Int. J. Environ. Res., 10(1):13-20, Winter 2016
ISSN: 1735-6865

Applying QuEChERS Method in Screening for Polychlorinated Biphenyls (PCBs) from Raw and Wastewater from Gaborone (Botswana) and Mafikeng (South Africa)

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Received 6 Jan. 2015; Revised 17 Apr. 2015; Accepted 30 Apr. 2015

ABSTRACT: Wastewater contains a variety of chemical substances owing to the different sources that contribute to affluent into sewage treatment plants. Raw and wastewater samples were obtained from the surface of water bodies at depths of 50 and 100 metres but at different locations in each sampling site. The water samples were subjected to extraction of polychlorinated biphenyls (PCBs) using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction kit before analysis using the Gas Chromatography Mass Spectrometer (GC-MS). The control samples had turbidity, temperature and pH values within the acceptable levels as per Botswana, South Africa and WHO drinking water standards. Turbidity values were rather higher than the set standards for raw and wastewater, with water samples from Modimola/Setumo dam having recorded the highest range of 25.0 – 200 Nephelometric Turbidity Units (NTU). The same sampling site also had water samples with pH higher at 9.01 to 9.78. Wastewater effluent in Notwane Sewage Treatment Plant, Gaborone Dam (both in Gaborone) and Disaneng Dam (South Africa) have polychlorinated biphenyls (PCBs) below detectable levels by the Agilent Gas Chromatography Mass Spectrometer (GC-MS). Only traces of PCBs were detected from wastewater from Modimola dam in Mafikeng. This may be due to the different industries in Mafikeng producing various chemicals compared to Gaborone. The water in Modimola dam therefore requires thorough treatment before it can be returned for domestic consumption as PCBs are toxic compounds that have been found to trigger cancer in humans and also affect the reproduction system resulting in low IQ.

Key words: Wastewater, Polychlorinated biphenyls, Recycling, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), Extraction

INTRODUCTION

Chemicals are used extensively and intensively in the technological society (Cycon *et al.,* 2013; Guillen *et al.,* 2012). Depending on their properties, the modes and extent of use, large amounts of these chemicals reach the environment, and have unpredictable environmental and health effects in the long term (Guillen *et al.,* 2012). Chemical pollution of soil and wastewater is a significant environmental problem (Raja *et al.,* 2009). The recalcitrant compounds, especially hydrocarbons and polymers such as polychlorinated biphenyls (PCBs) are highly toxic and can act as mutagens and carcinogens, within living organisms (Hennessee *et al.,* 2009; Mallick *et al.,* 2007).

However, in Botswana, the Integrated Resources Water Management (IRWM) focuses more on the salinity and general pollution (DWA, 2013) than POPs on water quality assessment guidelines although the Stockholm convention urges countries to consider the elimination of POPs in wastewater (EPA, 2009; UNEP, 2009). UNEP (2009) reported that in Africa, the key challenges in relation to management of POPs was mainly due to lack of knowledge and information on hazards, risks and safer alternatives; lack of legislation or reinforcement measures; weaknesses in the technical infrastructure; and a shortage of qualified human resources. These factors are known to account for the extremely minimal available data on PCBs (Ministry of Environment, Wildlife and Tourism of Botswana, 2008).

The Department of Environmental Affairs (DEA) of South Africa amended the National Environmental Management Act of 1998 (Act No 107, 1998) to include the facing out of the general use of polychlorinated biphenyls (PCBs) containing materials and PCBs contaminated materials by 2023 and 2026 respectively

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(DEA, 2013). According to a report by the Ministry of Environment, Wildlife and Tourism of Botswana (2008), large quantities of PCB containing equipment (40 000 tonnes) was still in use in the country by 2006 and thus the number of contaminated sources could just have been that high too. These setbacks for African countries were mitigated by the development of country specific IWRM (WHO and UNEP, 2006). This study was therefore aimed at establishing the state of raw and wastewater with regard to POPs, and main focus being the polychlorinated biphenyls (PCBs).

The presence of chemicals in the environment calls for quantification of such so as to come up with risk analysis posed by those chemicals (Urbaniak, 2013; Guillen *et al.,* 2012). Substances such as polychlorinated biphenyls (PCBs) and pesticides are found in wastewater. The general chemical formula for PCBs is $C_{12}H_{10-n}Cl_n$, where *n* is any number between 1 and 10 (WHO, 2000). PCBs have varying numbers of chlorines in their structure (EPA, 2013). All PCBs are lipophilic and the lipophilicity increases with the number of chlorines attached to the molecule, thus, their low solubility to water (WHO, 2000). PCBs are mostly hydrophobic and some are less hydrophilic (Muir and Lohmann, 2013; Nwinyi, 2011).

PCBs are very stable compounds and do not decompose readily (Rudel *et al.,* 2008). Furthermore, PCBs have a long half life (8 to 15 years) and are insoluble in water (Rudel *et al.,* 2008). The biodegradability of PCBs is also dependent upon the number of chlorine molecules it has (Anyasi and Atagana, 2011; EPA, 2013). The more chlorine molecules contained in a compound renders that compound less biodegradable (EPA, 2013). PCBs are lipophilic and thus are mostly hydrophobic (Muir and Lohmann, 2013). These properties result in bioaccumulation of these compounds as they do no dissolve in water and thus render them difficult to be biodegraded (EPA, 2013; Muir and Lohmann, 2013).

Literature review revealed that a lot of work on removal of these pollutants from activated sludge has been done and little or no work has been done on effluents. According to Ying *et al.,* (2009), although most research conducted on the removal of these substances have been carried out, it is mainly on soil and activated sludge and very little done on wastewater. Wastewater is allowed to flow out into the environment and may pose health risks to animals and humans (WHO and UNICEF, 2014). This also results in these compounds being added to the soils in the surrounding environment. These soils become polluted with PCBs which poses a risk not only to plants that grow in those soils, but also to human being using the soils for farming (McDougall, 2004). Since these compounds are not easily broken down, they accumulate in the environment (bioaccumulation) and can be passed from one trophic level to the other through feeding relations (Nabavi *et al*., 2013; Sapozhnikova and Lehotay, 2013; McDougall, 2004) thus the need to have these compounds completely eliminated from the environment.

Several methods have been developed and used to determine quantitatively, these pollutants in wastewater derived mainly from sewage treatment plants (Ying *et al.,* 2009). For these PCBs to be removed from the wastewater, this study aimed at screening for their presence in the water. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach is being used in determination of pollutants in several types of samples (Vera *et al*., 2013). This is mainly based on its salting-out extraction with a solvent, followed by a clean-up procedure (Vera *et al*., 2013). QuEChERS have been mainly used in the determination of pesticides in soils (Vera *et al*., 2013).

The use of QuEChERS has not been employed in the screening of these compounds in water but in tissue samples of either fish or plant material from polluted water bodies (Sadowska-Rociek *et al*., 2014; Sapozhnikova and Lehotay, 2013). This study therefore aims at closing this gap.

MATERIALS & METHODS

Water samples were obtained from Gaborone dam and effluent from the Notwane Sewage Treatment Plant in Gaborone, Botswana. The Notwane Sewage Treatment Plant was selected on the basis that it is the major wastewater treatment plant in Gaborone, receiving large volumes of wastewater from different industries, schools, medical facilities and households. Water samples were also obtained from Disaneng dam and the Setumo/Modimola Wastewater Treatment Plant effluent which is in Mmabatho, South Africa. Disaneng dam was chosen because of its raw water unlike Modimola/Setumo dam which has wastewater. These wastewater treatment plants receive wastewater from residential areas, industrial and agricultural sectors. Fig. 1 is a point map to show the sampling sites.

Ten (10) water samples from each site were collected for analysis using Gas Chromatography Mass Spectrometer (GC-MS). Tap water samples obtained from Gaborone and Mafikeng were used as negative controls. Samples were collected during the different seasons of the year. The samples were carried from sampling sites in cooler boxes filled with ice to keep samples at about 4°C. Physical parameters that included pH, temperature and turbidity of samples were determined immediately at the sampling sites during collection. Turbidity was measured using Orbeco Hellige

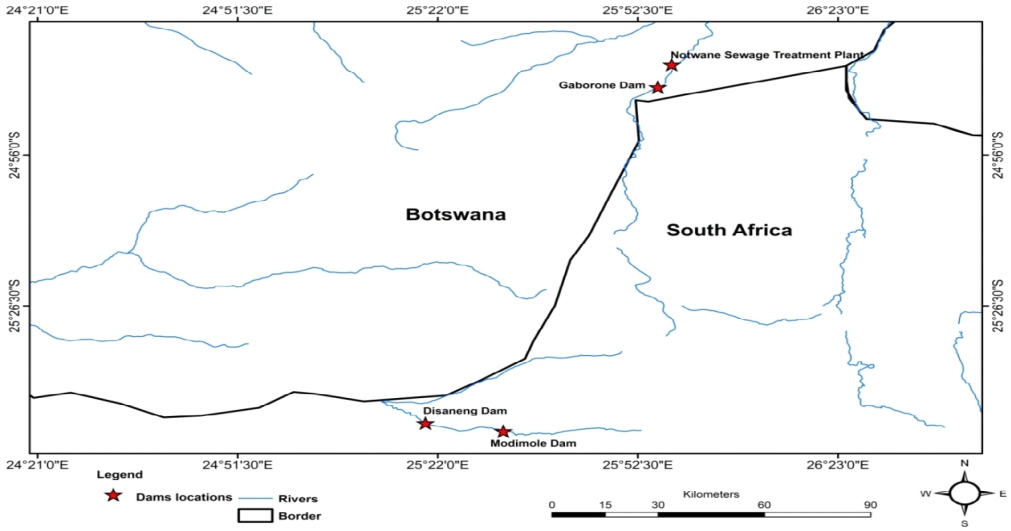


Fig. 1. A sketch map to show the sampling sites

(The sampling sites were in Gaborone, Botswana and Mafikeng area, South Africa so as to analyse the effect of the different industries in these two areas on components of wastewater/sewage effluent, in terms of PCBs composition.)

TB 200™Potable Turbidimeter from LASEC, South Africa. The pH and temperature (°C) were measured using Crison PH 25 – Spain supplied by LASEC, South Africa. The raw water samples were obtained from the surface of the water bodies at depths of 50 and 100 metres but at different locations in each dam. Gas Chromatography Mass Spectrometer (GC-MS) was used to detect the presence of PCBs in water samples (Kawaguchi *et al.,* 2012; Mallick *et al.,* 2007). To achieve this, 250 mL polyethylene (PET) bottles were washed with nitric acid to remove impurities. Composite water samples (250 mL) were prepared by mixing samples from the different depths collected from the four sample sites of a particular dam. The identities of resolved products were determined by comparing results with the profiles of GCMS reference compounds.

Quality control was set using the water samples and five randomly selected pesticides that were available from the supplier (Sigma-Aldrich, Germany). The pesticides utilised were Aldrin, 4,4-DDT, 4,4-DDD, 4,4-DDE and Dieldrin. Stock solutions of the pesticides were prepared by dissolving 5 mg in 1 Litre of HPLC grade hexane obtained from Sigma Aldrich, South Africa. Spiked PAH dissolved in saline and PCB standards were also prepared (SUPELCO Solutions Within™ - USA). GC-MS calibration standard solutions were concentrated in HPLC grade toluene (Sigma Aldrich, South Africa). Standard stock solutions (1 ppm and 5 ppb) were prepared in HPLC grade acetonitrile (Sigma Aldrich, South Africa). These were used to spike water samples in preparation for GC-MS analysis. QUECHERS kit-AOAC, part number 5982-5755, Agilent Technologies, South Africa was used in this study.

As a quality control measure, water samples were divided into two parts of 10 mL each. Each part was further divided into two and spiked individually with 1 ppm and 5 ppb of each pesticide (Aldrin, 4,4-DDT, 4,4-DDD, 4,4-DDE and Dieldrin) stock solution. However, the other portions were not spiked with the pesticides and recalcitrant compounds. The main rationale is that, upon GC-MS analysis, the pesticides and recalcitrant compounds should be detected in the spiked samples when compared to the unspiked water samples. In addition, this procedure was used to evaluate the effectiveness of the QuEChERS method in the extraction of recalcitrant compounds from water samples (Furlani *et al*., 2011).

To extract polychlorinated biphenyls from water samples, aliquots of 10 mL of water sample (both spiked and unspiked) were transferred into a 50 mL polypropylene centrifuge tube and 10 mL of HPLC grade acetonitrile added to it. The mixture was vortexed (model Vortex Genie 2) for 1 minute. Four grams of anhydrous $MgSO_4$ and 1g of NaCl were added to the mixture and vortexed for 1 minute. The mixture was transferred into a 15 mL polypropylene centrifuge tube and centrifuged for 5 minutes at 5000 rpm using a Labofuge 200 centrifuge (Heraeus Sepatech Germany) supplied by Phala Scientific (PTY) LTD, Gaborone. The centrifuged product was cleaned by transferring 4 mL aliquot of the upper layer (supernatant) into a polypropylene centrifuge tube containing 200 mg PSA and 600 mg anhydrous $MgSO_4$ (Furlani *et al*., 2011). This was vortexed for 30 seconds using a Vortex Genie 2 supplied by Scientific Industries. The product was then centrifuged for 3 minutes at 3500 rpm using the

Labofuge 200 centrifuge (Heraeus Sepatech Germany) supplied by Phala Scientific (PTY) LTD, Gaborone. A 4ml aliquot of the supernatant was transferred into a vial and concentrated by blowing with nitrogen gas until it dried up. The product was topped up to 0.5 ml with toluene (Furlani *et al*., 2011) in a vial and used for GC-MS analysis.

The vials were placed in an auto-sampler tray in Gas Chromatography equipment (model 7890A– Agilent Technologies and 5975C Mass Spectrometer Inert XL EI/CI MSD) with a Triple axis detector and samples were subjected to GC-MS analysis. The runs were performed under the following GC-MS conditions: 260°C Inlet –F temperature, 220°C oven temperature, 1.5 column 1 flow, 280°C Aux -2 temperature, 200°C MS source and 150°C MS quad (Brondi *et al*., 2011; Furlani *et al*., 2011).

RESULTS & DISCUSSION

The mean pH, turbidity and temperature of the water samples were determined and results are presented in Table 1. As shown in Table 1, samples obtained from Modimola dam had the highest turbidity (with mean values of 25.10 – 200.6) and pH (mean values of 9.01 – 9.78) values while those from Notwane Sewage treatment Plant had the highest temperature (mean values of 10.1 – 26.2) values. In addition, the turbidity values of both control samples (tap water) were 0.01 respectively compared to the results obtained for the environmental wastewater samples. Generally, the pH, turbidity and temperature values for the control water samples were within the acceptable standard reference values for drinking water.

The site which was found to have the most turbid water was Setumo/Modimola dam with a mean turbidity value ranging from 25.10 to 200.6 NTU while Gaborone dam had the least record of mean turbidity readings with a value range of 10.12 to 10.68 NTU. Modimola/Setumo Dam receives effluent from the neighbouring Setumo Wastewater Treatment Plant while Gaborone Dam (like Disaneng Dam) stores raw water. Water from Disaneng Dam was found to be more turbid than effluent obtained from the Notwane Sewage Treatment Plant in Gaborone. The samples from all sites had turbidity of >5.0 NTU above what is set out in the Target Water Quality Range (TWQR) (DWA-BW and DWA-ZA, 2013) while the control water samples were both at 0.01 NTU which is acceptable according to the set level. With both the raw water and wastewater from Gaborone being less turbid, this might be as a result of the fewer activities that occur at these sites while in Modimola/Setumo and Disaneng dams there is a lot of activity carried out there such as fishing which is done with the aid of engine boats. A large number of livestock also use the sites as drinking holes, a factor that was highlighted by Mulamattathil *et al*., (2014). All these may result in the addition of pollutants to the water bodies resulting in reduced light penetration.

The mean pH values for all the sites from which samples were obtained was found to range between

Table 1. Mean values of physical parameters (pH, turbidity and temperature) of water from the four sampling sites

Sample source	Mean Turbidity (NTU)	Mean pH	Mean Temperature (°C)
Gaborone Dam	10.12 – 10.68	8.26 – 8.64	9.2 – 24.5
Disaneng Dam	13.31 – 25.09	8.96 – 9.04	8.0 – 26.7
Notwane Sewage Treatment Plant	12.44 – 20.34	8.99 – 9.77	10.1 – 26.2
Modimola/Setumo Dam	25.10 – 200.6	9.01 – 9.78	9.0 – 24.2
Control – 1	0.01	7.78	24.4
Control – 2	0.01	7.88	26.1
EPA reference value for drinking water	=<1.0	6.5 – 9.5	25.0
EPA reference value for wastewater	=<5.0	5.5 – 10.0	= <40.0
WHO reference value for drinking water	=> 1 - =<5.0	6.5 – 8.0	25.0
WHO reference value for wastewater	5.0	6.5 – 8.0	30.0
BW and ZA reference value for drinking water	0.5 – 5.0	6.5 – 8.5(BW) 5.5 – 9.5 (ZA)	25.0
BW and ZA reference value for wastewater	25 - 30.0	6.5 – 8.5	30.0

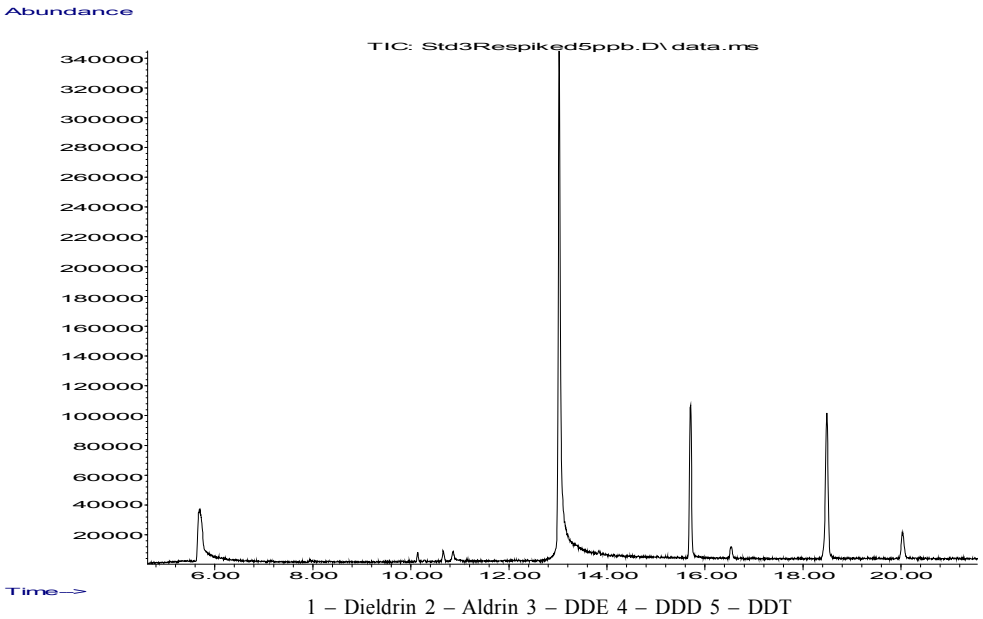
BW = Botswana; ZA = South Africa; WHO = World Health Organization; EPA = Environment Protection Agency; Control 1= Gaborone; Control 2= Mafikeng (Water samples from Modimola/Setumo Dam presented high turbidity values and pH, above the set standards for raw and wastewater. The control sample from both Gaborone and Mafikeng were found to have turbidity, pH and temperature within acceptable levels/standards as set by Botswana, South Africa and World Health Organization (WHO))

pH7.78 and pH9.78 (Table 1). This shows that the wastewater, raw water and the tap water from the sampling locations are slightly alkaline with a 0.28 pH value above the Target Water Quality Range (TWQR) which is between pH5.5 and pH9.5 (DWA-BW and DWA-ZA, 2013). Turbidity does not determine the microbial quality or give a clue on the chemical composition of water but may be an indication of increased pollution although the presence of some chemical pollutants may result in an increased or low pH in water. The results showed that the PCBs were below the detection limit of the instrument used. This

Table 2. Recalcitrant Compounds Screening results from the GC-MS

Sample	Sample Code	Aldrin (mg/kg)	Dieldrin (mg/kg)	4,4 DDD (mg/kg	4,4 DDE (mg/kg)	4,4 DDT (mg/kg)	PCB (mg/kg)
1	A1	ND	ND	ND	ND	ND	ND
2	A2	ND	ND	ND	ND	ND	ND
3	A3	ND	ND	ND	ND	ND	ND
4	A4	ND	ND	ND	ND	ND	ND
5	A5	ND	ND	ND	ND	ND	ND
6	B1	ND	ND	ND	ND	ND	ND
7	B2	ND	ND	ND	ND	ND	ND
8	B3	ND	ND	ND	ND	ND	ND
9	B4	ND	ND	ND	ND	ND	ND
10	B5	ND	ND	ND	ND	ND	ND
11	C1	ND	ND	ND	ND	ND	ND
12	C2	ND	ND	ND	ND	ND	ND
13	C3	ND	ND	ND	ND	ND	ND
14	C4	ND	ND	ND	ND	ND	ND
15	D1	ND	ND	ND	ND	ND	ND
16	D2	ND	ND	ND	ND	ND	ND
17	D3	ND	ND	ND	ND	ND	ND
18	D4	ND	ND	ND	ND	ND	Traces
Control 1	cont 1	ND	ND	ND	ND	ND	ND
Control 2	cont 2	ND	ND	ND	ND	ND	ND

ND = Not Detected; A = Gaborone Dam; B = Disaneng Dam; C = Notwane Sewage Treatment Plant; D = Setumo/ Modimola Dam; Control 1 = Gaborone; Control 2 = Mafikeng (PCBs levels in the water sample from Gaborone Dam. Disaneng Dam, Notwane Sewage Treatment Plant were below detectable level by GC-MS (7890A/5975C model))



1 – Dieldrin 2 – Aldrin 3 – DDE 4 – DDD 5 – DDT

Fig. 2. GC Chromatogram for pesticides used as a standard

(All the pesticides used in the quality control experiment were detected by the GC-MS used in this study. This was used as reference for the analysis of PCBs in water. This therefore shows that the PCBs were below detectable levels in wastewater. This might have been due to their lipophilic characteristic)

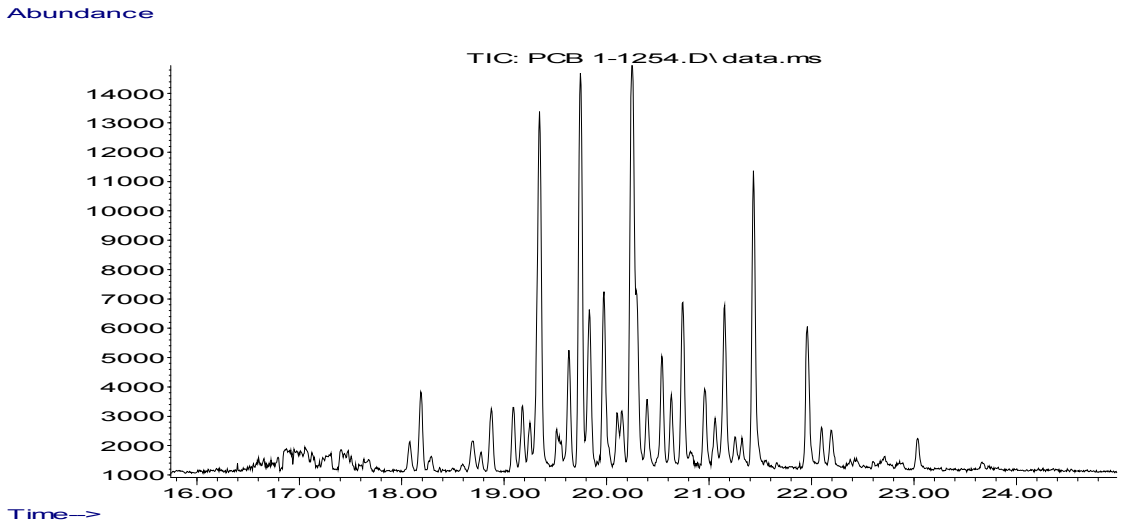


Fig. 3. GC Chromatogram for PCB Aroclor 1254 used as a standard

(The PCB standard used in the quality control experiment was detected by the GC-MS used in this study. This therefore shows that the PCBs were below detectable levels in wastewater)

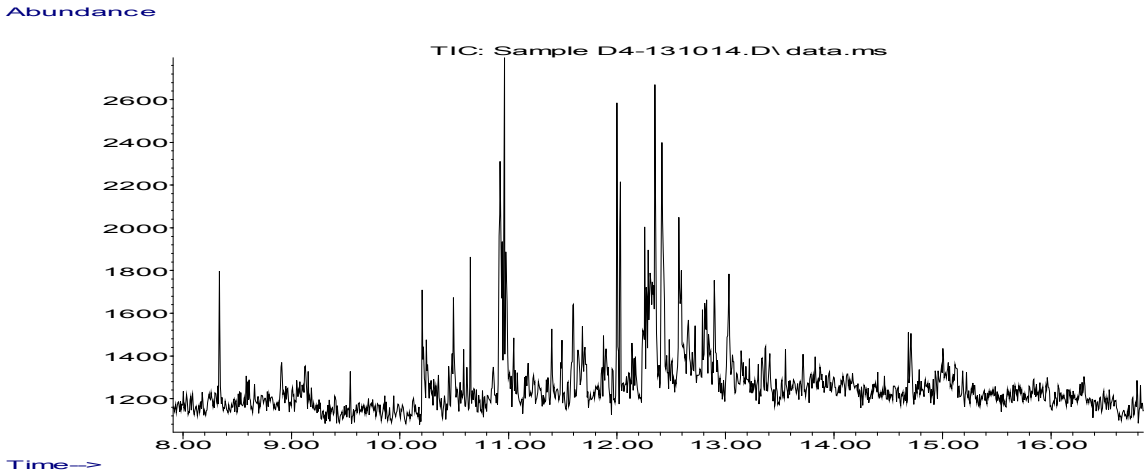


Fig. 4. GC chromatogram for water sample from Modimola/Setumo dam

(Only traces of PCBs were detected from wastewater collected from Modimola/Setumo Dam. Further analysis is required though, on other types of samples, other than water to verify the traces and for identification of those compounds)

was confirmed through the use of standards which indicated that the equipment detection limit for the compounds was 17.1 µg/L. This was obtained from the equipment detection limit using the results for the standard. The results are presented in Table 2. Although the study was targeting PCBs, none of the insecticides that were used for quality control were detected in the water samples. The results are also presented in Table 2.

The screening results were also shown in GC-MS chromatograms. These results are shown in Figs. 2 to 4. The chromatogram from the standards is shown in Figs. 2 and 3. The standard pesticides used in the study as standards are represented by peaks 1 to 5 in Fig. 2.

The results showed that the compounds were not detected. There were traces of PCBs from Modimola/ Setumo dam though, although the quantities could not be determined as the levels were below quantification and thus traces, as presented in Fig. 4.

The results indicate that since these compounds are highly lipophilic (WHO, 2000) they required certain surfaces to which they can adhere and require fats in which they dissolve. This explains why most studies have only been able to detect these compounds from fish, plant material and soils. This also explains why these compounds highly result in bioaccumulation (EPA, 2013; Nabavi *et al*., 2013) and thus very undesirable in the ecosystem. The implication from the

results obtained during this study may be that these compounds if they get attacked by bacteria, they are biodegraded as these will absorb the compounds and use them as they carbon sources thus biodegradation as was described by Azhari *et al*., (2010) and Vrchotova *et al*., (2013) or they get taken up into tissues of organisms found in the water bodies (Nabavi *et al*., 2013). The PCBs that manage to pass through the wastewater treatment plant un-degraded, they will be contained in the effluent which has a low bacterial population and as a result they will end up into the soils where the effluent is released, or sediments of dams and rivers into which effluent reach thus polluting the environment (WHO and UNICEF, 2013).

CONCLUSIONS

The targeted recalcitrant compounds, the poly-chlorinated biphenyls (PCBs), were not detected in the water samples from the studied sites. Only traces (un-quantifiable levels) of PCBs were detected from waste-water sample from Modimola/Setumo dam. The use of QuEChERS procedure to extract PCBs from water may not to be very effective as these compounds are highly lipophilic and thus higher levels of these compounds would be required for them to be extracted from water. PCBs although they get access into wastewater from the different industries, their lipophilic property make them not be easily isolated directly from the water. There is need to analyse for these compounds in the living organisms and soils where the wastewater is discharged to ensure that the compounds are not re-introduced into the environment through effluent. The physical parameters of drinking water in the two (2) regions where the study was conducted are within the global acceptable standards.

ACKNOWLEDGEMENTS

I would like to thank Dr Kwenga Sichilongo and Professor CN Ateba for the technical support during the study; the Department of Water Affairs (DEA) and Water Utilities Corporation (WUC) for granting me the permit to collect and use water sample from the waste-water treatment plants. My gratitude goes to Depart-ment of Biological Sciences, North-West University-Mafikeng Campus and Dr NP Sithebe for financial sup-port towards this study.

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