REMOVAL OF SELECTED CHLORINATED PHENOLIC COMPOUNDS FROM WATER SOURCES IN VAAL TRIANGLE USING HPLC, MACADAMIA NUTSHELL ACTIVATED CARBON AND SOLID PHASE EXTRACTION

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DECLARATION

I, Sechaba Machedi declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Technology (M-Tech): Chemistry at Vaal University of Technology, Vanderbijlpark. It has not been submitted before for any degree or examination at any other University.

(Signature of Candidate)

--------------- 10------------ December -------- 2017

DEDICATION

This is dedicated to my family, daughter and wife. They kept me going.

ACKNOWLEDGEMENTS

ABSTRACT

In this study, analytical method for determining the chlorinated phenols in water was developed using High Performance Liquid Chromatography. The following four compounds which are 2, 4, 6- Trichlorophenol (2, 4, 6 TCP), 3-chlorophenol (3CP), 2, 4- Dichlorophenol (2, 4 DCP) and 4-chloro-3-methylphenol (4C3MP) were identified and quantified with a High Performance Liquid Chromatography (HPLC). The validation parameters tested were,: linearity, trueness, precision, detection limit of quantitation, sensitivity, specificity, selectivity. The linear calibration ranges of five standard solution from 1-10 ppm. The linearity ranges between 0.9298-0.9813. The activated carbon based on the waste macadamia nutshell activated carbon (MAC) was investigated for its potential uses as an adsorbent for chlorinated phenols removal and compared with grafted macadamia nutshell activated carbon (GMAC). The adsorbent was characterized with Fourier transform infrared spectrophotometer (FTIR), scanning electron microscope (SEM) and thermo gravimetric analysis (TGA). The parameters such as pH, temperature, contact time, concentration and adsorbent were investigated by adsorption technique. The strata C18E has been used before for the same reason and therefore the research was based on mimic the functional group of solid phase extraction (SPE) into macadamia activated carbon (MAC). The functional groups in SPE C18E are benzene and octadecyl. MAC was grafted with strata C18E functional groups to compare its potential with the SPE. The pseudo-first-order and pseudo-second-order kinetic models were applied to verify the experimental data. The pseudo-second order exhibited the best fit for the kinetic studies for MAC adsorption. Chemical removal of chlorinated phenols from wastewater is necessary to reduce harmful products on the environment and human health. Chlorinated phenols have been previously listed as some of the highest priority contaminants and as well as mainly important capability carcinogenic toxins released from chemical plants. Their availability in water

supplies was perceived by their bad taste and smell. The acceptable chlorinated phenols concentration in portable water is 1 (mg/l) base on the approval of world health organization. The permanent checking of chlorinated phenols in environmental samples has a greater significance and stresses highly effectiveness, common selectively and great sensitively methods.

The maximum uptake of Phenol using weighed mass of MAC was found to be 78 % and for GMAC was 84% for both 2,4,6TCP. t=250 min, pH=5, C_o=1mg/l, T = 25 ^{o}C and m = 0.3 g/l were the optimum condition for Phenol-MAC system and GMAC system. Over all analysis of equilibrium model analysis indicates the fitness of Langmuir isotherm model to Phenol-MAC adsorption system, suggesting a monolayer adsorption of phenol on the surface of MAC. Phenol adsorption capacity of MAC was found to be decreasing with increase in temperature suggesting that the adsorption process was exothermic in nature, which was further supported by the negative values of change in enthalpy. Characterization of MAC and GMAC confirmed the mesoporous texture, highly carbonaceous nature and a higher effective surface area of 912 m^2/g. The highest phenol uptake capacity of GMAC was found to be 28.0049 mg/g. The optimal conditions for various process parameters are t = 250 min, pH=5, C_o=1mg/l, T = 25 ^{o}C and m = 0.3 g/l were the optimum condition for Phenol-GMAC system. Like Phenol-MAC system, the kinetics studies confirmed that Phenol-GMAC adsorption system can be described by pseudo- second-order kinetics model. Equilibrium model analysis indicates the fitness of Langmuir isotherm model to Phenol-MAC adsorption system, suggesting a monolayer adsorption of phenol on the surface of GMAC. Phenol adsorption capacity of GMAC was found to be decreasing with increase in temperature suggesting that the adsorption process was exothermic in nature, which was further supported by the negative values of change in enthalpy. The negative values of Gibb's free energy suggested that adsorption of phenol onto GMAC was a spontaneous process.

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LIST OF ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
SPE	Solid Phase Extraction
MAC	Macadamia Activated Carbon
GMAC	Grafted Macadamia Activated Carbon
CP	Chlorophenol
DCP	Dichlorophenol
TCP	Trichlorophenol
CMP	Chloro-methylphenol
T	Temperature
min	Minute
mg/L	Milligram per litre
Ppm	Parts per million
Mg	Milligram
L	Litre
C	Concentration
V	Volume
q_e	Amount at equilibrium
q_t	Amount at time
m	Mass
C_e	Concentration at equilibrium
EPA	Environmental Protection Agency
USEPA	US Environmental Protection Agency

WHO   World Health Organisation

RSD   Relative Standard Deviation

%R   Percentage recovery

-OH   Hydroxyl group

C-   Carbon

UV   Ultraviolet

FTIR   Fourier Transform Infrared spectroscopy

TGA   Thermogravimetric analysis

SPME   Solid-Phase Microextraction

CHAPTER 1

1. Introduction

1.1. Background

It is a well-known fact that global pollution has increased dramatically over the past couple of centuries. Our environment is filled with chemicals which pollute air, water, soil and food. The effects of industrialization, technology, and infrastructure have raised a concern, contaminating our resources, the environment, human health, and affecting quality of life. It is essential to find solutions to the pollution problem however it will be difficult to accomplish due to less information about the causes, levels, and effects of pollution. (Michael K. Sofoniou G and KouimtziSb, 2000).

The human race is influenced by the quality of water resources in the natural environment. In order to guarantee a secure and healthy life, the water consumed by everyone must be free from harmful chemical substances. The water released into the environment by water purification plants and private organizations should be regularly accessed and examined to maintain complete yielding to water framework act. Thus, treated waters should be safe for consumption and free from harmful compounds to guarantee a healthy life (Bagheri, Mohammadi and Salemi, 2004; Çoğ, 2007). However, there are environmental agencies for water which have given standards to ensure that water is free from contaminants, thus making safe water consumption possible. Water treatment requires adequate analysis and monitoring which includes determination of low level contaminants. The maximum level of contaminants present in drinking water should be so small that the effect is harmless to human life. Thus analytical methods are important to quantify the contaminants. (Jüri Ruut, Health Protection Inspectorate, Tartu, 2007)

Phenols and chlorinated phenols derivatives are harmful substances found in water (Nielsen et al., 1991). They are used in many industrial activities for the production of pesticides, insecticides, herbicides, and synthetic products (Nielsen et al., 1991; Ohlenbusch, Kumke and Frimmel, 2000). They are also used in the bleaching process of paper manufacturing. Purified chlorinated phenols exist as colourless crystalline solids, with the exception of 2-chlorophenol which is a clear liquid, while the technical grades may be light tan or slightly pink due to impurities (Gaylord and Jayne, 1964; Hasselgren and Tronstad, 1975).

Phenols which contain certain amount of pesticides and wood preservatives can lead to specific health damages even at the lowest concentration levels. Nevertheless, it is important to be aware of phenols and substituted phenols in environmental and biological samples. (Calace N, Nardi E, Petronio BM, 2002). However, phenolic compounds are not only generated by human activity, but they are also formed naturally, e.g. during the decomposition of leaves or wood. As an outcome, they are often found in the aquatic environment. (Ohlenbusch, Kumke and Frimmel, 2000). Phenol and its vapor are corrosive to the eyes, the skin and the respiratory tract.

Repeated or prolonged skin contact with phenol may cause second and third degree burns due to phenol's caustic and defatting properties. Phenols and its derivatives (phenolic) are considered to be among the most recalcitrant and hazardous contaminants due to their high toxicity for human life, aquatic life and others (Salame and Bandosz, 2003). Phenols and phenolic compounds find their way into the environment through a number of ways including   coal conversion processes, coke ovens, petroleum refineries, phenolic resin manufacturing, herbicide manufacturing, fiberglass manufacturing and petrochemicals (El-Ashtoukhy et al., 2013; Veeresh GS, Kumar P, 2004; Jadhav and Vanjara, 2004) European Union (EU) legislation has set a maximum allowed phenol concentration of 0.5 µg/L in tap water (Jüri Ruut, Health Protection Inspectorate, Tartu, 2007).

1.2. Description of the phenol compounds

Phenols are categorized as organic compounds related to alcohols, but they form stronger hydrogen bonds. The hydroxyl (-OH) group which is attached to a C (carbon) atom is part of an aromatic ring that characterizes the phenolic compound. The structural formula of phenol which is (C6H5OH), represents only a phenol as a simplest member of phenols, and there are phenols with structure C6H4OH-R, where R represents some groups like (Fig1.1) CH3, and NO2 (Ela et al., 2011). However, phenols in water are more soluble than alcohols and have a higher boiling point. Phenols are white solids at room temperature or highly toxic colourless liquids. (Montizaan et al., 1994).

1.2.1. Background on 2, 4, 6 Trichlorophenol
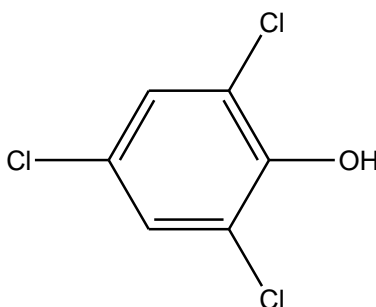
Figure 1. 1: showing the structure 2, 4,6 trichlorophenol

2,4,6-TCP is a chlorinated phenol that survives at room temperature as colourless to yellow crystal by means of a strong phenolic smell. It has been utilized mainly in several pesticides formulation and as a wood stabilizer. The methods that are possible for a human to be exposed to 2, 4, 6-TCP are inhalation, ingestion and dermal contact. People may be exposed 2, 4, 6-TCP in aquatic environment because of the chemical plants or industries that are discharging wastewater to dam and river without treating their effluent water. The 2, 4, 6-TCP has been identified in samples of river water, chemical plant effluent wastewater, treatment and tap water. In 1988 and 2005 over 88% of 2, 4,6-TCP was released to

underground injection well according to the U.S Environmental Protection Agency's Toxics Release Inventory. The compound may volatilize from water by means of a half-life of 20 days in a river or 150 days in a lake model. (Fattahi and Assadi, 2007)

1.2.2. Background on 3-Chlorophenol
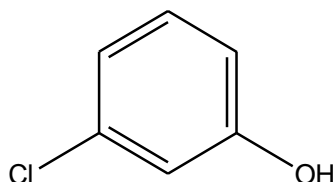
Figure 1. 2: showing the structure 3 chlorophenol

3-Chlorophenol (CP) is used to formulate chemical such as an antiseptic for animals and catalysts. The Special Health Hazard Substance have listed 3-chlorophenol because it is corrosive in the environment. The main nonpoint cause of toxic waste of 3-chlorophenol comes from the product of pesticides and chlorination of wastewater containing phenols. Evaporation half-life for 3-chlorophenol from 0.38 cm water bottomless 1.35-1.6 hours and 12.8-17.4 hours depending on the substitution of the chlorine to phenol chemical structure (Chiou and Martin, 1980; Shan-Li Wang a, Yu-Min Tzoua, Yi-Hsien Lua, 2007)

1.2.3. Background on 2,4-Dichlorophenol
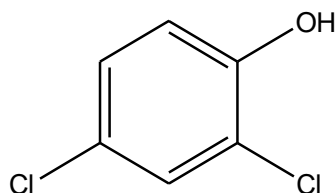
Figure 1. 3: Showing the structure 2,4-dichlorophenol

2,4-DCP is crystalline (sand-like) and is colourless solid, used in production of other chemical and act as an intermediate in making preservatives, disinfectants and herbicides.

Approximated in 1977, water emissions of 2,4-DCP were 741,000 pounds from U.S. production facilities (Scow et al. 1982). Volatilization of 2,4-DCP from water is usually slow. Liquid (molten) 2,4-DCP is readily absorbed through the skin and contact with large amounts may be fatal (Kintz, Tracqui and Mangin, 1992). "Accidental death caused by the absorption of 2,4-dichlorophenol through the skin". Arch.Toxicol 66 (298–9.) Solid 2,4-DCP does not readily absorb through skin and has a lower NFPA H=3 rating (versus H=4 for molten 2,4-DCP). This is primarily caused by instantaneous renal failure, liver damage and various other organ failure. (Anisuzzaman et al., 2015)

1.2.4. Background on 4-Chloro-3-methylphenol
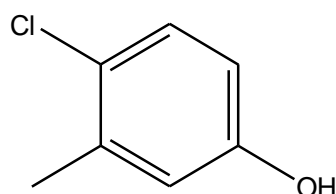
Figure 1. 4: Showing the structure 4-chloro-3-methylphenol

4-Chloro-3-methylphenol is a pinkish to white crystalline solid with a phenolic odour. Melting point 64-66°C. Shipped as a solid or in a liquid carrier. Soluble in aqueous base. Toxic by ingestion, inhalation or skin absorption. Used as an external germicide. Used as a preservative in paints and inks. The half-life of 4-C-3-MP is estimated for 21days in surface water. (Frasch et al., 2010; Peñalver et al., 1999)

Figure 1. 5: Phenols Structures (Ela et al., 2011)

1.3. Problem statement

The organic compounds in wastewater are a problem to the environment and to humans due to phenolic compounds discharged to the aquatic environment, as a result of these applications, they are found in soils and sediments and this leads to wastewater and ground water contamination. Owing to high toxicity and persistence in the environment, both the US Environmental Protection Agency (USEPA) and the European Union have included some of them in their lists of priority pollutants.

1.4. Aim

The aim of this study was to determine the concentration of selected chlorinated phenolic compounds from water sources in the Vaal River using HPLC with a UV detector and the removal using activated macadamia nutshell charcoal.

1.5.   Objectives

1. To develop HPLC method using SPE for determination of selected chlorinated phenols.

2. To compare the adsorption capabilities of activated macadamia nutshell carbon for selected chlorinated phenols with grafted activated macadamia carbon.

3. To modify macadamia nutshell activated carbon with the SPE functional group (benzene octadecyl).

4. To assess the adsorption capability of macadamia nutshell activated carbon after adding the benzene octadecyl group.

1.6.   The value of this study

The value of the study will be to provide the levels and extend of pollution due to phenols and their derivatives. Furthermore, it will give information on the performance of

octadecyl grafted macadamia activated carbon in terms of its adsorption capabilities.

(recovery), and eliminates problems associated with incomplete phase separation and yields quantitative extractions that are easy to perform (KUIPERS, J., REUTER, N., DE WITTE, 2007).

CHAPTER 2

2. Literature review

2.1. Four chlorinated phenols

Phenol consists of an aromatic ring (aromatic hydrocarbon) and a hydroxyl function group. It is the compound of the family of phenols with a molecular weight of 94. It was supplied by Sigma Aldrich with a purity of 99 %. The selection of this compound as model organic pollutant results from its frequency in the residual waters coming from the diverse industries. It is also one of the most reported aromatic pollutants in the literature and is the aromatic compound used as model for several years in our laboratory (Suwanprasop et al., 2005; Gillespie, Karklins and Creanga, 2007; Rouessac, Ayral and Ayral, 2009) .The substituted phenols studied here are 2,4,6 Trichlorophenol (2,4,6 TCP), 3-chlorophenol (3CP), 4-chloro-3-methylphenol(4C3MP) and 2,4 Dichlorophenol (2,4DCP). These compounds were selected due to their release in the residual waters from the many industries (petrochemical, pharmaceutical, papermaker, plastic, food-processing, etc.).

2.2. Method of extraction

A method for simultaneous determination of 11 phenols in drinking water was developed and validated. The analytes were extracted by solid phase extraction (SPE) with an Agilent Bond Elut Plexa cartridge, and separated by HPLC using an Agilent Poroshell 120 column. Overall recoveries ranged from 87% to 108%, with RSD values between 1.4% and 6.7%. The method was simple and effective for the extraction, enrichment, and analysis of multiple phenol compounds in drinking water. (Zhai, 2012)

An efficient extraction technique should be such that it can produce good results within a short time with minimum operator involvement. It should also be cheap, and safe for both the analyst and the environment. Each of the techniques has its own advantages and the choice of extraction depends on several factors including capital cost, operating cost, sample

matrix simplicity of operation, sample throughput and the availability of a standardized method (Oluseyi et al., 2011). SPE is a technique that is becoming increasingly popular, because unlike liquid-liquid extraction (LLE) it does not require large volumes of toxic organic solvents, analysis time can be decreased significantly and on-line and/or automated procedures are easily designed. Another advantage of SPE over LLE is the wide variety of extraction conditions which may be used to achieve the desired separation and concentration. The great variety of types of sorbents commercially available has increased the use of SPE (Habib and Hadjmohammadi, 2008).

Chromatographic techniques are among the most useful analytical methods. Gas and liquid chromatography were used in the analysis of some organic compounds: phenol, hydroquinone, benzoquinone, and maleic and fumaric acids. The analytical way for the determination of these compounds in water samples was investigated. Solid-phase extraction (SPE) technique was used on the sample preparation step, different divinylbenzene-based sorbents were applied. Calibration curves of given compounds were linear over the ranges: 50–500 μg/mL for phenol and its acetic derivatives, 50–1500 μg/mL for benzoquinone in GC analysis, and 50–250 μg/mL for phenol, 40–1000 μg/mL for hydroquinone, and 4–4500 μg/mL for carboxylic acids in HPLC analysis. The LOD and LOQ of proposed analytical procedure were in the ranges of LOD: 0.042–23.83 μg/mL; LOQ: 0.138–78.64 μg/mL. (Bielicka-Daszkiewicz et al., 2012). (Spivakov, Malofeeva and Petrukhin, 2006) and (LI, Y., George, J.E., Mscarty, C.L. & Wendelken, 2006) further argues that the C18 sorbent can be classified as reversed-phase sorbent with octadecyl (C18), octacyl (C8), ethyl (C2) and phenyl or as normal-phase sorbent with cyanopropyl, aminopropyl and diol functional groups. Their interaction mechanisms are mainly based on hydrophobic interaction (van der Waals forces), thus these SPE packing provide high recoveries for nonpolar analytes. Nevertheless, silica-based sorbents are unstable at extremes pH (2 > pH >8), and they have relatively low capacity and low recovery for basic analytes. Several types of modifications

were used to immobilize different compounds on the surface of classical silica-base sorbents to increase their selectivity. Furthermore, extraction could also be performed with membrane disks containing C18-bonded silica (8 μm particles) on polytetrafluoroethylene or glass fiber supports.

In this study (Barcel, 1995) with solid-Phase extraction system using eight different sorbents packed in a precolumn was coupled on-line with a liquid chromatograph with UV detection. The method used 50-100mL of ground water; its performance was compared with that of an off-line method using Empore extraction disks and 1 L water samples. Phenol recoveries varied from < 20 to 100 % for concentrations in the range 0.1-10 μg/L at an acid pH. The presence of the phenols in water was confirmed by using thermospray LC-mass spectrometry in the negative ion mode. The stability of the phenols in water was studied at a 10 μg/L level in ground and estuarine water at acid pH (2.5-3) and at 4 ^{o}C for 1 month. The system was validated by various inter-laboratory exercises with samples containing 2, 4, 6-trichlorophenol and pentachlorophenol at concentrations from 0.1 to 0.5 μg/L.

This method development task may involve experimenting with many solvent mixtures. Methylene chloride is commonly used as an elution solvent for hydrophobic analytes. However, for one determination which includes preconditioning, eluting and rinsing, 45 ml of this solvent is required. For laboratories processing several samples, this volume will greatly multiply. Also, for reversed phase HPLC determinations, methylene chloride cannot be directly injected. Sample extracts must first be evaporated then reconstituted in a solvent compatible with reversed-phase liquid chromatography. Thus, methylene chloride will enter the atmosphere during this step. There is discussion of methylene chloride eventually being regulated because of its possible harm to the ozone layer. In addition, methylene chloride is a suspected carcinogen. Other solvents have been used to elute PAHs from C18 silica, including benzene, but they are also HPLC incompatible and/or harmful. (Zhao et al., 2005)

2.3. Method of adsorption

The adsorption and treatment of organic contaminants using activated carbon was investigated. Waste material was carbonized at 400 ^{o}C-500 ^{o}C and activated with acid at 800 ^{o}C to produce granular activated carbon (GAC). Adsorption of organics from the refinery waste on the activated carbon produced was examined at 28 ^{o}C. The experimental batch equilibrium data was correlated by Freundlich and Langmuir isotherms. The adsorption data fitted well into the Freundlich isotherm. Breakthrough time of about 1.5 hours was observed for the fixed bed adsorption process. The organic concentration expressed as chemical oxygen demand (COD) was reduced from an initial value of 378 mg/l to 142 mg/L for the first hour, 143 mg/l for the second hour, 152 mg/L for the third and fourth hours, and 156 mg/L for the final hour., which also compare favorably with the refinery effluent specification of 150 mg/L Results from the study shows that waste Nigerian bamboo can be converted into high capacity adsorbent and used for the remediation of polluted industrial waste waters (Ademiluyi et al ., 2009).

The adsorption equilibrium isotherms of five phenolic compounds from aqueous solutions onto granular activated carbon (GAC) were studied and modeled. Phenol (Ph), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (DCP), and 2,4,6-trichlorophenol (TCP) were chosen for the adsorption tests. To predict the adsorption isotherms and to determine the characteristic parameters for process design, seven isotherm models: Langmuir (five linear forms), Freundlich, Elovich, Temkin, Fowler–Guggenheim, Kiselev, and Hill–de Boer models were applied to experimental data. The results revealed that the adsorption isotherm models fitted the data in the order: Fowler–Guggenheim > Hill–de Boer > Temkin > Freundlich > Kiselev > Langmuir isotherms. Adsorption isotherms modeling showed that the interaction of phenolic compounds with activated carbon surface is localized monolayer adsorption that is adsorbed molecules are adsorbed at definite,

localized sites. Each site can accommodate only one molecule. The interaction among adsorbed molecules is repulsive and there is no association between them, adsorption is carried out on energetically different sites and is an exothermic process. Uptake of phenols increased in the order Ph < 2-CP < 4-CP < DCP < TCP, which correlates well with respective increase in molecular weight, cross-sectional area, and hydrophobicity and decrease in solubility and pKa. Additionally, for the four tested chlorophenols, it seems that the magnitude of adsorption is directly proportional to their degree of chlorination. (Oualid Hamdaouia, 2007).

Activated carbon based on the waste macadamia nut shells (MAC) was investigated for potential use as an adsorbent for phenol removal. The pseudo second-order kinetic model best described the adsorption process. The extent of the phenol adsorption was affected by the pH solution and the adsorbent dosage. Equilibrium data fitted well to the Langmuir model with a maximum adsorption capacity of 341 mg g^{-1}. The calculated thermodynamic parameters suggested that the phenol adsorption onto MAC was physisorptive, spontaneous and exothermic in nature. Phenol desorption from loaded adsorbent was achieved by using 0.1 mol L^{-1} NaOH, ethanol (100 %) and deionized water (Rodrigues et al., 2013).

There was no enough literature using macadamia nutshell activated carbon for phenol removal. In other studies ((Pakade et al., 2016)) information was regenerated several times without losing their adsorption capacity. The feasibility of quaternized activated carbon derived from *Macadamia* nutshells was explored in the removal of hexavalent chromium (Cr(VI)) from aqueous solutions. The amino-modified activated carbon was achieved by cross-linking native macadamia activated carbon (MAC) with epichlorohydrin and grafting diethylenetriamine and triethylamine (EDT) on the surface and the product was labelled as MAC-EDT. The adsorbent was characterized by Fourier transform infrared spectroscopy

(FTIR), Bruemer-Emmett-Teller (BET), scanning electron microscope (SEM) and theCHNSanalyzer. The optimum parameters for Cr(VI) removal found were pH (5), contact time (120 min) and sorbent mass (0.10 g) where removal efficiencies were >90% in all cases. Langmuir model fitted the data best and the adsorption capacity was 145.5 mg g-1 after surface modification with EDT. The Cr(VI) ion was found to be adsorbed more as compared to other anions, and the sorption order was: $SO_4^{2-} < PO_4^{3-}$ »$NO_3^- < Cr_2O_7^2$. The removal mechanism involved adsorption of Cr(VI) and/or subsequent reduction of Cr(VI) to Cr(III).

Macadamia nutshell biosorbents treated in three different activating agents [raw Macadamia nutshell powder (RMN), acid-treated Macadamia nutshell (ATMN) and base-treated Macadamia nutshell (BTMN)] were investigated for the adsorption of hexavalent chromium [Cr(VI)] from aqueous solutions. Fourier transform infrared spectroscopy spectra of free and Cr(VI)-loaded sorbents as well as thermogravimetric analysis revealed that the acid and base treatments modified the surface properties of the sorbent. Surface characteristics were also evaluated by the scanning electron microscopy and surface area analyzer. The optimum conditions for the adsorption of Cr(VI) by sorbents were pH 2, contact time 10 h, adsorbent mass 0.2 g and concentration 100 mg L-1. The equilibrium data were fitted into the Langmuir, Freundlich, Redlich–Peterson and Sips isotherms, and no single model could clearly explain the sorption mechanism. Maximum binding capacities of 45.23, 44.83 and 42.44 mg g-1 for RMN, ATMN and BTMN, respectively, were obtained. The kinetic data were analyzed using the pseudo-first, pseudosecond and Elovich kinetic models, and it was observed that the pseudo-second-order model produced the best fit for the experimental data. Macadamia nutshell sorbents showed potential as low-cost adsorbent for the removal of Cr(VI) from aqueous solution. (Pakade and Nchoe, 2016).

Activated carbon which was produced by chemical activation using zinc chloride from tea industry wastes (TIWAC) to adsorb phenol molecules from aqueous solution was tested by equilibrium, kinetic, and thermodynamic parameters (Gundogdu et al., 2012). Phenol adsorption on TIWAC took place with a high yield at pH values in the range 4 to 8. The optimum contact period was observed as 4.0 h and from the adsorption graphs plotted as a function of time; it was established that phenol adsorption on TIWAC conformed more to a pseudo second-order kinetic model. Additionally, it was determined that the adsorption rate is controlled by intraparticle diffusion as well as film diffusion. It was established that phenol adsorption on TIWAC can be better defined by the Langmuir adsorption model and its adsorption capacity was 142.9 mg·g−1 from the linear Langmuir equation. Temperature had an adverse effect on adsorption yield, and hence, the adsorption process was exothermic in their case. Moreover, increasing electrolyte concentration in the medium has a positive effect on adsorption yield. From the data obtained, it was concluded that the removal of phenol from aqueous solution by TIWAC produced from tea industry wastes with a very low cost took place with an extremely high performance.

Anirudhan and Ramachandran, 2014 used applicability of surfactant-modified bentonite (SMB) for removing 2,4,6-trichlorophenol (TCP) from water and petroleum refinery industry effluents through a batch adsorption process. The adsorbent was prepared from Na-bentonite by exchanging Na+ ions with the cationic surfactant, hexadecyltrimethyl ammonium (HDTMA) chloride. The adsorption capacity for TCP was significantly enhanced by 2.3 times through surfactant treatment of the bentonite. The effect of pH, initial concentration, contact time, adsorbent dose, ionic strength and temperature on adsorption of TCP was investigated. A strong dependence of the adsorption capacity on pH was observed, the capacity increased as the pH value decreased. The long alkyl chains of intercalated HDTMA increased the hydrophobicity of the adsorbent and provided particular affinity for

TCP molecules. The adsorbent showed high efficiency towards TCP and >99.0% removal was achieved from an initial concentration of 10 mmol/L at pH 3.0. The kinetics of the adsorption process was described by a pseudo-first-order model. Film diffusion was found to be the rate-limiting step. The adsorption isotherm was consistent with the Langmuir adsorption isotherm, and maximum monolayer capacity of the adsorbent was found to be 70.42 mmol/g at 30 8 °C, which was higher than that of commercial activated carbon (52.63 mmol/g). The adsorbed TCP was recovered by using 0.1 M NaOH. The regeneration process was carried out in four cycles and results indicate minimum loss in adsorption capacity.

Adsorption is an effective method for the treatment of phenol. In this work, phenol was removed from the wastewater by adsorption process using activated carbon generated from the dates' stone. Effects of operating parameters like initial pH (3 and 9), temperature (20 ^{o}C and 50 ^{o}C), adsorbent dosage (0.1g and 0.2g) and agitation time (1h and 2h) were investigated. The initial phenol concentration of 30 mg/L was reduced to the 0.3 mg/L corresponding to the removal efficiency of 99% at pH 3, 50 ^{o}C with 0.2 g activated carbon. Considering the results, it can be said that adsorption of phenol from wastewater using activated carbon generated from dates' stone can be used as an effective treatment method (Tezcan, 2017).

2.4. Method Validation

Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation program required depends entirely on the particular method and its proposed

applications. Typical analytical parameters used in assay validation include: Precision, Accuracy, Linearity Range, and Ruggedness Limit of detection, Limit of quantitation, Selectivity and Specificity. (Stockl et al., 2009).

2.4.1. Linearity

This is the method's ability to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. Linearity is determined by calculating the regression line using a mathematical treatment of the results (i.e. least mean squares) vs. analyte concentration (De Souza et al., 2007). Nilsson and Eklund,1999 adds by indicating that the coefficient of regression (R^2) is used to assess the acceptability of a calibration curve.

2.4.2. Accuracy

Accuracy is a measure of the closeness of test results obtained by a method to the true value. Accuracy indicates the deviation between the mean value found and the true value. lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. Accuracy and precision are not the same. A method can have good precision and yet not be accurate. The range for the accuracy limit should be within the linear range (Chan, 2008).

2.4.3. Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results (i.e. between 5 - 10). The precision is then expressed as the relative standard deviation (Chan, 2008).

2.4.4. Limit of Detection

The detection limit (DL) is a characteristic for the limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection is usually expressed as the concentration of the analyte in the sample, for example, percentage, parts per million (ppm), or parts per billion (ppb). For instrumental procedures that exhibit background noise, it is common to compare measured signals from samples with known low concentrations of analyte with those of the blank samples. The minimum concentration at which the analyte can reliably be detected is established using an acceptable signal - to - noise ratio of 2: 1 or 3 : 1. (De Souza et al., 2007; Chan, 2008).

2.4.5. Limit of Quantitation (LOQ)

This is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. The limit of quantification can be calculated and determined as the response that gives a signal to noise (S/N) ratio of 10:1 or as a function of

Relative Standard Deviation (RSD). The LOQ of a method is affected by both the detector sensitivity and the accuracy of the sample preparation at the low concentration of the impurities (Kelly et al., 2008; Chan, 2008). Most researchers calculate LOQ as 10% RSD or simply as 10 times the standard deviation of the error associated with detection of the analyte in the blank sample (Cuadros-Rodriguez et al., 2001).

2.4.6. Selectivity and Specificity

Selectivity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix. Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and/or impurities. Determination of this can be carried out by assessing the peak identity and purity. Diode array detectors can facilitate the development and validation of HPLC assays. Spectra data obtained from diode array detectors, effectively supplement the retention time data for peak identification, also spectral manipulation often provides information about the peak purity (Chan, 2008).

CHAPTER 3

3. Materials and Methods

3.1. Study Area

The Vaal River is the largest tributary of the Orange River in South Africa. The river has its source near Breyten in Mpumalanga province, east of Johannesburg and about 30 kilometres (19 mi) north of Ermelo and only about 240 kilometres (150 mi) from the Indian Ocean. (Times Comprehensive Atlas, 12th ed. Times Books, London, 2007).   It then flows westwards to its conjunction with the Orange River southwest of Kimberley in the Northern Cape. It is 1,120 kilometres (700 mi) long, and forms the border between Mpumalanga, Gauteng and North West Province on its north bank, and the Free State on its south. Three sampling points were selected alongside the Vaal triangle areas which are Sharpeville dam, Barrage River and Vaal River (Fig 3.1).The selection of the samples was guided by the purpose of the research. The study area selection was based on the presence of chlorinated phenols pollutants within the river water.
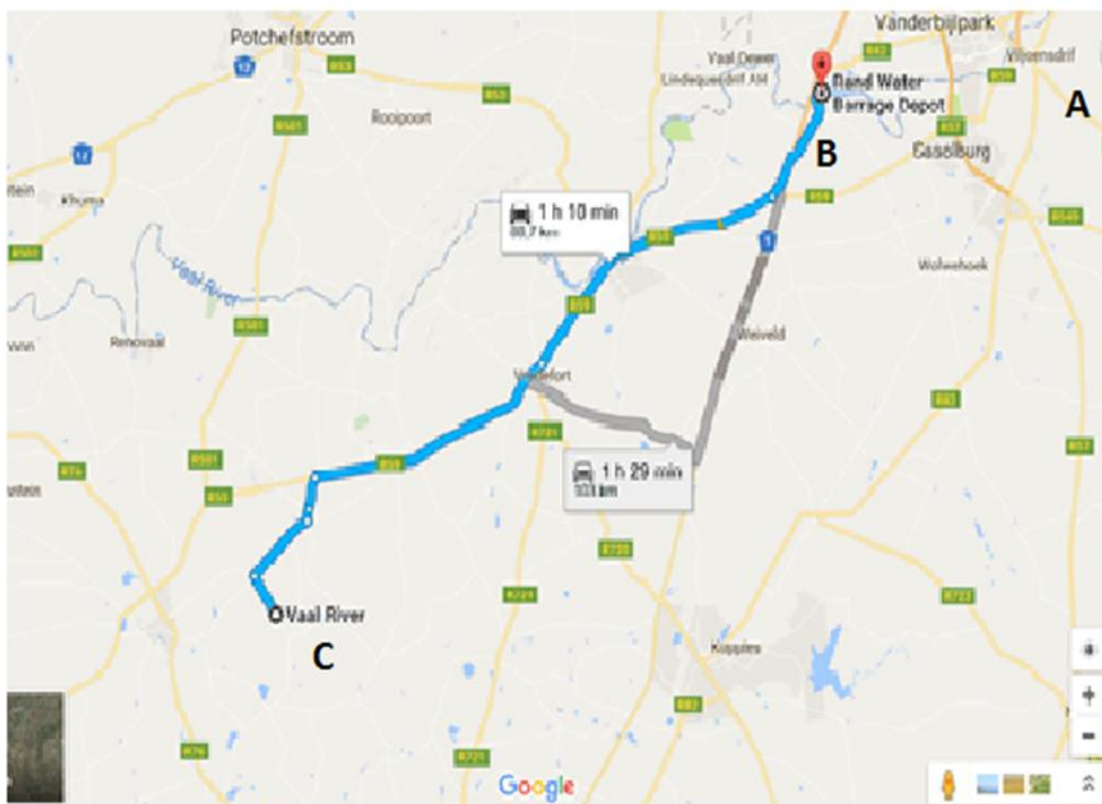
Figure 3. 1: Map showing the sampling points around Vaal river

All solvents and chemicals were purchased from Sigma-Aldrich, ultra-pure water was used for all dilutions and standard preparations. Table 3.1. lists the equipment used while Table 3.2. shows the chemicals and material used.

Table 3. 1. Apparatus

Filter membranes	Quartz filter and 20x25cm Whatmann 41 filter papers
Vials	Capacity is 2ml
Pipettes	Grade A
SPE Cartridges	Strata- X and C18
Beakers	Grade A
Analytical balance	220g max Sartorius
HPLC	Agilent 1260 Series

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SPE Vacuum Manifolds	Supelco Visiprep ^{TM} 24
Column(HPLC)	C18
Volumetric flasks	Grade A

Table 3. 2. Reagents

Manufacturer	Component	Purity
Sigma Aldrich	Acetone	99 %
Sigma Aldrich	Acetonitrile(HPLC Grade)	99.9%
Sigma Aldrich	Methanol (HPLC Grade)	>99 %
Sigma Aldrich	3 chlorophenol	98 %
Sigma Aldrich	4 Chloro-3-methylphenol	99%
Sigma Aldrich	2,4 dichlorophenol	99%
Sigma Aldrich	2,4,6 trichlorophenol	98%
Sigma Aldrich	octadecylamine	≥99 %
Filtatech	Macadamia activated carbon	N/A

3.2.   Sampling

The underlying principles of sampling are based upon the extrapolation of part of the sample population to obtain a representative sample. The population can be defined as the whole material whose properties are being investigated and a sample being a fraction of the population selected for analysis. Since the water analytical procedure could not be done in situ, it was important to obtain representative volumes of water from the sampling points.

The main objective of the sampling was to obtain suitable environmental organic matrix water samples for the purposes of studying the influence of the sample matrix on the selective determination of selected chlorinated phenols. The exact nature of the sample

matrix at each day of sampling was not known hence it was decided to obtain one representative sample from a mixture of all the samples collected.

3.2.1. Sampling plan

The sampling plan was designed taking into consideration the fact that the sample population is dynamic, that is, it changes over time hence it was decided to sample over seven months in order ensure that a more representative sample matrix was attained.

3.2.2. Sampling site selection

The sampling sites were selected by using the stratified sampling method which is an important sampling technique as it allowed the use of logic and judgement to obtain samples with desired properties of interest.**Error! Bookmark not defined.** This sampling technique was made possible by using previous pre-existing data gathered from water monitoring from these points which indicated that there were significant persistent organic pollutants to make up a significant organic matrix within the samples collected.[i] This enabled the population to be separated into three different strata or sub-groups whose properties formed a suitable organic matrix

This sampling technique was very useful, particularly because the aim of the research was not to study the properties of the whole population but to obtain a representative real sample matrix required to fulfil the main specific objectives of this research. Since the variables by which the samples were stratified are strongly correlated with the variable of interest, that is the sample matrix, the stratified sampling method proved to be the best sampling technique to be employed for this research.

Identified sources of contamination observed near the sampling points include

Site 1: agricultural land

The over-application of agrochemicals such as pesticides and herbicides in agricultural land was a potential threat identified on site 1.

Site 2: an urban settlement and water treatment works

The proximity of sampling site 2 to a water treatment works and an urban settlement exposed the water channel to a wide range of organic contaminants. Human activities have been identified as one of the contributors to organic pollution of freshwater bodies

Site 3: an industrial area

The industrial area situated upstream of site 3 is a source of synthetic organic micro-toxicants such as benzene hexachloride (BHC) isomers which are a by-product from industries such as paper and pulp factories and any other industrial processes which involve the use of chlorine bleaches.

The three sampling points were established downstream of the potentially polluting areas. The combination of socio-economic activities including urbanisation, industrial operations and agricultural production coupled with the naturally occurring toxicants within the same watershed created a complex sample matrix which is paramount for this research.

3.2.3. Sampling techniques

The samples were collected by a sub-surface grab method which is one of the simplest sampling techniques.

3.2.4. Sampling procedure

The main objective of sampling was to obtain representative environmental samples for the purpose of determination of chlorinated phenols. The identified sources of contamination observed near the sampling points were an urban settlement and industrial area. Industrial activities have been identified to be one of the contributors to organic pollution wastewater

from effluent plants discharged in rivers. Water samples were collected every month per site from Vaal River, Barrage and Sharpeville dam in morning at 8:00 and afternoon 16:00. A sub-surface grab sampling method was followed where samples were collected at 30 cm below the surface of the stream at 45 degrees angle to the direction of the flow. Samples from the three points were mixed to produce a composite sample per site. 4 L amber bottles with caps were thoroughly cleaned with soap and water, rinsed with tap water, then soaked overnight with dilute nitric acid (HNO_3) solution and later washed with deionized water several times in the laboratory. Bottles were further rinsed twice with sample water before filing them at the sampling points. An organic modifier, 10 ml acetonitrile was added to the samples before performing SPE procedures. Unspiked samples (i.e. "blank" samples) were also processed, in an identical manner to the spiked samples. Samples were transported to the lab in a cooler boxes filled with ice. The samples were then stored in the freezer at $0°C$ until they were used.

3.2.5. Sampling frequency

Samples were collected every month at midday between 5 January and 28 July 2016 from the selected three points and stored at a temperature of ≤ 5 degrees Celsius.

3.2.6. Sample size

Since the bigger the sample size, the lower the sampling error, it was decided to collect a significant sample size of four litres.

3.2.7. Sampling apparatus

Cooler box with ice, four litre glass amber bottles with caps and a water resistant work suit were the standard sampling apparatus used.

3.3. Determination of chlorinated phenols

Blank matrix neat sample. Consisted of the chlorinated phenols cocktail in methanol solvent. Deionised spiked water sample consisted of deionised water spiked with 1 mL of the 1 ppm chlorinated phenols cocktail. Low matrix water sample consisted of the representative real sample water spiked with 1mL of the 1 ppm chlorinated phenols cocktail. High matrix water sample consisted of the low matrix water sample spiked with 5ml of a 1ml solution which consisted of 4 persistent organic pollutants including chlorinated phenols pollutants which are 2,4,6 trichlorophenol (TCP), 3-chlorophenol (CP), 2,4 dichlorophenol(DCP) and 4-chloro-3-methylphenol(CMP). Blank neat individual standards consisted of individual chlorinated phenols standards in methanol.

3.3.1. HPLC Method Development

The high performance liquid chromatography (HPLC) is a technique of separation as well as qualitative and quantitative analysis of constituents. In this technique, a mobile phase passes through a column. The column can contain porous particles (filled column) or be equipped with a thin film (capillary column). At the initial time, the mixture to be separated is injected into the inlet of the column where it is diluted in the mobile phase which passes through the column. If the stationary phase has been well selected, the constituents of the mixture are unevenly retained during the passage through the column. A detector placed at the end of the column coupled with a recorder performs the chromatogram, a constant signal on the recorder for the base line is only shown in the presence of the mobile phase. During the exit of every separated constituent, its peak time (retention time) is recorded on the detector. In the given chromatographic conditions, the retention time characterizes qualitatively a substance. The area between a peak and the extension of the base line varies linearly with the concentration of constituents detected and isolated. The analysis of several standard solutions gives the standard curve characteristic of each constituent by plotting the peak area versus its concentrations. From the curve, it is then possible to measuring the area, to

determine the unknown concentrations of the compounds present in the injected mixture. (Alivelu Samala, Santhosh Pawar, Sowmya Manala, 2013)

Three solvents, namely, acetonitrile, methanol and acetone were used for method development of HPLC. The instrument which was used for this study was an Agilent HPLC model. Distilled water was spiked with a known concentration of selected chlorinated phenolic compounds to give prepared samples. The four stock solution of each 2,4,6 trichlorophenol (TCP), 3-chlorophenol (CP), 2,4 dichlorophenol(DCP) and 4-chloro-3-methylphenol (CMP) of 100 mg/L were prepared by dissolving each chlorinated phenols compounds with methanol in a volumetric flask. The standard solutions varying from 1 - 10 mg/L were prepared by taking aliquots from the stock solution and transferred into different volumetric flasks, diluting them with methanol. The standard solutions were analysed with the HPLC instrument to get the linearity, specificity, repeatability, detection limit, precision and accuracy.

3.3.2. Detection limit

The stock solution of 4 chlorinated phenols were prepared in each 1000 volumetric flask. Standard solution of different aliquots were prepared in each 100 volumetric flasks varying from 0.05 – 1ppm concentration to determine the lowest level/concentration that an instrument can be detect.

3.3.3. Repeatability

To get the peaks and its consistency in the instrument the standard solutions were prepared from the stock solution varying from 0.05 – 1ppm and were run in five replicates per concentration.

3.3.4. Linearity

The Linearity of the detector response was demonstrated by prepared five standard solutions ranging from 1 – 10 ppm. The linear correlation coefficient (r) should be 0.95 or greater, as agreed that it should exceed 0.99 to evaluate the linearity plot approach and r value.

3.3.5. Precision and accuracy

The precision of the analytical method is the closeness of a series of individual measurement of an analyte by the repeatability that was analysed five times with three different concentrations (low, medium, high). The sample solution of the 3 chlorophenol, 4chloro-3-methylphenol, 2,4 dichlorophenol and 2,4,6 trichlorphenol, 1g of each was prepared by weighing and dissolving them in 50 mL of mobile phase solution as solvent and injected repeatedly (5 injection). Five sample solution (1 ppm, 3 ppm, 6 ppm, 8 ppm and 10 ppm) were prepared for the same standardized sample solution preparation and injected five for each sample to calculate their %RSD from the data obtained.

3.3.6. Recovery

The recovery was determined by five determinations with three different concentrations (low, medium, high) in range of excepted concentration. The extraction efficiency (recovery) was determined at three different concentrations, adjusted by adding known amounts of 3 chlorophenol(3CP), 4 chloro-3-methylphenol(4C3MP), 2,4 dichlorophenol and 2,4,6 trichlorophenol to a distilled water sample. The standards were dissolved in methanol to obtain a 100 ppm stock solution, and 1, 5, and 8 ppm aliquots of this were mixed with 50 mL of distilled water (three replicates at each concentration). Steroids residues were concentrated on Strata C18 SPE cartridges. The SPE cartridges were conditioned with 10 mL methanol, equilibrated with 10 mL HPLC grade water, loaded with 20 mL filtrated sample, washed with 10 ml HPLC grade water and eluted with 30 mL methanol. After evaporation to dryness (rotary evaporator, 40 °C) extracts were reconstituted in 750 μL

methanol. The 750 µL methanol was concentrated to dryness (Eppendorf Concentrator Plus at 30 °C, V-AL mode) and reconstituted in 50 µL methanol.

3.3.7.   High Performance Liquid Chromatography

HPLC (Fig3.2) is a technique for separation, identification and quantification of components in a mixture.
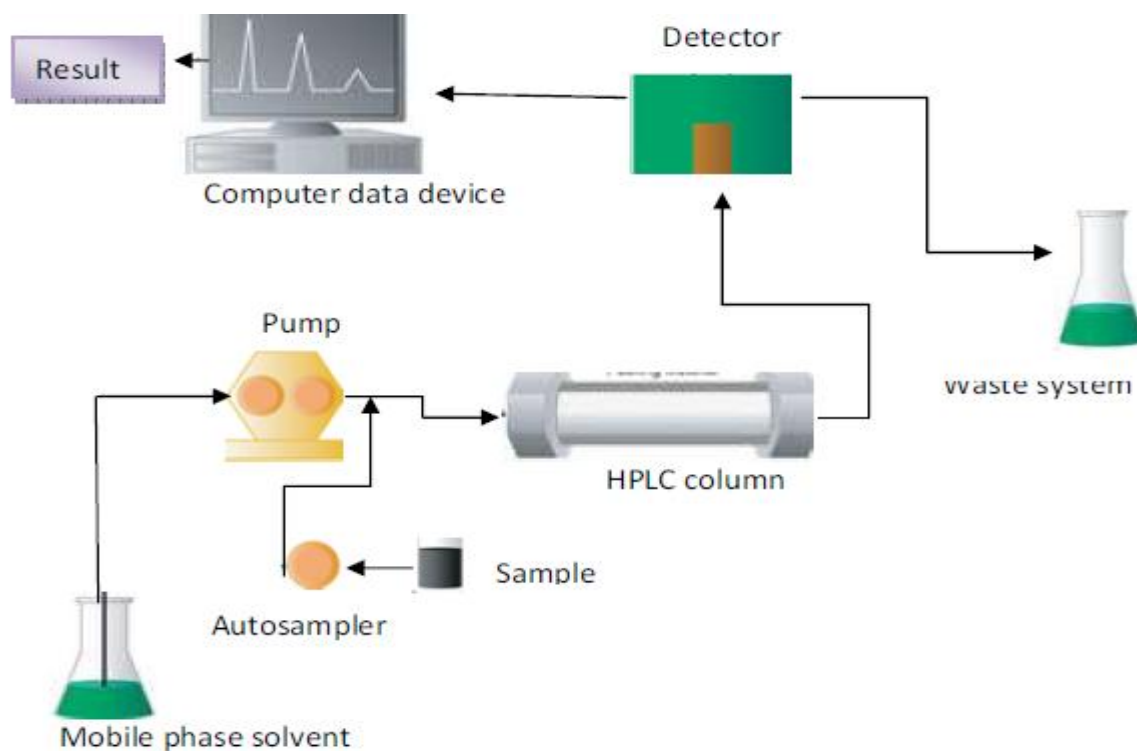
Figure 3. 2: Showing the schematic HPLC diagram (Bhanot, 2014)

The HPLC is especially suitable for compounds which are not easily volatilised, thermally unstable and have high molecular weights. The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column, the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively retained on the basis of physico-chemical interactions between the analyte

molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components. (Bhanot, 2014).

3.3.8. Mobile Phase

Mobile phase serves to transport the sample to the system. Essential criteria of mobile phase are inertness to the sample components. Pure solvents or buffer combinations are commonly used. The mobile phase should be free of particulate impurities and degassed before use (Bhanot, 2014).

3.3.9. Mobile Phase Reservoirs

These are inert containers for mobile phase storage and transport. Generally transparent glass bottles are used so as to facilitate visual inspection of mobile phase level inside the container. Stainless steel particulate filters are provided inside for removal of particulate impurities in the mobile phase if any (Bhanot, 2014).

3.3.10. Pump

Variations in flow rates of the mobile phase effect elution time of sample components and result in errors. Pumps provide constant flow of mobile phase to the column under constant pressure (Bhanot, 2014).

3.3.11. Injectors

Injectors are used to provide constant volume injection of sample into the mobile phase stream. Inertness and reproducibility of injection are necessary to maintain high level of accuracy (Bhanot, 2014).

3.3.12. Column

A column is a stainless steel tube packed with stationary phase. It is a vital component and should be maintained properly as per supplier instructions for getting reproducibility separation efficiency run after run (Bhanot, 2014).

3.3.13.Column Oven

Variation of temperature during the analytical run can result in changes of retention time of the separated eluting components. A column oven maintains constant column temperature using air circulation. This ensures a constant flow rate of the mobile phase through the column (Evaluation, 1996).

3.3.14. Detector

A detector gives specific response for the components separated by the column and also provides the required sensitivity. It has to be independent of any changes in mobile phase composition. Majority of the applications require UV-VIS detection though detectors based on other detection technique are also popular these days (Evaluation, 1996).

3.3.15. Data Acquisition & Control

Modern HPLC systems are computer based and software controls operational parameters such as mobile phase composition, temperature, flow rate, injection volume and sequence and also acquisition and treatment of output (Evaluation, 1996).

3.3.16. Standard and Sample Preparation

The chlorinated phenols stock solution were prepared in a volumetric flask by weighing 1g of each in 1000 ml. Each of the analyte namely, 3-chlorophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenols and 2,4,6-trichlorophenol were prepared by weighing 1g into 1000 ml volumetric flask and filled up to the mark respectively with a HPLC grade methanol. The

mixture was sonicated for 10 minutes. The final concentration required were prepared from a stock solution from an appropriate amount to give calibration standards. Loaded to the HPLC agilent for their determination.

3.3.17. Sample Analysis

In the investigation an Agilent 1260 HPLC with a UV-vis detector was utilised for all analysis on the three methods for the removal of chlorinated phenols. A cocktail of chlorinated phenols where prepared to get the parameters that are suitable for the analytes determinate. The parameters of the column is Thermo Scientific Acclaim 120, C18, 5μm (2.1 x 250 mm) analytical, mobile phase A: water, B: HPLC grade methanol-50% : 50%, Gradient : 10-54% B (0-4min), 54% B (4-12 min), 100% B (12-16min) and 10% B (16-20min). Flow rate: 2 ml/min. Injection volume: 2μL, Temperature at 20 ^{0}C, detection UV absorbance at 248,254 and 269 nm. Noise < 0.1 mAu, Backpressure system (0 - 400bar).

3.3.18. Solid Phase Extraction

Solid phase extraction is a sample preparation technique used for extracting semi volatile and non-volatile analytes from their matrices for subsequent chromatographic analysis. It is considered one of the most powerful techniques currently available for isolating trace amounts of organic compounds such as pesticides from water and other environmental samples (Poole, Gunatilleka and Sethuraman, 2000a). It entails the use of SPE cartridges which are packed with silica bonded to a particular analyte adsorbing phase. SPE can be compared to other extraction techniques like liquid-liquid extraction although it is advantageous in that it provides better selectivity and extraction efficiency (recovery) eliminates problems associated with incomplete phase separation and yields quantitative extractions that are easy to perform (Poole, Gunatilleka and Sethuraman, 2000a).

3.3.19. SPE cartridges used

The following C-18 – 500 mg solid phase extraction column was used (Sulpeco).

3.3.20. C18 cartridge

C18 (Isolute) is produced from trifunctional octadecyl silane sorbent has enhanced secondary silanol interactions which can be very useful for example in the extraction of basic compounds from aqueous solution. It has the average particle size of 50μm with irregular shape particles and the porosity of 60Å. It is applied to aqueous analytes and has a strong non-polar (hydrophobic) phase. (International Sorbent Technology, 1997). The functional group is displayed below in Figure 3.3.

$$\begin{array}{c}| \\ \backslash Si{-}O{-}Si{-}(CH_2)_{17}CH_3 \\ O \quad | \\ \backslash \\ Si{-}OH \\ / \end{array}$$
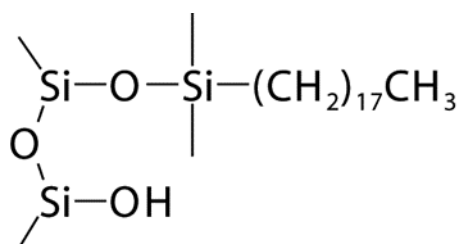
Figure 3. 3: The structure of C-18 sorbent

3.3.21. SPE Vacuum Manifold

Samples and cartridges were arranged in corresponding Visiprep ™ 24 vacuum manifold which enables analysts using solid phase extraction tubes to simultaneously prepare up to 24 samples (Fig 3.4). The manifold consists of a chemical-resistant cover, gasket, and base, a glass basin, a vacuum gauge and vacuum bleed valve, 24 flow control valves, 24 replaceable

solvent guide needles, the SPE condition listed in Table 3.3 and a rack for sample collection

vessels (base, 5 support plates, support rods, retaining clips) (Azevedo et al., 2001).
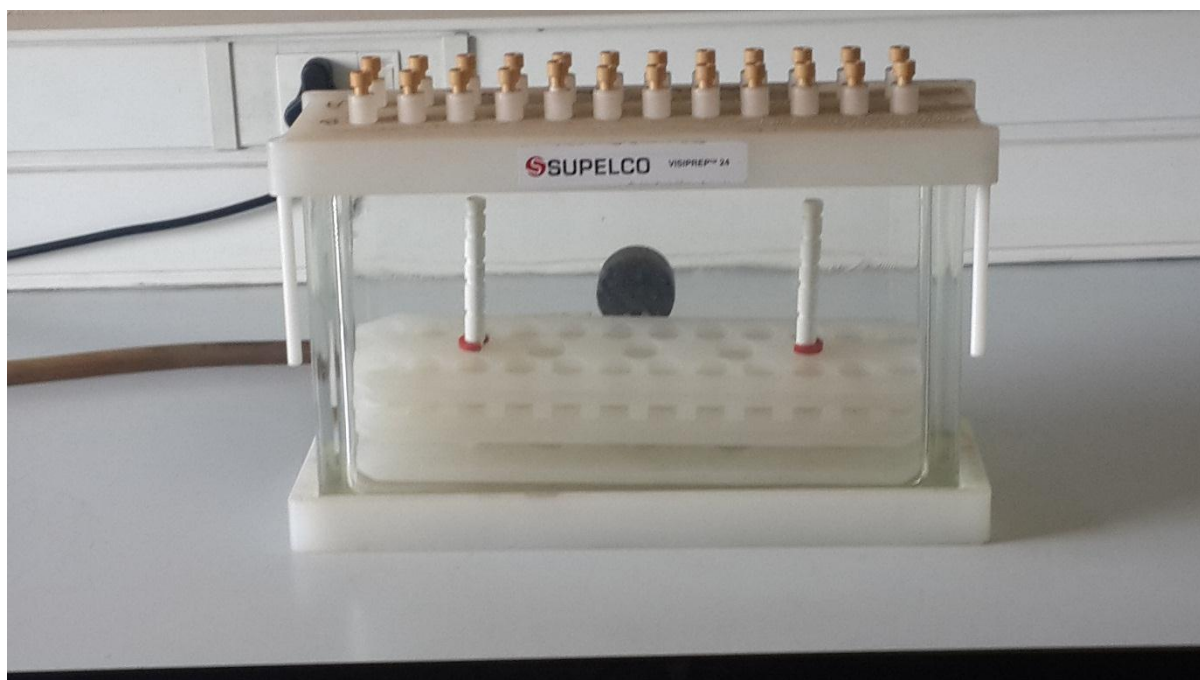
Figure 3. 4: Vacuum manifold Source: Supelco bulletin (1998)

Table 3. 3. SPE instrument conditions

Flow rates	mL/min
Condition	10
Load	20
Rinse	10
Elute	30
Condition air push	15

3.3.22. Method for SPE

Step 1 - Cartridge was conditioned with 10 millilitre of methanol. Step 2–Cartridge was conditioned with 10 ml of water. Step 3 –20 ml of the sample was loaded. Step 4 – Cartridge was rinsed with 10 ml of water. Step 5 – elution was done with 30ml of methanol. There were no air bubbles in the sample lines prior to starting; the sample of 20 ml was loaded on the SPE cartridges and were rinsed using 10 ml of water. The new cartridges were placed again in the sample lines to continue with another extraction experiments. In a 50 ml conical flasks the eluted solution was collected and pipette in a 1.5 ml HPLC vials for analysis.

3.4.   Assessment of adsorption capabilities

3.4.1.   Adsorption Process

Adsorption occurs when impurities from the solution accumulate at a solid-liquid interface. It considers chemical or physical process where a substance is collected at a boundary between phases (Yapar and Yilmaz, 2005). The adsorbent is the solid phase which removes the substance of interest from the liquid phase and is known as adsorbate. The adsorption increases with increasing molecular length which is more nonpolar depending on the hydrocarbon chain (Montgomery, 1985). The solubility decreases when the length of carbon chain increases. The adsorption of compounds decreases when increasing solubility of the solute in the liquid. The molecular size is an important factor on the adsorption process (Eckenfelder, 2000). The aliphatic hydrocarbons are less adsorbed than aromatic substituted compounds (Eddy, 2003).

3.4.2.   Adsorption Isotherms

The data of the adsorption equilibrium graph is plotted with mass in fluid on the x-axis and mass adsorbed on the y-axis at a uniform temperature in the form of an adsorption isotherm. (Choy, McKay and Porter, 1999).

3.4.3.   Langmuir adsorption isotherm

The Langmuir model assumes a monolayer coverage of a homogenous adsorbent surface by the adsorbate (Pakade *et al.*, 2016), and Langmuir isotherm equation is given below.

The following equation is a curvilinear Langmuir isotherm:

$$Q_e = \frac{bCe}{1+bCe} \qquad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots..3.1$$

The following equation is a linear form of Langmuir isotherm

$$\frac{Ce}{Qe} = \frac{Ce}{Qmax} + \frac{1}{bQmax} \qquad \dots\dots\dots\dots\dots\dots\dots.3.2$$

Qe : amount of adsorbate adsorbed by 1g of adsorbent (mg/g)

Qmax: maximum single layer adsorption capacity (mg/g)

Ce : amount of adsorbate left unadsorbed at equilibrium (mg/g)

b: adsorption enthalpy

The term R_L is another parameter for Langmuir isotherm model which is a nondimesional constant, characterized by this equation below:

$$R_L = \frac{1}{1+bCo} \qquad \dots\dots\dots\dots\dots\dots\dots\dots\dots............3.3$$

C_o : initial adsorbate concentration in aqueous solution.(mg/L)

b: Langmuir constant (L/mg)

R_L : nondimesional constant

3.4.4.   Freundlich Adsorption isotherm

Freundlich isotherm is a physical adsorption from liquids and can also be used for the adsorption of hydrocarbons on carboneous materials. (Pakade *et al.*, 2016).

The following equation defines Freundlich isotherm:

$$q_e = K_f \times C_e^{1/n} \quad \text{...} \quad 3.4$$

q_e : amount of material adsorbed by the adsorbent

K_f : constant associated with adsorption capacity (mg/g)

n : empirical parameter associated with adsorption intensity.

3.4.5. Adsorption Thermodynamics

The total energy change of the system (ΔH^o), method Gibbs free energy change (ΔG^o) and energy change which is the standard for entropy (ΔS^o) for the temperature required for the adsorption. The relationship between total energy change of the system, Gibbs free energy change and standard for entropy is expressed as follows:

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad \text{..} \quad 3.5$$

ΔG^o :standard Gibbs free energy change (kJ./mol)

ΔH^o : standard enthalpy change (kJ./mol)

ΔS^o : standard entropy change (kJ./mol.K)

T : absolute temperature (K)

The adsorption process completed at a definite temperature to get Gibbs free energy, its essential to get equilibrium constant (K_d).

$$K_d = C_a/C_e \quad \text{...} \quad 3.6$$

K_d : adsorption equilibrium constant

C_a :adsorbate concentration adsorbed on unit mass of adsorbent(mg/L)

C_e :adsorbate concentration remaining in the solution after adsorption (mg/L)

3.4.6. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis were performed to identify the thermal behavior of macadamia nutshell activated carbon, phosphoric acid impregnated shells and phosphoric acid impregnated chars. In TGA analysis, Shimadzu DTG-60H simultaneous DTA-TG apparatus

in VUT Chemistry Department was used. TGA experiments were conducted at the same experimental conditions with respect to carbonization and activation temperature, N_2 flow rate and heating rate.

3.4.7. Fourier Transform Infrared Spectroscopy (FTIR) analysis

In this study, commercial MAC was modified using chemical treatment. Hence, changes in the functional group should be compared. Both the commercial and modified activated carbon was analyzed by FT-IR Spectroscope (FTIR-100, Perkinlmer) to detect the surface functional group. A small amount of dry MAC was crushed into powder form and tested. The spectra were recorded from 4000 to 400 cm^{-1} (Shaarani and Hameed, 2011). The FT-IR spectra of powder macadamia activated carbon were measured by a Perkin- Elmer FT-IR system, Spectrum GX. The spectra were recorded from 4000 to 500 cm^{-1} with a resolution of 4 cm.

3.4.8. Scanning Electron Microscopy (SEM) analysis

SEM is an instrument which applied a narrow electron beam to scan over the surface of the specimen which is coated with a thin layer of metal. Secondary electrons are collected by a detector and produce a three-dimensional image on television screen. By using SEM, the morphology, pore structure, and structural changes of activated carbon can be observed (Chen et al., 2017). AC was prepared in granular form and placed at the sample placement. After the AC was coated, SEM was run to determine its characteristics.

3.4.9. Adsorbate

The adsorbate mixtures of various initial concentrations C_0 ranging from 1ppm -10 ppm were prepared. The chlorinated phenols mass in grams was weighed accurately in a 1000 ml volumetric flask and dissolved in small quantity with distilled water up to the made. The stock solution was prepared and stored in a 5 litre dark brown chemical bottle to avoid photo- oxidation to take place in a reaction form.

3.4.10. Adsorption Test

The MAC adsorbent was pulverized and sieved into particle size with the average diameter 0.096 mm using 80 ± 230 mesh. The MAC sample of 0.5 g was weighed in 250 ml series of capped volumetric flasks. In every flask that were in series 50 ml of phosphoric acid 0.1 mol/L and sodium hydroxide 0.1 mol/L were added in those flasks, and chlorinated phenols mixture were also added in those flasks with a concentration of 1ppm – 10 ppm at a pH of 7 and capped volumetric flask were stirred at speed of 300 rpm for 250 minutes. After 250 minutes the sample was filter using the No.1 Whatman filter paper. The analysis of chlorinated phenols filtrates were carried out using the HPLC Agilent. The adsorbed phenol amount q_e (mg/g) was calculated from this equation:

$$q\text{e} = \frac{(Co-Ce)V}{M} \qquad \text{................................3.7}$$

C_o : initial liquid phase concentration of chlorinated phenols (mg/L)

C_e : equilibrium liquid phase concentration of chlorinated phenols (mg/L)

V : volume of the solution (L)

M : amount of the adsorbent required (g)

The removal of chlorinated phenols expressed in percent is calculated as follows:

$$\% = \frac{(Co-Cf) \text{ x } 100}{Co} \qquad \text{................................3.8}$$

C_o is the initial (after adsorption) chlorinated concentration

C_f is the final (after adsorption) chlorinated concentration

All the tests were performed at room temperature $25\pm1^{0}C$ in a replica form to cover the reproducibility of the results, mean of the measurements is reported. The variables of the experimental ranges are indicated in Table 3.4.

Table 3. 4. The experimental parameter ranges as follows for macadamia activated carbon not grafted and MAC grafted.

Chlorinated phenols concentration(mg/L)	Contact time, minutes	Adsorbent dosage (g/L)	pH	Temperature ^{0}C
1, 3, 6, 8, 10.	0,50,100, 150,200, 250.	0.1, 0.2, 0.3, 0.4, 0.5.	2, 5, 7, 9, and 11	20, 25, 30, 35 and 40

3.4.11. Effect of initial chlorinated phenols concentration

The batch experiment of adsorption was carried at different initial concentrations (1, 3, 6, 8, and 10) mg/L for each analyte respectively with a dosage of 0.5 g of macadamia nutshell activated carbon at a room temperature of $25 \pm 1.0\ ^{0}C$ with 50 ml of chlorinated phenols at pH value of 7. The capped conical flasks were arranged in a numerical order of label 1 to 5. The sample mixture of each analyte was stirred for 250 minutes, 10 min at a speed of 300 rpm and filtered with No.1 Whatman filter paper (125 mm). HPLC was used to analyse the filtrates. This procedure was repeated for pH, contact time and dosage.

3.4.12. Effect of contact time

The batch experiment of adsorption was carried at a different contact time (50, 100, 150, 200, 250) minutes with a dosage of 0.5 g of the macadamia nutshell charcoal at a room temperature $25 \pm 1.0\ ^{0}C$ with 50 ml of chlorinated phenols at pH value 7. The capped conical flasks were arranged in a numerical order of 1 to 5. The mixture was stirred at a speed of 300 rpm and filtered. HPLC was used to analyse the filtrates.

3.4.13. Effect of adsorbent dosage

The batch experiment of adsorption was carried with (0.1, 0.2, 0.3, 0.4, and 0.5) g, of macadamia charcoal with a concentration of 10 mg/L of each analyte in 50 ml conical flasks at a room temperature $25 \pm 1.0\ ^0C$ at pH value 7. The in 50 ml he mixture was stirred for 6 hours at a speed of 300 rpm and filtered. HPLC was used to analyse the filtrate

3.4.14. Effect of pH

The batch experiment of adsorption was carried out at pH values of (2, 5, 7, 9 ,11) with a concentration of each analyte (10 mg/L) in a 50 ml conical flasks at a room temperature $25 \pm 1.0\ ^0C$. The mixture was stirred for 250 min at a speed of 300 rpm and filtered. HPLC was used to analyse the filtrates.

3.4.15. Effect of the temperature

The batch experiment of adsorption was carried out at temperature values of (20, 25, 30, 35, and 40) ^0C with a concentration of each analyte (10 mg/L) in a 50 ml conical flasks at a weighted mass of 0.5 g MAC. The mixture was stirred for 250 min at a speed of 300 rpm and filtered. HPLC was used to analyse the filtrates

3.5. Modification of macadamia nutshell activated carbon

3.5.1 Activated carbon

Activated carbon, also called activated charcoal, is a form of carbon processed to have small, low-volume pores that increase the surface area available for adsorption or reactions (Jafari Khoshnabadi, Lotfi and Vadi, 2015). Due to its high degree of micro porosity, just one gram of activated carbon has a surface area in excess of 3,000 m^2 (32,000 sq ft) (Dillon et al., 1989). An activation level sufficient for useful application may be attained solely from high surface area of macadamia activated carbon. However, further chemical treatment often enhances adsorption properties. Activated carbon is usually derived from charcoal and is sometimes utilized as biochar. Activated carbon with high surface area and pore volumes are

produced from a variety of macadamia nutshells. South Africa is the third largest producer of Macadamia in the world after Australia and Hawaii (Mogala, 2014). As the growth rate of Macadamia plantations steadily increases so also is the accumulation of Macadamia shells (Mogala, 2014). There appears to be no real use for waste Macadamia shells except for road compaction in farms and as a source of heat. Activated carbon is used as a sorbent in wastewater treatment for removing taste and odor-causing organic compounds from drinking water; it can also remove organic contaminants (Krishnaiah, et al., 2013; Monser, 2002). Activated carbon removes compounds via sorption both to the surface and within the granules or substrate matrix. The effectiveness of activated carbon is influenced by surface area, porosity, surface pH, and surface charge (Verla, A. W, Horsfall Jnr, M, E.N Verla, A.I. Spiff, 2012).

3.5.2. Grafting of macadamia nutshell activated carbon with the octadecyl amine functional group.

Water (0.81 L) and H_3PO_4 6N (0.09 L) were stirred in a 1 L flask, and outgassed for 1 h under N_2 bubbling. Weight 0.5 octadecylamine was introduced in the solution and stirred until complete dissolution. When dissolution was achieved, MAC (0.5 g) and sodium nitrite (0.01g) were quickly introduced and the action mixture was stirred between 1 and 8 h under N_2. In order to remove traces of unreacted amine, the grafted MAC were then washed in cycles using three different solvents (water, methanol and acetone) each washing cycle was applied for one day. Finally, the grafted MAC were dried in an oven for 24 hours.

CHAPTER 4

4.   Results and Discussion

4.1.   HPLC method for determination

The HPLC method validation was aimed to establish that the method is fit for the purpose. The validation parameters tested were, linearity, detection limit, sensitivity, accuracy, specificity, selectivity, robustness and ruggedness.

4.1.1   Calibration parameters

The linearity test solutions for the proposed method was obtained over the tested calibration ranges of 1 to 10 mg/l for 3-Chlorophenol (Fig4.1), 4-Chloro-3-methyl phenol (Fig4.2), 2,4-Dichlorophenol (Fig4.3) and 2,4,6-Trichlorophenol (Fig4.4) in a five-point concentration range. The working range as shown in Table 4.1 for all the components is wide enough to produce valid measurements. The results showed an r^2 of greater than 0.98 as shown in Figure 4.3. The calibration curves are plotted below.

linear curve CP

$y = 1.0659x + 2.9443$
$R^2 = 0.9481$
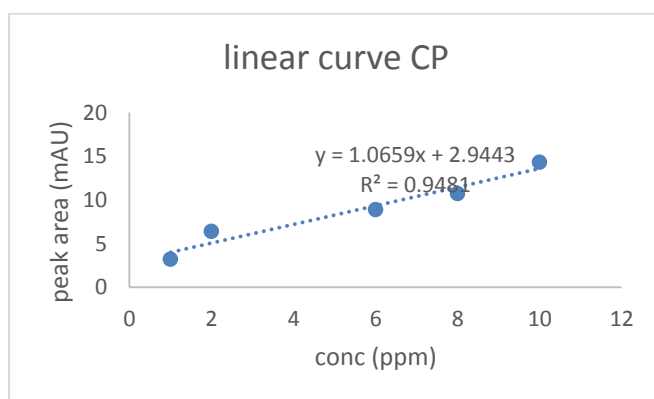
Figure 4. 1: Linear curve for Response versus Concentration for 3-chlorophenol

Linear curve 4C3MP

$y = 1.1598x + 1.4571$
$R^2 = 0.9734$

peak area (mAU) vs conc (ppm)
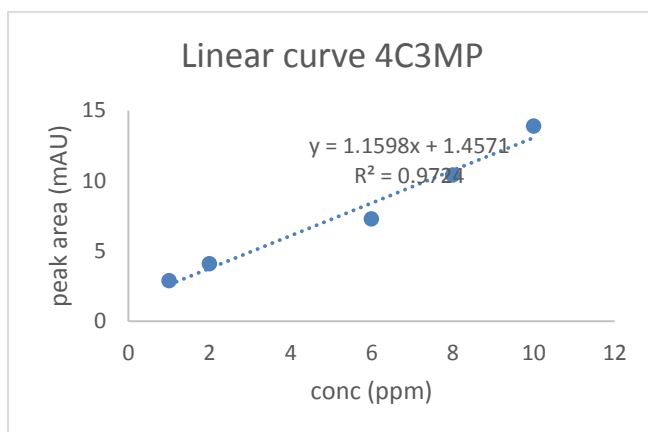
Figure 4. 2: Linear curve for Response versus Concentration for 4-chloro-3-methylphenol

Linear curve DCP

$y = 1.1125x + 2.3125$
$R^2 = 0.9813$

peak area (mAU) vs conc (ppm)
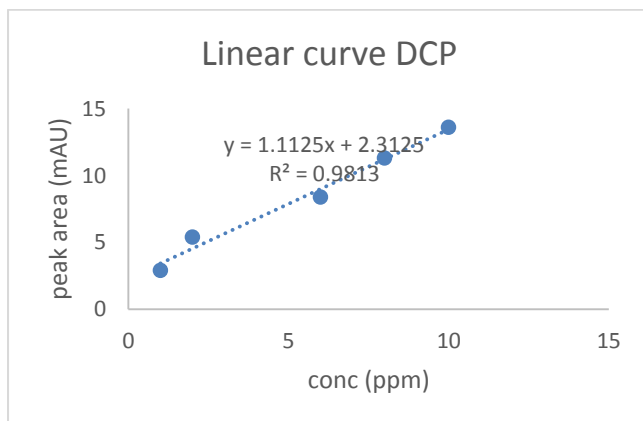
Figure 4. 3: Linear curve for Response versus Concentration for 2,4 dichlorophenol

Linear curve TCP

$y = 1.0616x + 2.6156$
$R^2 = 0.9298$

peak area (mAU) vs conc (ppm)
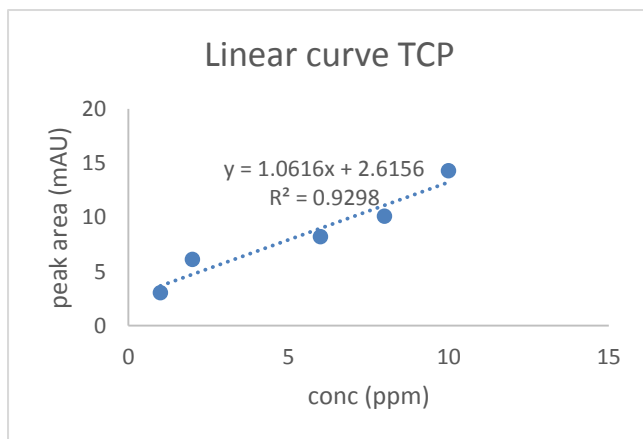
Figure 4. 4: Linear curve for Response versus Concentration for 2, 4, 6 trichlorophenol

Table 4. 1. Linearity data for 3-chlorophenol, 4-chloro-3-methylphenol, 2, 4-dichlorophenol and 2, 4, 6-trichlophenol

Chlorinated Phenols	Linearity range (mg/l)	Linear equation (y= mx+c)	Linearity (R^2)	Detection limits (mg/l)
3-Chlorophenol		y=1.0659x+2.9443		
	1-10		0.9481	0.051
4-Chloro-3-methyl phenol	1-10	y=1.1598x+1.4571	0.9724	0.201
2,4Dichlorophenol		y= 1.1125x+2.312		
	1-10		0.9813	0.496
2,4,6 Trichlorophenol	1-10	y= 1.0616x+2.615	0.9298	0.038

4.1.2. Accuracy results

Accuracy depends on two factors, trueness and precision.

4.1.3. Trueness results

To evaluate the trueness, the calculated amounts of the standards were compared to the measured amounts by reprocessing the calibration sample data as samples. The results obtained using the t-test for comparing experimental mean with a single true value are shown in Table 4.2.

Table 4. 2. Trueness data

Chlorinated Phenols	True value (mg/l)	Experimental mean (mg/l)	Standard deviation	%RSD
3-Chlorophenol	1	0.9740	0.0199	2.0429
4-Chloro-3-methyl phenol	1	0.9743	0.0242	2.4838
2,4Dichlorophenol	1	0.9984	1.9352×10^{-3}	0.1938
2,4,6 Trichlorophenol	1	0.9973	2.0651×10^{-3}	0.2071

The 4-Chloro-3-methylphenol showed the highest degree of trueness with % RSD values of 2.5%. As a quality control procedure, % RSD of less than 5% is considered to be valid. Accordingly, all analytes tested showed a percentage RSD of less than 5%.

4.1.4. Precision results

The repeatability precision for the 4 phenols are determined from five replicate analyses of the distilled sample spiked with 1mg/l standard is presented in Table 4.3.

Table 4. 3. Precision data

Chlorinated Phenols	Mean	STD Deviation	%RSD	T-value	Degrees of freedom	Precision limit
3-Chloro phenol	0.8260	0.0242	2.9297	2.26	4	0.0120
4-Chloro-3-methyl phenol	0.9514	0.0337	3.5409	2.26	4	0.0223
2,4Dichlorophenol	0.7672	0.0135	1.7596	2.26	4	0.0571
2,4,6						

| Trichlorophenol | 0.5051 | 0.01133 | 2.2438 | 2.26 | 4 |
| | | | | | 0.0968 |

Except for 4-Chloro-3-methyl phenol which showed the highest degree of repeatability with %RSD values of 3.5409 %, all analytes tested showed a percentage RSD of less than 5%.

4.1.5.   Specificity and Selectivity results

The identification of peaks as presented in (Fig 4.5, 4.6, 4.7, 4.8) was confirmed by injecting the chlorinated phenols individually and the retention times. The method performs very well on these compounds and there is no evidence of co-elution.
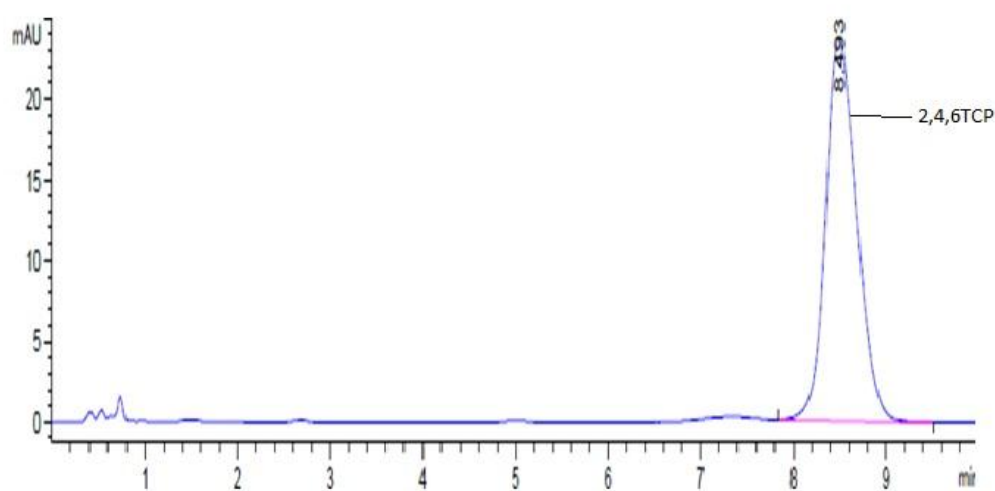
Figure 4. 5: Chromatogram of distilled water spiked with 1mg/l std of 2,4,6 trichlorophenol
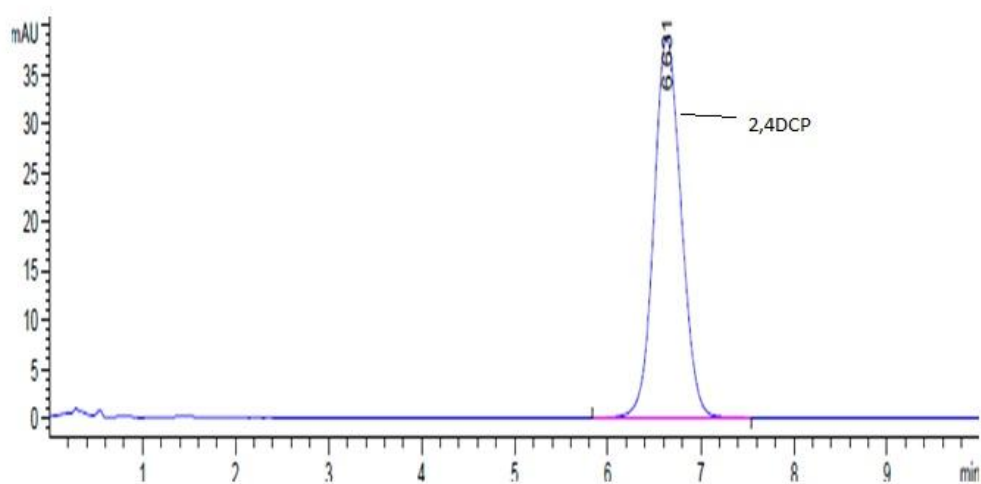
Figure 4. 6: Chromatogram of distilled water spiked with 1mg/l std of

2,4dichlorophenol
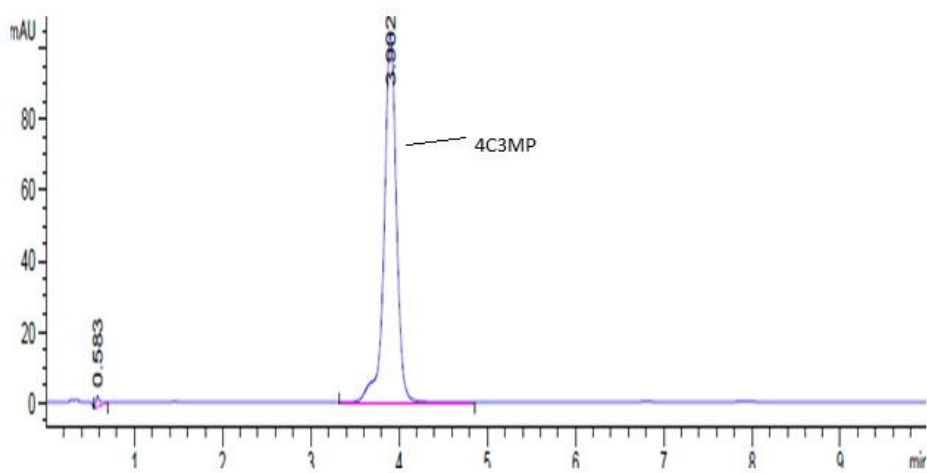
Figure 4. 7: Chromatogram distilled water spiked with 1mg/l std of 4-chloro-3-
methylphenol
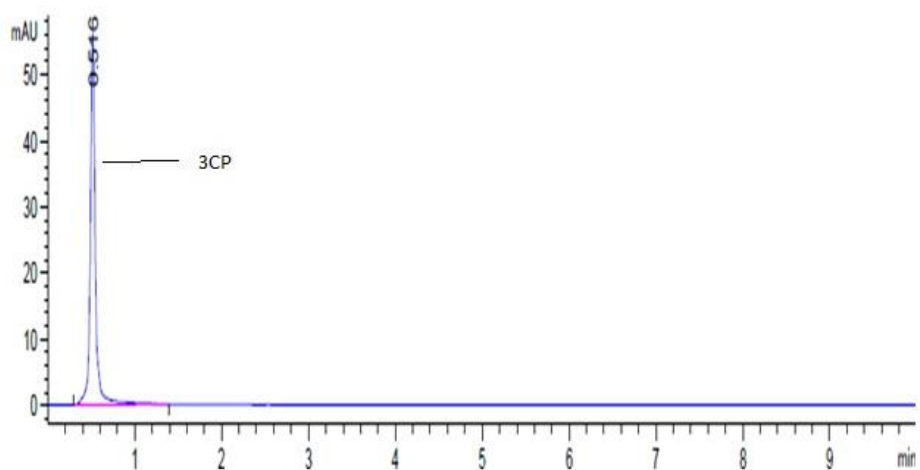
Figure 4. 8: Chromatogram of distilled water spiked with 1mg/l std of 3-chlorophenol

HPLC chromatogram of four (4) compounds of the chlorinated phenols standard mixture is shown Figure 4.9. The analytical method development was successful as shown by relatively narrow and well separated peaks of individual chlorinated phenols compounds.
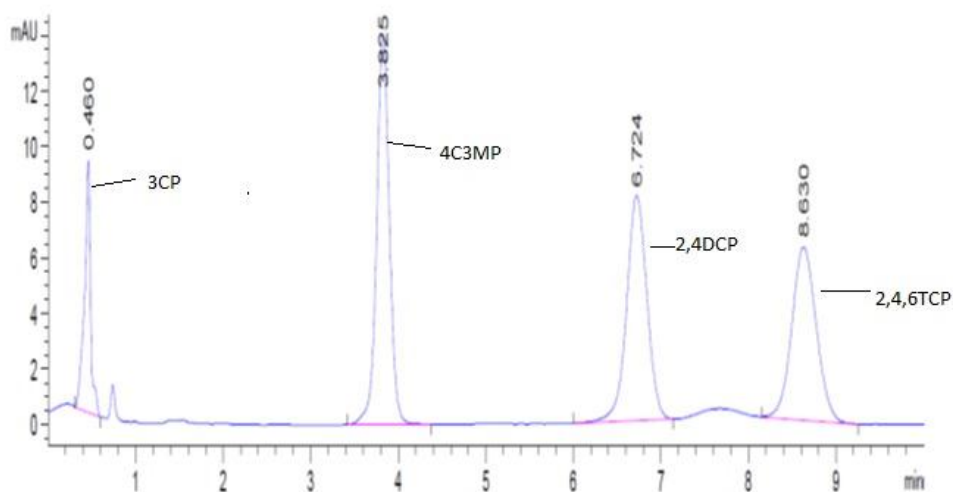
Figure 4. 9: Mixture of chlorinated phenols spiked with 1mg/l std

4.1.6. SPE method development

The HPLC method was validated by examining SPE variables such as one sorbent, type of solvents, and breakthrough volume. Solid-phase extraction (SPE) is an increasingly useful sample-preparation technique. With SPE, many of the problems associated with liquid-liquid extraction, such as incomplete phase separations, less-than-quantitative recoveries, use and disposal of large and expensive quantities of organic solvents. This technique is used most often to prepare liquid samples and extract semi volatile or nonvolatile analytes, but can also be used with solids that are pre-extracted into solvents. They are available in a wide variety of chemistries, adsorbents, and sizes so that it is necessary to select the most suitable product for each application and sample.

4.1.7. Efficiency of a concentration in a sorbent phase

The first parameter tested on the one cartridge, namely C-18 500 mg was the effect or the efficiency of the sorbent phase. The cartridge was first unconditioned in Figure 4.10 to check for the recovery of chlorinated phenols. The conditioned cartridge results with 10 ml methanol, and equilibrated with 10ml ultra-pure water are shown in Figure 4.10. The 10 ml deionized water was spiked with standard solution containing 1mg/l, 3 mg/l, 5 mg/l chlorinated phenols and was then loaded to the cartridge. When the analytes are retained, the elute was collected and injected on the HPLC to test for the sorbent phase efficiency.
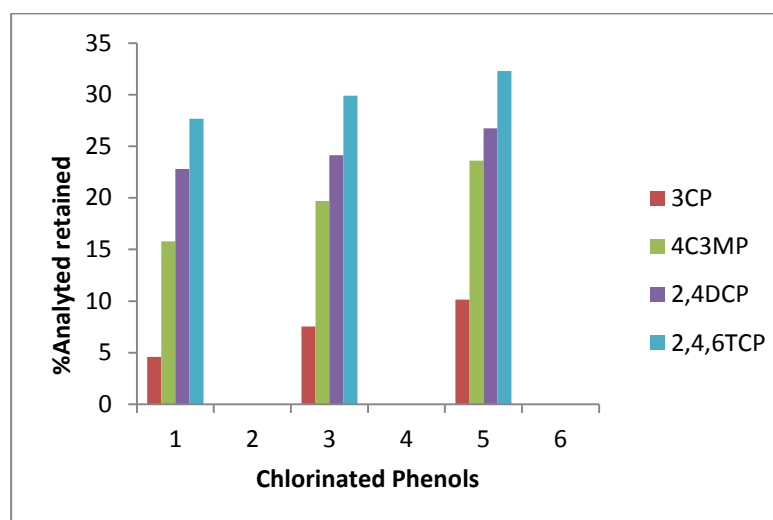
Figure 4. 10: Recovery of analyted using different concentration on unconditioned C18 cartridge

The results of HPLC analysis of the elute in Fig 4.11 indicate that there was significant analyte retention by the C18 cartridge, particularly indicate that significant quantities of the analytes were adsorbed by the solid phase, which means low and high concentration of elute was detected by the HPLC. Most notably is the 91% recovery of 2, 4,6Trichlorophenol in the elute extracted using the C-18 cartridge indicates that the concentration of the elute is about 5 mg/l.
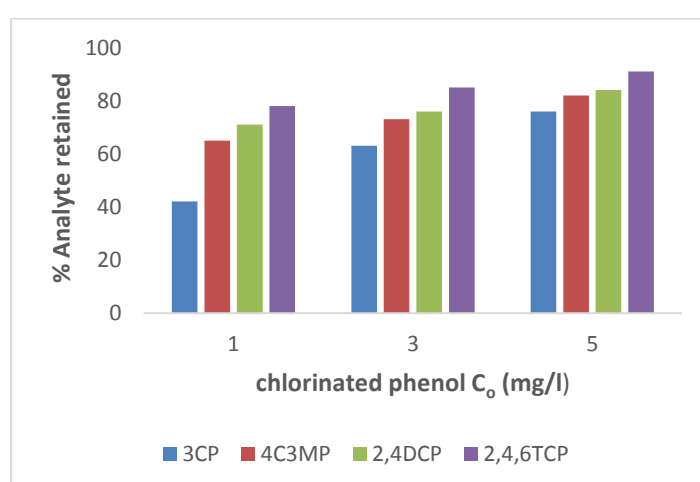
Figure 4.11: Recovery of analyted using different concentration on conditioned C18 cartridge.

4.1.8. Selection of the best elution solvent

Three solvents Acetone, Methanol and Acetonitrile were tested for best elution using C18 cartridge. The cartridge was conditioned with 10 ml ethanol, and equilibrated with 10 ml ultra-pure water. The sample blank spiked with standard solution containing 1mg/l chlorinated was then loaded to the cartridge, sorbent was dried under vacuum using vacuum manifold for 10 minutes and eluted with 30 ml solvent (sorbent was soaked for 10 min with elution solution before each elution). The elute was collected and analysed on HPLC.

4.1.9. Results of C-18 cartridge recovery values using different solvents

The chlorinated phenols compounds 20ml of a 1ppm of each solution was loaded onto a cartridge previously conditioned using 30ml methanol. The elute obtained after loading was collected and analysed by HPLC. The results of HPLC analysis of the elute indicate that there was significant analyte retention by all solvents, particularly the acetone and solvents whose results indicate that significant quantities of the analytes were adsorbed by the solid phase as shown by low analyte concentrations detected within the elute as compared to methanol solvent.

Most notably is that in the elute extracted using the C-18 cartridge indicating that there was up to 98% retention. It can therefore be deduced that it is essential to condition the cartridges before use, with the degree of conditioning depending upon the nature of the sorbent bed and the bed mass. These results are in sync with findings by (Poole, Gunatilleka and Sethuraman, 2000b) who postulated that the high surface tension of water often causes slow and uneven flow rates through solid phases when a cartridge is not conditioned first before loading the sample, resulting in low analyte recovery (Poole, Gunatilleka and Sethuraman, 2000b). From the results in Figure 4.12 it can be deduced that acetonitrile eluted more of the

analyte compared to acetone and methanol when C18 was used. The recovery values in % of C18 cartridge using different solvents follows below.
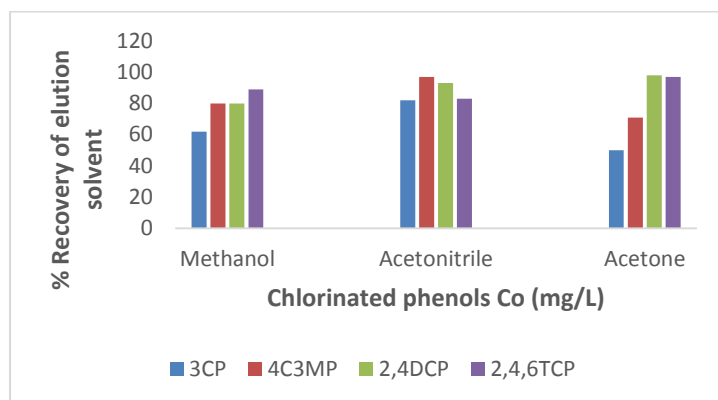
Figure 4. 12: Recovery of chlorinated phenol using different solvents

4.1.10. Effect of optimum volume

The optimum volume of different chlorinated phenols was determined using a spiked deionized water sample. According to (Poole et al., 2000b) the optimum volume is reached at the sample volume when the amount of analytes entering and leaving the solid phase becomes equal, due to saturation of the solid phase by analytes introduced. The optimum volume for the chlorinated phenol volume load curve theoretically take the form of a curve. The optimum chlorinated phenol loaded volume for a particular analyte is the volume which produces a 96% recovery. Figure 4.19 indicates that the 30 ml of spiked deionized sample volume proved to be more robust as it produced the highest recoveries for the chlorinated phenols analytes, compared to other sample volumes. One of the drawbacks of using optimum volume load curve for multi-residue analysis is that the optimum volume varies for each particular analyte. For this research, 30 ml was selected as the optimum volume load curve and was used for all further analyses.
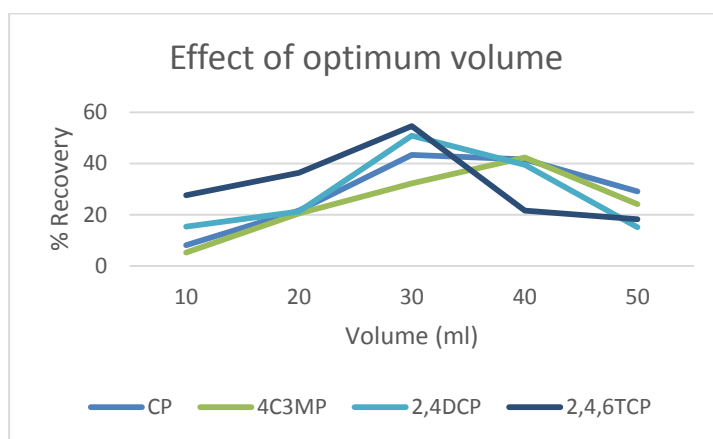
Figure 4. 13: The effect of optimum volume unconditioned C18 cartridge

In order to obtain reliable analytical results and a high enrichment factor, different sample volumes of 10, 20, 30, 40 and 50 ml containing 1mg/l of 3-chlorophenol,4chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol were passed through the C18-SPE cartridge at the optimal flow rate, respectively. The experimental results are shown in Fig 4.13 and Figure 4.14. Recoveries were acceptable for the extraction when the standard volume went up to 50 ml. When the volume of standard sample was more than 50 ml, the recoveries of chlorinated phenols decreased slightly. Considering the enrichment factor and stability, a standard sample volume of 30 ml was selected as the optimal sample volume. The volume of eluent is another factor that should be considered.

A series of experiments were designed and investigated through changing the volume of eluent methanol from 10 to 30 ml. The experimental results obtained are shown in Fig 4.14. It can be observed that the recoveries of four chlorinated phenols increased with the slight increase in the volumes of methanol from 10 and 30 ml. When the volume of methanol was >10 ml, the recoveries of chlorinated phenols increased slightly for 2, 4, 6-trichlorophenol. In the operation process of SPE, another 10 ml methanol was employed, passing through the C18 adsorbent packed cartridge in order to get rid of possible residual analytes and the results showed an increase and decrease were detected at 10 and 50 ml elution. Therefore, in subsequent experiments, 30 ml methanol was selected.
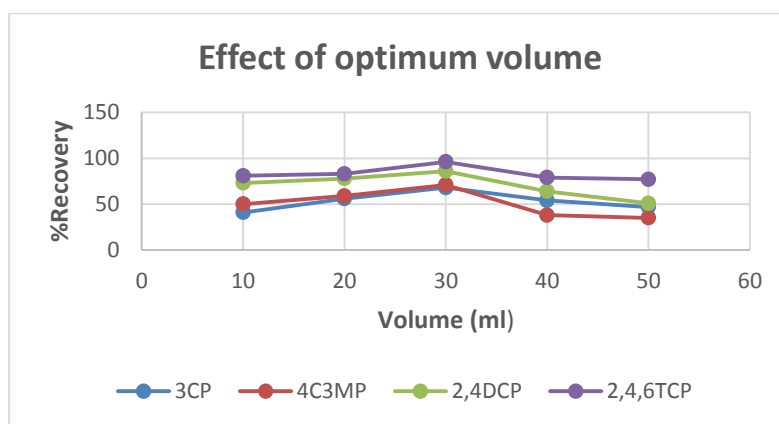
Figure 4. 14: The effect of optimum volume conditioned C18 cartridge

4.1.11.Determination of chlorinated phenols concentrations in deionized water samples

The chlorinated phenols compounds 20 ml of a 1ppm mixture solution was loaded onto a cartridge previously conditioned using 15 ml methanol. The elute obtained after loading was collected and analysed by HPLC. The results of HPLC analysis of the elute indicate that there was significant analyte retention by the cartridge, particularly the C-18 whose results indicate that significant quantities of the analytes were adsorbed by the solid phase as shown in in Figure 4.15, 4.16, 4.17, 4.18 by low analyte concentrations detected within the elute. It can therefore be deduced that that it is essential to condition the cartridge before use, with the degree of conditioning depending upon the nature of the sorbent bed and the bed mass. These results are in sync with findings by (Poole et al., 2000a) who postulated that the high surface tension of water often causes slow and uneven flow rates through solid phases when cartridges are not conditioned first before loading the sample, resulting in low analyte recovery.

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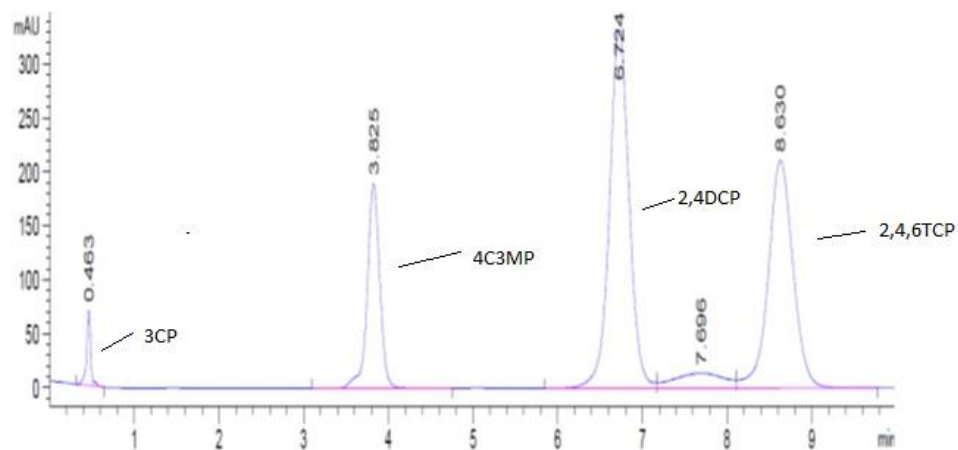
Figure 4. 15: Chromatogram mixture of chlorinated phenols spiked with 1mg/l std
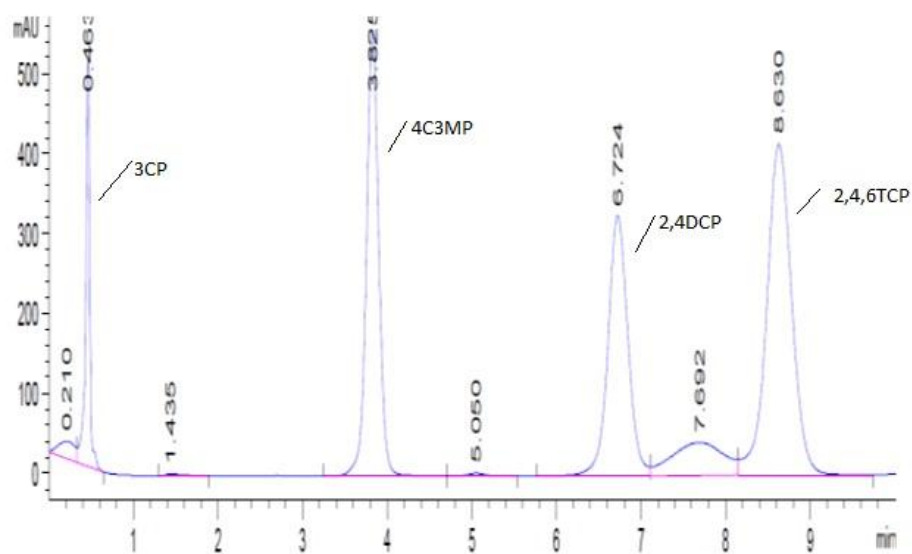
Figure 4. 16: Chromatogram mixture of chlorinated phenols spiked with 1mg/l std
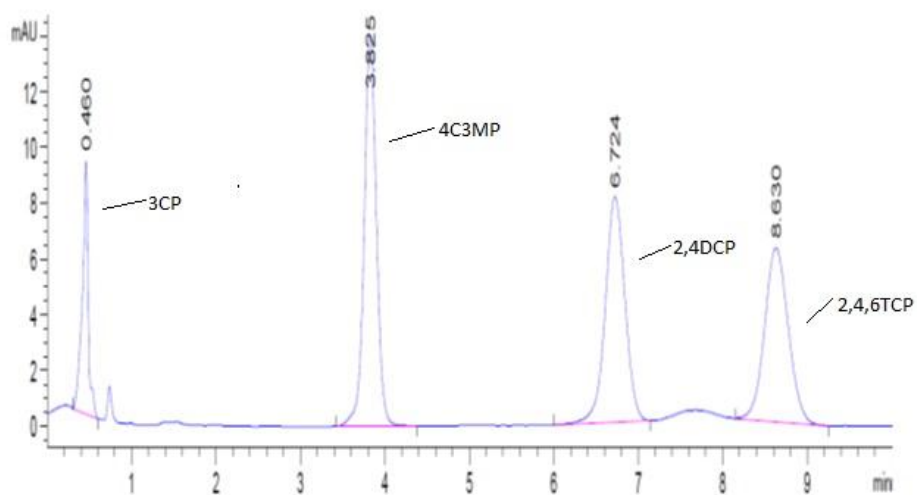
Figure 4. 17: Chromatogram mixture of chlorinated phenols spiked with 1mg/l std
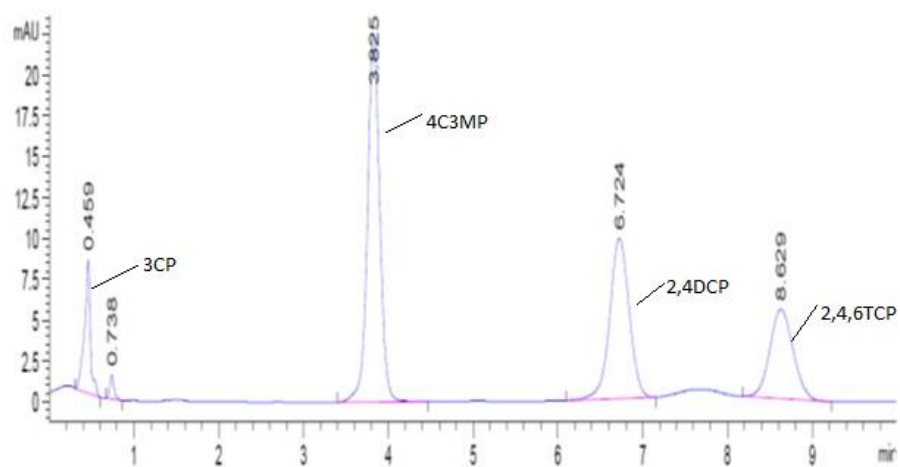
Figure 4. 18: Chromatogram cocktail of chlorinated phenols spiked with 1mg/l std

4.2. Adsorption capabilities of MAC before modification and after GMAC

4.2.1 THERMOGRAVIMETRIC ANALYSIS (TGA) for MAC and GMAC

The shape of TGA thermograms depends on the thermal behavior of the biomass which is related with chemical composition and chemical bonding of the structure. It should be considered that TGA can be used as a primary data for activated carbon production. Figure 4.19 and 4.20 shows the effect of temperature on the residual weight percent of the macadamia –nutshell activated carbon.
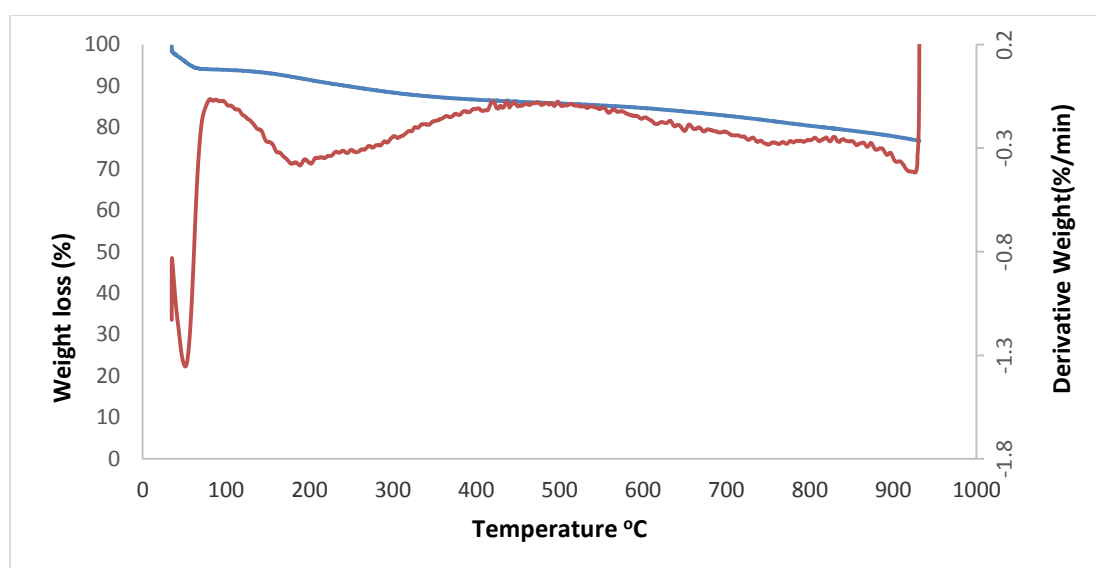
Figure 4. 19: TGA Curve of macadamia-nutshell activated carbon (MAC)
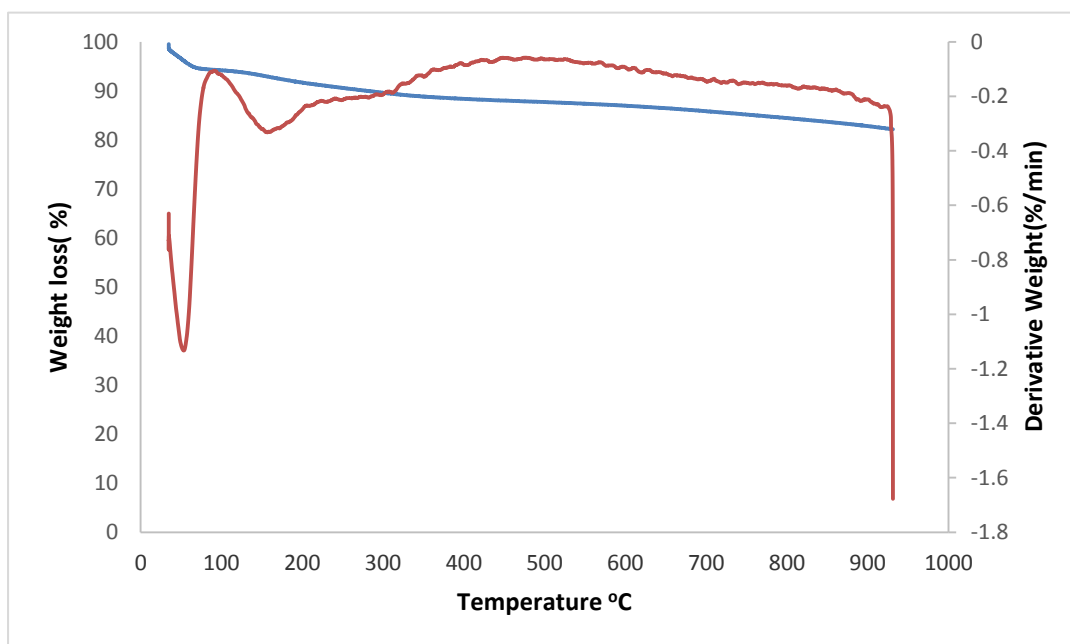
Figure 4. 20: TGA Curve of grafted macadamia-nutshell activated carbon (MAC)

The thermogram of macadamia nutshell activated carbon shows that the thermal composition of material starts to be noticeable after about 71 ^{o}C in Fig 4.19 and 69 ^{o}C in Fig 4.20. Weight loss observed prior to 71 ^{o}C corresponds to moisture content of activated material and was found to be 5.39 % and also shown in Fig 4.20 weight is 5.29%. After 71 ^{o}C, the main carbonization reactions started to dominate. The weight loss at the beginning ($<$ 150°C) was most likely due to the loss of moisture in macadamia nutshell activated carbon and the grafted MAC starts losing at $<$100 ^{o}C. The weight loss was observed around 71 – 482 ^{o}C for MAC and at 69 – 310 ^{o}C for grafted MAC which are likely associated with a loss of volatile matters. After that, a slight decrease of weight loss was still observed until 925.59 ^{o}C and 929 ^{o}C in Fig 4.20. DTA curve corresponded with the energy absorption while weight loss was reduced as shown in Fig. 4.19 and 4.20. In this stage, the main compound cellulose and hemicelluloses and partial lignin were lost from macadamia shell (Varhegi et al., 1997). Finally, the weight loss was slowly decreased in between 482 – 926 ^{o}C and 310 –

74

929 $^{\circ}$C (Kumar et al., 2007) . It was mostly associated the phase structure stable when compared with weight loss in both Fig 4.19 and 4.20.

4.2.2 Fourier transform infrared spectra (FTIR) for MAC and GMACFTIR Analysis.

The spectra of the macadamia activated carbon and grafted MAC were not similar, as shown in Figure 4.21 and 4.22. The same main bands appeared with more or less different shapes and relative intensities. The main band features were identical with the ones obtained for cellulose charcoals prepared at elevated pressures, at similar experimental conditions (Mok et al., 1992). The spectra indicate an alkyl aromatic activated carbon structure (3000-2800 cm^{-1} and 900-700 cm^{-1}) with numerous oxygen-containing functional groups: OH (3700-2000 cm^{-1}), COOH (a shoulder around 2600 cm^{-1}), (1700 cm^{-1}), C-O-(C) (1275 cm^{-1}), and C-O-(H) (1050 cm^{-1}). The most intense band of the spectra (1600 cm-^{1}) Fig 4.21 could due to an aromatic skeletal mode, significantly intensified by the presence of oxygen containing polar substituents. The biomass charcoals of the present work reveal a small amount of carbonyl groups (1700 cm^{-1}) than HY cellulose charcoals produced at conditions similar to the present work. The eucalyptus wood charcoal contained less residual OH (3700-2000 cm^{-1}) and aliphatic CH (3000-2800 cm^{-1}) than the macadamia nutshell charcoals Figure 4.21. The quantity of the aromatic CH groups (900-700 cm^{-1}) was approximately the same in all samples. The stronger carbonization in the bottom part of the reactor resulted in less oxygen-containing functional groups, less aliphatic CH, and more aromatic CH Figure 4.22.
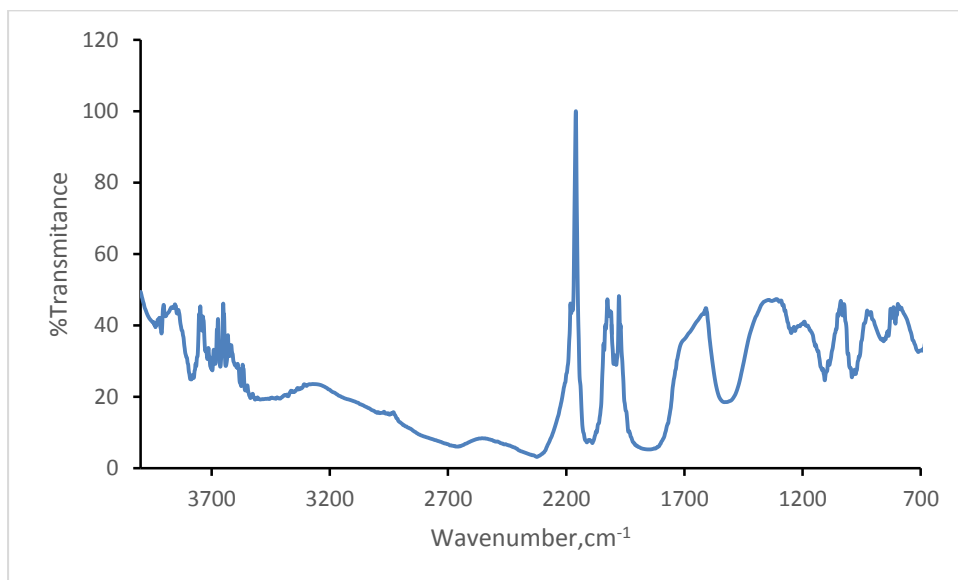
Figure 4. 21: FTIR-spectra of macadamia activated carbon
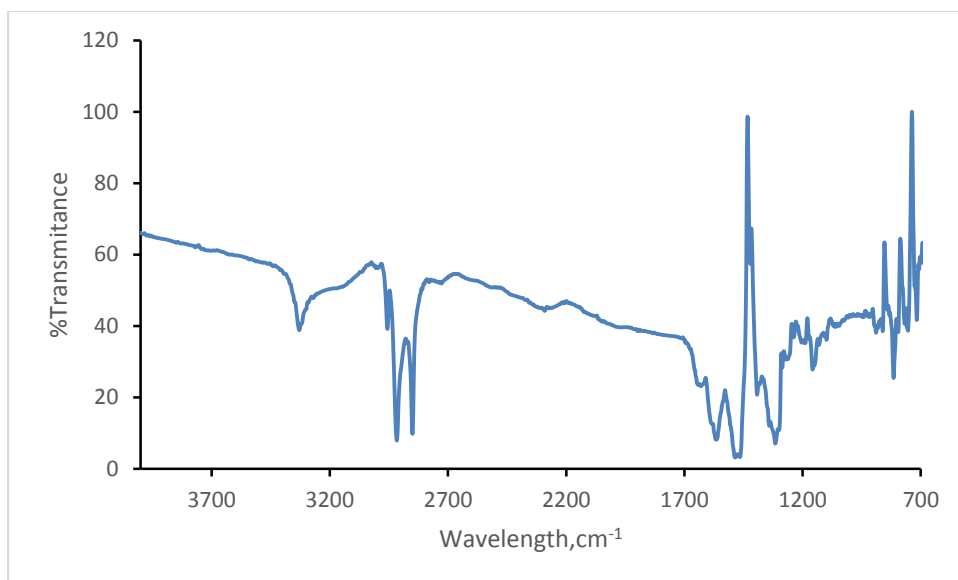
Figure 4.22: FTIR-spectra of grafted macadamia activated carbon

The adsorption capacity of activated carbon depends upon porosity and the chemical reactivity of functional groups at the surface. This reactivity creates an imbalance between forces at the surface as compared to those within the body, thus leading to molecular adsorption by the Van der Waals force. Knowledge on surface functional groups gives insight to the adsorption capability of the produced activated carbons. The FT-IR spectra are

recorded for qualitative characterization of the surface functional groups of the macadamia activated carbon (MAC) and grafted macadamia activated carbon (GMAC) in Figure 4.21 and 4.22, the O-H stretching is sharp and broad at 3600-3200 cm^{-1} and CO_2 stretching at 2328 cm^{-1} and from 2000 -1650 cm^{-1} bending aromatic compound overtone. The amide functional groups: N-H stretching of secondary amine and alkyl C-H stretching at 2950 - 2850 cm^{-1}, C=N stretching imine at 1690-1640 cm^{-1} and strong aliphatic ether stretching C-O at 1158 cm^{-1},with numerous C=C bending at 1000 – 754 cm^{-1}. The band of the spectra at 1649 cm^{-1} can be due to aromatic skeletal mode (Sun et al., 2001). FT-IR spectra of pyrolysis chars and activated carbons are quite similar (ILC, 1980). The macadamia activated carbons prepared from raw macadamia nutshell showing less intense infrared peaks compared to the grafted macadamia activated carbon. The activity of carbon increased due to the breakdown of functional group i.e. the intensity of infrared peaks decreased.

4.2.3. SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) was used to observe the morphology of macadamia nutshell activated carbon produced by phosphoric acid activation and activated carbon produced by potassium hydroxide activation.
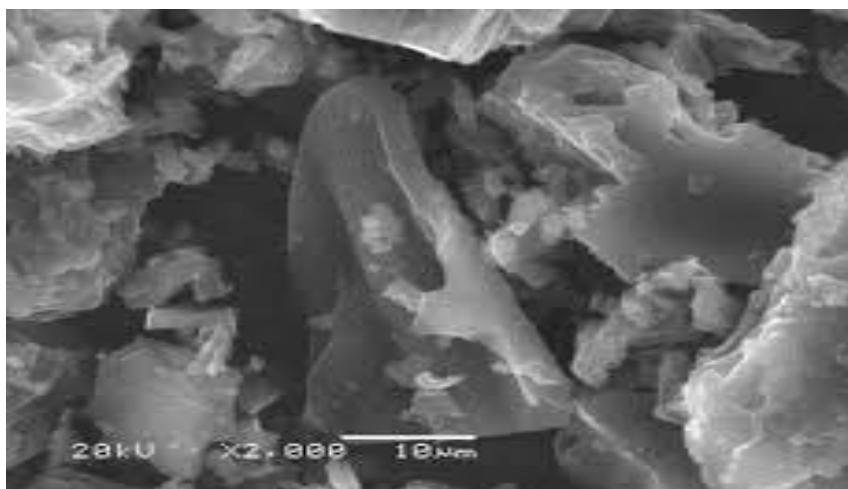
Figure 4. 23: SEM Micrographs of Macadamia Activated Carbon
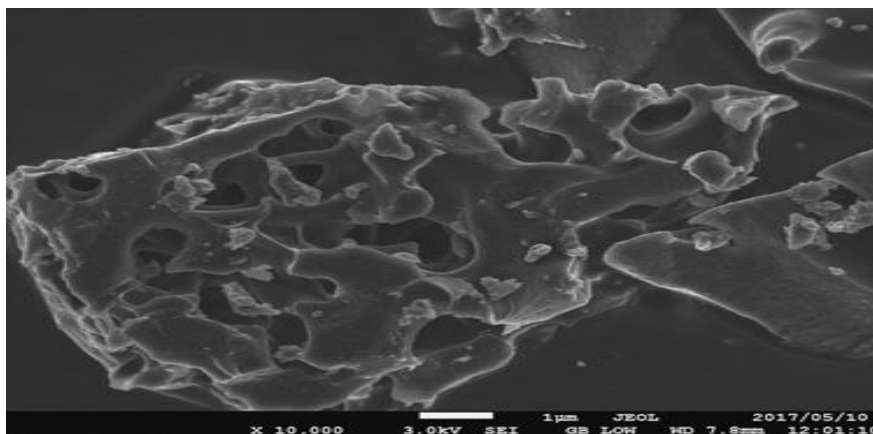
Figure 4. 24: SEM Micrographs of grafted Macadamia activated carbon

The micrographs of activated carbon sample (GMAC) produced in this research is given in Figure 4.24. This product achieved highest GMAC surface area value among the other product shown in Figure 4.23 and it was expected to have a higher porous texture in the micrographs of this activated carbon. The micrographs showed a heterogeneous and irregular texture with an eroded surface. Undoubtedly, these pores or cavities occurred by the thermal degradation, the phosphoric acid coated on the surface during carbonization and activation by leaving voids or pores. This porous texture of produced activated carbon is essential for taking in adsorbate molecules in adsorption processes comparing Fig4.24 with Figure 4.23 have a flaky long structure.

4.2.4. Method development

The ash content of activated carbon primarily depends on precursor material and these mineral matters are desired to be as low as possible to obtain an activated carbon of high quality. It is known that mineral content of activated carbon causes undesirable effects such as decreasing the activity and regeneration efficiency of activated carbon. In chapter 3 for the determination of selected chlorinated phenol in surface water samples the method was developed. Three solvents acetone, methanol and acetonitrile were selected for best elution

using macadamia activated carbon and grafted one. The solvents were chosen due to their difference in polarity. On reaching the column the sample components are selectively retained on the basis of physico-chemical interactions between the analyte molecules and the stationary phase. Detection techniques are employed for detection and quantification of the eluted components (Bhanot, 2014). Different concentrations were prepared by dilution of the 1 ppm standard solution. Three method were used to adsorb the chlorinated phenols from water which are solid phase extraction, macadamia activated carbon and grafted macadamia activated carbon to get the best technique from this three.

4.2.5. Effect of pH

The pH value of MAC is mainly based on the inorganic ingredients in the source material or added during manufacture. In this study, acidic pH values were obtained. This is due to phosphorus-containing compounds such as polyphosphates, which may form during impregnation (Puziy et al., 2002). The values are then controlled to be in the range of 5–7 by undergoing washing process with sodium hydroxide. According to (Lee and Morris, 1962), pH value below 8 is more favorable for adsorption of 3CP, 4C3MP, 2,4-DCP and 2,4,,6 TCP. The result of the study Figure 4.25 showed that pH changes did not significantly affect adsorption of chlorinated phenol onto MAC adsorbent from very low to moderately pH and started to reach equilibrium at and after pH=7 to 9 .The adsorption percentage (qe % values) of the adsorbent was relatively uniform from the pH of 7 to 9, and thereafter reached a constant value of the adsorbed % from pH 7 , the value decreased after 9 . This observation is expected, and stems from the fact that both the surfaces of the adsorbents and the chlorinated phenols are charged. Hence, change in hydrogen ion concentration is expected to have minimum effect on the adsorbent–chlorinated phenol interaction at high pH. However, at very high pH values, there was a significant reaching of descending in adsorption performance. This can be explained on the basis that increasing pH induced deprotonating of

the OH functional group on adsorbent surface and enhanced the p-electron-donor ability of the surface and thus strengthened p–p electron-donor–acceptor interactions of the aromatics (Ali et al., 2005).
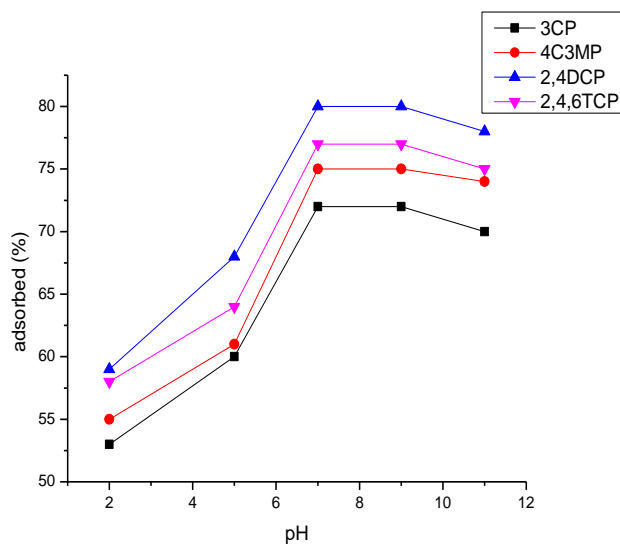
Figure 4. 25: Effect of pH on adsorption of chlorinated phenol onto MAC

4.2.6. Effect of concentration
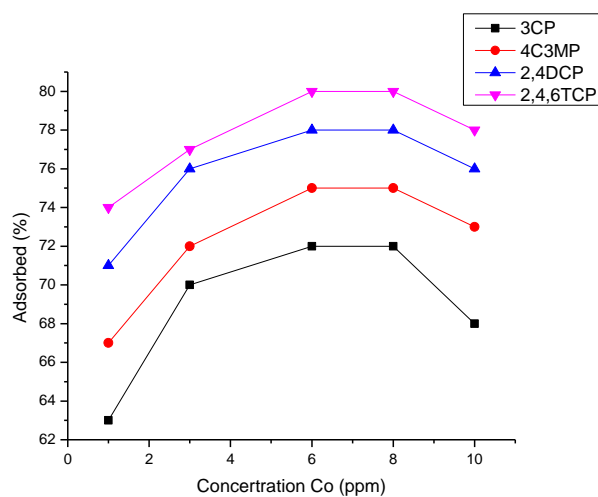
Figure 4. 26: Effect of initial concentration on adsorption of chlorinated phenol onto MAC

The initial concentration provides an important driving force to overcome all mass transfer resistence of the adsorbate between the aqueous and solid phases (Chemica, 2015). The effects of initial phenol concentration on adsorption were studied at the 25 ± 5 ^{o}C and 0.5 g/40ml adsorbent concentration for 250 min. As can be seen from Figure 4.26, when the initial chlorinated phenol concentration was at equilibrium and also the amount of the adsorbed chlorinated phenols was uniformed from 6-8 ppm, the removal of chlorinated phenols depends on the concentration. Similar trends have also been observed for methylene blue dye sorption onto bamboo-based activated carbon (Of et al., 2007), cotton waste (Ertaş et al., 2010) and fly ash (Basava Rao and Ram Mohan Rao, 2006). This decrease in proportion of removed phenol may be probably due to equilibrium shift during sorption process. A similar results has also been recorded for adsorption of Congo red from aqueous solution onto calcium-rich fly ash (Acemioglu, 2004). The initial phenol concentration provides an important driving force to overcome the mass transfer resistance of phenols between the aqueous phases and solid phases, therefore, decreasing initial concentration would enhance the adsorption capacity of phenol. These results are in accordance with obtained findings by other researchers (Cengiz and Cavas, 2008; Chemica, 2015).

The adsorbent dosage is an important parameter determining the capacity of the adsorbent (macadamia nutshell activated carbon for a given initial chlorinated phenol concentration. In order to determine the effect of adsorbent dosage on adsorption between 0.1 to 0.5 g/40ml adsorbent were used for adsorption experiments at fixed initial pH (pH = 5), initial phenol concentration (1 mg/ L), and temperature (25 ± 5 ◦C) for 250 min. increases with the adsorbent loading up to 0.5 g/ 40ml. This result can be explained by the fact that the sorption sites remain unsaturated during the sorption whereas the number of sites available for sorption site increases by increasing the adsorbent dose. The maximum adsorption efficiency of phenol onto macadamia nutshell activated carbon was found to be 74% with 2, 4,6-

trichlorophenol at adsorbent concentration of 0.5 g/40 ml. Also, as it can be seen from Fig 4.27, there was a significant increase in the percentage removal of phenol when the adsorbent concentration increases beyond 0.3 g/m l. This suggests that after a certain dose of biosorbent, the maximum adsorption is attained and, hence, the amount of pollutants remains slightly increasing, even with further addition of dose of adsorbent (Chakravarty, Sarma and Sarma, 2010).
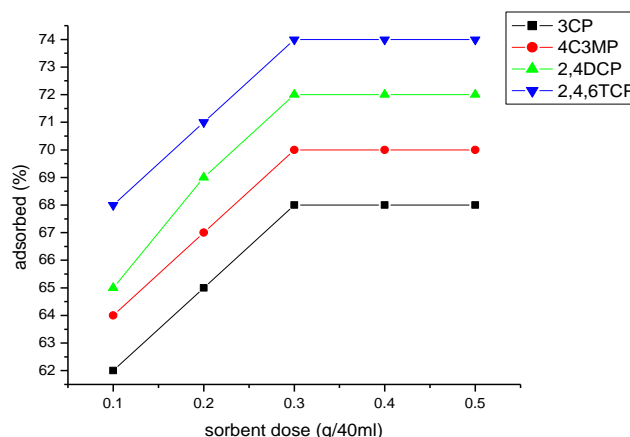
Figure 4. 27: Effect of adsorbent (MAC) dose on adsorption of chlorinated phenol

Also, when the adsorbent dosage increased, percentage removal of chlorinated phenol also increased but amount of adsorbed phenol, per g adsorbent (q_e) increased due to the fact that fixed phenol concentration 1 mg/L led to saturated active sites on adsorbent surface, and increase in the adsorbent concentrations caused particle aggregation (Calvete *et al.*, 2010). Thus, the adsorbent dose was maintained at 0.5 g/40 ml in all the subsequent experiments, which was considered to be the best relation between total adsorbed amount of phenol and percentage of removal.

Adsorption studies with 1 ppm chlorinated phenol solutions were conducted in the presence of solid macadamia nutshell activated carbon to determine the adsorbed percentage of the 3-chlorophenol, 4chloro-3-methylphenol, 2,4 dichlorophenol and 2,4,6 trichlorophenol The removal of phenol as a function of reaction time at different contact time with macadamia nutshell activated carbon is given in Figure 4.28 in terms of percent phenol removed and mg

phenol removed per gram of MAC, respectively. It is seen that phenol removal by the MAC and grafted MAC increases with increasing reaction time and reach equilibrium after 150 minutes. It should be noted, however, that there are different removal percentage value for all 4 chlorinated phenol. For example, no matter how long the reaction time, the maximum phenol removal that can be achieved by MAC and grafted MAC depend on the contact time of the adsorption process increase the uptake of the chlorinated phenol from 50 min to 150, showing an increase in adsorbed % for all the phenols, respectively. Complete phenol removal seems to be achievable for MAC and grafted MAC at 50 to 250 minutes only. On the other hand, a constant value is being achieved at 150, 200 and 250 minutes.
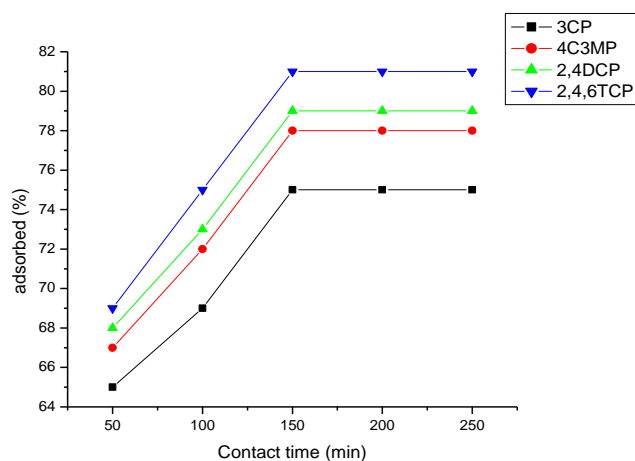
Figure 4. 28: Effect of contact time on adsorption of phenol onto MAC
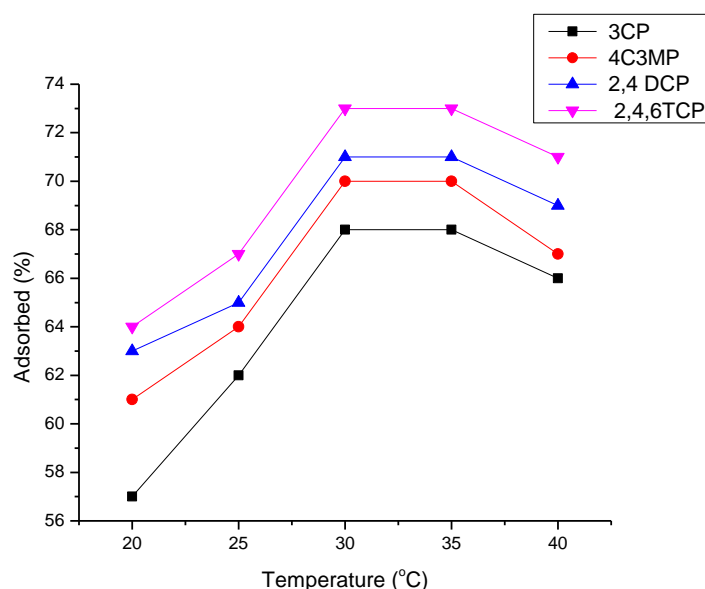
Figure 4. 29: Effect of temperature on adsorption of phenol onto MAC

It was found that the uptake of phenols by the sorbent adsorbed percentage start at increasing with the increase in temperature from 20 to 30 ^{o}C (Fig 4.29) reached equilibrium from 30 to 35 ^{o}C, indicating that the adsorption was an exothermic process (Al-Degs et al., 2008). According to (Hameed et al., 2017), the increase in the adsorption may be a result of increase in the mobility of the phenols with the increase in temperature. An increasing number of molecules may also acquire sufficient energy to undergo an interaction with active sites at the surface. Furthermore, increasing temperature may produce a swelling effect within the internal structure of the macadamia nutshell activated carbon, thereby enabling large phenols to penetrate further (Mohammed, 2013).

4.3.   Adsorption by macadamia nutshell activated carbon before modification

4.3.1.   Effect of pH on adsorption of chlorinated phenol onto MAC

It is well known that phenol adsorption onto activated carbon can occur via a complex interplay of electrostatic and dispersion interactions with three possible mechanisms namely (Altenor et al., 2009) π-π dispersion interaction between the phenol aromatic ring and the delocalized electrons present in the aromatic structure of the graphite layers (Ania, Parra and

Pis, 2002). Hydrogen bond formation (Armour, 1991) electron donor–acceptor complex formation at the carbon surface. Although in adsorption process electrostatic attraction exists between phenolate ions (C_6H_5O-) and adsorbent surface plays a very significant role, the oxygen of the surface carbonyl group acts as the electron donor and the phenol aromatic ring as the acceptor. Both aspects are determined by the solution pH.
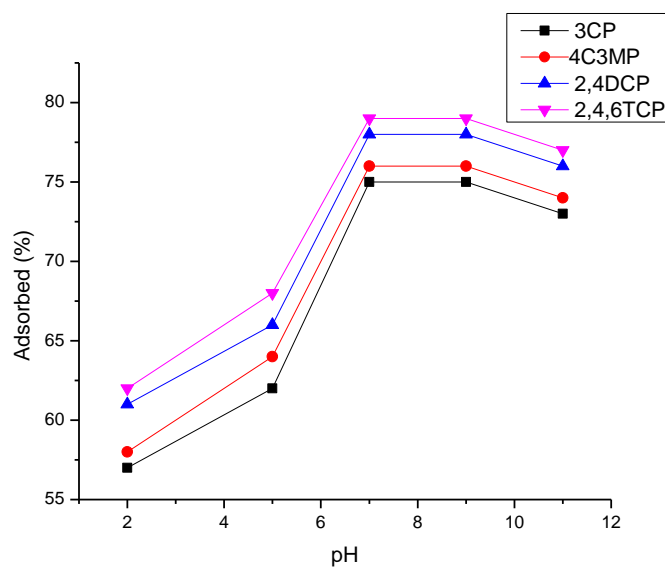
Figure 4. 30: Effect of pH on adsorption of chlorinated phenol onto MAC

Due to the amphoteric character of a carbon surface, its adsorption properties may be influenced by the pH value of the (Salame and Bandosz, 2003). The effect of initial pH on the adsorption of phenol was also evaluated at 25^0C at different initial pH values in the range of 2–11 for initial concentrations of 1 mg/l for chlorinated phenol solution adjusted by adding either 0.1M HCl or 0.1M NaOH. The variation of adsorption with different values of pH is shown in Fig.4.30.

4.3.2. Effect of initial concentration on adsorption of phenol onto MAC
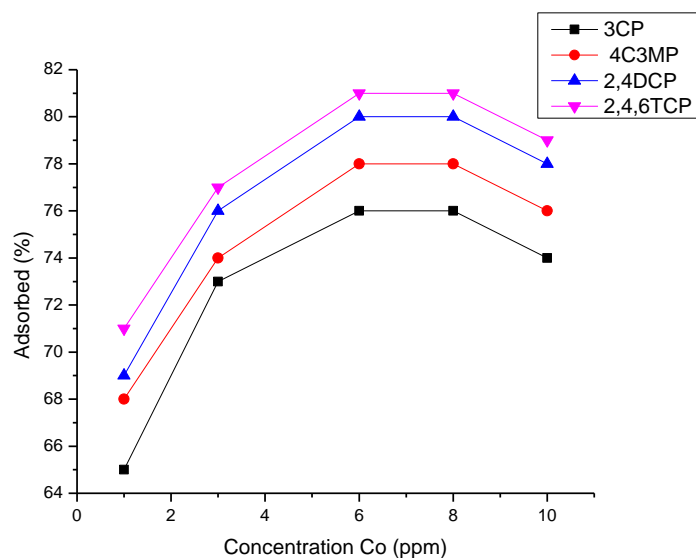
Figure 4. 31: Effect of initial concentration on adsorption of chlorinated phenol onto MAC

To study the effect of initial phenol concentration, experiments were carried out in different conical flasks with a fixed adsorbent dose of 0.5 g/l at varying phenol concentrations of, 1,3,6,8 and 10 mg/l. These flasks were agitated in shaker for 250 minutes keeping pH=5 fixed in all the samples. The chlorinated phenol samples were withdrawn from the shaker at above noted time. Post adsorption the supernatant was collected and filtered, first using 42 grade whatman filter papers and then by syringe driven 100 mm Whatman filter. Filtered supernatant was analyzed using HPLC. Fig 4.31 was plotted with qe (%) versus initial concentration. The adsorption data for the uptake of phenol versus at different initial concentrations is represented in Fig.4.31 above showing increasing value from 2 ppm up until 6ppm, it is evident that, 81% removal is possible with an initial phenol (2,4,6-TCP) concentration of 2-6 ppm and the equilibrium is reached at 6-8 ppm. However, with initial concentration of 6 ppm about 81% of trichlorophenol removal is becoming possible and equilibrium was in the approaching state in the middle of 6-8 ppm. The results with the initial concentration of 6 ppm have shown higher uptake for all the chlorinated phenol which is above 75 % of the total amount of phenol uptake was found to occur in the first 6ppm and

the adsorption process was almost tending towards equilibrium at the end (Vázquez et al., 2007). The higher sorption rate at the initial period (first 60 minutes) may be due to an increased in concentration gradients between adsorbate in solution and adsorbate on adsorbent surface, as time precedes this concentration gradient is reduced due to the accumulation of phenol particles in the vacant sites leading to a decrease in the sorption rate at the larger stages from 50 to 250 minutes (Uddin, Islam and Abedin, 2007).

4.3.3 Effect of adsorbent (MAC) dose on adsorption
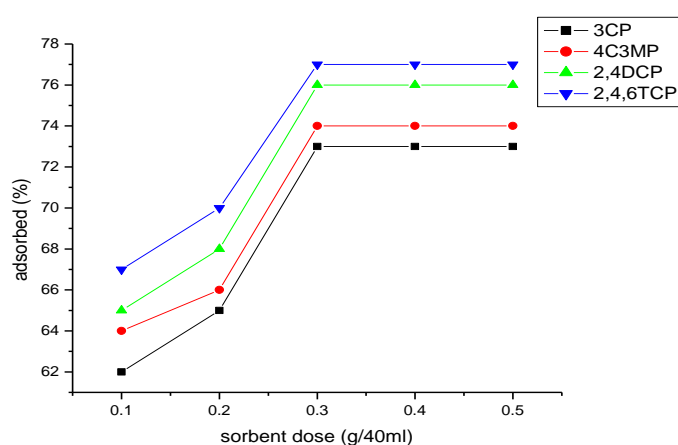
Figure 4. 32: Effect of sorbent dosage on adsorption of chlorinated phenol onto MAC

In order to find out the appropriate adsorbent dose of MAC, this study was done. The effect of MAC on the amount of removal of phenol solution was obtained by contacting 40 ml of phenol solution of initial concentration of 1 mg/l with different weighed amounts (0.1,0.2, 0.3, 0.4 and 0.5 g) of MAC in stopper conical flasks. Each sample was then agitated for 250 minutes at a constant 120 rpm at the natural pH of solution. The supernatants were then filtered using Whatman filter paper 42 grade and subsequently with syringe driven Whatman Millipore filter having pore size 0.45μm. The phenol concentration in the filtered supernatant was then analyzed as before. A plot of q_e % removal was plotted on different axis against adsorbent dose. Fig 4.32 shows the effect of adsorbent (MAC) dosage on the removal of phenol. The percentage removal of phenol increased with the increase in

adsorbent dosage. This can be attributed to increased adsorbent surface area and availability of more adsorption sites from the increase adsorbent dosage. But amount of phenol adsorbed per unit mass of MAC reaches equilibrium with increase in adsorbent dosage at 0.3 g, because for the same phenol concentration we got large number of adsorption sites with the increment of MAC dose (Uddin, Islam and Abedin, 2007).

4.3.4. Effect of Contact time for adsorption of Phenol onto MAC
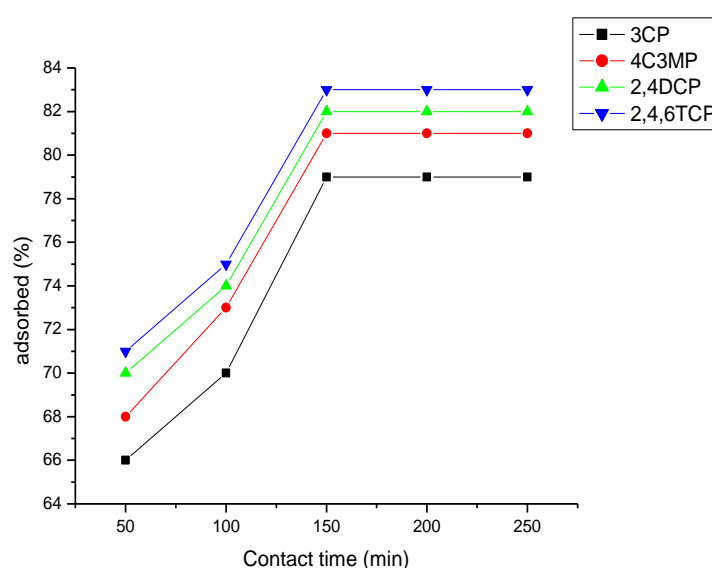
Figure 4. 33: Effect of contact time on adsorption of chlorinated phenol onto MAC

To explain the effect of contact time on adsorption of phenol (Co =1 mg/L), the batch experiments were carried out in a series of conical flasks with a constant MAC dosage of 0.5g/L in all the samples. These flasks were agitated in water bath shaker for 50, 100, 150, 200 and 250 minutes keeping pH at the natural point in all the samples. The samples were withdrawn from the water bath shaker at predetermined time intervals. The supernatant was collected and filtered using syringe driven 0.45μm Millipore Whatman filter. The concentration of phenol in supernatant was measured for all the samples. The qe was evaluated for all the samples and a Fig 4.33 was plotted between q_e % versus time, the results showed that equilibrium time required for the adsorption of phenol onto MAC was

250 min. However, the results also indicated that up to 60% of the total amount of phenol uptake was found to occur in the first 50 min. The higher sorption rate at the initial period (first 50 min) may be due to an availability of large number of vacant sites on the adsorbent at the initial stage, as a result there was an increased in concentration gradients between adsorbate in solution and adsorbate on adsorbent surface (Uddin, Islam and Abedin, 2007). Thus tends to increase in phenol sorption at the initial stages. As time precedes this concentration gradient is reduced due to the accumulation of phenol particles in the vacant sites leading to an equilibrium stage in the sorption rate at the larger stages from 150 to 250 minutes.

4.3.5. Effect of temperature on adsorption of Phenol onto MAC
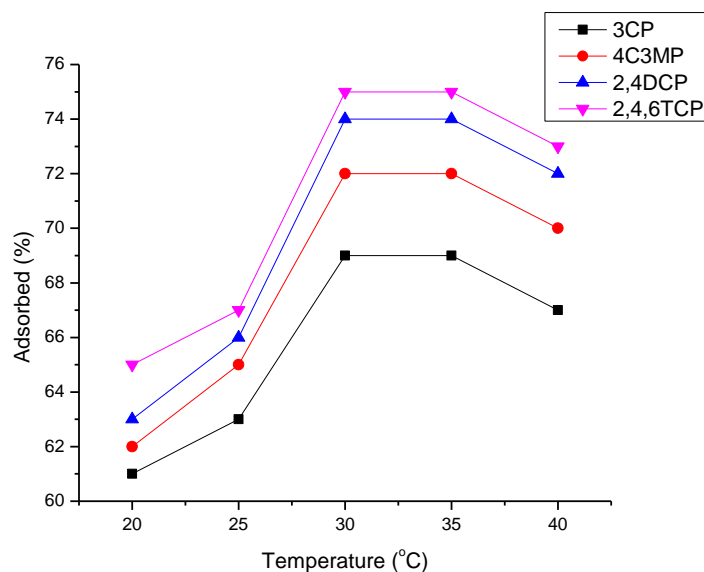
Figure 4. 34: Effect of temperature on adsorption of chlorinated phenol onto MAC

To explain the effect of temperature on adsorption, phenol solutions of same concentration namely, 3-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol were prepared at 1ppm. All sample solutions were maintained at pH at the natural for all the sample and 0.5 g/ 40ml MAC was added to each solution, then the

samples were agitated for 250 min at different temperatures 20, 25, 30, 35 and 40 ^oC respectively. A plot of uptake capacity of MAC against initial concentrations at different temperatures was plotted shown in Fig.4.34, it is evident from Fig. 4.29 that the value of maximum adsorption capacity qe increase with temperature thus suggesting that adsorption is favored at lower temperatures. At high temperature kinetic energy of adsorbate phenol is so high that they do not bind with the active sites available on the MAC surface. However if the process was endothermic in nature than only the sorption capacity would increase because of inter molecular pore diffusion (Weber, 1963).

4.4. Adsorption after modification of MAC

4.4.1. Effect of pH on adsorption of chlorinated phenol onto GMAC
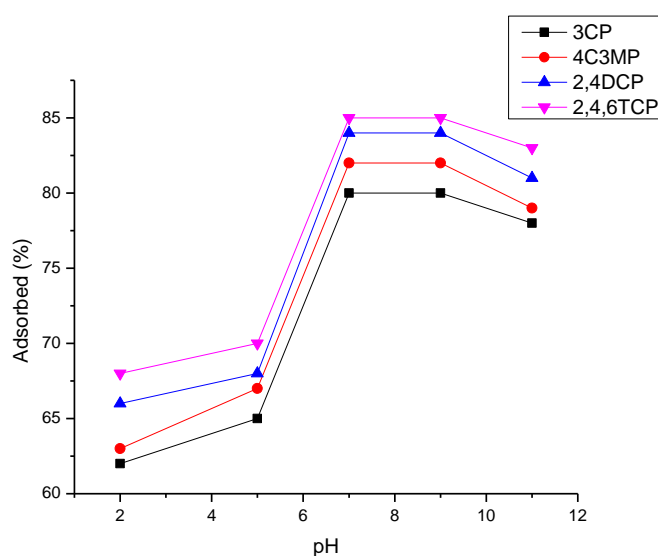
Figure 4. 35: Effect of pH on adsorption of phenol onto grafted MAC

It is evident from Fig 4.35 that the uptake of phenol increases with the increase in pH, and attain to the maximum value at pH = 7 thereafter, the uptake of phenol decreases with decreasing in pH uptake. Further, increase in pH causes again increase in phenol uptake. In this study the amount of phenol adsorbed is highest at pH= 7 followed by 9, next to that the uptake of phenol reaches equilibrium at pH = 7-9. pH also affects the degree of ionization of

phenol in adsorption medium. In this study, the amount adsorbed increase at both high and low pH values. The pKa value for phenol is 9.89. Phenol could be expected to become negatively charged phenolate ion above this pH and repulsion between the surface layer and the anionic phenolate results in reduced adsorption. The low pH value was obtained by using an acid solution. It introduced additional protons in the solution, which competed for the carbonyl sites, so the adsorption was reduced at low pH (Podkościelny and László, 2007).

4.4.2. Effect of initial concentration on adsorption of phenol onto GMAC
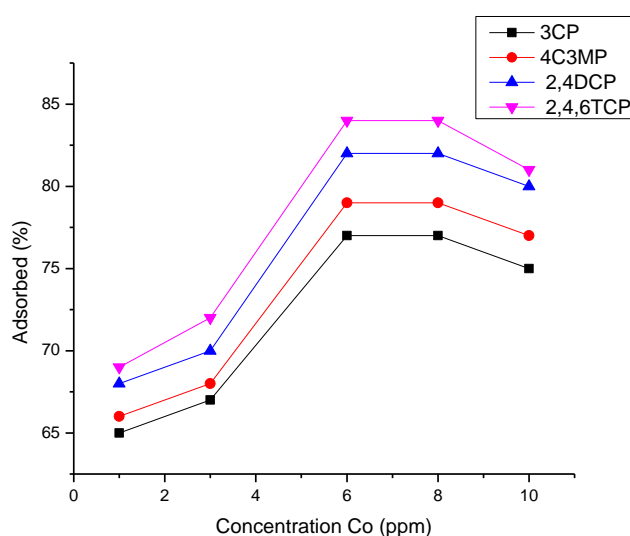
Figure 4. 36: Effect of initial concentration on adsorption of phenol onto grafted MAC

The effect of C_o on the adsorption on grafted MAC as a function of time is shown in Fig 4.36. At time 250 min the adsorbed % of adsorbent increased with increasing C_o. The C_o provides the necessary driving force to overcome the resistances to the mass transfer of phenol between the aqueous and the solid phase. The increase in C_o also enhances the interaction between phenol and grafted MAC. Therefore, an increase in C_o of phenol enhances the adsorption uptake of phenol (J.S Zogorski, 1978)

4.4.3.   Effect of adsorbent (GMAC) dose on adsorption
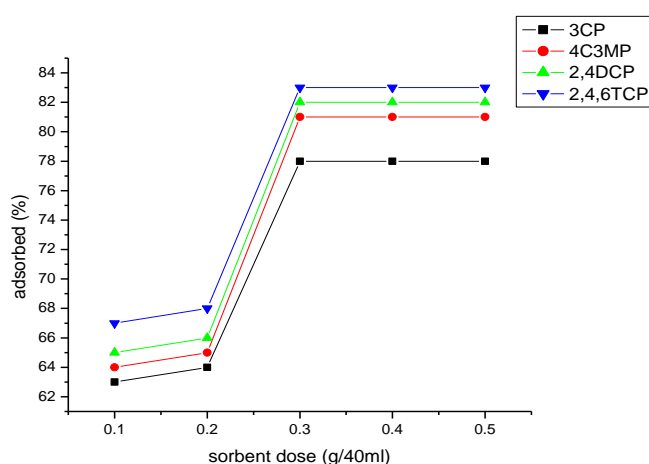
Figure 4. 37: Effect of sorbent dosage on adsorption of phenol onto grafted MAC

The adsorbent dose is also an important parameter in adsorption studies because it involves effective removal of adsorbate and cost as well with it, i.e. if the amount of adsorbent is less then we will not find an effective removal of the adsorbate, secondly if we will be using more amount of adsorbent then though our goal will be achieved but the process will not be a cost effective one. For these reasons the study of effect of adsorbent dose on adsorption is a must for all adsorption works. Here the adsorbent is the grafted macadamia activated carbon (GMAC). In order to study the effect of adsorbent dosage, the GMAC dosages were varied from 0.1 to 0.5 g/L and placed into series of the 100 mL conical flasks containing 50 ml of 1 mg/L chlorinated phenol solutions. These samples were agitated for 250 min at 120 rpm at the pH = 5. A plot of qe % removal was plotted on different axis against adsorbent dose. Referring to Fig 4.37 above we obtain, It was found that with increase in adsorbent dose percentage removal is increased because large amount of adsorption sites were found, in contrast the uptake of phenol is gradually increasing because of two reasons, firstly for the same amount of phenol large number of adsorption sites are found and secondly large amount of adsorbents in the big available space clump together thereby not limiting the path

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of diffusion and thus adsorption (Singh et al., 2008a). The corresponding point on x-axis of the intersection point of these two curves give the optimal dose of adsorbent and here we found the optimal dose for MAC as 1 mg/l of phenol solution.

4.4.4. Effect of Contact time for adsorption of Phenol onto GMAC

To investigate the effect of contact time on adsorption of phenol (Co =1mg/L), the batch experiments were carried out in a series of conical flasks with a constant grafted MAC dose of 0.5 g/L in all the samples. These flasks were agitated in water bath shaker for 50, 100, 150, 200 and 250 minutes at a constant 120 rpm at the natural   pH in all the samples. The samples were withdrawn from the multi stirrer at predetermined time intervals. The supernatants were then filtered using Whatman filter paper 42 grade and subsequently with syringe driven Whatman Millipore filter having pore size 0.45μm. The concentration of phenol in supernatant was measured for all the samples. The qe was evaluated for all the samples and a graph was plotted between q_e %versus time as shown in Fig.4.38.
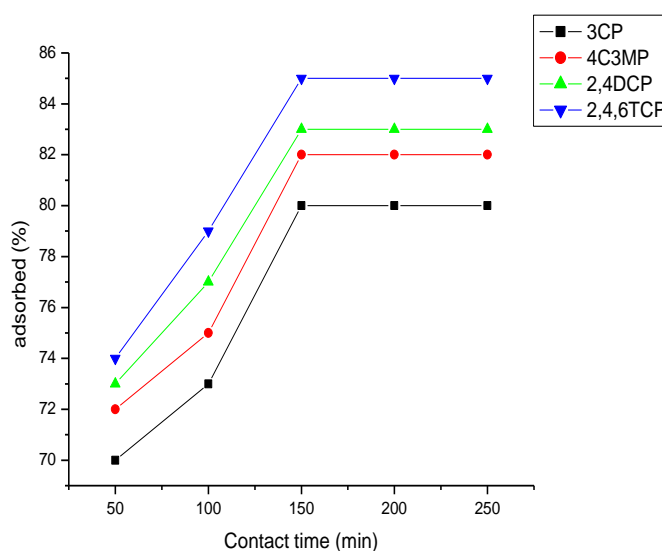
Figure 4. 38: Effect of contact time on adsorption of phenol onto grafted MAC

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Fig.4.38 depicts that 70% of the total amount of phenol uptake was found to occur in the first 50 minutes and the adsorption process was almost tending towards equilibrium at the end of 150 minutes. The higher sorption rate at the initial period (first 50 minutes) may be due to availability of large number of vacant sites on the adsorbent at the initial stage, as a result there was an increase in uptake between adsorbate in solution and adsorbate on adsorbent surface, thus tends to increase in phenol sorption at the initial stages. As time precedes this concentration gradient is reduced due to the accumulation of phenol particles in the vacant sites leading to reach equilibrium in the sorption rate at the larger stages from 150 to 250 minutes (Sandro Altenor et al., 2009).

4.4.5. Effect of temperature on adsorption of Phenol onto GMAC
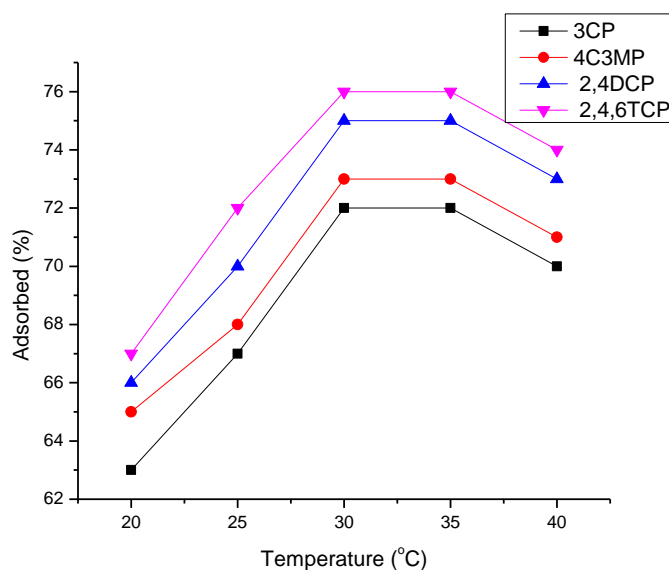
Figure 4.39: Effect of temperature on adsorption of phenol onto grafted MAC

For analyzing the effect of temperature on adsorption, phenol solutions of the same concentration of 1 ppm were prepared to determine the chlorinated phenol at different temperatures from 20, 25, 30, 35 and 40 °C. All sample solutions were maintained at natural a pH and 0.5 g/L grafted MAC was added to each solution, then the samples were agitated for 250 min at different temperatures 20, 25, 30, 35 and 40 °C respectively. A plot of uptake

adsorbed % of grafted MAC against temperature at different temperatures was plotted shown in Fig 4.39, It is evident shown in Fig 4.34 that the value of maximum adsorption capacity qe increase with temperature thus suggesting that adsorption of phenol on grafted MAC is endothermic. The increased uptake of phenol with increase in initial concentration suggests that both physiosorption as well as chemisorption is taking place (Singh et al., 2008b).

4.4.6. Adsorption kinetics study

Kinetics models are used to examine the rate of the adsorption process and potential rate controlling step. In the present work, the kinetic data obtained from batch studies have been analysed by using pseudo-first-order and pseudo-second-order models for 3-chlorophenol, 4- chloro 3-methyl phenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol in this study (Uddin, Islam and Abedin, 2007). The first order equation of Langergren is generally expressed as follows, (Dash S. N., 2010).

$$\frac{dq}{dt} = k_1(qe - q)\ldots\ldots\ldots\ldots\ldots\ldots.4.1$$

ectively, and k_1 is the rate constant of pseudo-first-order sorption (min-1). The linearized form of aboWhere qe and q are the amounts of phenol adsorbed (mg/g) at equilibrium and at time t (min), respve equation is given as,

$$\text{In (qe- q) = Inqe –k1t}\ldots\ldots\ldots\ldots..4.2$$

A plot of ln(qe-q) against t should give a linear relationship with the slop k1 and intercept of ln qe.

The pseudo-second-order kinetic rate equation is expressed as follows

$$\frac{dq}{dt} = k_2 (q_e - q)^2 \ldots\ldots\ldots\ldots\ldots.4.3$$

Where k_2 is the rate constant of pseudo-second-order sorption (g mg-1min-1). The linearized form of above equation becomes,

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \ldots\ldots\ldots\ldots\ldots\ldots\ldots...4.4$$

If the second order kinetic equation is applicable, the plot of t/q against t should give a linear relationship. The qe and k_2 can be determined from the slope and intercept of the plot (Uddin, Islam and Abedin, 2007).

$$q_e = k_f C_e^{1/n} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..4.5$$

Where k_f and n are the Freundlich constants that indicate adsorption capacity and adsorption intensity, respectively. The linearized form of Freundlich isotherm can be written as,

$$\text{In}q_e = \text{In}k_f + \frac{1}{n}\text{In}C_e \ldots\ldots\ldots\ldots\ldots4.6$$

The value of K_f and n can be calculated by plotting ln q_e versus ln C_e.

The linearity test solutions for the proposed method was obtained over the tested calibration ranges of 50 to 250 minutes for 3-Chlorophenol (Fig4.40), 4-Chloro-3-methyl phenol (Fig4.41), 2,4-Dichlorophenol (Fig4.42) and 2,4,6-Trichlorophenol (Fig4.43) in a five-point time range. The working range as shown in Table 4.9 for all the components is wide enough to produce valid measurements. The results showed an r^2 of greater than 0.98 as shown in
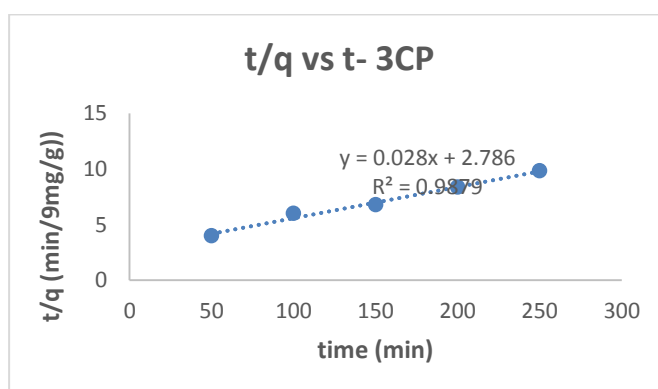
Figure 4. 40: Linear curve for 3 chlorophenol (3CP) on macadamia activated carbon
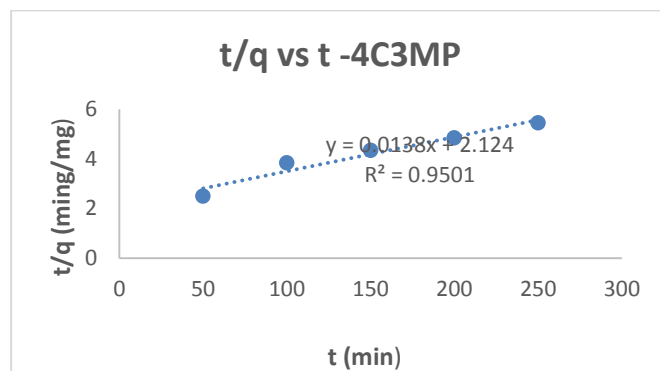
Figure 4. 41: Linear curve for 4chloro-3methylphenol (4C3MP) on macadamia activated carbon
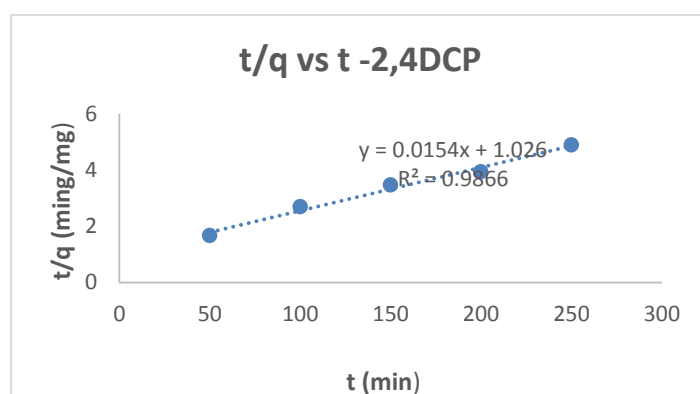
Figure 4. 42: linear curve for 2,4 dichlorophenol (2,4DCP) on macadamia activated carbon
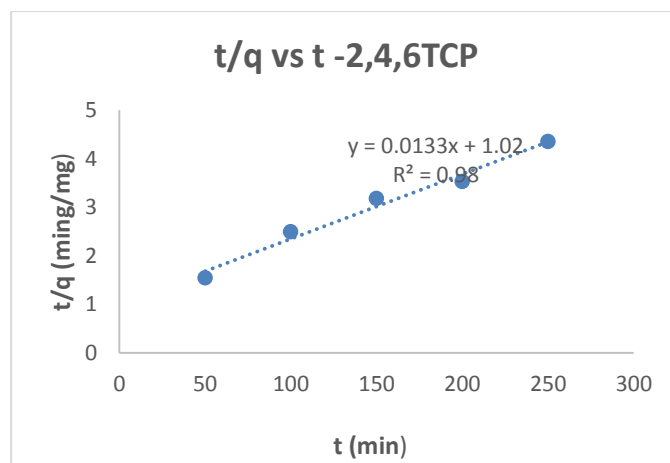
Figure 4. 43: Linear curve for 2,4,6 trichlorophenol (2,4DCP) on macadamia activated carbon

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Table 4.4. Pseudo first and second order parameters for 3chlorophenol, 4chloro-3
methylphenol, 2,4 dichlorophenol and 2,4,6 trichlorophenol sorption onto
macadamia activated carbon

Analyte	Equation	K_1 (min^{-1})	R^2	K_2 (gmg^{-1}min^{-1})
3 Chlorophenol	y=0.028x+2.786	0.028	0.9879	2.815×10^{-4}
4Chloro-3-methyphenol	y=0.0138x+2.124	0.0138	0.9501	8.967×10^{-5}
2,4 Dichlorophenol	y=0.0154x+1.026	0.0154	0.9866	2.312×10^{-4}
2,4,6 Trichlorophenol	y=0.0133x+1.02	0.0133	0.98	1.734×10^{-4}

4.4.7. Langmuir isotherm model and Freundlich isotherm model

4.4.8. Langmuir isotherm model

The most widely used isotherm equation for modelling of the adsorption data is the Langmuir equation, which is valid for monolayer sorption onto a surface with a finite number identical site and is given by following equation,

$$q_e = \frac{q_o k_L C_e}{1+k_L C_e} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots.4.7$$

Where q_o and K_L are Langmuir parameters related to maximum adsorption capacity and free energy of adsorption, respectively. C_e is the equilibrium concentration in the aqueous solution and q_e is the equilibrium adsorption capacity of adsorbent. The linearized form of Langmuir equation can be written as,

$$\frac{1}{q_e} = \frac{1}{q_o} + \frac{1}{q_o k_L} + \frac{1}{C_e}\ldots\ldots\ldots\ldots.4.8$$

The Langmuir constant q_0 and K_L can be calculated by plotting $1/q_e$ versus $1/C_e$.

4.4.9. Freundlich isotherm model

The Freundlich model is an empirical equation based on sorption on heterogeneous surface.

It is given as, $\quad q_e = k_f C_e^{\frac{1}{n}}$ 4.9

The data for heat adsorption on initial and equilibrium concentrations for
macadamia activated carbon is shown in Fig 4.44
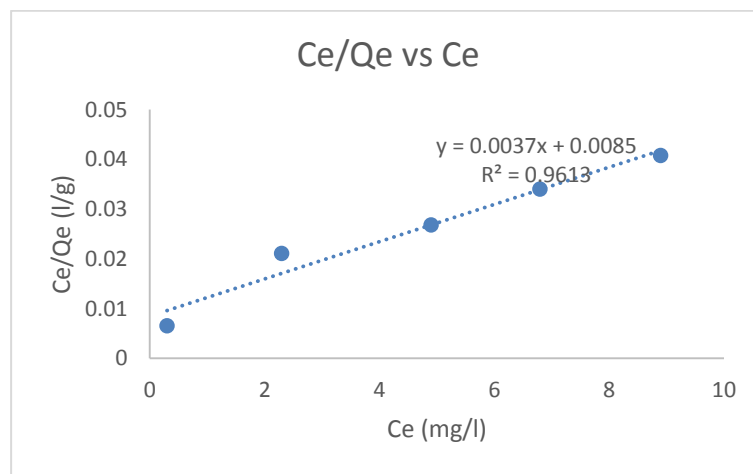
Figure 4. 44: linear curve for Ce/Q vs Ce for macadamia activated carbon

4.4.10. Thermodynamic parameters of adsorption

The Langmuir constant K changing with temperature can be used to calculate the enthalpy (ΔHo), free energy change (ΔGo) and entropy change (ΔSo). The free energy change of the adsorption is given as,

$$\Delta G^o = -RT\ln K ....4.10$$

The temperature dependence of the free energy change is given by the following equation,

$$d\left(\frac{\Delta G^o}{T}\right) = \frac{\Delta H}{T^2} dT \quad ....4.11$$

the equilibrium constant can be expressed in terms of enthalpy change of adsorption as a function of temperature.

$$\frac{d(lnk)}{T} = \frac{\Delta H^o}{RT^2} dT \quad ....4.12$$

If the process is endothermic (o $\Delta H > 0$), the equilibrium constant increases with temperature, if the process is exothermic (o $\Delta H < 0$), the equilibrium constant decreases as temperature is raised can also be written as the follow equation,

$$\frac{dlnk}{d(^1/_T)} = \frac{-\Delta H^o}{R}.....................4.13$$

Assuming that, o ΔH is approximately independent of temperature, the slope of ln K versus 1/T plot is equal to ΔH R in Fig 4.45. The change of adsorption entropy can be determined from the standard equation for Gibbs free energy which is $\Delta G^o = \Delta H^o - T\Delta S^o$ shown in Table 4.11. Gibbs free energy indicates the degree of spontaneity of the adsorption process and the higher negative value reflects a more energetically favorable adsorption

Table 4. 5. Gibbs free energy change parameters of chlorinated phenols on macadamia activated carbon adsorption system

ΔS^o (J/mol)	ΔH^o (J/mol)	ΔG^o (J/mol)
17.69	-0.189	-836.82
		-801.97
		-902.96
		-983.95
		-1063.67
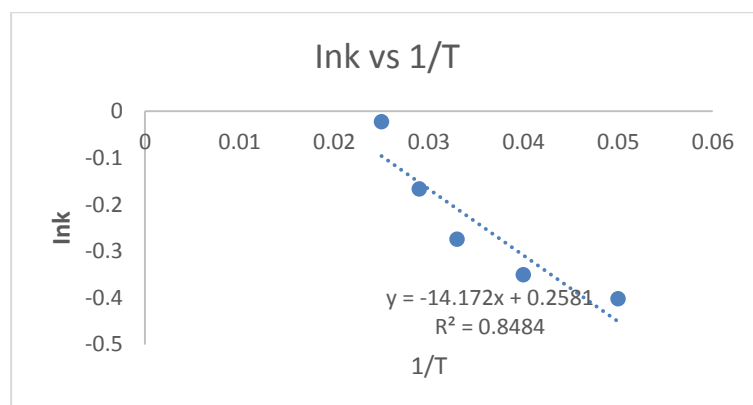
Figure 4. 45: lnK vs 1/T curve for macadamia activated carbon

Figure 4.46 shows the linear plots of In q_e versus C_e and is used to determine the value of q_{max} (mg/g) and b (L/mg). The data obtained were listed in Table 4.12 for all adsorbents. The Langmuir constants q_{max} and b are related to the adsorption capacity (amount of adsorbate adsorbed per unit mass of the adsorbent to complete monolayer coverage) and energy of adsorption, respectively. The essential characteristics of the Langmuir isotherm may be expressed in terms of a dimensionless constant separation factor or equilibrium parameter.
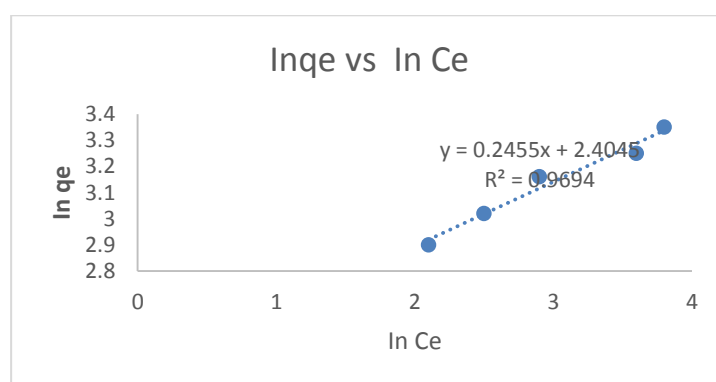
Figure 4. 46: InQe vs InCe curve for macadamia activated carbon

Table 4. 6. Langmuir and freundlich isotherm parameters for macadamia activated carbon

	Phenols		Langmuir model			Freundlich model	
		q_{max}	b	R^2		n	K_f
R^2							
		(molg^{-1})	(Lmol-1)				
MAC	3CP	76	0.7059	0.9879	0.6729	0.841	0.9694
	4C3MP	78	0.7843	0.950		0.469	0.09
0.9613							
	2,4DCP	80	0.8889	0.9866		0.741	0.031
0.8484							
	2, 4,6TCP	81	0.9412	0.98		0.144	0.004
0.9721							

4.4.11. Grafted macadamia

4.4.12. Adsorption kinetics study for Phenol-MAC system

For the kinetics study pseudo-first-order and pseudo-second order models were considered. The calculated q_e, k_1, k_2 and the corresponding linear regression coefficient R^2 values are presented in Table 4.13 .The applicability of the kinetic model is compared by judging the correlation coefficient R^2 and in agreement between calculated and the experimental q_e values. In light of these considerations, we can conclude that the pseudo-second order is predominant. Similar kinetics results have also been reported for adsorption veral paper dealing with organic solutes on activated carbon. (Hamdaoui and Naffrechoux, 2007); (Guedidi et al., 2017) The various results obtained from this study are represented in Table 4.13.

4.4.13. Pseudo-first-order kinetics of Phenol-MAC system

The linearized form of pseudo-first-order kinetics is given by equation 4.1 of chapter-4. From the equation 4.1 it is clear that, a plot of $\ln(q_e - q)$ against t should give a linear relationship with the slop k_1 and intercept of $\ln q_e$. The Pseudo-first-order kinetics of Phenol-GMAC system is given below,

4.4.14. Pseudo-second-order kinetics of Phenol-MAC system

The linearized form of pseudo-second-order kinetics is given from the equation 4.1, the plot of t/q against t should give a linear relationship. The q_e and k_2 can be determined from the slope and intercept of the plot The Pseudo-second-order kinetics of Phenol-MAC system is shown in Fig 4.47, 4.48, 4.49 and 4.50.
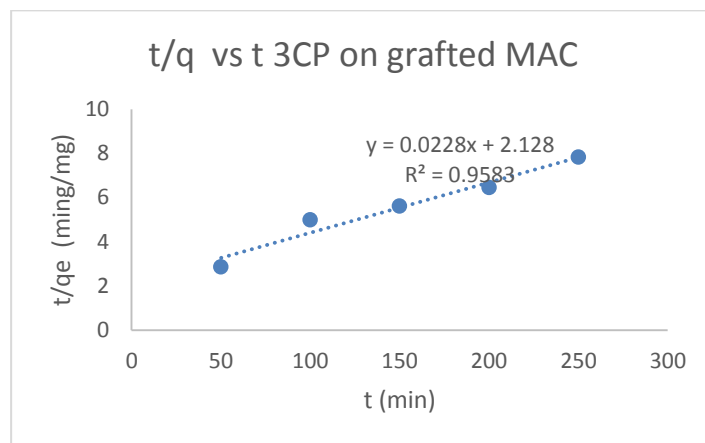
Figure 4. 47: Linear curve for 3chlorophenol (3CP) on grafted macadamia activated carbon
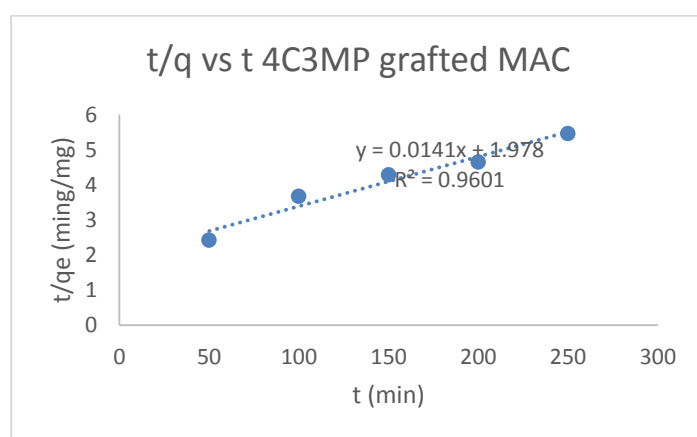
Figure 4. 48: Linear curve for 4chloro-3-methylphenol (4C3MP) on grafted macadamia
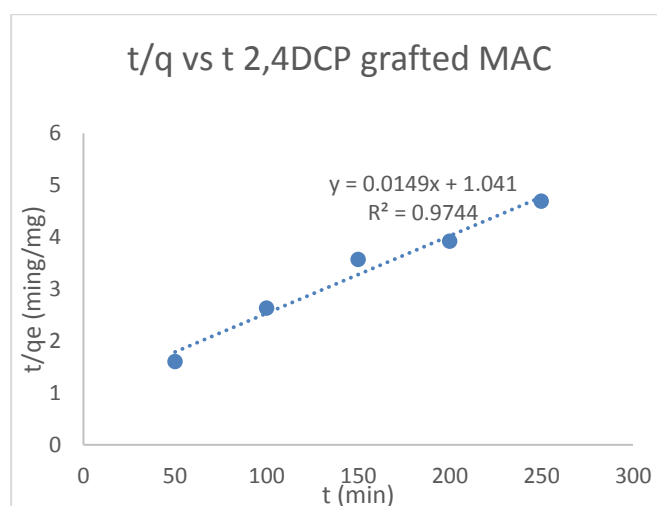
activated carbon

Figure 4. 49: Linear curve for 2,4dichlorophenol (2,4DCP) on grafted macadamia
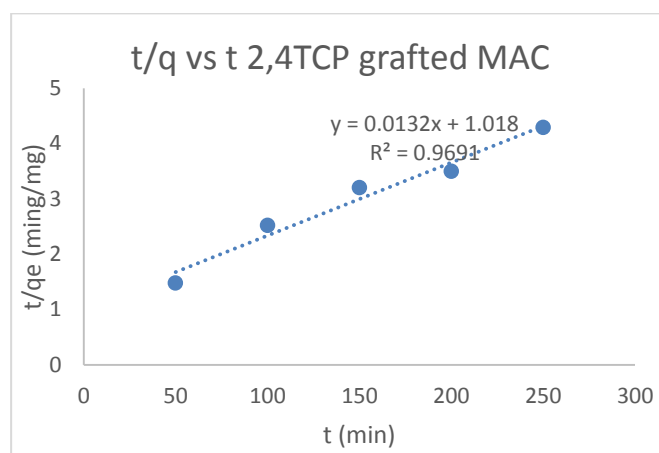
activated carbon

Figure 4. 50: Linear curve for 2,4,6 trichlorophenol (2,4,6TCP) on grafted macadamia activated carbon

Table 4. 7. Pseudo first and second order parameters for 3chlorophenol,4chloro-3-methylphenol, 2,4 dichlorophenol and 2,4,6 trichlorophenol sorption onto grafted macadamia activated carbon

Analyte	Equation	K_1 (min^{-1})	R^2	K_2(gmg^{-1}min^{-1})
3 Chlorophenol	y=0.0228x+2.128	0.0228	0.9583	2.443x 10^{-4}
4Chloro-3-methylphenol	y=0.0141x+1.978	0.0141	0.9601	1.005x 10^{-4}
2,4 Dichlorophenol	y=0.0149x+1.041	0.0149	0.9744	2.133x 10^{-4}
2,4,6 trichlorophenol	y=0.0132x+1.018	0.0132	0.9691	1.711 x 10^{-4}

Figure 4.51 shows the linear plots of C_e/q_e versus C_e and is used to determine the value of q_{max} (mg/g) and b (L/mg). The Langmuir constants q_{max} and b are related to the adsorption capacity (amount of adsorbate adsorbed per unit mass of the adsorbent to complete monolayer coverage) and energy of adsorption, respectively. The essential characteristics of the Langmuir isotherm may be expressed in terms of a dimensionless constant separation factor or equilibrium parameter.
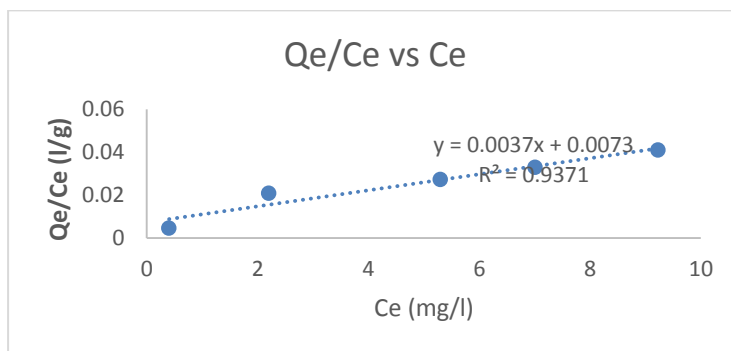
Figure 4. 51: linear curve for Ce/Q vs Ce for grafted macadamia activated carbon

Thermodynamic parameters such as the free energy change ΔG, enthalpy change (ΔG) and entropy change (ΔS), were calculated from the variation of the thermodynamic equilibrium constant , K_o with temperature shown in Fig 4.52 . The values of K_o and other thermodynamic parameters for the adsorption process were calculated using reported methods (Doğan, Alkan and Onganer, 2000).), and values of ΔH, ΔG, and ΔS for initial concentrations 20, 25, 30, 35 and 40 ^{o}C and shown in the Table 4.14 . The negative values of ΔG indicate the feasibility and spontaneous nature of chlorinated phenol onto macadamia activated carbon. The magnitude of ΔG, increased with increasing temperature, revealing that the degree of spontaneity increased at higher temperatures. The positive values of ΔS reflect an increased degree of disorderliness at liquid/solid interface during the adsorption of phenols onto macadamia activated carbon. The exothermic nature of chlorinated phenols by macadamia activated carbon was confirmed by the negative values of ΔH. The enthalpy value for a sorption process may be used to distinguish between chemical and physical sorption. For chemical sorption, values of enthalpy change range from 83 to 830 kJ/mol, while for physical sorption they range from 8 to 25 kJ/mol. The low values of ΔH give clear evidence that the interaction between chlorinated phenol and macadamia activated carbon was weak.

Table 4. 8. Gibbs free energy change parameters of chlorinated phenols on macadamia
activated carbon adsorption system

ΔS^o (J/Kmol)	ΔH^o (J/mol)	ΔG^o (J/mol)
0.061	-0.031	-894.95
		-803.44
		-897.95
		-993.43
		-1062.08
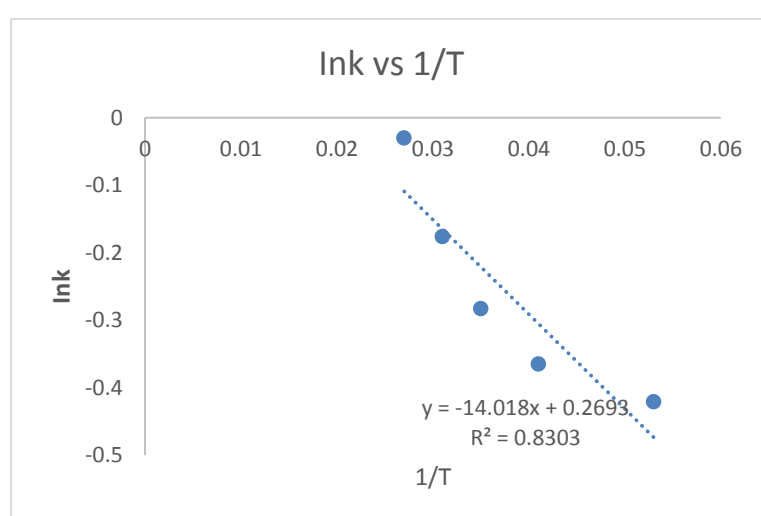
Figure 4. 52: Linear curve for lnK vs 1/T for grafted macadamia activated carbon

Figure 4.52 shows the linear plots of In q_e versus C_e and is used to determine the value of q_{max} (mg/g) and b (L/mg). The data obtained were listed in Table 4.15 for all adsorbents. The Langmuir constants q_{max} and b are related to the adsorption capacity (amount of adsorbate adsorbed per unit mass of the adsorbent to complete monolayer coverage) and energy of adsorption, respectively. The essential characteristics of the Langmuir isotherm may be expressed in terms of a dimensionless constant separation factor or equilibrium parameter.
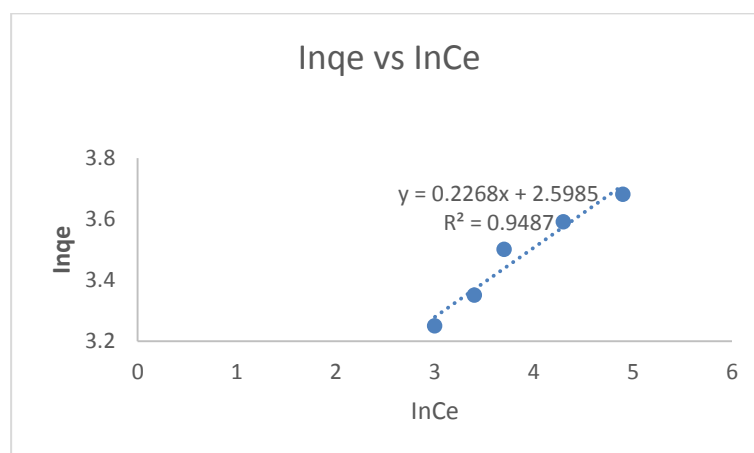
Figure 4. 53: Linear curve for InQe vs InCe for grafted macadamia activated carbon

Table 4. 9. Langmuir and freundlich isotherm parameters for grafted macadamia activated carbon

Phenols R^2		Langmuir model				Freundlich model	
		q_{max} (molg^{-1})	b	R^2		n	K_f
GMAC	3CP	77	0.7477	0.9583	0.642	0.0088	0.9487
	4C3MP	79	0.8362	0.9601		0.377	0.0019
0.9504							
	2,4DCP	82	1.0169	0.9744		0.383	0.0032
0.9371							
	2, 4,6TCP	84	1.2000	0.9691	0.421	0.0044	0.8303

4.4.15. Application of the developed method

This research is based on SPE, MAC and GMAC process and the methods were validated differently to test water samples on 4 compounds of chlorinated phenols using phenols calibration mix. However, the aim was to determine the adsorption capabilities for SPE, MAC and GMAC process and to get a suitable adsorbent replace SPE because is expensive. Before any analysis, the accuracy of calibration curves was checked to ensure they are still valid with a five standard solution of 1 mg/l, 3mg/l and 5mg/l. The results from the analysis

of phenols from standard solutions to unconditioned and conditioned cartridge using this validated method can be seen in Fig4.10 and 4.11.

4.4.16. Solid phase extraction water samples

Fig 4.54 above shows a chromatograph of the chlorinated phenols obtained during the HPLC run. Visible in this figure are 4chloro-3-methylphenol, 2,4 dichlorophenol and 2, 4,6 trichlorphenol all in concentration of approximately 1ppm. They are shown in the order of increasing retention time
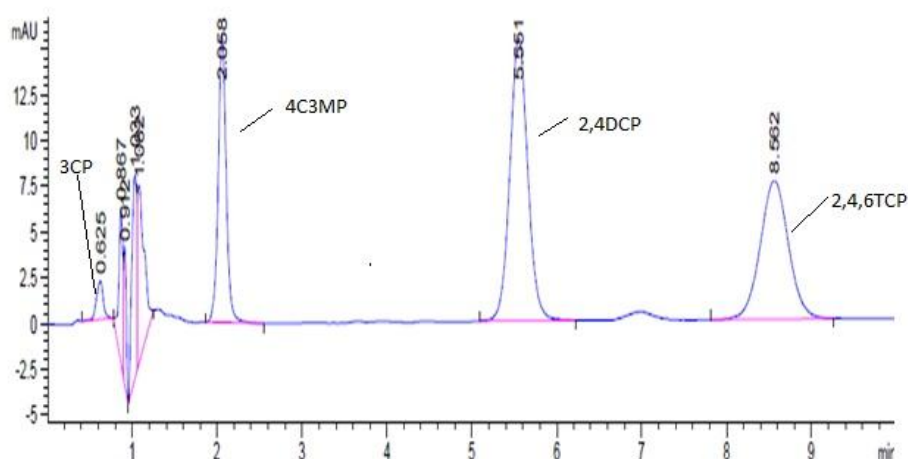
Figure 4. 54: Mixture of chlorinated phenols Sharpeville dam spiked with 1mg/l std

The chromatogram in Figure 4.55 refers to that obtained from a spiked 1mg/l standard solution in vaal river water samples. The peak at the beginning of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes. Thus, the baseline is smooth due to lower concentration of spiked water sample with four standard solution of 1 mg/l.
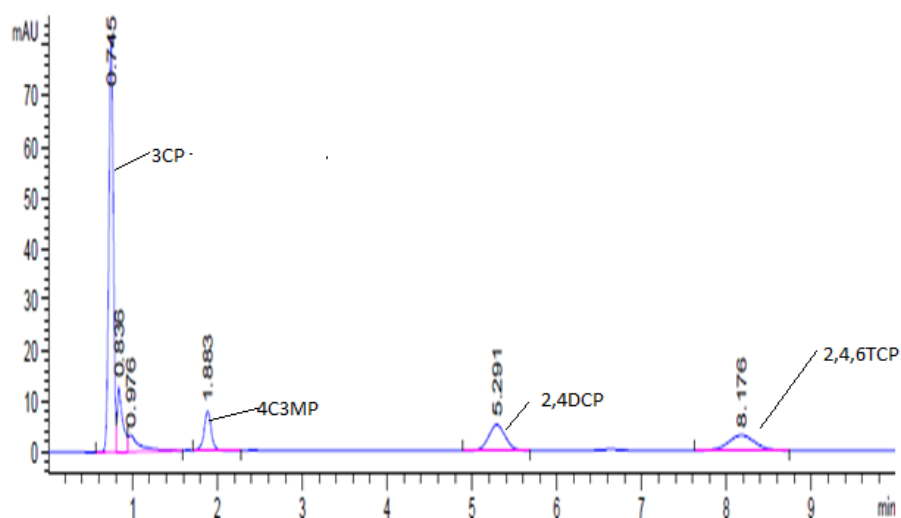
Figure 4. 55: Mixture of chlorinated phenols vaal river spiked with 1mg/l std

Figure 4.56 shows a typical chromatogram obtained from a standard sample containing the 4 phenols from water sample with a standard solution of 1 mg/l. The baseline is smooth due to lower concentration of the standard solution of chlorinated phenol.
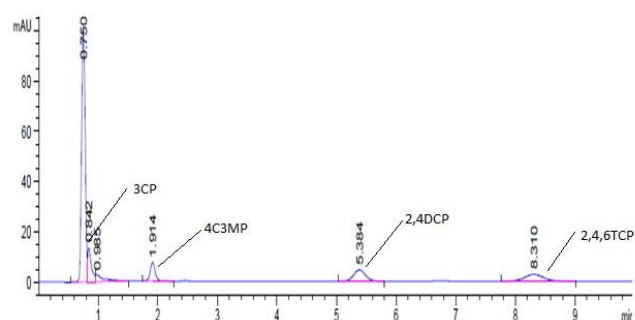
Figure 4. 56: Mixture of chlorinated phenols at barrage river spiked with 1mg/l std

4.4.17. Macadamia activated carbon water samples

Figure 4.57 shows typical chromatogram from spiked wastewater sample. However, the baseline is smooth from that obtained standard samples due to low concentration of standard solution 1 mg/l used in the experiment. The peak at the beginning and last peak of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes.
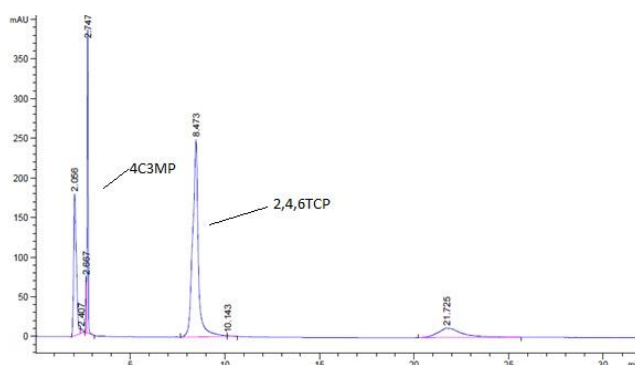
Figure 4.57: Mixture of chlorinated phenols at Sharpeville dam spiked with 1mg/l std

Figure 4.58 shows typical chromatogram from spiked Vaal river wastewater sample. However, the baseline is quite different from that obtained from a standard samples due to low concentration of standard solution 1 mg/l used in the experiment. The peak at the beginning of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes.
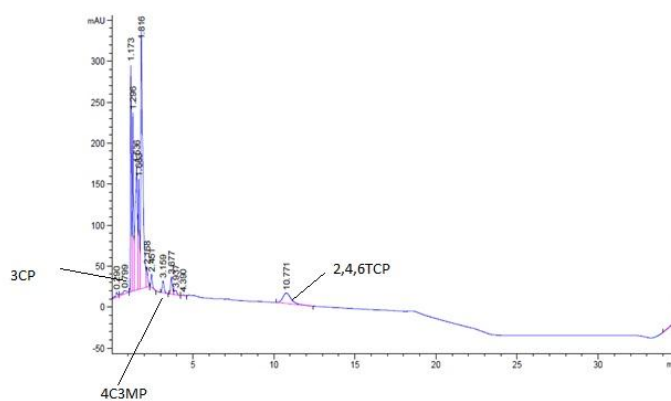
Figure 4. 58: Mixture of chlorinated phenols at Vaal river spiked with 1mg/l std

Thus, the nominal value of concentration of each phenols were 1 mg/l but the results are less than 1 mg/l indicating that there are some serious interferences between matrix and analytes Fig 4.59. Thus, recovery for this analysis needs to be calculated because recovery for this matrix is different. The chromatogram of wastewater shows that there are no phenols present within the working range.
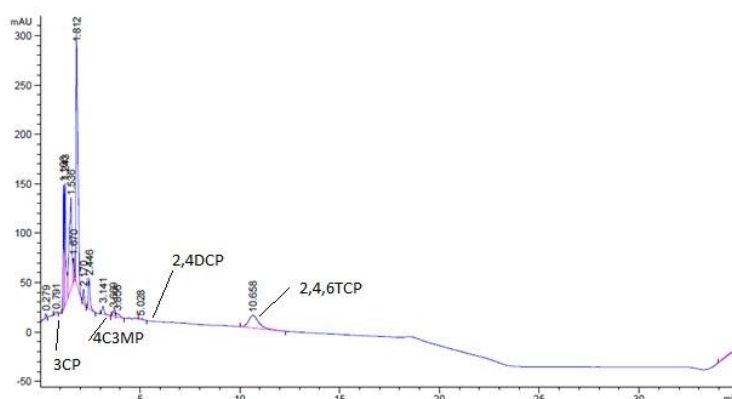
Figure 4.59: Barrage river sample spiked with 1mg/l chlorinated phenols standard

4.4.18. Grafted Macadamia activated carbon water samples

Chlorinated present in Fig 4.60 the water samples were identified by comparing their retention times with those of the known standards. Both the standards and the samples were analyzed under the same operating conditions. Identification of the unknown chlorinated phenols in the sample was carried out by spiking the wastewater sample by a known standard solution of 1mg/l.
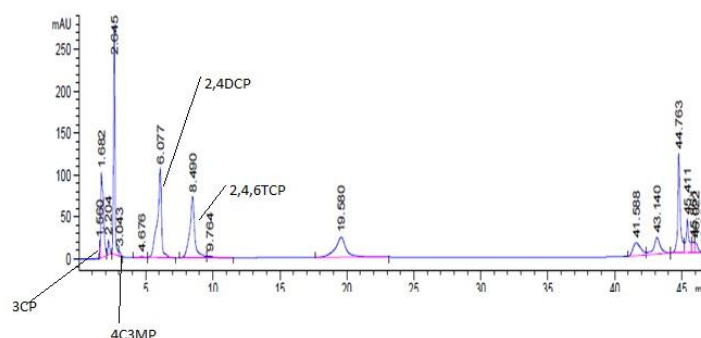
Figure 4. 60: Sharpeville dam sample spiked with 1mg/l chlorinated phenol
Standard

A total of two chlorinated phenol compounds were identified and quantified in the Vaal river sample (Figure 4.61) namely 4chloro-3-methylphenolhenanthrene, and 2,4chlorophenol at a very low concentration. Lowest chlorinated phenol levels were recorded for 2,4,6 trichlorophenol The chromatogram showing the Vaal river sample after adsorption with GMAC procedure is shown in Figure 4.61.
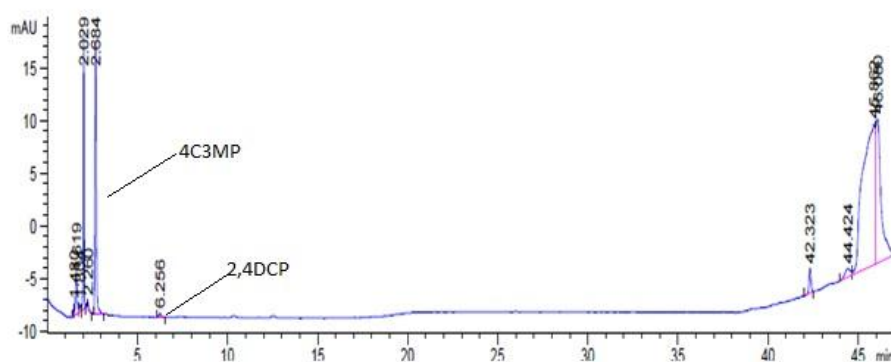
Figure 4. 61: Showing Vaal river sample spiked with 1mg/l chlorinated phenols standard

Figure 4.62. Shows typical chromatogram from spiked barrage river wastewater sample. However, without the baseline that is obtained from a standard samples due to low concentration of standard solution 1 mg/l used in the experiment. The peak at the beginning and last of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes.
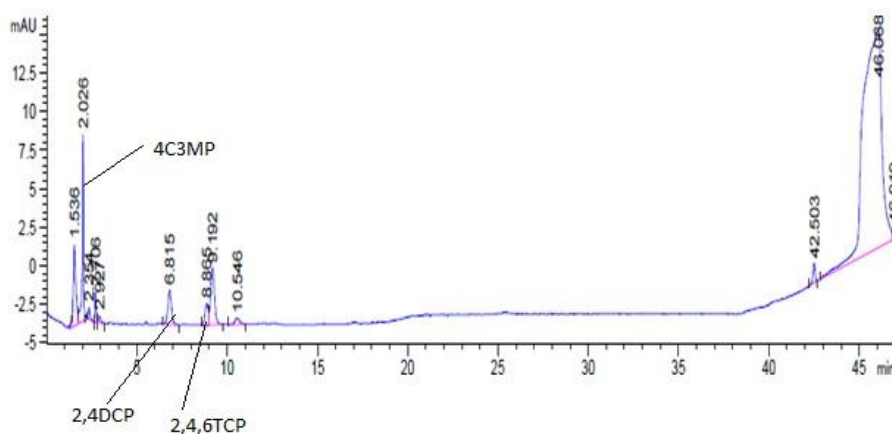
Figure 4. 62: Barrage river sample spiked with 1mg/l chlorinated phenols standard

Conclusion

From the results obtained, a suitable method for the determination of selected phenols using HPLC was successfully developed. It was also discovered that macademia nutshell charcoal grafted with octadecyl functional group which is the functional group found in the C-18 SPE used in this study can be used instead of C-18 SPE since it shows results that are comparable with those found when using C-18 SPE. The advantage with MAC is that it is cheap and biodegradable. A total of two chlorinated phenol compounds namely, 4-chloro-3-methylphenolhenanthrene and 2,4-chlorophenol were obtained at a very low concentration from Vaal River and they may be associated with the industries that are found around and near the river.

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CHAPTER 5

5. Conclusions

The following conclusions can be drawn from the present work:

This study is based on the SPE, MAC and GMAC process, and the method employed was validated for distilled water using chlorinated phenol calibration mix which aims at checking whether there are other adsorbents that could be used for the developed method as well as other matrixes. However, for the validated method three different adsorbents were analyzed to determine their properties. The ability of each adsorbents to retain analytes and the results achieved show that C18, polar plus, MAC and GMAC are suitable for determination of phenols from water samples using HPLC with a UV-detector but C18 can be use due to excellent recoveries of analytes which were between the working ranges of 80-98% shown in Table 4.10. Thus, the validated method was concluded to be suitable for analyzing different matrixes which gave excellent recoveries within the working range. It can also be concluded that phenols are not present in lake water but the validated method is suitable for analyzing different water matrixes. Samples were collected from Sharpeville dam, Barrage River and Vaal River thus, every sample was analyzed twice for accuracy. As regards waste water, the recoveries were very low due to interferences between matrix and analytes. A different approach is needed for determining concentrations. Thus, one approach might be to calculate recovery for each waste water sample separately. The experimental results show that phenols are not present in water within the working range of concentrations 0.003-0.25 mg/l. In comparison with the result from the experiments obtained from spiked water samples and that of standard samples, it show that the concentration was for 2, 4, 6-trichlorophenol which supports the conclusion that the method is suitable for the determination of 2, 4, 6-trichlorophenol and the rest of phenols in water using HPLC with a UV-detector. 40 Referring to the research questions of this study, the developed method is

trustworthy and suitable for the determination of phenols in water. The method was checked for accuracy using internal quality control (checking the calibration curves and recovery testing). This research has ignited in me a further interest and motivation in method development and validation not only in the environmental sector but also in forensic sciences. It has vastly enhanced my knowledge in the field of environmental analysis, research study which will enable me to overcome future challenges in working life. The maximum uptake of Phenol onto MAC was found to be 83 % and for GMAC was 77%. t=250 min, pH=5, Co=1mg/l, T= 25 ^{o}C and m= 0.3 g/l were the optimum condition for Phenol-MAC system and GMAC system. The kinetics studies confirmed that Phenol-MAC adsorption system can be described by pseudo- second-order kinetics model. Over all analysis of equilibrium model analysis indicates the fitness of Langmuir isotherm model to Phenol-MAC adsorption system, suggesting a monolayer adsorption of phenol on the surface of MAC. Phenol adsorption capacity of MAC was found to be decreasing with increase in temperature suggesting that the adsorption process was exothermic in nature, which was further supported by the negative values of change in enthalpy. The negative values of Gibb's free energy suggested that adsorption of phenol onto MAC was a spontaneous process. Characterization of MAC and GMAC confirmed the mesoporous texture, highly carbonaceous nature and a higher effective surface area of 912 m2/g. The highest phenol uptake capacity of GMAC was found to be 28.0049 mg/g. The optimal conditions for various process parameters are t=250 min, pH=5, Co=1mg/l,T= 25 ^{o}C and m= 0.3 g/l were the optimum condition for Phenol-GMAC system. Like Phenol-MAC system, the kinetics studies confirmed that Phenol-GMAC adsorption system can be described by pseudo- second-order kinetics model. Equilibrium model analysis indicates the fitness of Langmuir isotherm model to Phenol-MAC adsorption system, suggesting a monolayer adsorption of phenol on the surface of GMAC. Phenol adsorption capacity of GMAC was found to be decreasing with increase in temperature suggesting that the adsorption process

was exothermic in nature, which was further supported by the negative values of change in enthalpy. The negative values of Gibb's free energy suggested that adsorption of phenol onto GMAC was a spontaneous process.