prestige Laboratory Supplies (Pty) Ltd. The organophosphate pesticide, Parathion was purchased from Industrial Analytical, South Africa. For the extraction of Parathion in the soil and sediment samples, an ultrasonicator (LASEC South Africa PTY LTD, single frequency 40 Hz, 6 L) was used.

### 4.2.2 Sample collection and preservation

Sediment and soil samples were collected following standardized protocols (US EPA) from Buffalo River estuary, which is located in East London city of the Eastern Cape Province, South Africa and a nearby dumpsite, respectively. The apparatus/equipment used were a grab sampler washed with tap water and soap, then rinsed with deionized water and air-dried prior sampling, stainless steel spoon/auger and aluminium foil. Each sample was covered with clean aluminium foil to prevent possible contamination. The samples were labeled appropriately, preserved on ice and transported immediately to the laboratory for further analysis. These precautions were taken to prevent the samples from microbial degradation. Sampling equipment was cleaned between consecutive sampling sites to prevent potential cross-contamination (Pieters, 2007).

Each bulk soil and sediment sample was air dried, thoroughly mixed, ground with an agate mortar and pestle and then passed through a 2 mm sieve. Any material retained on the sieve was discarded. Total Organic Carbon content of the soil and sediment, as well as the percentage moisture were estimated using gravimetric methods (Ramamoorthi and Meena, 2018).

### 4.2.3 Particle size determination

Sieves were stacked on top of each other in ascending degrees of coarseness (250 µm, 500 µm, 1 mm), and 30 g of each sediment and soil samples (air-dried) was placed on the top sieve. The nest of sieves was completed by a well-fitting pan at the base and a lid at the top. The nest of sieves was agitated for about 5 minutes. Each sieve from the nest was carefully removed without losing material, and the weight of material on each sieve and the collecting pan was determined. For each sieve in the nested series, the mass and percentage of sample retained on the sieve were recorded (Brittain, 2002).

### 4.2.4 Determination of Total Organic Carbon: Gravimetric method (Loss on ignition)

Five grams of sediment and soil that passed through a sieve of 2 mm mesh were further dried at 105 $^{o}$C for 24 hours and weighed after being cooled in the desiccator. Then, the samples were subjected to calcinations for 5 hours at 500 $^{o}$C in order to decompose their organic contents (Burns *et al.*, 2003). The samples were thereafter allowed to cool down in a desiccator and weighed. The percentage difference between the initial and final mass was taken to be the organic matter (OM) portion of the sample, as shown in the following equations (Ramamoorthi and Meena, 2018). Organic carbon (OC) levels were obtained by multiplying the percentage organic matter contents with 0.58, since OM is believed to contain approximately 58% of organic carbon (Equations 4.1 & 4.2).

$$\text{OM\%} = \frac{W105 - W500}{W105}(100) \qquad (4.1)$$

$$\text{OC\%} = (\text{OM\%})\,(0.58) \qquad (4.2)$$

### 4.2.5 Oil and Grease

Sediment and soil samples were air dried, crushed and sieved for grain size distribution using a standard set of stainless sieve. Ten grams of each sample was transferred into paper extraction thimbles. Total oil and grease (TOG) was extracted using soxhlet apparatus for 4 hours with n-hexane. A rotary evaporator was used to concentrate the organic extracts, which was later filtered through Whatman filter paper containing 1 g anhydrous sodium sulfate. The solvent was further evaporated in a rotary evaporator to determine the weight of the dry extract (Mohsenzadeh *et al.*, 2010). The concentration of n-hexane extractable material (HEM) in the sample was calculated using equation 4.3.

$$HEM\left(\frac{mg}{kg}\,wet\ weight\right) = \frac{\text{gain in weight of flask(mg)} \times 1000}{weight\ of\ wet\ solid(g)} \qquad (4.3)$$

### 4.2.6 Ultrasonic extraction procedure for pesticides

Four grams (4 g) of sediment/soil was ground with $Na_2SO_4$ in the ratio 1:1 (w/w) for sediments and 4:1 (w/w) for soil, after which they were transferred to extraction tubes. Ten milliliters (10 ml) of dichloromethane, acetone, ethyl acetate, and cyclohexane mixture in the ratio 3:2:1:1 (v/v/v/v) was used for the extraction of the samples by ultrasonication (LASEC South Africa PTY LTD, single frequency 40 Hz, 6 L) for 15 minutes with 3-s pulses. Samples were then vortexed for 5 min, and then centrifuged for another 5 min to separate the extract from the pellets. This procedure was repeated twice more without vortexing, each time with an additional 10 ml of extraction solvent (Lyytikäinen *et al.*, 2003).

### 4.2.7 Preparation of the microparticulate system

The β-CD-chitosan-alginate system was synthesized using a modified version of Ivancic *et al.*, (2016) method. β-CD was weighed and placed in a mortar followed by chitosan and distilled water, this mixture was kneaded until a paste was formed. Sodium alginate was added after the paste was kneaded for an additional 30 minutes. The resulting mixture was ground and 20 ml of 1 M $CaCl_2$ was added to form a paste. The mass ratios used for the system were 9:5:5 (w/w/w) (Ivancic *et al.*, 2016).

### ++++++++++++++++++++4.2.8 Instrumental analysis

#### 4.2.8.1 +++++++++++++++++

#### -µECD analysis

The capillary column used was a DB-5 (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The injector and detector temperatures were both 250 °C. The column oven temperature was initially set to 80 °C and held for 2 min, then increased to 160 °C at a rate of 10 °C $min^{-1}$ and finally increased to 265 °C at a rate of 3 °C $min^{-1}$ and held at this temperature for 15 min. Helium was used as carrier gas and the injection was splitless with a purge time of 0.75 min. The injection volume of the samples was 1 µL (de Souza Pinheiro, and de Andrade, 2009).

#### 4.2.8.2 Morphology of particles, FTIR analysis

The surface features of the synthesized β-CD-chitosan-alginate system were determined using scanning electron microscope (SEM) by placing a small sample of the powder on a carbon double sided tape on a stub and coated with Au/Pd for a clearer image. Fourier transform infrared (FTIR) spectroscopy was used to observe the vibrations of the materials, this was achieved by placing a small amount of the sample on a Perkin Elmer Universal ATR sampling accessory spectrum 100 FT-IR spectrometer (Ojemaye and Okoh, 2019).

### 4.2.9 Quality assurance and Quality control (QA/QC)

All glassware used were washed with detergent, rinsed with distilled water, then dried at 105 ºC for 4 hours. They were thereafter cooled and rinsed with acetone (HPLC grade) procured from Prestige Laboratory Supplies (Pty) Ltd. Recovery studies were carried out in order to establish the ability to generate accuracy and precision, where a mixture of the OP standards together with 1-Bromo-2-nitrobenzene used as surrogate standard, purchased from Industrial Analytical, South Africa, were spiked into 1 g of pure sand from SAINS AGENCIES. The sand was extracted as described in section 4.2.6, the final extracts were later analyzed with GC- µECD as described in section 4.2.8.1, in which the syringe was automated to wash 2 times pre-injection and 3 times post injection with both acetone and hexane to prevent cross-contamination. The recovery percentages ranged from 57.78 to 102% for the analytes. The calibration curves were plotted using the peak areas and concentrations for a mixture of standards prepared with a 9:1 ratio of hexane and acetone (HPLC grade purchased from Prestige Laboratory Supplies (Pty) Ltd), with concentrations ranging from 60 to 1280 µg/L (10 concentration levels). The limit of detection and quantification (LOD and LOQ) were calculated using 3.3* (Sy/S) and 10* (Sy/S) respectively, where Sy is the standard deviation of the response of the curve and S is the slope of the calibration curve (Zhang *et al*., 2021; Moosavi and Ghassabian, 2018).

## 4.3 Statistical Analysis

The following statistics analysis were followed in this study of Ops in the Buffalo River estuary: descriptive statistics, distribution percentage, composition pattern of OPs in soil, Pearson correlation and sediments and physicochemical parameters, were all calculated using Microsoft Excel 2016. The percentage distribution of OPs in two seasons were calculated and compared and all samples below LOD were denoted as zero throughout this study.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 Calibration and Linearity

The calibration curves plotted for the four OPs were linear with correlation coefficients ($R^2$) ranging from 0.9935 to 0.9972 (Table 4.1). The chromatographic peaks of OPs are shown in Appendix 2.

**Table 4.1**: Calibration and linearity of the selected OP pesticides

| OPs | RT | $R^2$ | Regression equation | LOD (µg/L) | LOQ (µg/L) | RSD |
|---|---|---|---|---|---|---|
| **Ronnel** | 20.099 | 0.996 | y = 348.4x | 0.13 | 0.39 | 2.04 |
| **Malathion** | 21.240 | 0.9972 | y = 68.07x | 0.75 | 9.06 | 3.50 |
| **Parathion** | 21.414 | 0.9935 | y = 3.4723x | 2.99 | 2.27 | 1.02 |
| **Dursban** | 21.851 | 0.9958 | y =417.72x | 0.09 | 0.27 | 1.54 |

### 4.4.2 Limit of detection, limit of quantification and relative standard deviation

To measure the sensitivity of the instrument, limits of detection (LODs) and limits of quantification (LOQs) were calculated as described in section 4.2.9. The relative standard deviations (RSDs) were also estimated in order to determine the instrument's precision from 10 replicate injections of a middle level calibration standard. The results summarized in Table 4.1 show that the RSDs for the samples were all less than 11% (USEPA, 2007), and the LODs and LOQs of the OPs ranged from 0.09 to 2.99 and 0.274 to 9.06 µg/L, respectively.

### 4.4.3 Recovery of OP pesticides in sediments and soil

The results obtained for the OP pesticides' recovery studies using the two ratios (3:2:1:1 and 2:1:1:1) of dichloromethane, acetone, ethyl acetate, and cyclohexane mixture are given in Table 4.2. These ratios of solvents were chosen after a number of optimizatio processes. Pure sand samples were fortified with pesticides' standards at 960 µ/L level and the recoveries ranged from 66.0 to 279% for the samples extracted with 2:1:1:1 ratio and 57.78 to 102% for the samples extracted with the 3:2:1:1 ratio. All the OPs extracted with 3:2:1:1 solvent ratio, yielded good recoveries except for malathion, which had a recovery percentage below 70%. This could be due to either the lower vapour pressure of this pesticide (Kashi *et al*., 2021), or poor extraction efficiency. A similar shortcoming was reported in article by Kuet and Seng (2003), where a low recovery percentage of 17.3% for the polar pesticide Methamidophos was obtained due to strong retention of this pesticide by the polar Solid Phase Extraction (SPE) sorbent quaternary amine/aminopropyl SAX/($NH_2$).

**Table 4.2**: OP recovery from spiked sand

| | **3:2:1:1** | | **2:1:1:1** | |
|---|---|---|---|---|
| **Congener** | **% Recovery (960µg/L)** | **SD** | **% Recovery (960µg/L)** | **SD** |
| **BNB** | 86.4 | 17.88 | 279 | 36.5 |
| **Ronnel** | 90.5 | 18.69 | 130 | 29.75 |
| **Malathion** | 57.8 | 11.96 | 136 | 29.75 |
| **Parathion** | 102.1 | 21.08 | 66.0 | 32.75 |
| **Dursban** | 84.9 | 17.54 | 122 | 30 |

## CONCLUSION

The determination of four organophosphate pesticides by means of GC-µECD was validated. The GC-µECD gives appropriate sensitivity, detection and precision. The RSD was $\leq$ 11% showing that the response of the instrument was reliable. The OPs had good recoveries that were within the acceptable range except for one (malathion). Based on these results, the method is validated and considered good enough for the extraction of OP pesticides from soil and sediment samples.

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# CHAPTER FIVE

## Spatial distribution and health risk assessment of OP pesticides in environmental matrices from Buffalo River Estuary, South Africa

## ABSTRACT

Contamination of waterbodies such as the Buffalo River in Eastern Cape, South Africa through agricultural, industrial and anthropogenic activities has attracted much attention, due to the fact that these water-bodies are of importance to human livelihoods. Samples of sediments and soil in the neighbourhood were collected in two seasons, and analysed for some physicochemical properties, including particle size, total organic carbon and pH, in order to assess how they influence the behavior and fate of contaminants such as OP pesticides in the environment. In winter, the particle size percentages of the sediment and soil samples ranged from 34.4 to 79.4%, 15.4 to 23.9% and from 7.77 to 38.3% for coarse, medium and fine fractions, respectively. While in spring the particle size for the coarse, medium and fine mesh fractions ranged from 37.4 to 74.6%, 14.1 to 25.1% and 12.4 to 35.0% respectively. Finer deposits had the highest values of total organic carbon. Both the organic carbon percentages and oil and grease (n-Hexane extractable material) increased from winter to spring, thus, suggesting an increase in human activities and domestic waste influx in the area. The OP pesticides' concentrations ranged from 0.0023 to 63.8 µg/g in winter and from 0.0017 to 36.6 µg/g in spring, with Malathionbeing the predominant congener and Dursban being the least dominant in both seasons. There was no clear seasonal pattern when comparing the concentrations of OPs in the soil and sediment samples for both seasons. The risk quotient (RQ) method was used to assess the risk of OP exposure and the results showed that Dursban is capable of posing a potential high risk to organisms that dwell in the sediments at the levels determined in this study, because the RQs for both seasons were above ten.

**Keywords**: HEM, Organic matter, Moisture content, Risk Quotient

## 5.1 INTRODUCTION

The contamination of lotic ecosystems caused by agricultural, industrial and anthropogenic activities has attracted the attention of researchers, authorities and scientists, due to the fact that these waterbodies are of social and economic importance to human livelihoods. OP pesticides can be released into different environmental matrices from many sources, including agricultural and urban runoff. Physicochemical properties of sediments and soil including particle size, total organic carbon and pH can influence the behavior and fate of contaminants such as OP pesticides in the environment (Barhoumi *et al*., 2019; Ahmed *et al*., 2017). Toxic chemicals released into these waterbodies sink down to the bottom sediments and in time their concentrations accumulate to greater values, and may therefore become resistant to biodegradation, thus, posing a huge pollution problem (de Andrade *et al*., 2019; Arslan-Alaton *et al*., 2009).

Contamination of water by OPs can lead to serious health problems such as loss of appetite and memory, nausea and runny stomach to the public that uses the water resource and is exposed to the contaminant (Van Ginkel *et al*., 1996; Adeniji *et al*., 2019; Chigor *et al*., 2013, Eskenazi *et al*., 1999). OPs such as Dursban, are one of the constituents in insecticides used in residences, and have been banned for domestic use in some countries such as


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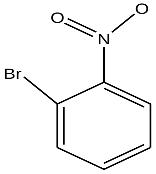
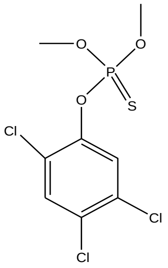
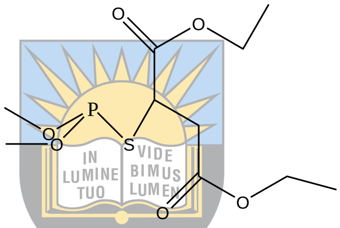
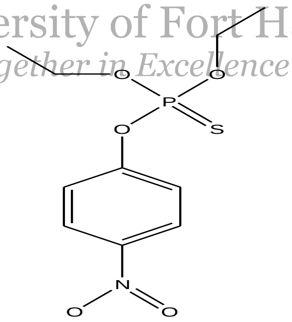
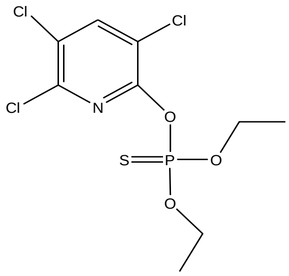
Spain, as they were detected in newborn babies that are more vulnerable than adults, and could develop neurodevelopmental disorders when exposed to OPs (Llop *et al*., 2017). OPs persist and accumulate in sediments and these can change to more harmful compounds than the initial one, due to various environmental and biological processes, hence, it is important to understand the trends of these contaminants in environmental matrices and the risks associated with them (Ccanccapa *et al*., 2016).

The Buffalo River is located in one of the most popular regions in the province of Eastern Cape, South Africa, providing water to over 300,000 residents. The Buffalo River estuary is 6.5 km long and approximately 2.4 to 7.4 m deep. It receives effluents from large rivers and industries around, including Vukani petroleum, BP and Total South Africa. The Buffalo River has been reportedly polluted by contaminants such as Polycyclic aromatic hydrocarbons (PAHs) (Adeniji *et al*., 2019) and heavy metals such as cadmium, zinc and lead (Fatoki and Mathabatha, 2001**)**. This chapter aims to determine the spatial distribution and health risk assessment of OP pesticides in environmental matrices from Buffalo River Estuary, South Africa. Table 5.1 shows the properties of the congeners investigated in this study.

**Table 5.1**: Congeners, structures and physicochemical properties of the OPs under study

| **Congeners** | **IUPAC ID** | **Structure** | **Chemical formula** | **Molar mass (g/mol)** | **Boiling point** | **Melting point** |
|---|---|---|---|---|---|---|
| BNB | 1-bromo-2-nitrobenzene |  | $C_6H_4BrNO_2$ | 202.01 | 258.0°C | 43.0°C |
| Ronnel | Dimethoxy-sulfanylidene-(2,4,5-trichlorophenoxy)λ$^5$-phosphane |  | $(CH_3O)_2PSOC_6H_2Cl_3$ | 321.5 | | 41°C |
| Malathion | Diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate |  | $C_{10}H_{19}O_6PS_2$ | 330.4 | 156-157°C | 28°C |
| Parathion | Diethoxy-(4-nitrophenoxy)-sulfanylidene-λ$^5$-phosphane |  | $C_{10}H_{14}NO_5PS$ | 291.26 | 375°C | 61°C |
| Dursban | *O,O*-Diethyl *O*- (3,5,6-trichloropyridin-2-yl) phosphorothioate |  | $C_9H_{11}Cl_3NO_3PS$ | 350.59 | 160°C | 43 °C |

## 5.2 Materials and Method

### 5.2.1 Study Area

The Buffalo river estuary is located in the mouth of the Buffalo River in East London (EL) in the Eastern Cape Province, South Africa. The Buffalo River is a catchment area that is situated at 33º1´43´´S, 27º51´51´´E.

Shown in Figure 5.1 are the selected sampling site, map (Figure 5.2) and their coordinates, which were 200 m apart.

Buffalo river estuary (B) and the dumpsite where soil samples for this study were collected (C).

**Table 5.2**: Description of selected sampling sites in the Buffalo River estuary

| **Site** | **Depth (m)** | **Longitude** | **Latitude** | **Description** |
|---|---|---|---|---|
| Sed1 | 7 | 27.85996º E | 33.02943º S | Nearshore, shallow water |
| Sed2 | 13 | 27.87698º E | 33.02628º S | Downstream |
| Sed3 | 22 | 27.88257º E | 33.02540º S | Second creek outlet |
| Sed4 | 7 | 27.89234º E | 33.02353º S | First creek outlet |
| Sed5 | 47 | 27.89486º E | 33.02322º S | Located under the Buffalo bridge |
| Sed6 | 16 | 27.89746 º E | 33.02325º S | upstream |
| Soil | 2 | 26.84952 º E | 32.79251 º S | Dumpsite nearby the river |

Figure 5.2: Map of Buffalo River estuary sampling sites.

### 5.2.2 Standards and Reagents

The standards and reagents used in this study were mentioned in detail in Chapter 4; Section 4.2.1

### 5.2.3 Sample collection and preservation

The collection and preservation of the sediment and soil samples were described in Chapter 4; Section 4.2.2

### 5.2.4 Extraction

The extraction of OP pesticides was carried out as described in Chapter 4; Section 4.2.6

### 5.2.5 Instrumental Analysis

The OPs under study were analyzed with GC-μECD as described in Chapter 4; Section 4.2.8.

### 5.2.6 Quality assurance and Quality control (QA/QC)

QA and QC were carried out as described in Chapter 4; Section 4.2.9.

### 5.2.7 Risk Assessment bf

To determine the risk of OP exposure into aquatic organisms, the risk quotient (RQ) method was used (equation 5.1), where the ratio of the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC) was calculated (Ogbeide *et al*., 2015).

$$RQ = MEC/ PNEC \quad (5.1)$$

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Impact of physicochemical properties on concentrations of OPs in Buffalo River estuary

The physicochemical parameters of soil and sediment samples are summarized in Table 5.3. In winter, the MC ranged from 44.0 to 73.1% for sediments, with point 4 being the highest and point 5 being the lowest. OC ranged from 0.06 to 0.37% with point 1 having the highest and point 5 having the lowest percentage; while the oil and grease varied from 1.77 to 10.28 mg/kg, point 4 recorded the highest and point 5 the lowest concentrations. In spring however, the MC of the sediments were in the range of 57.1- 63.4%, point 3 had the highest value but point 5 recorded the lowest. OC ranged from 0.45 to 1.95%, with point 1 having the highest and point 6 the lowest concentration. In the same vein, the oil and grease varied from 2.65 to 4.28 mg/kg, with point 3 having the highest value and point 6 the lowest.

In the soil samples, the determined physicochemical properties were lower in winter except for the HEM, which significantly decreased in spring from 2.6 to 1.63 mg/kg and this can be due to natural evapour ation processes and decrease in anthropogenic activities (Sørheim *et al*., 2020; Yogaswara and Wulandari *et al*., 2021). Comparing the physicochemical parameters of the sediments from different seasons, it can be seen that points 1,3 and 4, which were outlets of the first and second creeks (point 3 and 4 respectively), constantly had the highest values compared to the other points. The organic carbon percentages and oil and grease (n-Hexane extractable material) increased from winter to spring, this is evidence of the increase in human activities and domestic waste (Yogaswara and

Wulandari *et al.*, 2021). These results were expected as the higher temperatures in spring could lead to more precipitation, and the rains in this season can therefore bring about increased runoff from urban, residential and industrial resources into the river (Fatoki and Mathabatha, 2001).

**Table 5.3**: Physicochemical properties of soil and sediment samples from Buffalo River and the neighbourhood dumpsite

| | Winter | | | | Spring | | | |
|---|---|---|---|---|---|---|---|---|
| | **%MC** | **%OM** | **%OC** | **HEM** (mg/Kg) | **%MC** | **%OM** | **%OC** | **HEM** (mg/Kg) |
| **Sed1** | 63.9 | 0.16 | 0.37 | 3.10 | 60.1 | 3.57 | 1.95 | 3.51 |
| **Sed2** | 58.9 | 0.63 | 0.10 | 3.04 | 61.1 | 1.29 | 0.746 | 2.83 |
| **Sed3** | 63.0 | 0.26 | 0.15 | 8.40 | 63.4 | 2.48 | 1.44 | 4.28 |
| **Sed4** | 73.1 | 0.40 | 0.23 | 10.3 | 62.7 | 1.78 | 1.03 | 4.11 |
| **Sed5** | 44.0 | 0.10 | 0.06 | 1.77 | 53.8 | 1.47 | 0.852 | 2.66 |
| **Sed6** | 54.0 | 0.13 | 0.08 | 2.90 | 57.1 | 0.778 | 0.451 | 2.65 |
| **Soil** | 1.05 | 0.16 | 0.09 | 2.06 | 13.5 | 2.90 | 1.68 | 1.63 |

MC- Moisture Content, OM- Organic Matter, OC- Organic Carbon, HEM- n-Hexane Extractable Material

Figure 5.3 shows the particle size percentages of the sediment and soil samples. The values ranged from 10.3 to 79.4 in winter and from 12.4 to 74.6 in spring. The highest percentages of mass retained were in the 500 µm mesh, which collects coarse to medium sand for both seasons. Point 1 had the highest percentages of mass retained in the 63 µm mesh, which collects fine sand and silt and also had the highest percentage of organic matter in both winter and summer, this confirms the relationship between surface area and adsorption of organic pollutants to particulate matter and sediments in the aquatic areas (Prado *et al.*, 2021). Point 5 had the lowest percentages of OC and particle size (63 µm) in both season.

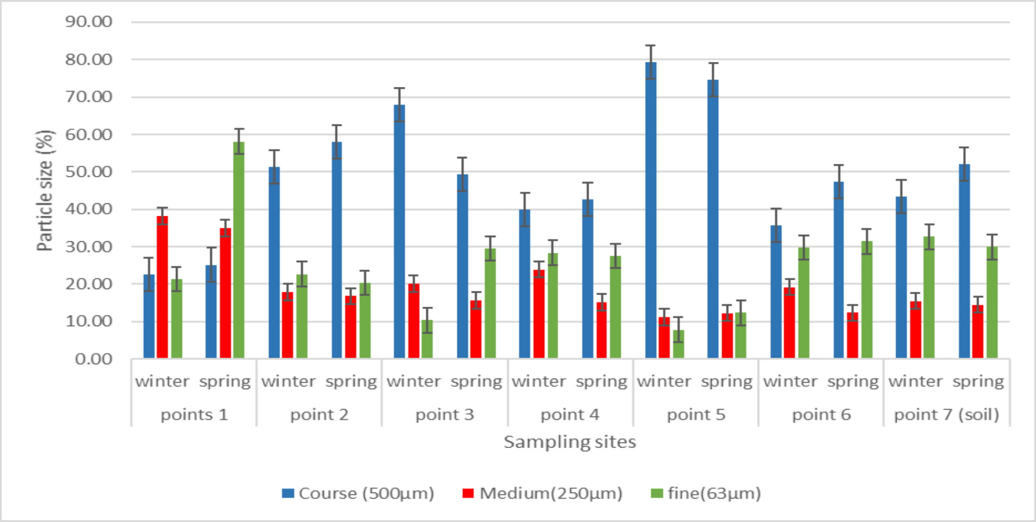


Figure 5.3: Particle size of sediment and soil samples from Buffalo River estuary and nearby

The percentages of OP concentrations in the Buffalo River estuary for winter and spring are shown in figure 5.4. Malathionhad the highest percentage of all the pesticides in this study with 63 and 77% in winter and spring, respectively. The high concentrations of Malathionin spring may be due anthropogenic activities, as this pesticide is an active ingredient in mosquito repellents which are most active in warmer seasons (Okanga *et al.*, 2013). In both seasons, the second highest concentrations were in Parathion which was 15% in winter and 8% in spring, Dursban had the lowest concentrations in both seasons.

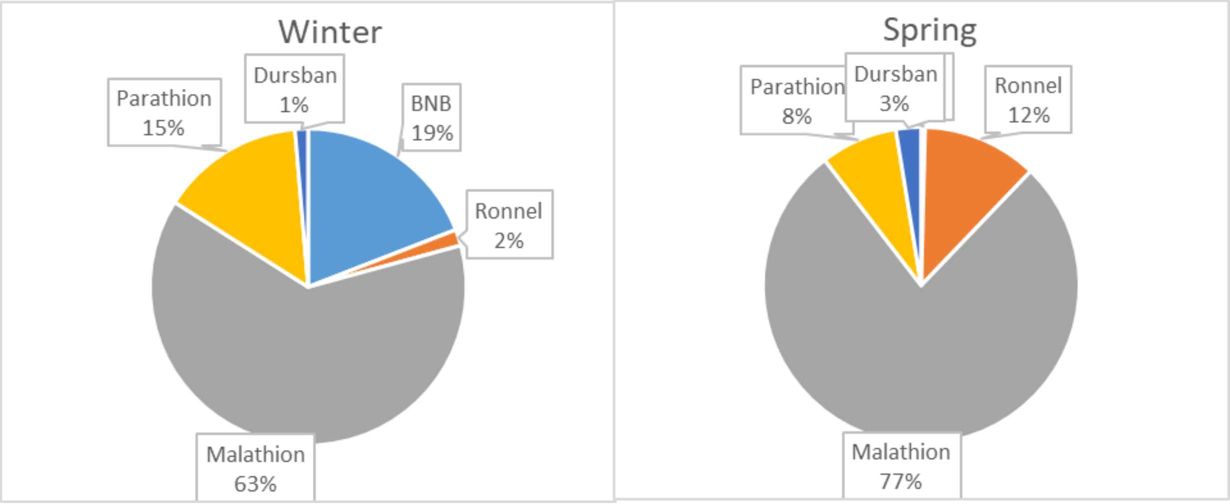


Figure 5.4 Pie-chart diagrams showing the percentages of average OP concentrations in winter and spring in the Buffalo river estuary

Concentrations (µg/g) of OP pesticides in sediment and soil samples were calculated using the formulas in appendix 3. The OP concentrations ranged from 0.0023 to 63.8 µg/g in winter and from 0.0017 to 36.6 µg/g in spring, with Malathionbeing the predominant congener and Dursban being the least dominant in both seasons. The concentrations of the OP pesticides in sediments surrounding a manufacturing plant in North China, investigated in a study by Ren *et al*. (2019) were in the range of 340 to 270,000 µg/g, which is higher than the values obtained in this study. However, the results in this study were found higher when compared to the concentrations obtained for OPs in the Sundays River estuary, South Africa in a study by Olisah e*t al.* (2021), with levels ranging from 3.30 to 8.07 µg/g for sediments, being the lowest concentrations of all the matrices investigated in that study. The levels of OP pesticides in this study were also lower compared to those reported in a study by Ezemonye *et al*. (2015) where the distribution of pesticide residues in sediments from Ogbesse River, Edo State, Nigeria were assessed.

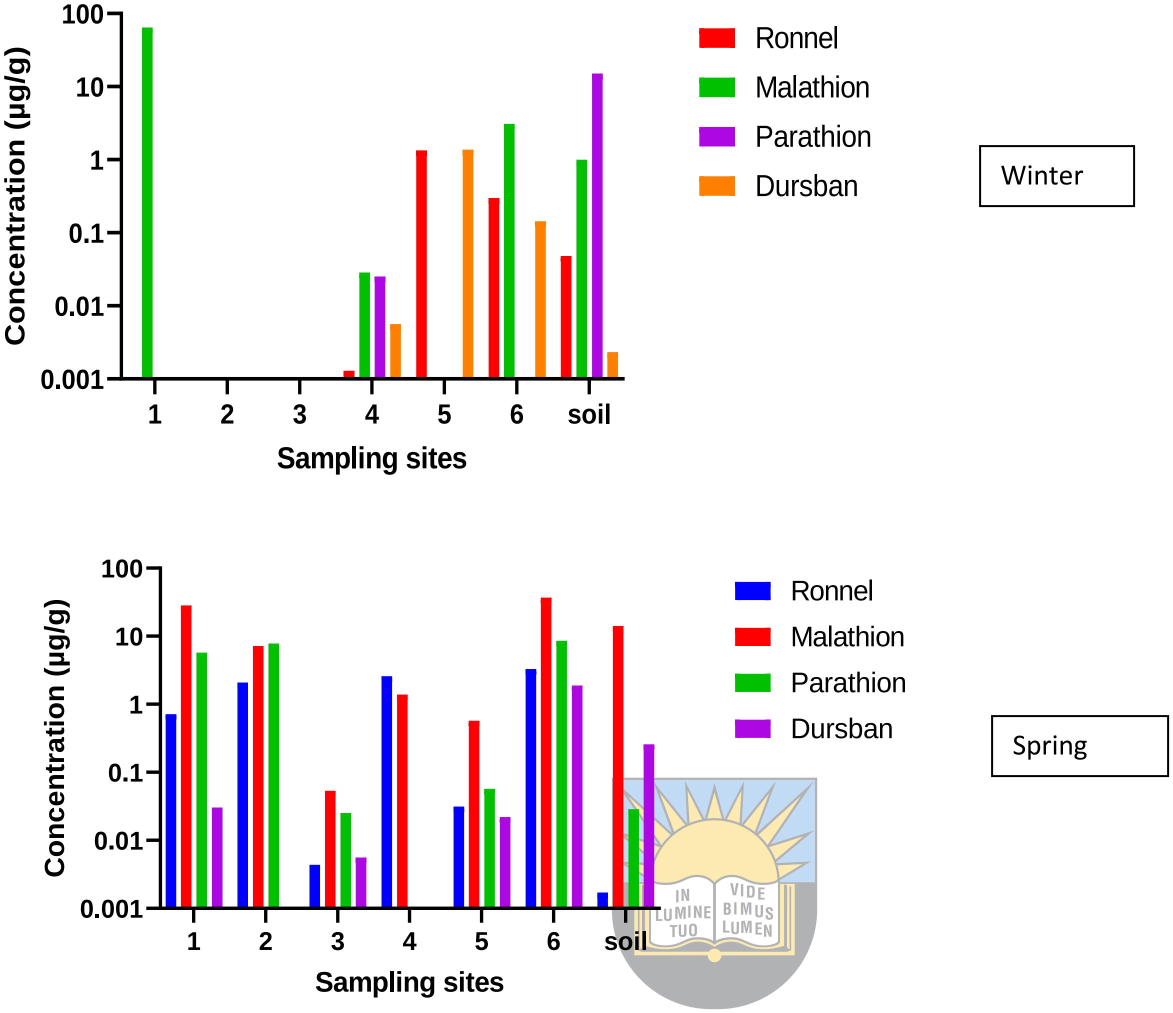


Figure 5.5: $\text{Log}_{10}$ concentrations of four OP pesticides in sediment (1-6) and soil (7) samples from Buffalo River estuary for winter and spring (µg/g)

Pearson product-moment correlation was used to evaluate the association between the analytes and physicochemical properties of the organophosphate pesticides in winter and spring. Correlated data can be negative if the magnitude of variable is changing in the opposite direction of another, and it can be positive if the magnitude of the variables is changing in the same direction. The Pearson correlation coefficient ranges from –1 to 1, where -1 is considered weak correlation and 1 is considered strong correlation (Schober *et al*., 2018). The correlation of the two seasons' physicochemical parameters is shown in Tables 5.4 and 5.5. In winter, it can be observed that HEM had a moderate correlation with MC and weak correlation with OM and OC, while in spring (Table 5.5), HEM had a moderate correlation with MC and weak correlation with OM and OC, this implies that an increase in HEM will result in an increase in MC and a decrease in OC and OM, this is because an increase in OC is attributed to an increase in water retention capacity (Manns *et al.*,2016). Parathion had weak correlation with every other variable except for Malathionin winter, whereas in spring, Parathion correlated very weakly with malathion, this can be attributed to the fact that products containing Parathion are mostly used to control pests found in crops, such as corn and in S.A the season for this crop is June to August, while Malathioncan be found in products used in the control of mosquitos which are most active in the spring season (Durand, 2013; Fan *et al.,* 2011; Okanga *et al.,* 2013). Very strong correlation was observed between Dursban and Ronnel in spring and in winter, implying that an increase in Dursban resulted in an increase in Ronnel. They are possibly from the same sources in the environment.

**Table 5.4**: Correlation of analytes and physicochemical properties of Buffalo Estuary (spring).

| | MC | OM | OC | HEM | Ronnel | Malathion | Parathion | Dursban |
|---|---|---|---|---|---|---|---|---|
| MC | 1 | | | | | | | |
| OM | -0.41503 | 1 | | | | | | |
| OC | -0.41503 | 1 | 1 | | | | | |
| HEM | 0.751477 | 0.105421 | 0.105421 | 1 | | | | |
| Ronnel | 0.367202 | -0.66887 | -0.66887 | 0.114036 | 1 | | | |
| Malathion | -0.10362 | 0.002547 | 0.002547 | -0.31613 | 0.404489 | 1 | | |
| Parathion | 0.206334 | -0.3729 | -0.3729 | -0.15905 | 0.626364 | 0.717392 | 1 | |
| Dursban | 0.04569 | -0.52517 | -0.52517 | -0.30891 | 0.597464 | 0.745641 | 0.554629 | 1 |

MC-Moisture content; OM-Organic Matter; OC-Organic Carbon; HEM- n-Heaxane Extractable Material

**Table 5.5**: Correlation of analytes and physicochemical properties of Buffalo Estuary (winter).

| | MC | OM | OC | HEM | Ronnel | Malathion | Parathion | Dursban |
|---|---|---|---|---|---|---|---|---|
| MC | 1 | | | | | | | |
| OM | -0.159 | 1 | | | | | | |
| OC | 0.476082 | -0.30727 | 1 | | | | | |
| HEM | 0.58 | -0.53597 | 0.341598 | 1 | | | | |
| Ronnel | -0.15702 | -0.08395 | -0.47194 | -0.42186 | 1 | | | |
| Malathion | 0.22681 | 0.012676 | 0.837109 | -0.20033 | -0.21641 | 1 | | |
| Parathion | -0.92523 | 0.012037 | -0.24339 | -0.31738 | -0.17149 | -0.16113 | 1 | |
| Dursban | -0.12874 | -0.08335 | -0.42298 | -0.38073 | 0.992911 | -0.19546 | -0.1859 | 1 |

MC-Moisture content; OM-Organic Matter; OC-Organic Carbon; HEM- n-Heaxane Extractable Material

The results for risk assessment in sediments using the RQ method are shown in Table 5.6. The risk assessment calculated was only for Dursban, as it was the only OP pesticide with a $\text{PNEC}_{\text{sediment}}$ that could be found from literature. The RQs for both worst case scenarios where the maximum concentration of Dursban was used and the normal case, where the mean concentration was used were higher than ten (>10) in both winter and summer. These results indicate that Dursban can pose a potential high risk to organisms that dwell in sediments. The high values of RQs are a result of the high concentrations of the Dursban in the sediments (Peris *et al*., 2022).

**Table 5.6**: RQs for the worst-case (RQ-Max) and the normal (RQ-mean) contamination scenarios

| **Pesticide (Dusban)** | **$\text{PNECs}_{\text{ed}}$** | **MEC-mean (µg/g)** | **MEC-max (µg/g)** | **RQ-mean** | **RQ-max** |
|---|---|---|---|---|---|
| **Winter** | 8.21 | 1372 | 1364 | 167 | 166 |
| **Summer** | 8.21 | 1911 | 1883 | 233 | 229 |

## Conclusion

The highest detected congener was Malathion and lowest was Dursban in both winter and spring. Point 1 together with the sampling sites that were outlets of the first and second creeks (3 and 4) had the highest percentages of MC, OC and HEM compared to the other sites. The levels of OPs reported in this study poses a potential health risk to the municipalities that fall under this river catchment and all the users of the water, including the industries and businesses around the Buffalo River estuary that contribute to the high levels of pollution. It is therefore imperative to employ sustainable strategies to control and reduce the pollution entering this river for the benefit of all direct and indirect users.

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# CHAPTER SIX

## Synthesis of polymer modified cyclodextrin for the removal of Parathion from soil and sediment samples

### ABSTRACT

Pesticides are widely used in the agriculture industry, and while they are important in the success of the agricultural industry, they have been found to be toxic, not only to insects but also to non-target organisms. In South Africa, there are more than 500 registered pesticides, including organophosphates. One of the most commonly used OP pesticides is Parathion, an organophosphate pesticide that is moderately toxic and binds tightly to soil particles. In this study, an inclusion complex (IC) between β-cyclodextrin, chitosan and alginate was synthesized and analyzed using different techniques. When comparing the different FTIR spectra, it was noted that some peaks either shifted, disappeared or decreased in intensity, such as the C=O peak at 1743 $cm^{-1}$ visible in the cyclodextrin FTIR spectrum that is not visible in the synthesized material.  These were a confirmation of the formation of the inclusion complex as the chitosan and alginate are encapsulated in the cyclodextrin cavity. The scanning electron microscope (SEM) and the energy dispersive X-ray spectroscopy (EDX) were used for the morphology and elemental composition of the IC, where it was seen that all three micrographs had irregularly shape rough surfaces. The CD and alginate complex had a porous morphology rougher surface when compared to CD and chitosan, and CD, chitosan and alginate IC. The adsorption capacity of modified cyclodextrin in this study was found to be highest at pH 2 (99.19%), when 3 mL of the adsorbent was used for 30 minutes. Based on the results of this study, the synthesized modified cyclodextrin can be used for the removal of organophosphate pesticides in solid matrices.

**Keywords**: Parathion, synthesis, FTIR, EDX, SEM, Adsorption

## 6.1 INTRODUCTION

Several harmful chemicals are found in the environment, these include pesticides which are synthetic compounds that have through their application and use become environmental contaminants (Friend, 1999). While pesticides play a vital role in the high productivity achieved in agriculture, they are toxic and often reach non-target organisms by means of long-range transportation (Van Dyk and Pletschke 2011). South Africa (S.A) is dependent on pesticide used for the maintenance of not only its ever-growing agriculture industry but also for human health. As one of the largest importers of pesticides, S.A has more than 500 pesticides registered with PAN (Pesticide Action Network, 2010), these include organophosphate (OP) pesticides, which have been reported to be the most used pesticides in conventional farming (Quinn *et* al., 2011; Jaipieam *et al*., 2009). OPs disperse in the environment through drainage, drift and run-off, hence, residues of OPs can be found in different environmental matrices including soils and sediments (Nasrabadi *et al*., 2011). Organophosphates are a product of the esterification reaction between phosphoric acid ($H_3PO_4$) and alcohol (OH), that have carbon-phosphorous bonds (Figure 6.1). OPs inhibit the activity of cholinesterase (ChE) enzyme by irreversible phosphorylation, which can be fatal not only to pests and insects but also to mammals (Jaga and Dharmani, 2003).

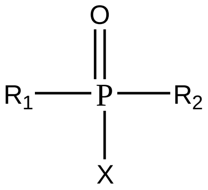


Figure 6.1: General structure of organophosphates

There are different OPs that are used worldwide, including chlorpyriphos, malathion, and Dursban to name a few (Fenik *et al.*, 2011). In order to regulate the exposure to these pesticides, many detection methods have been employed, including chromatography and enzymatic methods (Kaushal, *et al.*, 2021). One of the most commonly used OP pesticides is Parathion, an organophosphate pesticide that is moderately toxic and binds tightly to soil particles (Kulshreshtha *et al*., 2017, Herriage and Pope, 2020). Parathion undergoes photodegradation and is converted to paraoxon (Figure 2), an active metabolite in Parathion (Figure 6.2) when exposed to sunlight as it decomposes at 200 °C (Herriage and Pope, 2020). Because of their low solubility, pesticides are difficult to remove from the environment; therefore, surfactants, commercial adsorbents and organic co-solvents have been utilised to enhance the solubility of pesticides, however all these have their limitations and so cyclodextrins (CD) have been considered as alternatives to solubility enhancement and remediation of pesticides (Petrović *et al*., 2011).

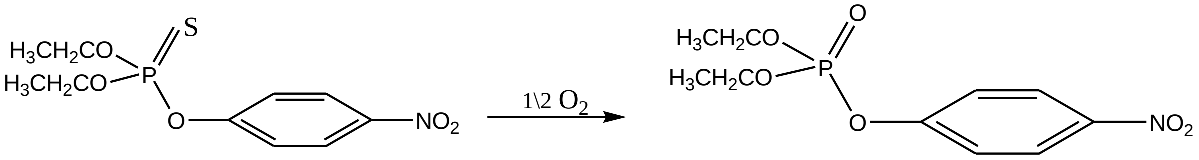


Figure 6.2: Oxidation of Parathion to Paraoxon

CDs are oligosaccharides that result from the treatment of starch with amylase of cyclodextrinase (*Bacilluss macerans*). CDs are named in accordance with the number of their glucose residues, so CDs with six, seven and eight glucose units are called α-, β- and γ-CD, respectively (Bender and Komiyama, 2012; Davis and Brewster, 2004). The outer surface of CDs is hydrophilic due to the presence of the hydroxyl functional groups while their inner cavity is hydrophobic owing to the methylene groups present. Depending on the size and stoichiometry of a guest molecule, different CDs form inclusion complexes (IC) with different molecules in their hydrophobic cavity (Jacob and Nair, 2018, Dodziuk, 2006). The nanoparticles of chitosan and alginate can be used to form complexes with CDs for different purposes such as water remediation or drug delivery. The natural biopolymer chitosan is derived from chitin, which is a component of crustacean outer skeletons and it has active hydrophilic groups (-OH and $NH_2$) that provide interactive sites for binding such as hydrogen and covalent bonding (Paul and Sharma, 2004, Jindal, 2022). Although chitosan has several good properties such as being non- toxic, biodegradable and its haemostatic activity that is a result of the positive charges on its backbone, it also has its limitations because of its poor solubility in neutral and basic media (Ibrahim and El-Zairy, 2015, Aranaz *et al*., 2021). Alginate is a natural anionic polymer that originates from brown seaweed, which is able to form a reversible gel by ionically crosslinking with cations such as $Ca^{2+}$ (Lee and Mooney, 2012, Zhang *et al*., 2010). Research has shown that adsorption into the surface of natural polymers and their derivatives can remove toxic pollutants from water (Benettayeb *et al*., 2022). Many different methods can be used to form complexes, these include the freeze-dried, co-precipitation and kneading methods (Barbosa *et al*., 2019). The aim of this study was to develop a method for the removal of Parathion in contaminated soil and sediment from the Buffalo River estuary, using modified cyclodextrin. The objectives of this study was to synthesize and characterise polymer modified cyclodextrin by forming inclusion complexes between β-cyclodextrin, chitosan and sodium alginate and characterising them using FTIR, SEM and EDX.

## 6.2 Experimental

### 6.2.1 Materials

β-cyclodextrin, chitosan, sodium alginate, and calcium chloride were all purchased from Sigma-Aldrich, South Africa. Chemicals such as dichloromethane, acetone, ethyl acetate and cyclohexane were all purchased from Prestige Laboratory Supplies (Pty) Ltd, South Africa. Pure sand (50-70 mesh) was purchased from Sains Agencies, South Africa. The organophosphate pesticide (Parathion) was purchased from Industrial Analytical, South Africa. For the extraction of Parathion in the soil and sediment samples, an ultrasonicator (LASEC South Africa PTY LTD, single frequency 40 Hz, 6 L) was used.

### 6.2.2 Quality Assurance

All glassware used were washed with detergent, rinsed with distilled water, dried at 105 ºC for 4 hours and later cooled and rinsed with acetone (HPLC grade). The standard used in this study was kept in the freezer at temperatures below 5 ºC so as to avoid degradation. In order to determine the optimum pH, time, concentration and dose for the removal of OPs, Parathion was spiked into 1 g of pure sand. The sand was then extracted as described in section 2.6, the final extracts were then analyzed with GC- µECD as described in section 2.7, in which the syringe was automated to wash two times pre-injection and 3 times post injection correspondingly with acetone and hexane, in order to prevent cross-contamination (Zhang *et al*., 2021; Moosavi and Ghassabian, 2018).

### 6.2.3 Characterization

The surface features of the synthesized β-CD-chitosan-alginate system were determined using scanning electron microscope (SEM) by placing a small sample of the powder on a carbon double sided tape on a stub and coated with Au/Pd for a clearer image. Fourier transform infrared (FTIR) spectroscopy was used to observe the vibrations of the materials, this was achieved by placing a small amount of the sample on a Perkin Elmer Universal ATR sampling accessory spectrum 100 FT-IR spectrometer (Ojemaye and Okoh, 2019).

### 6.2.4 Synthesis of the β-CD-chitosan-alginate system

The β-CD-chitosan-alginate system was synthesized using a modified version of Ivancic *et al.,* (2016) method. β-CD was weighed and placed in a mortar followed by chitosan and distilled water, the mixture was kneaded until a paste was formed. Sodium was then added after the paste was kneaded for an additional 30 minutes, the resulting mixture was milled and 20 mL of 1 M $CaCl_2$ was added to form a paste. The mass ratios used for the system were 9:5:5 (Ivancic *et al.,* 2016).

### 6.2.5 Removal of pesticide from soil and sediments samples

The soil and sediment samples were contaminated using 5 mL of 100 mM pesticide solution, then allowed to dry for 72 hours. Three millilitre (3 mL) of 5% β-CD-chitosan-alginate system was added to the samples, shaken overnight and extracted (Fenyvesi *et al.*, 1996). The effect of pH, time and concentration were investigated for adsorption experiments, where the pH was adjusted from 2 to 10 by the addition of either HCl or NaOH solution to the pesticide solution while the effect of time was conducted from 0 to 360 minutes. Similarly, the effects of dose and concentration were determined at three different doses (1, 3 and 5 mL) and four different concentrations (120, 100, 80, 60 mM).

### 6.2.6 Extraction of Parathion from soil and sand samples

Four grams (4 g) of sediment/soil was ground with $Na_2SO_4$ in the ratio 1:1 wt/wt for sediments and 4:1 wt/wt for soil and were transferred to extraction tubes. Ten millilitres (10 mL) of dichloromethane, acetone, ethyl acetate, and cyclohexane mixture in the ratio 3:2:1:1 vol/vol/vol/vol was used for the extraction of the samples by ultrasonication using a Tekmar Sonic Disruptor, model TM 501 (Cincinnati, OH) for 15 minutes with 3-s pulses. Samples were then vortexed for 5 minutes and then centrifuged for 5 minutes to separate the extract from the pellet. This procedure was repeated twice more without vortexing, each time with an additional 10 mL of extraction solvent.

### 6.2.7 GC-μECD analysis

The capillary column was used for the analysis of the final extracts. The injector and detector temperatures were both 250 °C. The column oven temperature was initially set to 80 °C and held for 2 min, then increased to 160 °C at a rate of 10 °C $min^{-1}$ and finally increased to 265 °C at a rate of 3 °C $min^{-1}$ and held at this temperature for 15 min. Helium was used as carrier gas and the injection mode was splitless with a purge time of 0.75 min. The injection volume of the samples was 1 μL (de Souza Pinheiro, and de Andrade, 2009).

### 6.2.8 Data Analysis

The analysis of data and plotting of graphs were carried out with OriginPro2020b software.

## 6.3 Results and discussion

### 6.3.1 Characterization

Different techniques were used to investigate the effectiveness of the modified CD, and characterization was carried out to confirm the formation of the inclusion complex. Figure 6.3 shows FTIR spectra of CD, chitosan and sodium alginate IC (A), CD and chitosan (B), CD and sodium alginate (C), Chitosan (D), sodium alginate (E) and CD (F). The peaks at 3200-3300 cm$^{-1}$ observed from B, C and D are associated with the O-H bond and indicate the presence of water molecules and the presence of hydrogen bonds within the synthesized material. The band observed at 3370 cm$^{-1}$ in chitosan (Figure 6.3A) shows the presence of amine group. The C-H stretch can be seen at 2881, 3000, 2935, 3000 and 2932 cm$^{-1}$ in all the spectra except for D which is the synthesized system.

Comparing the different spectra in Figure 3, it can be noted that some peaks disappeared, shifted or decreased in intensity, these are peaks such as the C=O that is seen in Figure 6.3F at 1743 cm$^{-1}$ that is not visible at the obtained synthesized material, it is also notable that the peak at 813 cm$^{-1}$ associated with the Na-O stretch in sodium alginate (Figure 6.3E ) is not visible in Figure 3D (the obtained system), all these lead to the suggestion that the inclusion complex was successfully synthesized as the chitosan and alginate are encapsulated in the cyclodextrin cavity (Ojemaye *et al.*, 2017, Ivancic et al., 2016, Thurein *et al.*, 2018). The morphology of CD and sodium alginate (Figure 6.4A), CD and chitosan (Figure 6.4B) and CD, chitosan and sodium alginate inclusion complex (Figure 6.4C) were studied using Scanning Electron Microscope (SEM) (Figure 6.4) at same magnification. Comparing the three micrographs, it can be seen that all three have irregularly shape rough surfaces. The CD and alginate complex (Figure 6.4A) has a porous morphology rougher surface when compared to Figures 4B and C.  The amorphous structure of the three complexes shows no significant change in the morphology of the adsorbent.

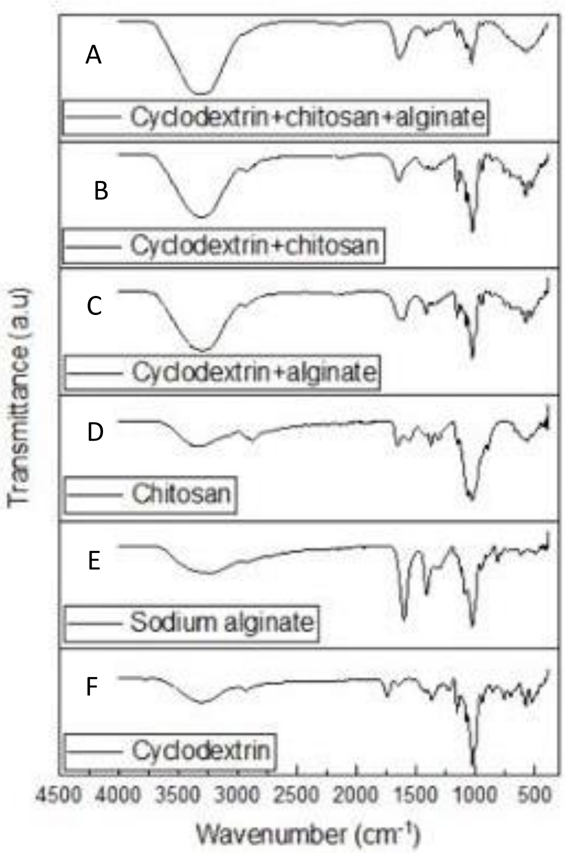


Figure 6.3: FTIR spectra of (A) CD, chitosan and sodium alginate IC, (B) CD and chitosan, (C) CD and sodium alginate, (D) Chitosan, (E) sodium alginate and (F) CD.

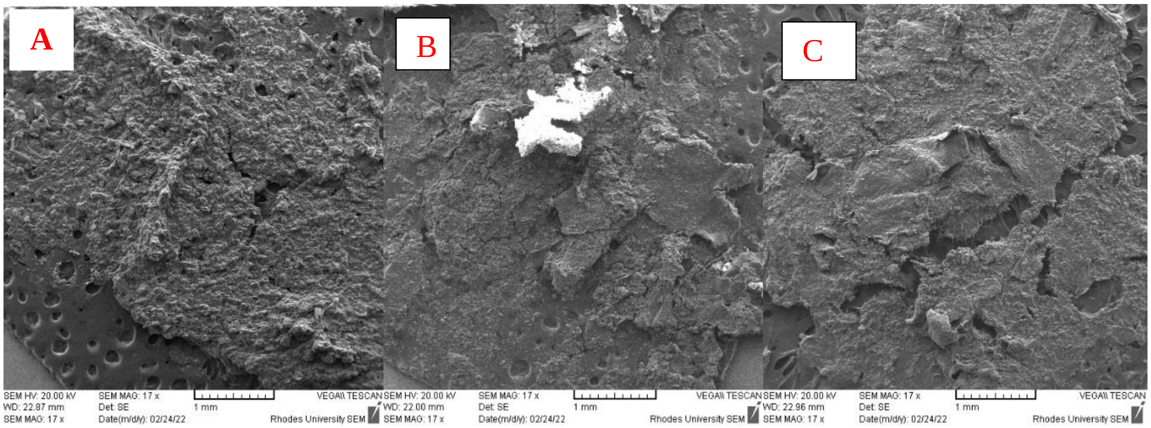


Figure 6.4: SEM micrograms of (A) CD and sodium alginate, (B) CD and chitosan and (C) CD, chitosan and sodium alginate IC.

The elemental composition in each synthesized material were studied using energy dispersive X-ray spectroscopy (EDX) (Figure 5). In Figure 6.5A, the peaks corresponded to the elements C, N, O, Na and Ca with percent weight composition of 32.02, 9.14, 48.34, 10.18 and 0.32 % respectively. The peaks in Figure 6.5B corresponded to the elements C, N, O, Na and Cl with weight percent of 45.43, 3.52, 50.68, 0.21 and 0.16 respectively. In a study by Yadav *et al* (2020) where β-CD was modified using activated charcoal and alginate polymer for the removal of cationic dye, the SEM results showed a rough porous structure and large surface area indicating suitable binding sites for the methylene blue dye molecules. The EDX results showed the disappearance of Mn(II) that came from the chemical composition of the activated charcoal after adsorption by the nanocomposite.

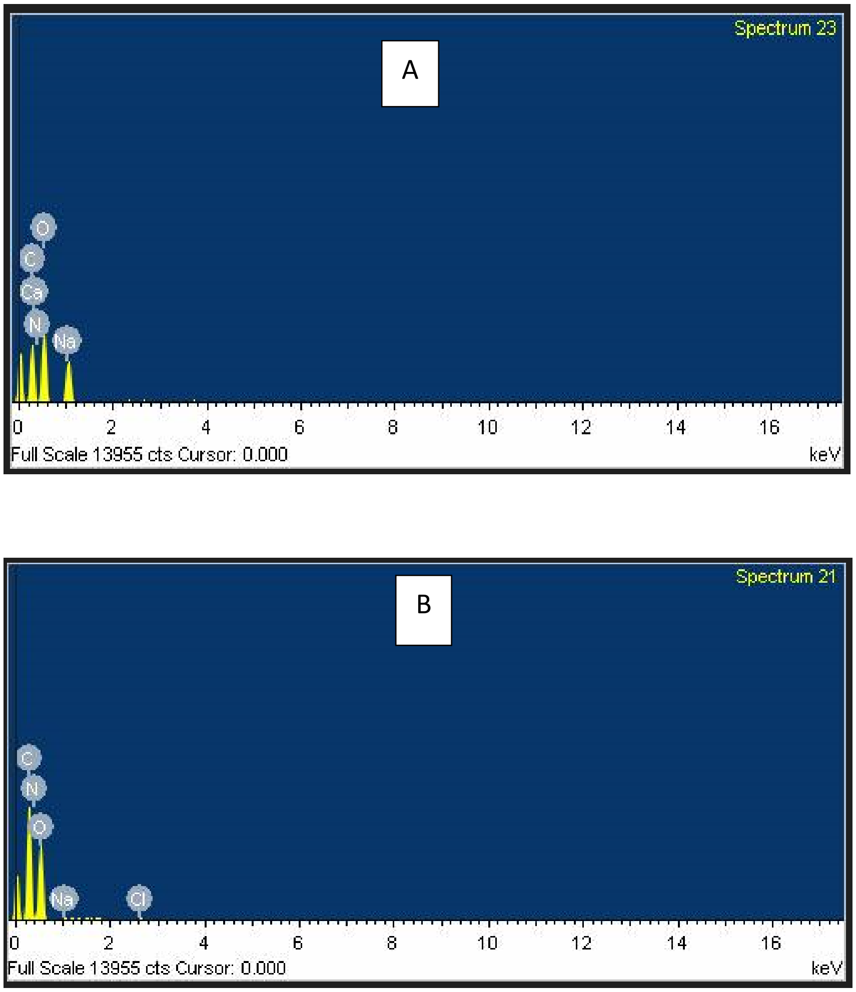


Figure 6.5: EDX images of CD-Alginate (A) and CD-Chitosan (B)

### 6.3.2 Adsorption experiments

To determine the efficiency of the synthesized adsorbent CD-chitosan-alginate for the removal of organophosphate pesticide, Parathion from soil and sediment, different parameters such as concentration, time, pH and dose were varied in order to find the optimum conditions for the removal of this pesticide from sediment/soil.

#### 6.3.2.1 Effect of pH

Effect of pH on the synthesized adsorbent was observed at a pH range of 2-10 at 150 rpm, 3 mL of 60 mM adsorbate concentration. The result showed that the adsorption of Parathion from the sediment was highest at pH 2 and lowest at pH 6 (Figure 6.6). The adsorption percentage fluctuated from pH 2 with no clear pattern, as such subsequent adsorption experiments were at this pH.

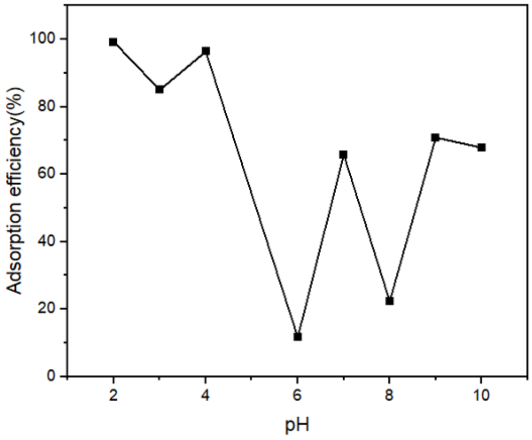


Figure 6.6: Effect of pH on adsorption of Parathion

#### 6.3.2.2 Effect of time

Figure 6.7 shows the effect of time on the adsorption of Parathion, where the adsorption was taken from different time intervals. A rise in the adsorption percentage was observed as the time increased from 10 to 30 minutes, and the optimum adsorption takes place at 15 minutes; while from 30 minutes, a decrease in the adsorption efficiency was observed. At durations above 60 minutes, the adsorption of parathion remained constant. The initial increase in the adsorption efficiency is an indication of the availability of active sites in the adsorbent leading to a strong interaction between the adsorbent and the adsorbate (Ojemaye and Okoh, 2019), while the gradual decrease of the adsorption efficiency indicates weak interactions between the adsorbent and the adsorbate due to fewer active sites on the adsorbent.

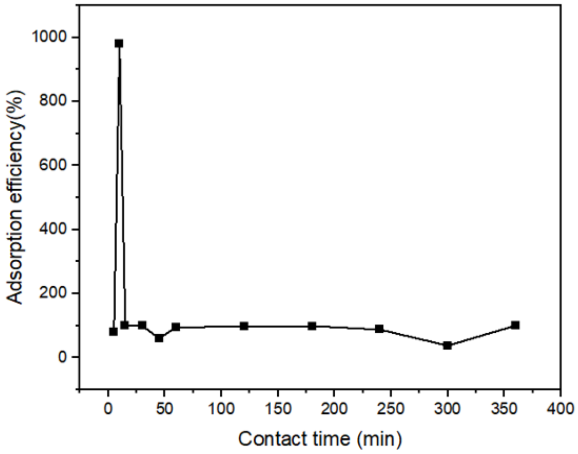


Figure 6.7: Effect of time on adsorption of Parathion

### 6.3.2.3 Effect of concentration

The effects of concentration and dose on the adsorption were conducted as presented in Figures 6.8 and 6.9 respectively. The effect of the dose was investigated by keeping the concentration of the adsorbate constant at 60 mM and varying the volume of the adsorbent (1, 3 and 5 mL) (Figure 6.9), while for the effect of concentration two concentrations, 80 and 100 mM were used while keeping the dose constant at 3 mL, linear graphs with a positive gradient were obtained for both, meaning that the adsorption increased constantly with an increase in dose and an increase in concentration.

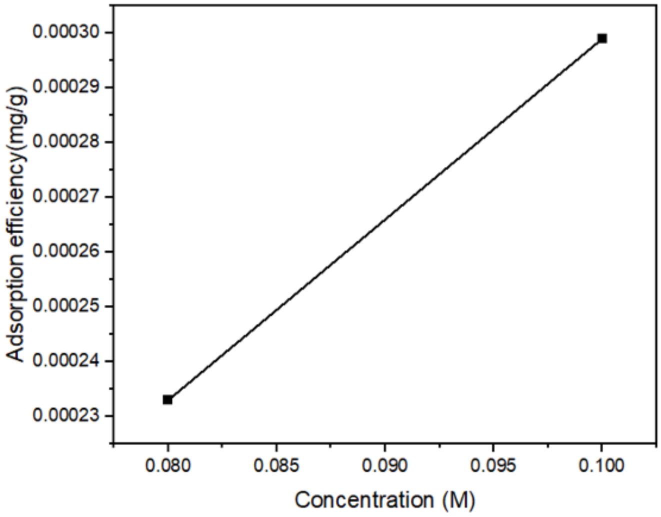


Figure 6.8: Effect of concentration on adsorption of Parathion

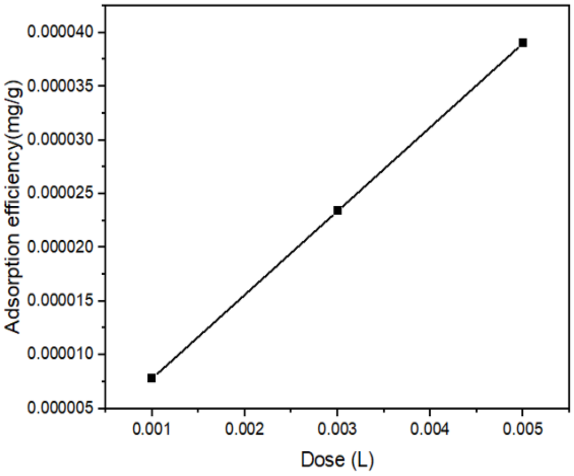


Figure 6.9: Effect of adsorbent dose on the adsorption of Parathion

### 6. 3.2.4 Adsorption Isotherms

To determine the process of adsorption of Parathion onto the CD-chitosan-alginate IC, three isotherm models, namely Langmuir, Freundlich and Temkin equations were used over two concentrations (0.08 and 0.1 M). The isotherm parameters obtained are shown in Table 6.1. Langmuir equations that assumes the surface as homogenous can be represented by the equation:

$$\frac{Ce}{qe} = \frac{1}{qmax * b} + \frac{Ce}{qmax} \qquad (6.1)$$

Where, $q_{max}$ is the maximum adsorption capacity in mg/g and b is the Langmuir constant in L/mg, the Langmuir isotherm parameters were calculated using the linear equation obtained from the plot of 1/qe against 1/Ce (Liu *et al*., 2019). In the Freundlich isotherm, a monomolecular layer is formed by the adsorbate on the surface of the adsorbent and can be represented by the equation:

$$\log(qe) = \log Kf + \frac{1}{n}\log C \qquad (6.2)$$

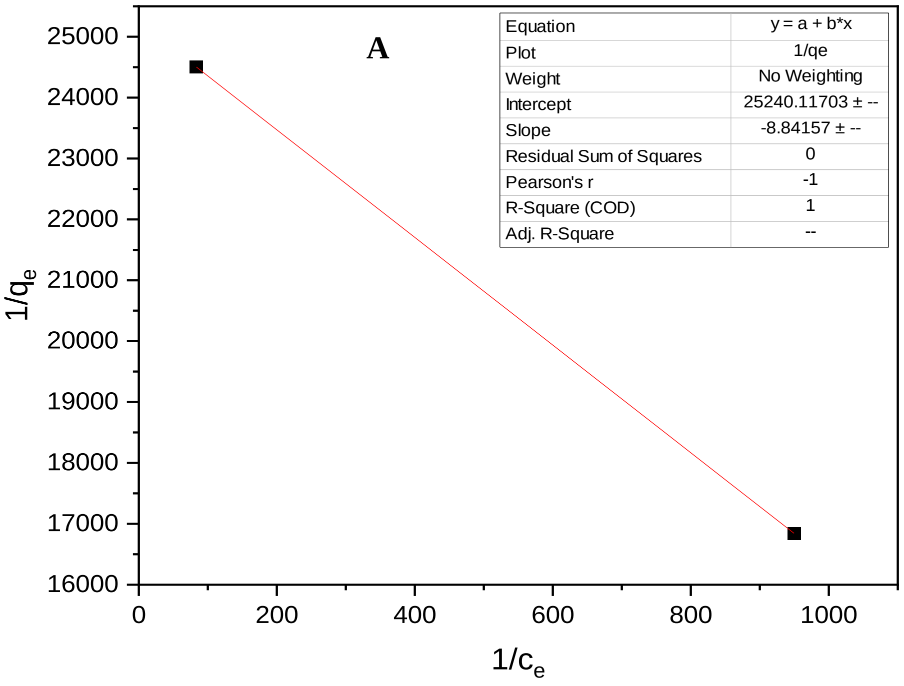
Where $K_f$ is the Freundlich constant in mg/g $(L/mg)^{1/n}$ and n is the heterogeneity factor, where the values obtained for $\frac{1}{n}$ measure the intensity and determine the shape of the Ce vs qe plots (Singh and Singh, 2016).

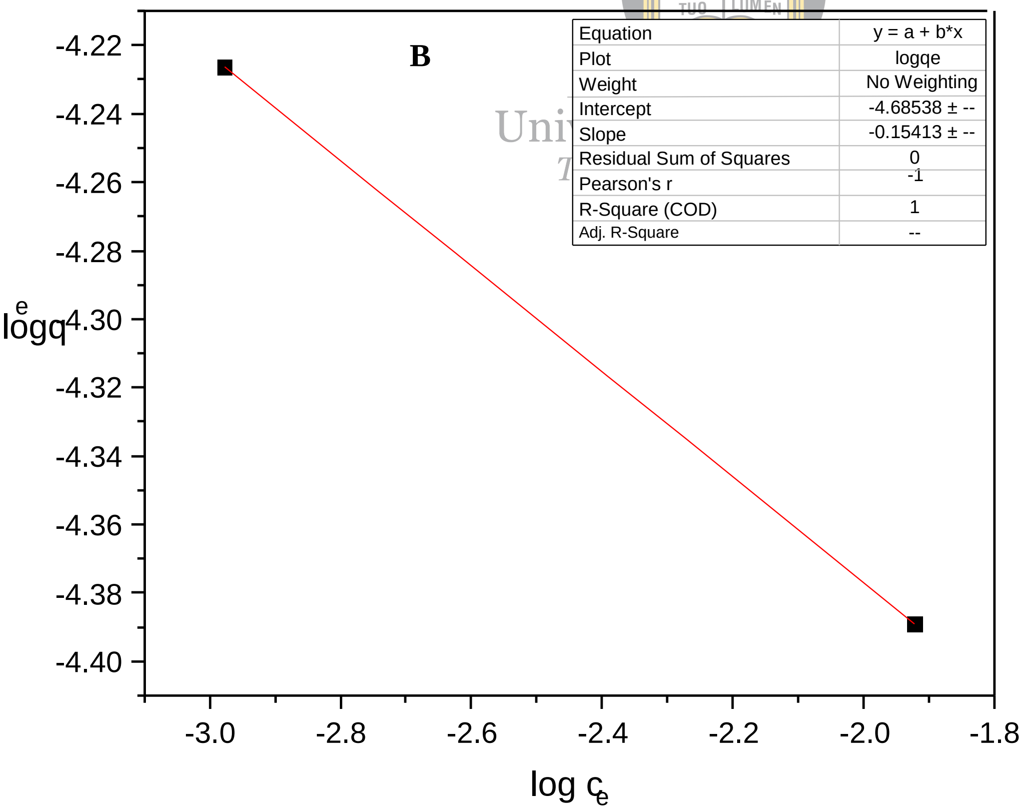
The Temkin adsorption isotherms are represented by the equation:

$$qe = BlnKT + \text{Bln Ce} \qquad (6.3)$$

Where B is the Temkin constant in J/mol and $K_T$ is the equilibrium binding constant in L/g. The Temkin parameters shown in Table 1 were obtained by plotting the graph of $q_e$ against $lnC_e$.

Comparing the three models, it was found that all the isotherms fitted the experimental data with $R^2$ =1, which is a good mathematical approximation to describe the adsorption equilibrium. However, the Langmuir adsorption isotherm gave a negative $K_L$ value while the Frendlich and Temkin isotherms gave positive values of $K_f$ and $K_T$ as shown in Table 6.1. The negative $K_L$ obtained is an indication that at a specific point where the adsorbent mass is increased, the adsorption capacity reaches a specific limit and adsorption does not follow Langmuir assumptions (Perwitasari *et al*., 2021). The Freundlich isotherm gave a negative value of the adsorption intensity (1/n), meaning the adsorption is not favoured. The Temkin isotherm was used to estimate the heat sorption process and it was found to be less than 1, which means that physical adsorption occurs (Ayawei *et al*., 2017).


A
Equation y = a + b*x
Plot 1/qe
Weight No Weighting
Intercept 25240.11703 ± --
Slope -8.84157 ± --
Residual Sum of Squares 0
Pearson's r -1
R-Square (COD) 1
Adj. R-Square --
$1/q_e$
25000
24000
23000
22000
21000
20000
19000
18000
17000
16000
0
200
400
600
800
1000
$1/c_e$


B
Equation y = a + b*x
Plot logqe
Weight No Weighting
Intercept -4.68538 ± --
Slope -0.15413 ± --
Residual Sum of Squares 0
Pearson's r -1
R-Square (COD) 1
Adj. R-Square --
$\log q_e$
-4.22
-4.24
-4.26
-4.28
-4.30
-4.32
-4.34
-4.36
-4.38
-4.40
-3.0
-2.8
-2.6
-2.4
-2.2
-2.0
-1.8
$\log c_e$

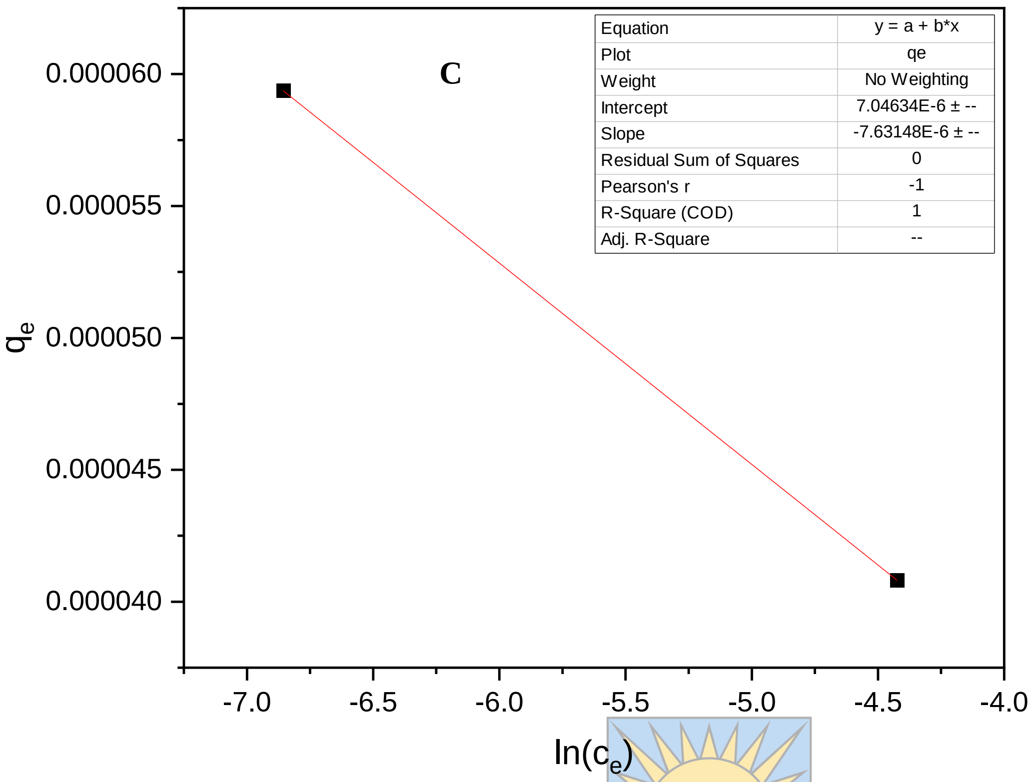


Figure 6.10: Langmuir (A), Freundlich (B) and Temkin (C) adsorption Isotherms for Parathion onto CD-chitosan-alginate IC (pH: 2, adsorbent dose: 3 mL and time: 30 minutes).

**Table 6.1:** Parathion adsorption isotherms' parameters.

| Adsorption isotherms | Parameters | Values |
|---|---|---|
| Langmuir isotherm | $K_L$ (L/mg) | -2854.709857 |
| | $q_{max}$ (mg/g) | $3.962 \times 10^{-5}$ |
| | $R^2$ | 1 |
| Freundlich isotherm | $K_F$ ((mg/g)(L/mg)$^{1/n}$) | $2.064 \times 10^{-5}$ |
| | n | -6.488 |
| | $R^2$ | 1 |
| Temkin isotherm | $K_T$ (L/mg) | 2.518 |
| | B (J.mol$^{-1}$) | $-7.631 \times 10^{-6}$ |
| | $R^2$ | 1 |

### 6.4 Removal of Parathion from real soil and sediment samples from the Buffalo River estuary and a nearby dumpsite

Removal of Parathion from soil and sediment samples was performed at the optimised conditions of pH 2, 60 mM while using 3 ml of 5% CD-chitosan-sodium alginate solution for 30 minutes, using the method described in section 2.5. Table 6.2 shows the initial and final concentrations of the soil and sediment samples taken from six sites from the Buffalo River estuary, before and after the removal. The total average percentage of adsorption efficiency is 68.78% for sediments, with the highest adsorbed percentage observed in points 1 and 2, followed by points 5 and 6, respectively.

**Table 6.2**: Initial and final concentrations of Parathion from sediment and soil samples

| **Parathion** | **Sed1** | **Sed2** | **Sed3** | **Sed4** | **Sed5** | **Sed6** | **Soil** |
|---|---|---|---|---|---|---|---|
| $C_i$ (mM) | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| $C_f$ (mM) | ND | ND | 41.92 | 53.57 | 9.10 | 7.81 | 38.09 |
| % adsorbed | 100 | 100 | 30.13 | 10.72 | 84.83 | 86.98 | 36.52 |

$C_i$- initial concentration, $C_f$- final concentration

## Conclusion

In this report, β-CD was modified with natural polymers to form an inclusion complex. CD-chitosan-sodium alginate IC was synthesized for the removal of a commonly used organophosphate pesticide, Parathion, from sediment and soil samples. Characterization showed that this complex was synthesized successfully and the different adsorption experiments carried out, ascertained the ideal conditions for the removal of Parathion from solid samples. In light of the results obtained from this study, it can be concluded that the adsorbent used in this study is efficient and can be used to remove organophosphate pesticides especially, Parathion from sediment and soils at the optimised conditions.

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# CHAPTER SEVEN

## GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

### 7.1 Discussion

South Africa's economy depends greatly on the agriculture industry, as such more than 3000 pesticides belonging to 700 different brand names are currently legally registered in South Africa and are used for agriculture and domestic purposes (Olisah *et al*., 2022). OP poisoning is the most common in many developing countries including South Africa, and these pesticides continue to be accessible in the market and are not monitored properly (Razwiedani and Rautenbach, 2017). In a study by Zhang *et al*., 2009, it was reported that in areas where OPs are used, suicide rates are usually high because exposure to these pesticides can lead to mortality due to mental disorders and other conditions such as depression and anxiety. As a result of the geographic and seasonal patterns of pesticide use in a particular region, pesticide residues are widespread in rivers and estuaries (Sud and Kaur, 2012). There has been evidence that the water quality of the available sources has declined in South Africa as a result of the increase in pollution by industries such as agriculture and urbanization (Musingafi, 2014).

In this study, four organophosphate pesticides, namely, Ronnel, malathion, Parathion and Dursban were extracted from sediment and soil samples and analysed using GC-µECD. These pesticides are used by farmers and their residues are commonly detected in non-target sites. The recovery for these pesticides were generally within the accepted range of 70 to 120% (Kuet and Seng, 2003). The recoveries were ranging from 84.88 to 102.07%. Although, Malathion had a recovery of 57.78% that was below the acceptable range, this could be due to poor extraction efficiency. These results indicate that the method used in this study can be employed to quantify organophosphate pesticides. The calibration curves plotted for the four OPs were linear with correlation coefficients ($R^2$) ranging from 0.9935 to 0.9972 and the relative standard deviation (RSD) was generally less than 11 (USEPA, 2007). Based on the results obtained, the method used in this study can be utilized to measure organophosphate levels. The limits of detection and quantification (LOD and LOQ) were calculated using 3.3 (Sy/S) and 10* (Sy/S), respectively (Zhang *et al*.,2021; Moosavi and Ghassabian, 2018). The LOD and LOQ of the

OPs ranged from 0.09 to 41.76 and 31.46 to 216 µg/L, respectively. The total average percentage of adsorption efficiency for sediments was 68.78% and 36.52% for soil.

### 7.2 Conclusion

In this study, the occurrence of OP pesticides in sediments from the Buffalo River estuary was investigated using GC-µECD instrument and the methods used were validated. GC-µECD gives appropriate sensitivity, detection and precision. The RSD was ≤ 11, and the samples were extracted using an ultrasonicator, which saves time by allowing extraction of multiple samples at a time. The solvents used for extraction of OPs in this study were dichloromethane, acetone, ethyl acetate, and cyclohexane, in different ratios because they gave the best recovery when compared to other solvents like ethyl acetate and methanol. In the same manner, the ratio (3:2:1:1) used for extraction in this study gave best recovery than 2:1:1:1. The dominant pesticide found in the Buffalo river estuary was malathion, a pesticide commonly used in controlling insects that destroy fruits and vegetables. Points 1,3 and 4 of the study area had the highest MC, OC and HEM; thus, it can be concluded that the source of pollution in points 3 and 4 could be the creeks at the sites. The oil and grease was the only physicochemical property that decreased from winter to spring and this can be due to natural evapour ation processes and decrease in anthropogenic activities. Characterization of the synthesized CD-chitosan-sodium alginate IC showed that the complex was successfully synthesized for the removal of Parathion from sediment and soil samples. The different adsorption experiments carried out, ascertained the ideal conditions for the removal of Parathion from solid samples. Considering the adverse effects of OP pesticides their sale and use should be monitored by appropriate government agencies so as to ensure environmental laws are obeyed.

## 7.3 Recommendations

- GC-MS should be employed in confirmation and validation of the results obtained from GC-µECD.
- Further investigation is needed in order to discover the exact sources of OPs in the Buffalo River estuary.
- Water samples and other matrices should be considered in future studies.
- Monitoring of the river may be mandatory so as to safeguard the mariculture activities taking place.

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# APPENDICES

## Appendix 1

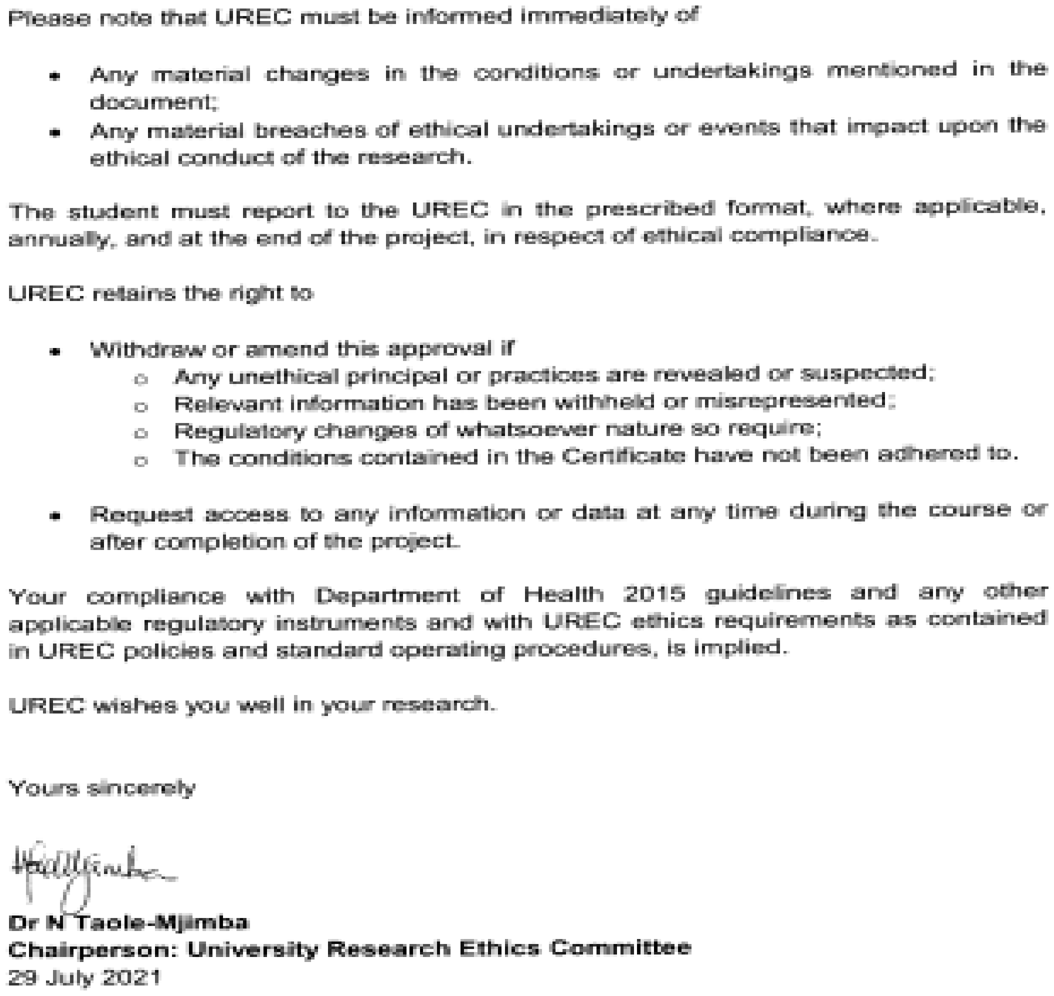
University of Fort Hare
*Together in Excellence*

### ETHICS CLEARANCE
### REC-270710-028-RA Level 01

| | |
|---|---|
| Project Number: | OKO011SMDE01 |
| Project title: | **Organophosphate and Carbamate Pesticides in the environmental matrices of the Buffalo River estuary and their removal using modified cyclodextrins.** |
| Qualification: | Masters in Chemistry (Full Dissertation) |
| Student name: | Nonkululeko Landy Mdeni |
| Registration number | 201506727 |
| Supervisor: | Prof O Okoh |
| Department: | Chemistry |
| Co-supervisor: | N/A |

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby grant ethics approval for OKO011SMDE01. This approval is valid for 12 months from the date of approval. Renewal of approval must be applied for BEFORE termination of this approval period. Renewal is subject to receipt of a satisfactory progress report. The approval covers the undertakings contained in the above-mentioned project and research instrument(s). The research may commence as from the 15/06/21, using the reference number indicated above.

Note that should any other instruments be required or amendments become necessary, these require separate authorisation.



Please note that UREC must be informed immediately of

- Any material changes in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The student must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

UREC retains the right to

- Withdraw or amend this approval if
    - Any unethical principal or practices are revealed or suspected;
    - Relevant information has been withheld or misrepresented;
    - Regulatory changes of whatsoever nature so require;
    - The conditions contained in the Certificate have not been adhered to.
- Request access to any information or data at any time during the course or after completion of the project.

Your compliance with Department of Health 2015 guidelines and any other applicable regulatory instruments and with UREC ethics requirements as contained in UREC policies and standard operating procedures, is implied.

UREC wishes you well in your research.

Yours sincerely

**Dr N Taole-Mjimba**
**Chairperson: University Research Ethics Committee**
29 July 2021

## Appendix 2

Physicochemical parameters calculations

1. **Particle size (%)**

= (mass of material in sieve/ initial mass of sediment) * 100%

2. **Percent organic matter**

$$OM\% = \frac{W105 - W500}{W105}(100)$$

OC% = (OM%) (0.58)

3. n-Hexane extractable material (HEM)/ oil and grease

$$HEM\left(\frac{mg}{kg}\,wet\ weight\right) = \frac{\text{gain in weight of flask(mg)} \times 1000}{weight\ of\ wet\ solid(g)}$$

## Appendix 3

Calculations showing how final concentrations of individual analytes were obtained in this study

1. Concentration of the analyte (C, in µg/L) = A - B

Where:

A = Average concentration of analyte obtained from the instrument (µg/L)

B = Average concentration of blanks

2. Final concentration of analyte (µg/L) = C * D/E

Where:

C= Concentration of the analyte in µg/L

D= Final volume of extract in L

E= Initial mass of extracted sediment/ soil sample in g

For example, Parathion concentration extracted from Buffalo River estuary sediment sample in winter (Point 4) was calculated thus.

Parathion

$$(\mu g/g) = \left(\frac{216.63 - 115.9\mu g}{L}\right)\left(\frac{0.001L}{g}\right)$$

$$= \left(\frac{100.73\mu g}{L}\right)\left(\frac{0.001L}{g}\right)$$

$$= 25.18\mu g/g$$

## Appendix 4

Chromatographic peaks at 1280 µg/L for the analytes

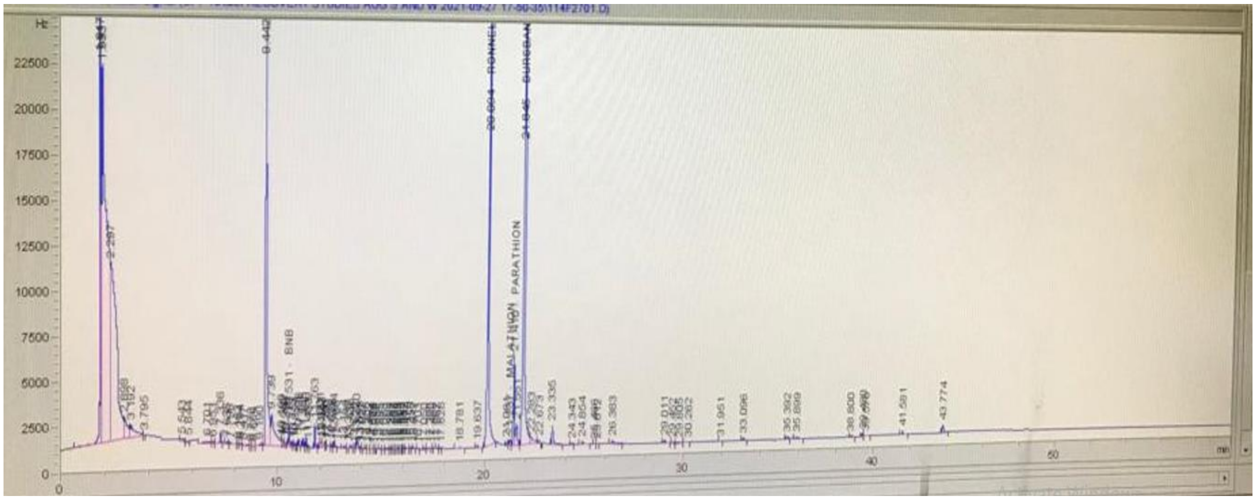


Figure 1: Chromatogram peaks at 1280 µg/L for the surrogate standard and OPs

## Appendix 5

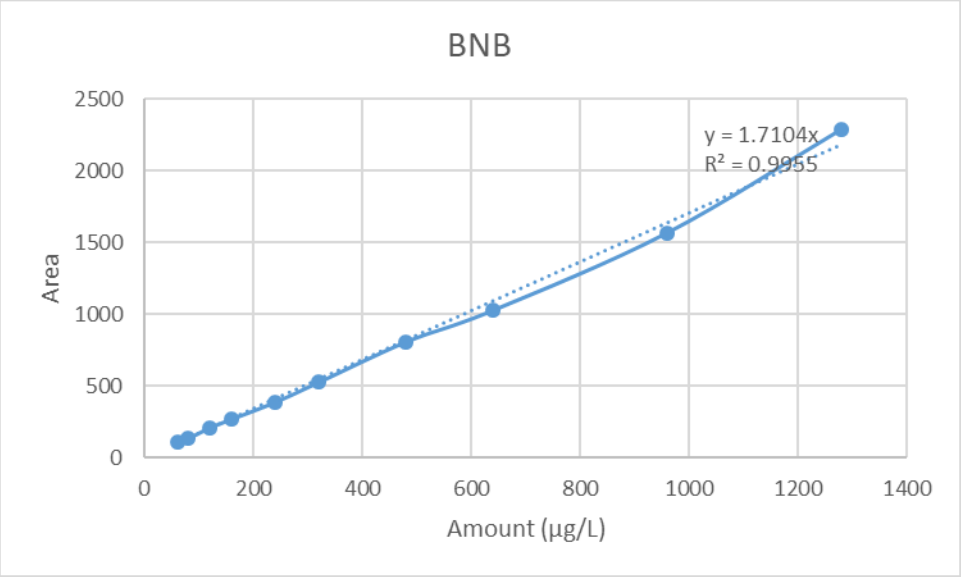


Figure 2: Regression coefficient for BNB on the calibration of OPs

## Appendix 6

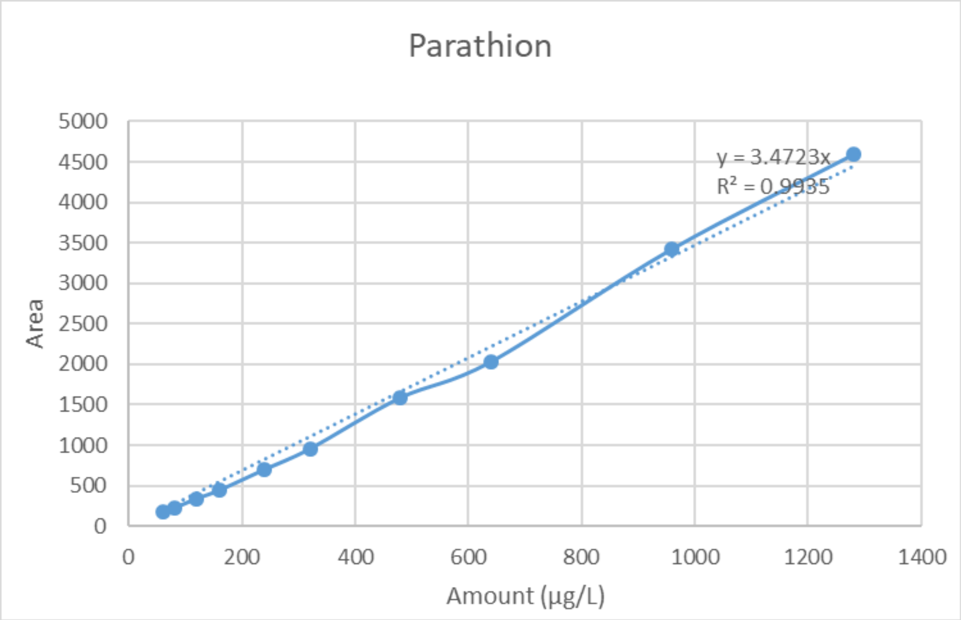


Figure 3: Regression coefficient for Parathion on the calibration of OPs

## Appendix 7

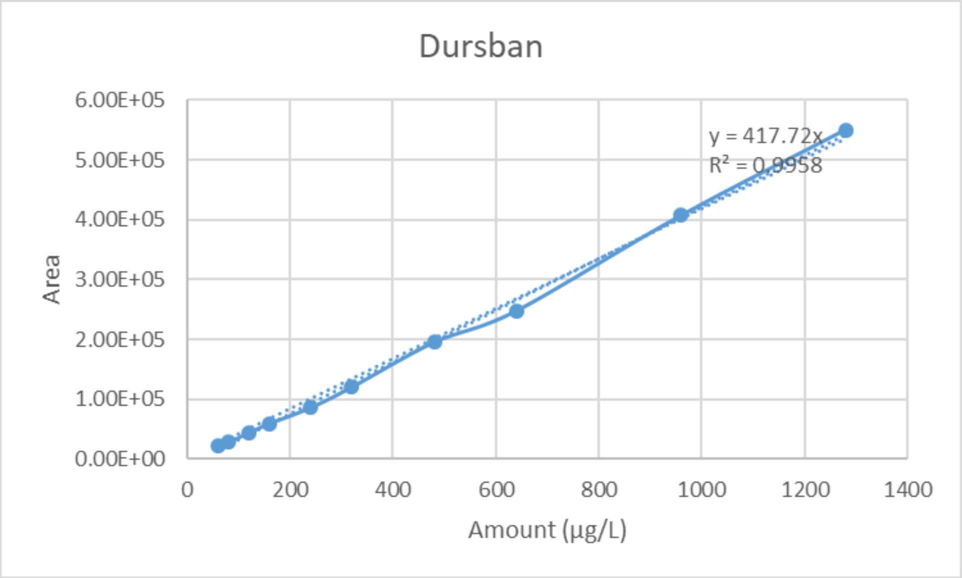


Figure 4: Regression coefficient for Dursban on the calibration of OPs

## Appendix 8

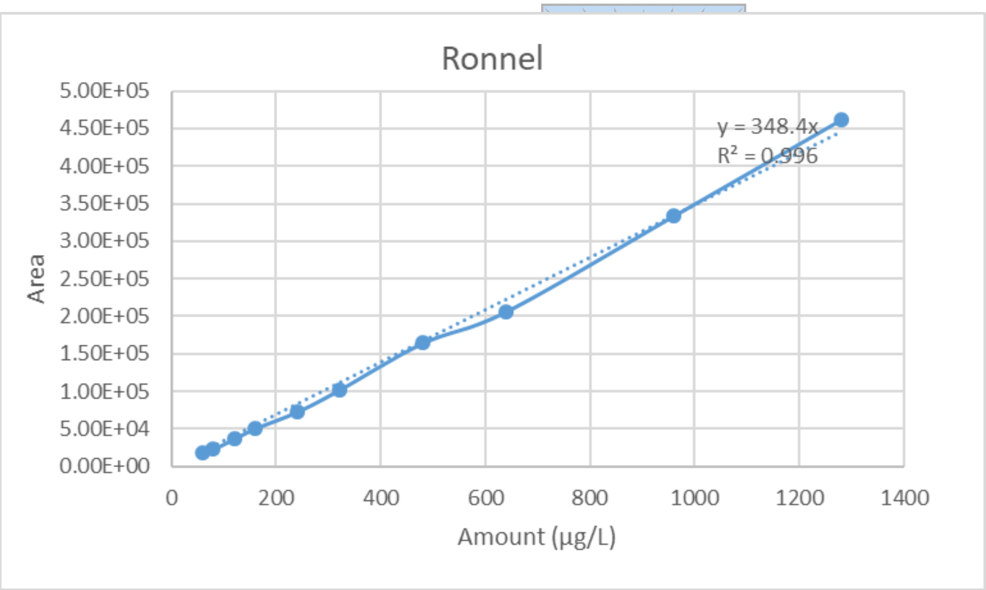


Figure 5: Regression coefficient for Ronnel on the calibration of OPs

## Appendix 9

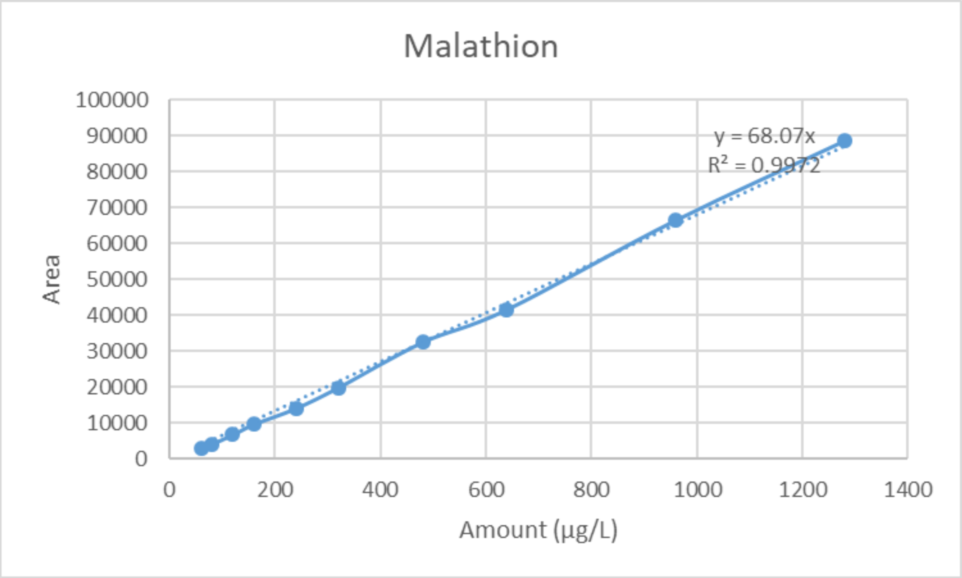


Figure 6: Regression coefficient for Malathionon the calibration of OPs

## Appendix 10

Calculations showing how the adsorption capacity was calculated both

Adsorption capacity (mg/g) = $(C_i – C_f \ / m)* V$

Adsorption capacity (%) = $(C_i – C_f / C_i)*100$

Where: $C_i =$ Initial concentration in mM/ M

$C_f$ = Final concentration in mM/ M

m = mass of adsorbent in g

V = Volume of adsorbent in L/ ml

## Appendix 11

Effect of adsorbent/ dose on the adsorption capacity of Parathion from solid samples

| Adsorbent (L) | pH | Time (min) | Sediment (g) | Adsorbate Concentration (M) | Amount of CD-Chitosan-Alginate (µg/L) | Adsorption efficiency (mg/g) |
|---|---|---|---|---|---|---|
| 0.001 | 2 | 30 | 1 | 0.06 | 7.80296 | $7.80*10^{-6}$ |
| 0.003 | 2 | 30 | 1 | 0.06 | 0 | $2.34*10^{-5}$ |
| 0.005 | 2 | 30 | 1 | 0.06 | 0 | $3.90*10^{-5}$ |

## Appendix 12

Effect of concentration on the adsorption capacity of Parathion from solid samples

| Adsorbent (L) | pH | Time (min) | Sediment (g) | Adsorbate Concentration (M) | Amount of CD-Chitosan-Alginate (µg/L) | Adsorption efficiency (mg/g) |
|---|---|---|---|---|---|---|
| 0.003 | 2 | 30 | 1 | 0.08 | 11.9783 | 0.000233 |
| 0.003 | 2 | 30 | 1 | 0.1 | 1.05306 | 0.000299 |

**Appendix 13**

Effect of pH on the adsorption capacity of Parathion from solid samples

| Adsorbent (ml) | pH | Time (min) | Sediment (g) | Adsorbate Concentration (mM) | Amount of CD-Chitosan-Alginate (µg/L) | Adsorption efficiency (%) |
|---|---|---|---|---|---|---|
| 3 | 2 | 30 | 1 | 60 | 4.07 | 99.2 |
| 3 | 4 | 30 | 1 | 60 | 18.4 | 96.4 |
| 3 | 6 | 30 | 1 | 60 | 347 | 31.8 |
| 3 | 7 | 30 | 1 | 60 | 319 | 37.2 |
| 3 | 8 | 30 | 1 | 60 | 45.0 | 91.2 |
| 3 | 9 | 30 | 1 | 60 | 148 | 70.9 |
| 3 | 10 | 30 | 1 | 60 | 163 | 67.9 |

**Appendix 14**

Effect of time on the adsorption capacity of Parathion from solid samples

| Adsorbent (ml) | pH | Time (min) | Sediment (g) | Adsorbate Concentration (mM) | Amount of CD-Chitosan-Alginate (µg/L) | Adsorption efficiency (%) |
|---|---|---|---|---|---|---|
| 3 | 2 | 10 | 1 | 60 | 4.07 | 0 |
| 3 | 2 | 30 | 1 | 60 | 4.03 | 99.2 |
| 3 | 2 | 45 | 1 | 60 | 247 | 51.4 |
| 3 | 2 | 60 | 1 | 60 | 25.2 | 95.0 |
| 3 | 2 | 120 | 1 | 60 | 14.8 | 97.1 |
| 3 | 2 | 180 | 1 | 60 | 7.51 | 98.5 |
| 3 | 2 | 300 | 1 | 60 | 61.5 | 87.9 |
| 3 | 2 | 360 | 1 | 60 | 278 | 45.4 |