Human health risk assessment of selected endocrine disrupting phenolic compounds in potable water and treated wastewater effluent in the Western Cape, South Africa

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ABSTRACT

Water resources are under threat by industrial and agricultural pollution due to the release of organic contaminants such as phenol into water bodies. This reduces water quality, thereby decreasing the availability of clean water. Samples of the Stellenbosch wastewater treatment plant effluent tap water, and four brands of bottled water were analyzed for the two phenolic compounds; 4-CP and 2,4-DCP using the HPLC/DAD. The phenolic compounds were both below the regulatory limits in all the samples analyzed. The concentrations of 2,4-DCP in the WWTP effluent, tap water and bottled water brands ‘A’, ‘B’, ‘C’and ‘D’ ranged from; ND-5.40×10^{-6}, ND- 1.90×10^{-5}, ND-1.31×10^{-5}, 3.68×10^{-6} - 1.37×10^{-5}, ND-6.85×10^{-6} and 6.28×10^{-6} - 1.47×10^{-5} respectively. Corresponding values for 4-CP were 4.04×10^{-6} - 5.61×10^{-5}, 9.96×10^{-6} - 1.90×10^{-5}, ND-5.81×10^{-6}, ND-6.95×10^{-6}, ND-9.78×10^{-6} and 8.90×10^{-7} - 6.74×10^{-6} (mg/L) respectively.

Keywords: Endocrine disruptors, 4-CP, 2,4-DCP, waste water effluent, bottled water, tap water

1. INTRODUCTION

Water has a dual responsibility, it can be considered as a pivotal sanitation parameter as well as an economic factor (Adeleye, 2016). A secure, reliable, economical, and easily accessible water supply is paramount for sound health (Hunter et al., 2010). The removal of micro constituents in a wastewater treatment plant can serve as an imperative component in ensuring safe, reliable, affordable and easy access of water supply because the effluents from the WWTPs are typically discharged into surface waters, such as rivers (Wagner, 2000; Edokpayi et al., 2017). The presence of chemicals and micro-pollutants is a serious barrier faced by treatment plants, as pollutants reduce the adequacy of treated wastewater to be used as a source of drinking water. Research has shown that South Africa's new traditional wastewater treatment solutions are insufficient to treat wastewater prior to reuse or discharge (Adeleye, 2016; Edokpayi et al., 2017; Afolabi et al., 2018). The study focused on two phenolic compounds, 4-chlorophenol and 2,4-dichlorophenol. They were chosen due to the ubiquitous occurrence of chlorinated phenolic compounds from various industrial sources. These environmental pathways include manufacturers of preservatives, pesticides and dyes, and pulp and paper industries and other phenol-based compounds (Olaniran & Igbinosa, 2011; Xu et al., 2017). The compounds pose some of the most threatening and persistent organic pollutants due to their vast industrial applications which have given rise to accumulation in the environment, and hence, a grave concern (Ghaffar et al., n.d.). Majority of these compounds include by-products of industrial processes as well as pharmaceutical, pesticide, paint and solvent production, wood, paper, and pulp processing (Allaboun & Al-Rub, 2016; Xu et al., 2017). Due to the toxicity of chlorophenols coupled with their persistence in the environment, methods of their elimination are urgently required (Movahedyan et al., 2008).

1. MATERIALS AND METHODS

1.1. Choice and cleaning of glassware

The choice of amber glass bottles for sample collection, the use of glassware instead of plastic and high purity solvents were necessary measures required for the development of a reliable analytical protocol. All glassware was thoroughly washed with liquid detergents and rinsed properly with tap water. Then, rinsed thoroughly with distilled water and left to drain and dried placed upside down overnight, prior sampling day.

1.2. Chemical and standards

The chemicals, materials, and reagents were purchased from trusted vendors and suppliers (Supelco and Sigma Aldrich). The standards for the toxicity were purchased from MicroBioTests, Belguim and were prepared according to UCI (2003) and used according to manufacturer's instructions. Phenolic compounds (2,4-dichlorphenol (99%) and 4 chlorophenol) were obtained from Sigma Aldrich (South Africa). C 18-E cartridges consisting of 500 mg/12 ml of adsorbent (Seupelco, South Africa) and a newly launched kinetex C18-100A column (150 mmx4.6 mm i.d., 5 µm particle size) was used.

1.3. Instrumentation and chromatographic conditions

High performance liquid chromatography (HPLC) manufactured by Waters Corporations (United State of America) includes a terminal solvent delivery system, an auto sampler and photodiode array detector attached to an analytical workspace was utilized for the identification and separations of the phenolic compounds. Separations were achieved using Sulpelco C18-E column (25cm x 0.46 cm i.d) and the elution of the compounds, using binary gradients, were optimized. Compound identification was conducted against the retention time values and the UV-spectral of the target analytes. A gradient mobile phase of Milli Q water ("A") and 0.1 % phosphoric acid, acetonitrile and 0.1 % phosphoric acid ("B") was used for the chromatographic separation flow- rate of 1.0 ml/min.& quot. Detection was conducted at 280 nm for all the target analytes. The chromatographic system was conditioned by allowing the solvents through for 30 minutes so that a stable baseline signal was obtained. Once the chromatographic system was conditioned with mobile phases, the chromatograms were obtained by injecting 20 ml of the standards and analytes in question (while the temperature was maintained at 25°C).

1.4. Sampling procedure and storage

Tap water samples were collected from the Cape Peninsula University of Technology, Bellville Campus laboratory and four brands of bottled water were bought from local grocery stores. Samples were collected in sterile 500 mL amber bottles from the sampling stations at the Stellenbosch WWTP and preserved in the ice chest to maintain the integrity of the samples. To minimize water quality changes between sampling and analysis, the samples reached the laboratory within 24 h and were analysed within seven days. Water samples were collected in two replicates from the WWTP including one blank sample that served as the control sample. Water samples were filtered by passing through 0.22 µm polyethersulphone membrane syringe filters to remove possible debris and particles before storage in the refrigerator (at 4 0 C) in the laboratory.

1.5. Phenol extraction materials and chemicals

The effluent was filtered using the 0,22 µm pore size nylon filters prior extraction. This was to avoid any blockages in the SPE. For the conditioning of the cartridges; 8.5 ml n-haxane:acetone (50:50 v/v), 8.5 ml methanol and 15 ml Milli-Q purified water, 5ml and 10ml glass pipette were required, along with the pipette bulb. C18-E cartridges for the extraction of the analyte was used along with hydrochloric acid for the adjusting of the pH and the nitrogen to blow to dryness the analytes under gentle pressure. The vacuum and pump were used for the analyte extraction process environment and sample filtration along with the vial glass for the collection of the analyte of interest. A hot plate was used to assist in the process of drying effectively.

1.6. Extraction procedures and analysis for phenols in water

The pH of the water samples was reduced to a pH of 2.5 with hydrochloric acid prior to channeling it through the conditioned cartridge. Water samples were spiked with a mixture of phenolic standard with a known concentration. After allowing the samples to go pass the cartridges, 5 ml of Milli-Q water was passed through and left on the vacuum manifold for 30 minutes to dry (-70kPa). Thereafter, the desired analyte was held back then eluted with 3.5 ml of methanol, 3.5 ml of n-hexane: acetone (50:50 v/v) into a glass flask respectively. Following this, it was blown to dryness using a gentle flow of nitrogen. Aliquots from the solution were analysed by direct injection into the HPLC system in single injection. Recovery studies will be conducted as well, while the concentration of the target analytes were determined by external calibration standards.

2. RESULT AND DISCUSSION

2.1. Phenols occurrence in WWTP effluent and potable water samples

Existing HPLC methods that were previously published by Opeolu et al., 2010 and Akharame et al., 2020 were adapted for the qualitative and quantitative analyses of the phenolic compounds. A typical chromatogram, calibration curves and data for the method used are presented in Figures 1, 2a, 2b and Table 1 respectively. The retention times were 11.7 and 14.1 for 4-CP and 2,4-DCP, respectively. The R^2 values for both calibrations were >0.99 indicating the suitability of the method for analysis (Table 1). In this study, selected phenolic compounds 4-CP and 2,4-DCP in Stellenbosch wastewater effluent, tap water and four brands of bottled water were analysed. The results obtained from the analysis of potable water samples and wastewater treatment plant effluent are presented in Table 2.

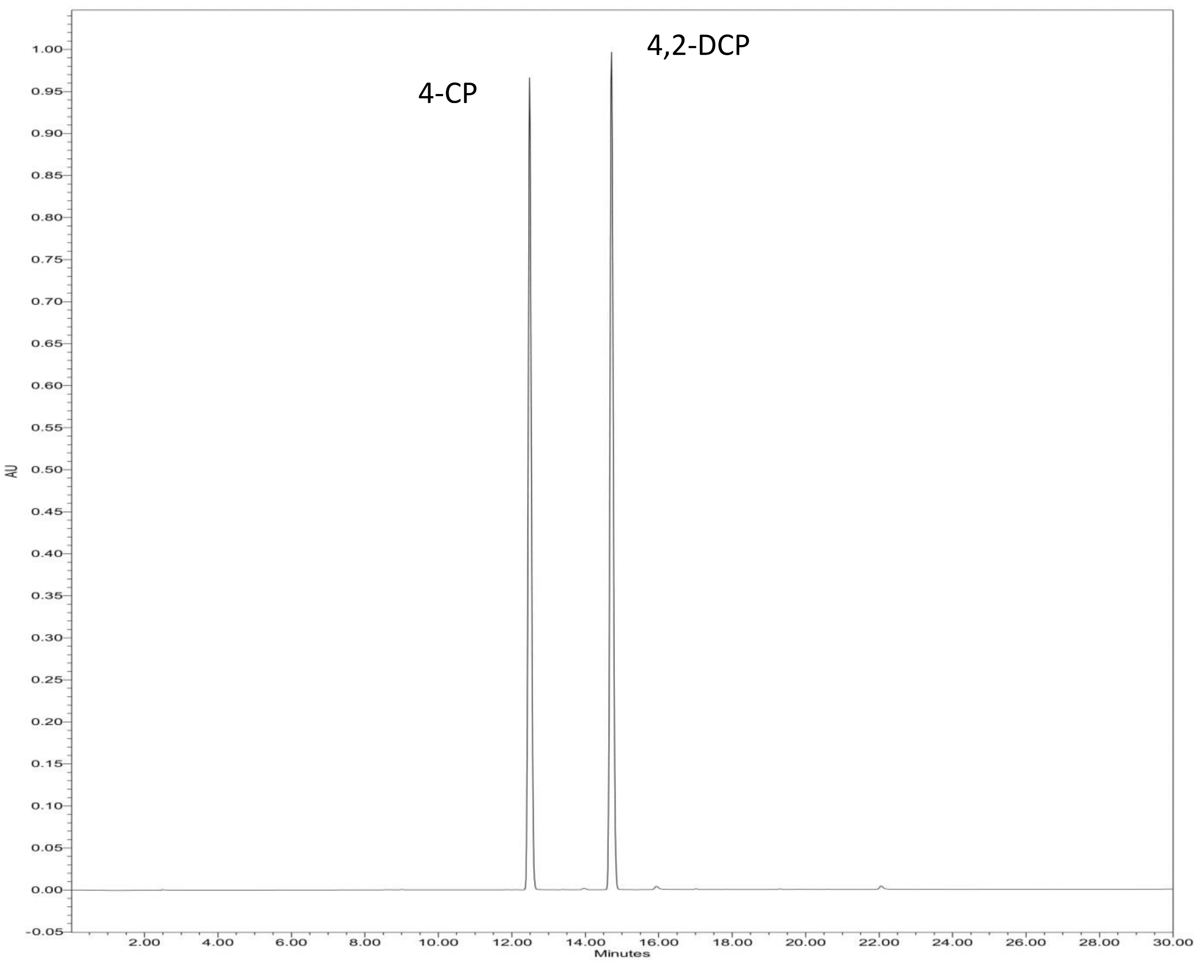

Figure 1: Chromatogram of 4-CP and 2,4-DCP

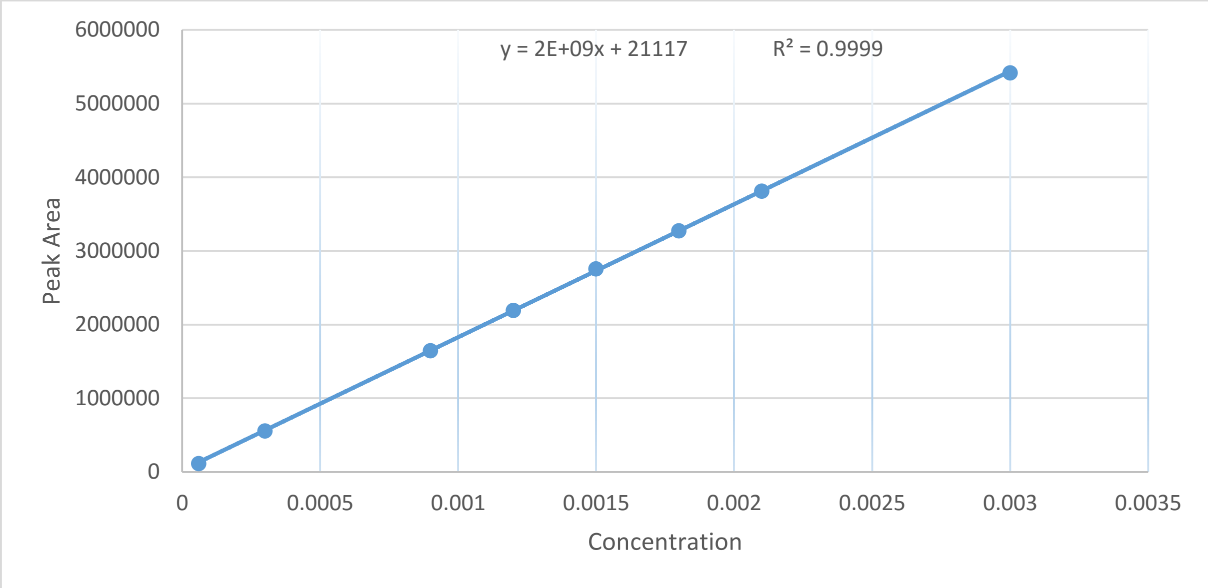

Figure 2a: Calibration curve for 4-CP

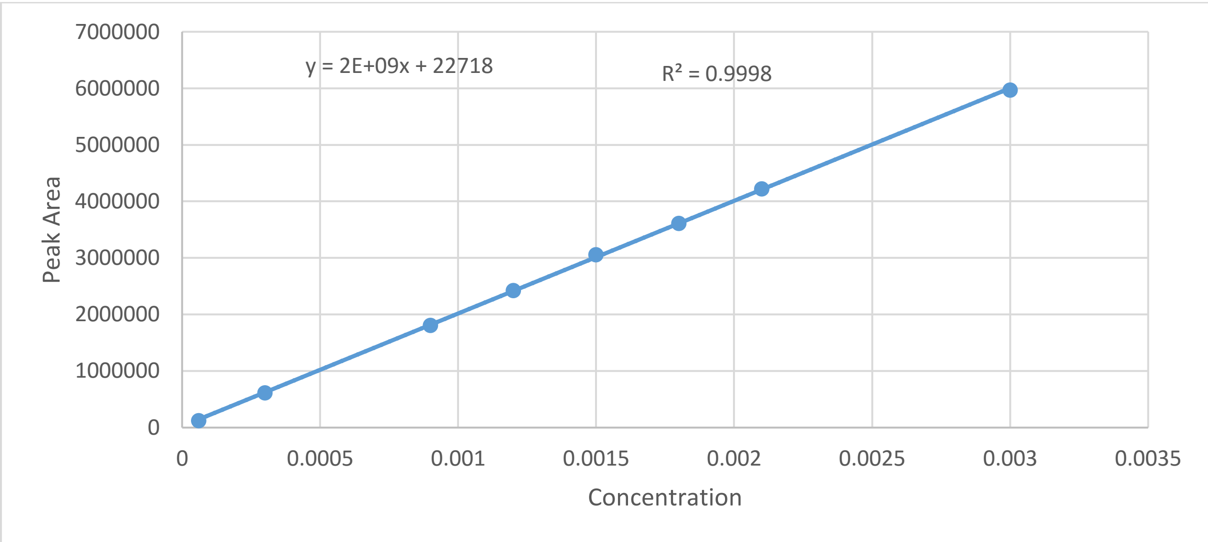

Figure 2b: Calibration curve for 2,4-DCP

Table 1:Calibration data for 4-CP and 2,4-DCP

4-CP			**2,4-DCP**		
Concentration (M)	**Peak Area**	**Retention Time**	**Concentration (M)**	**Peak Area**	**Retention time**
0.00006	113374	11.715	0.00006	123054	14.059
0.0003	557496	11.767	0.0003	614290	14.122
0.0009	1648622	11.761	0.0009	1805903	14.119
0.0012	2193595	11.762	0.0012	2421714	14.118
0.0015	2759050	11.723	0.0015	3058599	14.073
0.0018	3276298	11.739	0.0018	3610620	14.7
0.0021	3815336	11.708	0.0021	4224544	14.07
0.003	5420296	11.719	0.003	5969507	14.08

Table 2: Levels of phenolic compounds in potable water and WWTP effluent samples in mg/L (mean ± SD, n=3)

Sample	**4 CP**			**Mean**	**SD**	**2,4 DCP**			**Mean**	**SD**
	Batches					Batches				
	1	2	3			1	2	3		
WWTP influent	$1,04\times10^{-4}$	$1,149\times10^{-4}$	$6,43\times10^{-5}$	$9,43\times10^{-5}$	$2,66\times10^{-5}$	$9,81\times10^{-5}$	$1,72\times10^{-4}$	$6,39\times10^{-5}$	$1,11\times10^{-5}$	$5,51\times10^{-5}$
WWTP effluent	5.61×10^{-5}	1.27×10^{-5}	4.04×10^{-6}	2.43×10^{-5}	2.79×10^{-5}	ND	ND	5.40×10^{-6}	1.80×10^{-6}	3.12×10^{-6}
Bottle water Brand A	ND	9.32×10^{-7}	5.81×10^{-6}	$2,25\times10^{-6}$	3.12×10^{-6}	ND	3.68×10^{-6}	1.31×10^{-5}	5.60×10^{-6}	6.77×10^{-6}
Bottle water Brand B	ND	6.95×10^{-6}	3.42×10^{-6}	$3,46\times10^{-6}$	3.48×10^{-6}	5.56×10^{-6}	1.37×10^{-5}	3.68×10^{-6}	7.66×10^{-6}	5.35×10^{-6}
Bottle water Brand C	ND	ND	9.78×10^{-6}	3.26×10^{-6}	5.64×10^{-6}	8.80×10^{-7}	ND	6.85×10^{-6}	2.58×10^{-6}	3.73×10^{-6}
Bottle water Brand D	1.97×10^{-6}	8.90×10^{-7}	6.74×10^{-6}	3.20×10^{-6}	3.11×10^{-6}	1.47×10^{-5}	8.37×10^{-6}	6.28×10^{-6}	9.77×10^{-6}	4.36×10^{-6}
Tap water	9.96×10^{-6}	1.90×10^{-5}	ND	$9,65\times10^{-6}$	9.50×10^{-6}	6.23×10^{-6}	1.90×10^{-5}	$5,97\times10^{-6}$	$9,27\times10^{-6}$	$5,49\times10^{-6}$

For wastewater treatment, the Stellenbosch wastewater treatment plant makes use of the membrane bioreactors. This system uses a combination of biological treatment methods for suspended growth, generally activated sludge, with membrane filtration equipment, typically membranes for low-pressure microfiltration (MF) or ultrafiltration (UIF). To carry out the critical solid-liquid separation function, the membranes are used. (AMTA, 2016; Nqombolo *et al*., 2016). Therefore, the levels of 2,4-DCP in WWTP effluent are expected to be low due to this process of treatment used because the 2,4-DCP compound in drinking and wastewater is a by-products of water treated by chlorination (Park & Kisok, 2018) .

All samples were initially spiked with a known concentration of 0.0009 M of the analytes; the value was then subtracted from the result to obtain the actual concentration of phenolic compounds in the water samples. The concentrations of both phenolic compounds detected in the Stellenbosch WWTP effluent were below the limit set by the DWAF (0.01 mg/L). The concentration ranged between 4-CP4.04×10^{-6} mg/L - 5.61×10^{-5} mg/L and 0-5.40×10^{-6} mg/L for 4-CP and 2,4-DCP, respectively. The occurrence of the compounds at trace levels could be due to the compounds having been used either as raw materials or intermediate products in the agro-chemical industry and wood preservation (Santana *et al*., 2002; Ozkaya, 2005). Chlorophenols are also produced in pulp bleaching processes as metabolites of agricultural pesticides, due to inefficient removal of these congeners from wastewater treatment plants waste effluent and as by-products of the chlorination of drinking water (Heberer & Stan, 1997). The Stellenbosch WWTP is in the Boland region of Western Cape, South Africa- an area that is popular for its agricultural prowess as numerous commercial farms abounds. These farms mostly grow grapevines used to produce different types of wines. The presence of the low levels 2,4-DCP may possibly be from the agro-chemical usage from these farms as there was no chlorine treatment taking place in the WWTP. The actual possible sources of 2,4-dichlorophenol contamination into water sources may need further investigation.

There is limited information in the literature about 4-CP and 2,4-DCP occurrence in WWTP effluent (Buchholz & Pawliszyn', 1993; Kurniawan & Lo, 2007; Dilaver & Kargi, 2009; Saraji & Marzban, 2010; Olujimi, 2012). However, in the case of 4-CP the Wine production in the vicinity of the WWTP is a possible major contributor to the levels of phenolic compounds detected in effluent samples. Due to the toxicity of phenolic compounds in drinking and surface waters to aquatic and human lives, the European Commission (EC) and the United State Environmental Protection Agency (USEPA) have classified some of them as EDCs (Olujimi, 2012). Four brands of bottled water were investigated for 4-CP and 2,4-DCP. The American Food and Drug Administration (FDA) has established that in bottled drinking water, the phenol concentration does not surpass 0.001 mg/L (ATSDR, 2008b). Standard guidelines for general phenolic compounds were set at 2 mg/L by WHO for drinking water (Enderlein *et al*., 1996; ATSDR, 2006). The concentrations of 4-CP and 2,4-DCP in the bottled water denoted as brand "A" to brand "D are presented in Table 4.2. Brand "A" in the first month of sampling 4-CPneither of the two compounds were detected in the samples analysed. Subsequent analyses showed that water samples were tainted with the two phenolic compounds (9.32×10^{-7} and 5.81×10^{-6} mg/L -4-CP and 3.68×10^{-6} and 1.31×10^{-5} mg/L -2,4 DCP respectively).

The values were however below the regulatory limits of the FDA for phenols in bottled water. For Brand "B" in the 4-CP was not detected in the samples but 2,4-DCP (0.00000556) was present at levels below the FDA limits. The second and thirdbatches had 4-CP and 2,4-DCP levels ranging from 0.00000342 mg/L - 0.0000137mg/L; the values were also below the FDA regulatory limits. For Brand "C", 4-CP was not detected in the first two batches, but the last batch was contaminated at levels the FDA. Concentrations of 2,4-DCP below the FDA limit were detected in the first (0.000000880 mg/L) and the third (0.00000685 mg/L) batches of samples analysed but not in the second batch. In Brand "D", both 4-CP and 2,4 DCP were detected in all samples for the three batches, but the levels (ranging from 0.000000890 mg/L to 0.0000147 mg/L for both compounds) were below the FDA regulatory limit Olujimi (2012), reported the occurrence of USEPA 11 priority phenols in three brands of bottled water and found a mean concentration of 5.13 µg/L. However, Steiner *et al*. (2007) for the priority pollutants reported that 2,4-DCP did not occur in both the spiked and un-spiked samples. For 4-CP, The USEPA's guideline for potable water is ≤ 0.3 mg/L phenol, protecting human health from the potential adverse effects of phenol exposure by drinking water and/or consuming contaminated plants and animals (Younis & Rafati, 2004). The limit surface water (lakes, streams) sources is 3.5 mg L^{-1} (EPA, 2002). In the European Community, for every pollutant the maximum allowable concentration of phenols in drinking water is 0.1 µg.L^{-1} (Khalid, 2011). For all samples analyzed, the levels of both 4-CP (ND-0,0000190 mg/L) and 2,4 DCP (0,00000597 -0,0000190 mg/L) were found to be below the set limits and standards in the µg/L and mg/L levels. A similar study by Izawa *et al*. (2015) reported that the concentrations of phenols in tap water ranged from 0.01–0.20 mg/L (there were no specific values for the individual phenolic compounds). Possible sources of the low level 4-CP and 2,4-DCP in the tap water may be from the chlorination process carried out to disinfect the water. Moreover, it may come from the source water which is obtained from dams that may have been contaminated with agricultural run-offs. These dams are mostly filled by run-offs during rainfall in the winter seasons when farming activities are at their peaks.

3.2. Non-carcinogenic and carcinogenic risk assessment

Human health risk assessment was conducted to provide an indication of possible carcinogenic effects of the phenolic compounds exposed to humans. The calculations assumed exposure duration of 10 years, a body weight of 70 kg and life expectancy of 70 years (Table 2). Hazard quotient (HQ) was calculated and used to determine the non-carcinogenic health. A sample was considered to possess non-carcinogenic adverse effects if the HQ > 1 and non- carcinogenic adverse effect when the value is <1. The mean values of both phenolic compounds measured in the respective water samples were used for exposure concentration. The average daily dose (ADD), hazard quotient (HQ) and cancer risk of samples are presented in Tables 4.9a and 4.9b. The HQ value for all samples were <1. All samples therefore are classified to possess non-carcinogenic adverse effects risk for a lifetime exposure.

Table 4. 3: Cancer risk assessment using mean concentrations of 4-CP and 2,4-DCP of samples

Sample	**ADD**	RfD	**HQ**	SF	**CR**	**Comment**
4-CP						
WWTP-4CP-Influent	6.15E-07	3.00E-01	2.05E-06	1.10E-02	6.76E-09	Non-carcinogenic adverse effect
WWTP-4CP-Effluent	1.58E-07	3.00E-01	5.28E-07	1.10E-02	1.74E-09	Non-carcinogenic adverse effect
BW A-4CP	2.35E-05	3.00E-01	7.82E-05	1.10E-02	2.58E-07	Non-carcinogenic adverse effect
BW B-4CP	3.61E-05	3.00E-01	1.20E-04	1.10E-02	3.97E-07	Non-carcinogenic adverse effect
BW C-4CP	3.40E-05	3.00E-01	1.13E-04	1.10E-02	3.74E-07	Non-carcinogenic adverse effect
BW D-4CP	3.34E-05	3.00E-01	1.11E-04	1.10E-02	3.67E-07	Non-carcinogenic adverse effect
Tap Water	1.01E-04	3.00E-01	3.35E-04	1.10E-02	1.11E-06	Non-carcinogenic adverse effect
2,4-DCP						
WWTP-2,4DCP-Influent	7.24E-08	3.00E-01	2.41E-07	1.10E-02	7.96E-10	Non-carcinogenic adverse effect
WWTP-2,4DCP-Effluent	1.17E-08	3.00E-01	3.91E-08	1.10E-02	1.29E-10	Non-carcinogenic adverse effect
BW A-2,4DCP	5.84E-05	3.00E-01	1.95E-04	1.10E-02	6.42E-07	Non-carcinogenic adverse effect
BW B-2,4DCP	7.99E-05	3.00E-01	2.66E-04	1.10E-02	8.79E-07	Non-carcinogenic adverse effect
BW C-2,4DCP	2.69E-05	3.00E-01	8.97E-05	1.10E-02	2.96E-07	Non-carcinogenic adverse effect

BW D-2,4DCP	1.02E-04	3.00E-01	3.40E-04	1.10E-02	1.12E-06	Non-carcinogenic adverse effect

The cancer risk values for all samples tested were also below the regulatory limits set by national and international bodies. The regulatory limit set by DWAf (1984) for phenols in WWTP effluent is 0.01 mg/L. A lifetime exposure of 2 mg/L is not expected to cause an adverse health effect. The US FDA advisory limit is 0.001m/L in bottled water (ATSDR, 2008b). The European Union limit is 0.5 µg/L for total phenols and 0.1 µg/L for individual compounds (Fattahi *et al.,* 2007; Sanlana *et al.,* 2009). Khalid (2011) reported that below 0.3 mg/L, no harm to aquatic life was observed. The regulatory limits by the US-EPA for 4-CP is 5.5 µg/L (EPA, 1990) and 0.02 mg/L for 2,4-DCP (EPA, 2018). The WHO limit is 0.04 mg/L in water. None of the samples analyzed had a cancer risk (CR) value that exceeded any of the regulatory limits. The possibility of the samples causing cancer is therefore slim.

3. CONCLUSION

The WWTP treatment process showed considerable effectiveness for removal; the membrane bioreactors system replaces the old system fixed medium (stone) trickling filter systems to treat the influent. The influent was also tested for any of the phenolic compounds in question, and it also displayed low concentrations of the phenolic compounds.

This investigation is an introductory study into the human health risk assessment of selected endocrine disrupting phenolic compounds in potable water and treated wastewater effluent samples. The study has provided some insight into the possible human health risks associated with the occurrence of the 4-CP and 2,4-DCP in potable water and effluent samples. However, other questions such as the actual reasons for the toxicities observed remain unanswered. The ecological health studies were carried out at population and organism levels. Cellular and molecular level studies may provide greater clarity on some of these questions.

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