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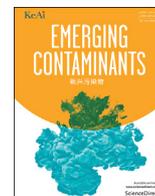
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Suspect and non-targeted screening of chemical pollutants in Botswana's aquatic environments

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ABSTRACT

Chemical pollution in the aquatic systems of Botswana has been sparsely studied despite its potential ecological importance. Here, we perform a study of water samples collected from 13 locations distributed across Botswana to obtain the first overview of the nature and distribution of chemical contaminants across the country's aquatic environment. High resolution mass spectrometry was applied using non-targeted and suspect screening methods to qualitatively analyse samples. A total of 114 contaminants of emerging concern (CECs) were identified including 68 (59.6 %) pharmaceuticals and pharmaceutical metabolites; 16 (14.2 %) pesticides; 13 (11.4 %) psychoactive compounds and metabolites; 11 (9.7 %) industrial chemicals and intermediates and lastly, 5 (4.4 %) personal care products. Allopurinol, 3,4-dimethylmethcathinone, and diazolidinyl urea represented the most commonly detected pharmaceutical, psychoactive drug and personal care product, respectively. The pesticide dodemorph and three industrial chemicals (stearamide, pthalic acid and bis(2-ethylhexyl) phthalate) were detected in all samples obtained. 90 CECs were detected in receiving water (from 7 sample locations), 75 in wastewater (from 3 sample locations) and 60 in surface water (from 9 sample locations). Of the compounds detected, only 8 had been identified in environmental samples acquired in Botswana previously. We discuss the variations in the nature and frequency of chemical pollutants detected in this work in a geographical context. The results indicate that Botswana's aquatic systems are subject to pollution, despite wastewater treatment and that in order to mitigate potentially harmful effects on both human and aquatic ecosystems, more investigations are required to correctly identify, track and tackle the sources of pollution.
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1. Introduction

Chemical pollution is of increasing global concern due to the ever-increasing number and diversity of registered chemical substances in production worldwide. Aside from priority pollutants that are listed by the EU Water Framework Directive or the US EPA, several contaminants of emerging concern (CECs) are currently being evaluated for potential future regulation [1]. CECs include among other compounds pharmaceuticals, pesticides, flame retardants, illicit drugs, perfluoroalkyl substances (PFAS) and personal care products (PCPs) [2,3]. After human consumption or use, CECs enter the sewer system and wastewater treatment plants (WWTPs) which are often not designed to efficiently remove CECs [4]. As a result, these chemicals and their transformation products are released into aquatic systems where they could potentially have a negative impact on the natural environment and human health [5–7].

Although there are an increasing number of reports of CECs globally, these investigations are predominantly conducted in developed countries [8]. This situation results mainly from the low availability of advanced analytical instrumentation in middle-to-low income countries. Southern Africa is one such geographical area, where very few investigations on CECs have been conducted. A recent review by Selwe et al. provides an overview of the limited studies of CECs that have been conducted across the Southern African Development Community [9].

Botswana is a land-locked Southern African country that offers a geographical profile with respect to chemical pollution that is valuable for detailed study. The country is thinly populated, meaning that population centres are generally well separated, potentially providing a clear panorama on which to understand links between population and distribution of chemicals within the broader aquatic environment. Furthermore, it has a semiarid climate, with rainfall generally restricted to the summer months, and limited to certain areas of the country [10]. The sparsity of rainfall should therefore allow for well-controlled tracking of the progress of chemical pollutants, including CECs, across the country's aquatic system. It is also notable that the Okavango Delta is located within Botswana. This is a vast, inland aquatic area of outstanding importance for biodiversity [11,12], and represents the world's largest inland wetland [13]. The monitoring of CECs in this region should be a key part of conservation activities. Given these considerations, it is surprising that very few studies of chemical pollution in Botswana have been conducted to date [14–17]. In particular, recent measurements of chemicals in aquatic sources have only been performed at wastewater treatment plants and a borehole [14,15], so information on levels of CECs across the broader aquatic landscape of Botswana are currently unavailable.

In a recent publication, we presented a study analysing water samples from a single wastewater treatment plant located in the Glen Valley, which is adjacent to the capital city of Gaborone [14]. Wastewater provides a valuable, non-intrusive avenue to assess public health such as community based usage of pharmaceuticals, personal care products, and even illicit drugs [18,19]. This was the first study of CECs in Botswana to apply high-resolution tandem mass spectrometry (HRMS) to water samples across the country. Out of 129 identified compounds, our work identified 28 compounds via suspect screening, of which 26 were pharmaceuticals including two illicit drugs. Of note, two antiretrovirals, abacavir and tenofovir, were detected in the influent and effluent sources, demonstrating that wastewater treatment plants are potentially significant pipelines of chemical pollution to the natural environment in Botswana. However, the boarder implications of the study were clearly limited given that samples had only been obtained at a single geographic location.

Herein we present a new study of chemical pollution in Botswana where water samples have been analysed from 13 distinct geographical locations across the whole of Botswana (Fig. 1), including measurements near the city of Maun which is near the key Okavango Delta region. By obtaining samples from a diverse selection of areas, we are able to gain insight into the presence of CECs in urban versus rural areas, and in locations specifically linked to tourism and agriculture. Furthermore, through sampling at the geographically dispersed locations, we are able to obtain a first overview of how the accumulation of wastewater can lead to the prevalence of chemicals in other environmental compartments. The broader survey of CECs obtained in the study is an essential precursor to future quantitative measurements, via more expensive targeted analysis [20], since it allows for informed prioritization of specific chemical substances.

LC-HRMS/MS analysis is used as the key analytical technique in this work, with both suspect screening and non-targeted analysis (NTA) approaches to compound identification. HRMS allows for simultaneous suspect screening and non-targeted analysis, thus enabling the detection of compounds within a sample without any prior information on substances present via comparison with mass spectral libraries [21,22]. The detected HRMS features are subjected to scrutiny against multiple thresholds such as peak intensity, blank subtractions and peak alignments before they are compared to openly accessible spectral databases [22,23]. This overall approach to characterising chemical pollution within a sample is

advantageous as the sample can be qualitatively screened for an unrestricted number of chemicals and transformation products. This includes compounds which may currently be classified as lower priority (and hence may not feature in all curated databases), and also compounds which have not been detected previously.

2. Material and method

2.1. Chemicals and reagents

Standards for calibrations of accurate masses and monitoring of variations of intensities comprised of Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-Hydroxy- Δ9-tetrahydrocannabinol (THC-OH) and cannabidiol (CBD) purchased from Cambridge Bioscience Ltd (Cambridge, UK). MDMB-FUBINACA and JWH-018 standards were purchased from Merck Life Science Ltd (Dorset, UK). Abacavir was purchased from Fluorochem Ltd (Glossop, UK). Liquid Chromatography-Mass spectrometry grade methanol, water, formic acid and ammonium formate for instrumental analysis were obtained from Fisher Scientific (Loughborough, UK). All reagents were of analytical grade.

2.2. Sample collection

A total of 19 grab samples (500 ml) were collected in duplicate from 8 different villages and cities in Botswana in June 2022. The samples included 3 effluent wastewater (WW) samples that were collected within or immediately beyond the WWTP, 7 samples from receiving waters (RW), where RW is defined as a body of water such as a river or pond, into which treated or untreated wastewater or effluent is discharged, and 9 samples from surface waters (SW), which did not have any direct link to a wastewater source. The different locations covered areas with a range of human activities such as tourism, agriculture, mining and industry. The samples were collected in pre-washed, high-density polyethylene bottles and transported in cooler boxes containing ice. Upon arrival at the laboratory, they were filtered under gravity using Fisherbrand™ Grade 122 filter paper (pore size 17–30 μm) from Fisher Scientific (Loughborough, UK), and kept in the freezer for 21 days before extraction.

Fig. 1 (above) shows the locations where samples were collected. Table 1 lists the sampling locations as a function of Global Position System (GPS) coordinates and provides further location details such as population size and local human activities.

2.3. Solid-phase extraction

Characterisation of the chemical profile of a sample using HRMS is dependent on the generation of an analyte mass signal above the noise generated by the matrix. To enhance this, sample pre-concentration and clean-up can be used to decrease detection limits and reduce the impacts of matrix effects. Solid-phase extraction (SPE) is beneficial when used in conjunction with LC-HRMS\MS for pre-concentration of analytes which would otherwise not have been detected [24,25]. Contaminants of the matrix which may give rise to interferences can also be removed during the selective filtration process in this way [26]. In this work, solid-phase extraction was performed as follows: Cartridges were conditioned with 5 ml MeOH followed by 5 ml ultrapure water. Waters Oasis® HLB (6 cc, 200 mg) SPE cartridges from Waters Cooperation (Milford, MA, USA) were used to concentrate the 500 ml samples under vacuum at a flow rate of 3–5 ml per minute. These cartridges were suitable for the non-targeted approach owing to their capability of good recovery and performance within a wide range of pH-values. The cartridges were allowed to dry for

Fig. 1. Map of Botswana showing the distribution of sampling locations (indicated by blue pins).

Table 1

Sampling locations, including GPS locations, with details of population number, human activities at the location and type of aquatic source sampled. Where two entries are listed for one location, samples were sourced from different positions at that source.

Location	Classification (Population)	Human Activities	GPS Coordinates	Sample Type[a]	Sample Code
Lecheng	Village (3,352)	Agriculture	S:22.671, E:27.215	SW	SC_001
					SC_002
Palapye	Town (52,398)	Mining	S:22.539, E:27.174	WW	SC_003
				RW	SC_004
Mmadinare	Village (13,087)	Agriculture	S:21.857, E:27.723	SW	SC_005
Francistown	City (102,444)	Metropolitan	S:21.219, E:27.536	RW	SC_007
Kasane	Town (9,013)	Tourism	S:17.799, E:25.183	RW	SC_008
					SC_009
			S:17.789, E:25.198	SW	SC_010
					SC_011
Maun	Town (85,293)	Tourism	S:19.928, E:23.512	SW	SC_012
					SC_013
			S:19.957, E:23.412	WW	SC_014
					SC_015
Ghanzi	Town (55,884)	Agriculture	S:21.709, E:23.412	RW	SC_016
Mogobane	Village (2,926)	Agriculture	S:24.977, E:25.697	SW	SC_017
Gaborone	City (244,107)	Metropolitan	S:24.645, E:25.935	SW	SC_018
			S:24.618, E:25.963	RW	SC_020
			S:24.627, E:25.960	RW	SC_023

[a] RW = Receiving water, SW = surface water and WW = wastewater.

10 min under airflow, then frozen and shipped to the University of York for analysis. The contents of the cartridges were extracted using 5 ml MeOH, followed by 5 ml 5 % Ammonium Hydroxide in MeOH. The extracts were dried under nitrogen and reconstituted in 1 ml 80:20 water: methanol solution before LCMS injection. All glassware was salinized to reduce the adsorption of analytes to the

glass surfaces [27,28]. This was done by rinsing glassware with a solution of 10 % dichlorodimethylsilane (DCDMS) in toluene followed by two toluene and two methanol rinses.

2.4. Ultra High-Performance Liquid Chromatography (UHPLC) and HRMS

Analyte separation was performed using a Dionex Ultimate 3000 Ultra High-Performance Liquid Chromatography (UHPLC) system with a Cortecs® T3 2.7 μm (2.1 × 150 mm) column and a Waters VanGuard™ 2.7 μm (2.1 mm × 5 mm) column guard and mobile phases A (0.01 M formic acid in HPLC grade water) and B (0.01 M ammonium formate in HPLC grade methanol). The elution gradient used a flow rate of 0.3 mL/min consisting of binary mobile phases (A: B) beginning with 95 % (A) linearly decreased to 5 % (A) over 20 min and held for two additional minutes. The mobile phases were rapidly returned to starting conditions (95 % A) over 0.2 min and held for 5.8 min to allow for re-equilibration of the column between sample injections.

HRMS was performed on an Orbitrap™ Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.), with an electrospray ionization source (ESI). A full scan-data dependent MS2 (ddMS2) acquisition was obtained in positive mode under the following MS parameters: sweep gas flow rate: 1 (arb); sheath gas flow rate: 50 (arb); aux gas flow rate: 10 (arb); ion transfer tube temperature: 325 °C; vaporizer temperature: 350 °C; MS1 detector: Orbitrap; MS1 scan range: $100-1000$ m/z; MS1 maximum injection time: 50 ms; MS2 detector: ion trap; HCD collision energies: 15, 30, and 45 %. The measurements were limited to positive ion mode due to time and cost restraints for accessing the HRMS instrument.

2.5. Suspect screening and non-targeted workflows

Suspect screening was performed using Thermo Scientific™ Compound Discoverer 3.3™ software. All positively charged ions with mass to charge ratios between 100 and 1000 m/z were considered including differentially ionized species ($[2 M + H]^+$, $[M+H]^+$, $[M+2H]^{+2}$, $[2 M + K]^+$, $[M+K]^+$, $[2 M + Na]^+$, $[M+Na]^+$, $[2 M + NH_4]^+$, $[M + NH_4]^+$). The most intense M species of this family of ions was reported for any given compound M, to allow for differences in ionization efficiency for different compounds. Features with accurate masses within 5 ppm were then grouped together provided they had similar retention times (0.5 min). A blank correction removed all features detected in samples and blanks unless peak areas in samples were greater than ten times the peak area in the blank. Background features were discarded, and these were defined as all features with a peak area lower than 1.0×10^5, a minimum chromatographic signal to noise ratio below 100, and a peak rating ratio below 5 (the built-in peak rating function gives peaks a quality rating from 0 to 10). This approach was adapted from a recent protocol from Pereira et al. [29]. Duplicate technical replicates were performed for each sample, and peaks must have met all criteria in both replicates to be further analysed. A graphical summary of this workflow is provided in the supplementary data (Fig. S1).

Following this, elemental compositions of the features were predicted using the measured accurate mass. All compositional predictions with a mass accuracy exceeding ±5 ppm were discarded. The subsequent fragment ions had to meet the minimum signal to noise ratio set to 100. The remaining suspect ion accurate masses were then matched with annotations to theoretical, exact masses found in a customised suspect mass list (±5 ppm) adapted from the NORMAN substance database (suspect screening), while spectral library matching was carried out through the mzCloud™ database (NTA). An automated R-script was developed to discard all

detections that did not satisfy the previously described thresholds. This approach was developed following similar approaches available from the recent literature [1,29,30], and significantly reduces data analysis time and efficiency of the workflow.

Features with accurate masses within 5 ppm of the annotated structure were confirmed at Level 5 confidence, according to the well-established Schymanski scheme [31]. Those with an unequivocal molecular formula (isotopic patterns) were confirmed at Level 4. Level 3 detections had the same criteria but with MS^2 data to provide structural information. Level 2b detections were confirmed using matches against compounds in the mass lists while Level 2a detections were confirmed by comparing the sample data to the mzCloud™ spectral library. This library was selected for spectra matching due to its quality curation with spectra generated on orbitrap™ instrumentation, the same used in this study. Only those detections with at least 70 % match confidences were selected. Candidate detections which had available reference standards were purchased and used to confirm level 1 detections.

2.6. Quality control and method validation

The column was preconditioned by running 10 injections (5 h) of a quality control sample that was prepared by pooling 50 μL from each sample. Additionally, this quality control sample was injected in triplicate after every 8 sample injections. These injections were used to monitor any potential changes in column conditions and were used by Compound Discoverer 3.3 to account for retention time drift (Fig. S2). Solvent blanks, also run after 8 sample injections, were used to determine instrument background and monitor carry-over between sample injections. Lastly, a mixture of the internal standards (n = 5) was spiked into a sample matrix at an elevated concentration, analysed and run through the non-targeted workflow to confirm accurate annotations by the software.

3. Results and discussion

The full sampling campaign identified a total of 2393 unique compounds with Level 4 confidence, based on the Schymanski model [31]. Of those, 1801 unique compounds were identified at Level 3 confidence of which 1441 were CECs. 114 CECs were identified at Level 2b, with 34 additional CECs identified at Level 2a, highlighting the importance of non-targeted analysis for these compounds since they would not have been detected using a suspect screening method.

Fig. 2 illustrates the distribution of the detected CECs between the various chemical categories, illustrating that pharmaceutical compounds are the most prevalent, followed by pesticides and natural products. Chemicals classified as either natural products or
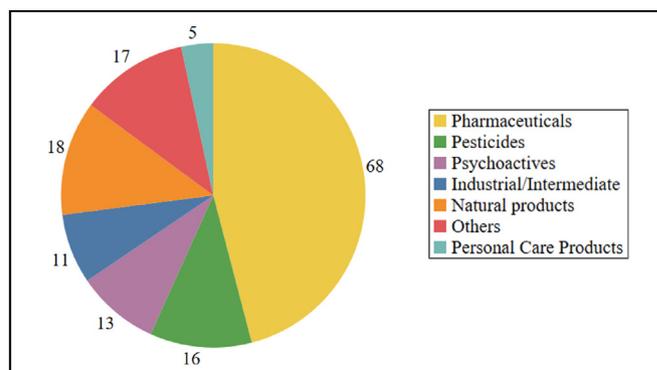

Fig. 2. Distribution of the detected CECs between different chemical categories.

others will not be discussed further here as they are not considered to be CECs (Fig. S3 provides a heat map for these compounds giving additional information for the interested reader.). Out of the CECs detected at Level 2 confidence, 68 (59.6 %) were pharmaceuticals and pharmaceutical metabolites; 16 (14.2 %) were pesticides; 13 (11.4 %) were psychoactive compounds and metabolites; 11 (9.7 %) were industrial chemicals or related intermediates and 5 (4.4 %) were personal care products.

The distribution of the CECs in the different water environment types is illustrated in Fig. 3. Analysis showed that 95 CECs were detected in receiving water (RW: 7 sample locations), 75 in wastewater (WW: 3 sample locations) and 60 in surface water (SW: 9 sample locations). The relatively lower number of chemicals in SW is consistent with these environments being unconnected to direct sources of either treated or untreated wastewater. Importantly, only three WW samples were collected in this study, compared to seven RW samples. It is therefore unsurprising that the number of compounds detected in RWs is higher than in WWs in this particular overview study, where our focus is on gaining a broad picture of contamination rather than a detailed understanding of quantitative pollution at each location.

The prevalence of the different chemical groups overall within the three sample types (WW, RW, SW) shown in Fig. 3 is similar to the overall distribution of the detected contaminants (Fig. 2), although it is notable that fewer pesticide compounds were detected in the SW samples compared to the WW and RW ones. This could be as a result of high household usage of pesticides to deal with mosquitoes and other insects in the household, as well as cockroaches in the sewage systems. Furthermore, the lower number of pesticides detected in SW may result from the limited number of sources that contribute to the distribution of pesticides in SW, as compared with the multiple sources that contribute to the WW and RW.

Fig. 4 provides a further level of detail, giving an overview of the

CEC distribution per sample. While there are common trends observable for all of the samples, e.g. pharmaceuticals are detected in greater numbers than other categories of chemicals, the individual sample profiles do show significant variations in terms of the extent of CECs present. The greatest difference in sample profiles appears for the RW samples, with the number of compounds varying from 58 (SC_007) to 21 (SC_004) for this group. The origin of this difference may be related to the large numbers of RW sites (n = 9) in relation to the number of WW sites (n = 3) that were chosen for the study (Table 1).

A tentative link between population size and number of contaminants was observed between population size and number of contaminants was observed in both the RW and SW samples. Gaborone (SC_020 & SC_023) and Francistown (SC_007) showed the highest number of detections, while samples from less populated villages such as Palapye (SC_004) and Kasane (SC_009) were associated with fewer detections. However, different trends were observed in the WW samples collected, where more detections were observed in samples from Palapye (SC_003) than in those from Maun (SC_014 & SC_015). Although this is against the population trends, it could be explained by the fact that there are more houses that are directly connected to the four sewage systems that were completed in 2022 in Palapye, while many residents in smaller villages around Maun still rely on pit latrines that are not linked to the WWTP [32,33]. It should also be noted that different wastewater treatment strategies are employed in the various locations [34], and thus, removal efficiencies may vary, and this further complicates the correlation between population size and the number of chemicals detected. Long-term studies to investigate removal efficiencies of each WWTP are necessary to accurately evaluate their role in mitigating chemical pollution of environmental waters as has been done in neighbouring South Africa [35].

Two samples were collected from the same location along the seasonal Thamalakane river in Maun during low-flow (SC_012) and high-flow (SC_013) periods. Interestingly, the number of detections increased in the latter sample by eight chemicals which could be an indication of additional pollution upstream or the possible influence of nearby run off. Future sampling at various points along this river, together with quantitative analysis are required to identify sources and monitor trends along this river.
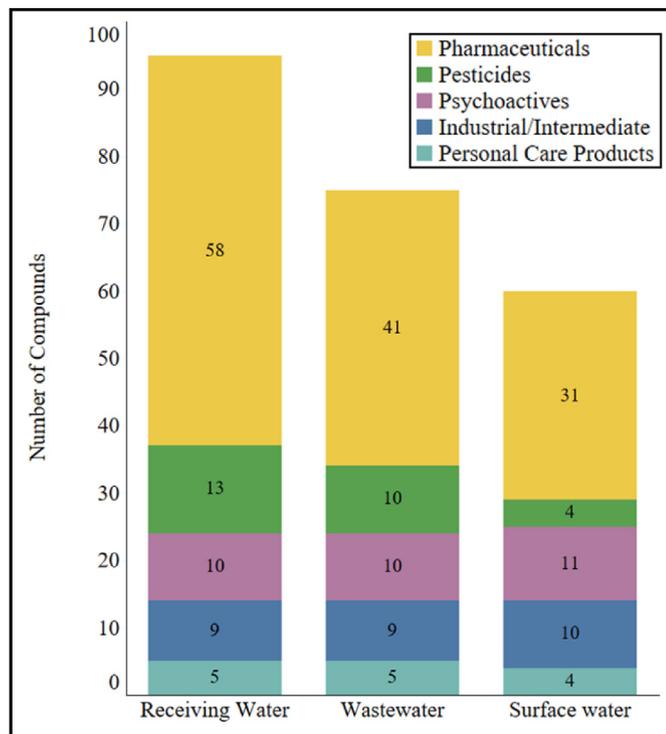

Fig. 3. Distribution of the detected CECs in the three distinct water environment types. Colour indicates contaminant category as defined in the legend.
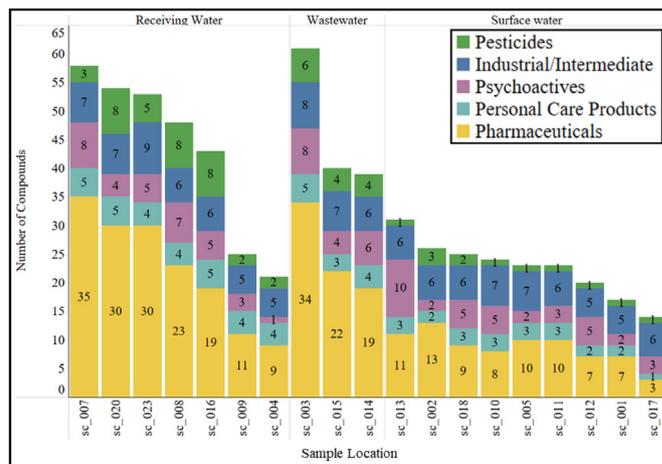

Fig. 4. Spatial distribution of contaminants of emerging concern in each sample. Colour indicates contaminant category as defined in the legend.

3.1. Pharmaceuticals and pharmaceutical metabolites

The data presented in Figs. 2 and 3 revealed that pharmaceuticals and pharmaceutical metabolites are the most prevalent CECs present in the samples studied. Inspection of the data for the individual samples (Fig. 4) shows that the number of unique pharmaceuticals and pharmaceutical metabolites detected per sample varied from 3 (sample SC_017) to 35 (SC_007). In line with the overview data (Figs. 2–4), a higher number of pharmaceuticals were detected in receiving water (58) compared to wastewater (41), likely due to the greater number of RW locations sampled. The WW sample (SC_003) had 61 chemical detections as compared to the corresponding RW sample (SC_004) which had 21 chemical detections. This could be justified by the fact that the RW is much more dilute as compared to the WW, but it could also be sign of the environmental degradation of the chemical pollutants studied here. However, this observation could also reflect the impact of secondary sources of pollution into the RW locations. This is further supported by the fact that only six of the detected pharmaceuticals (allopurinol, eugenol, gamolenic acid, griseofulvin, pregabalin and triethanolamine) were detected in our previous wastewater-based study. Indeed, the importance of secondary sources of pollution is evidenced by our observation of the 32 pharmaceutical compounds that were detected in surface waters (Fig. 3).

Fig. 5 presents a heat map to provide information on the relative abundance of the individual pharmaceuticals and metabolites across distinct locations. A broad overview of Fig. 5 data reveals that most of the pharmaceutical compounds were detected in WW and RW samples, with lower detections in the SWs. This supports the hypothesis that WWTPs are a major pathway of CECs such as pharmaceuticals into the environment [14].

In relation to individual chemicals, allopurinol, a medication used to treat high uric acid levels associated with gout was detected with the highest frequency of 79 %. It is one of the most widely prescribed pharmaceuticals, having recently been added to the World Health Organization (WHO) Model List of Essential medicines [36]. Allopurinol has previously been identified as a molecule that has an almost ubiquitous presence in the urban water cycle at elevated concentrations due to its high biological stability [37], and its metabolite oxypurinol has been suggested as a potential marker for domestic wastewater in the environment [38]. Atenolol, a beta blocker that is used to treat high blood pressure, is the second most prevalent chemical with a 73.3 % detection frequency. A group of three further chemicals are also present at a high frequency of ~63 %, namely olodaterol, paraxanthine, and 2-Amino-6-methyl mercaptopurine.

Examples of other pharmaceutical CECs detected include antibiotics (e.g., azithromycin and erythromycin); antiretrovirals (e.g., atazanavir, lopinavir and ritonavir); and antifungals (e.g., griseofulvin and fluconazole). The presence of such pharmaceuticals in the environment is a source of concern given that there is still little known regarding the breakdown of these compounds as well as their ecological impact in relation to issues such as antibiotic resistance.

Although most of these compounds have been reported previously in the aquatic systems of neighbouring South Africa [20,39] and in other sub-Saharan countries [40], to the best of our knowledge, this is the first report of these chemicals in Botswana with the exception of allopurinol, D-camphor, griseofulvin, guanine, L-threoninol, pregabalin and triethanolamine which were detected in our previous study on wastewater [14]. It is notable that detected pharmaceuticals are observed in higher quantities in samples collected from highly populated areas such as Gaborone (SC_020) and Francistown (SC_007) as compared to those samples collected in less populated villages such as Lecheng (SC_001) and Mogobane

(SC_017).

3.2. Pesticides

The distribution of pesticides across the individual sampling sites is illustrated by the heat map in Fig. 6. A total of 16 pesticides were detected, with at least one pesticide compound being detected in each water sample. 10 pesticides were detected in WWs, while 13 pesticides were detected in RWs all sampled in towns near in rural areas. This could suggest that RWs are impacted by indirect pollution sources such as landfill leachate, runoff or direct discharge of waste. The SW may also receive runoffs from local sources but to a lesser extent than the RW that receive runoffs from many sources. A further 4 pesticides were detected in SWs. A fungicide, dodemorph, was detected in all samples collected potentially indicating high persistence. Indeed, this is supported by a recent study in China which showed that dodemorph undergoes negative removal during wastewater treatment due to deconjugation of its transformation products [41]. Generally, the number of pesticides detected were higher in samples collected from the RW in more populated cities and towns such as Kasane (SC_008), Ghanzi (SC_016) and Gaborone (SC_20) as compared to SW samples collected from lesser populated villages such as Lecheng (SC_001), Mmadinare (SC_005) and Mogobane (SC_017).

We note that Wania and co-workers have previously studied pesticide contamination of air and soil samples around Botswana from May 2006 to May 2007 [17]. Overall, concentrations found in air were low, with α-endosulfan and lindane being most abundant, with concentrations in soils being extremely low, with dieldrin being the only pesticide found at a significant level. While the study led to the conclusion that arid soils in Botswana are not a major reservoir of organic pesticides, the results found here indicate that this may be due to transfer of these compounds from soil into aquatic systems by means of rainwater runoffs and wind-generated transport of soil particles. The different identities of the pesticides detected in our study compared to the work of Wania and co-workers may either reflect changes in usage of individual pesticides over time, or differences in relative persistence in soil compared to water.

3.3. Psychoactive compounds

Psychoactive chemicals were detected in all of the water samples, with the number per sample varying from 1 (SC_004) to 10 (SC_013). Eleven chemicals were detected in surface water, with a similar number (10) in wastewater and receiving water. Fig. 7 presents the distribution of psychoactive compounds as a function of the sampling sites.

3,4-dimethylmethcathinone (3,4-DMMC) and 4-fluoro pentedrone had the highest detection frequencies of 89 % and 79 %, respectively. Both of these compounds are members of the synthetic cathinones family or "bath salts" that are commonly used substances of abuse and have been detected in wastewater in other parts of the world [42,43], 4-fluoro pentedrone was recently reported to be present in groundwater in Denmark [44]. Other psychoactive chemicals that were detected include cannabinoids (cannabigerol, JWH-133) and their metabolites (AB-CHMINACA M4) and anabolic steroids (methenolone, methandienone) and their metabolites (4-androstenediol). It is noted that the highest number of psychoactive compounds were detected in a sample collected from Maun (SC_013), which is a holiday destination for both local and international tourists.

In our recent review, we highlighted that there is very little literature on the presence of illicit drugs in aquatic systems in southern Africa [9]. However, the detection of a wide range of

Fig. 5. Heat map showing spatial distribution of detected pharmaceuticals in the various samples. Colour indicates the peak area (log10 transformed) as defined in the legend. Compounds detected only once have been removed to simplify the data presentation (A heatmap with all detected pharmaceuticals can be found in Fig. S4 for the interested reader).

psychoactive compounds in this work is evidence that modern substances of abuse such as amphetamines, cannabinoids, cathinones and opioids are used in communities in Botswana and this is supported by recent reports of confiscation of these drugs by local law enforcement [45]. Other studies carried out in Botswana showed that marijuana is the most common recreational drug used by young people [46], but despite this, there is limited knowledge on the use and prevalence of it in the country. This is significant given that, unlike pharmaceuticals, there are no prescription data that can be used to monitor the usage of illicit drugs as they are typically imported or produced in makeshift laboratories [47]. Based on our findings, investigations can now be directed at

specific locations to quantify the presence of these cannabinoids and other illicit drugs to monitor usage trends.

3.4. Industrial chemicals and intermediates

Industrial chemicals and intermediates were common constituents of the aquatic samples studied, with at least five detections of industrial/intermediate in each sample. Fig. 8 presents a heat map to illustrate the distribution of these compounds across the samples. Nine compounds were detected in RW and WW, while 10 chemicals were detected in SW. The observation of these compounds is concerning given that exposure to industrial chemicals is
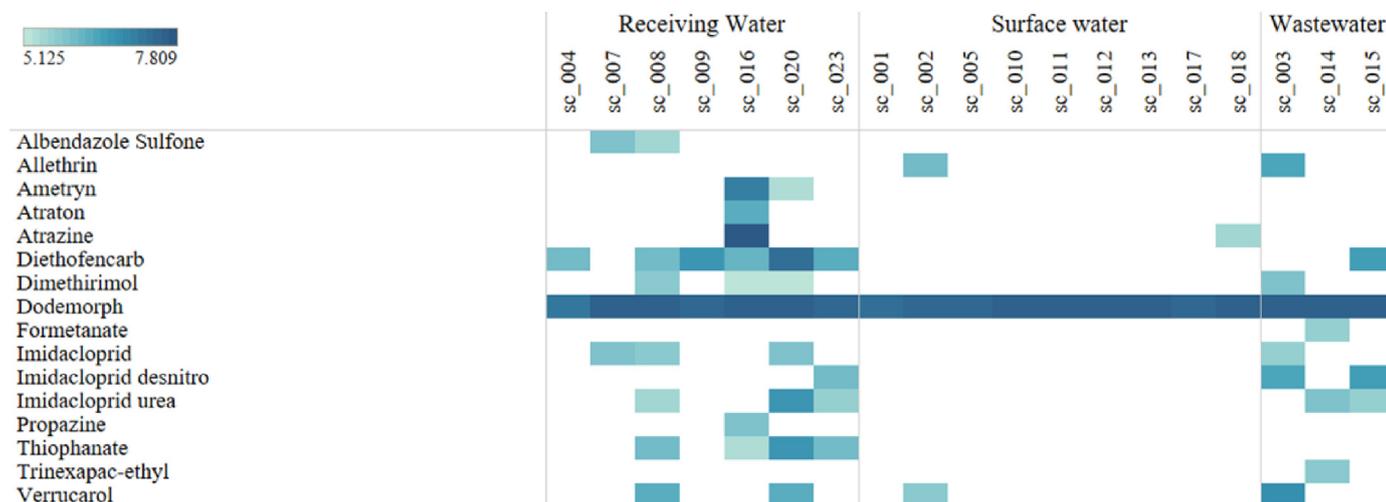
Fig. 6. Heat map showing spatial distribution of detected pesticides in the various samples. Colour indicates the peak area (log10 transformed) as defined in the legend.
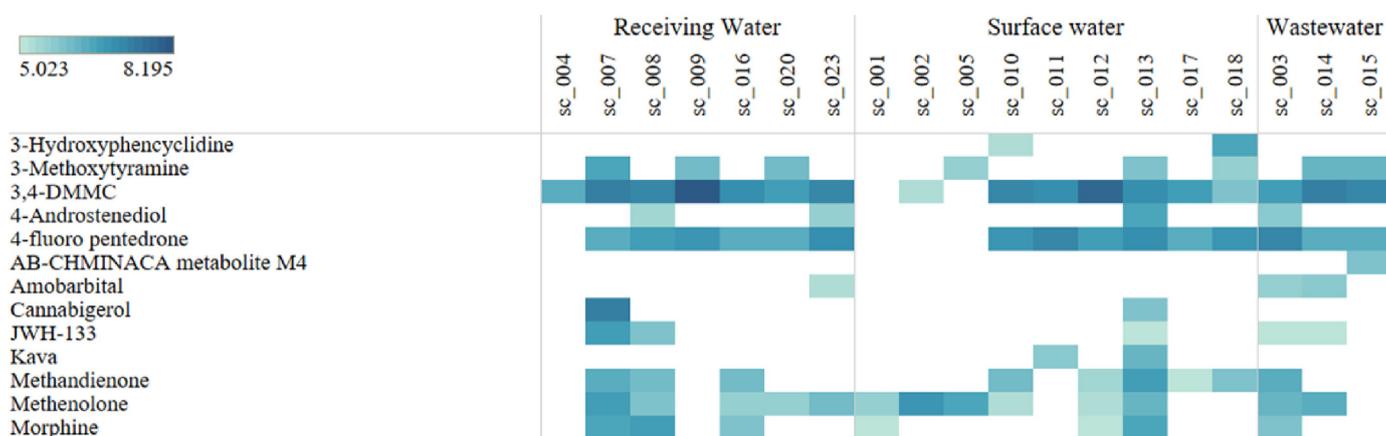

Fig. 7. Heat map showing spatial distribution of detected psychoactive substances in the various samples. Colour indicates the peak area (log10 transformed) as defined in the legend.
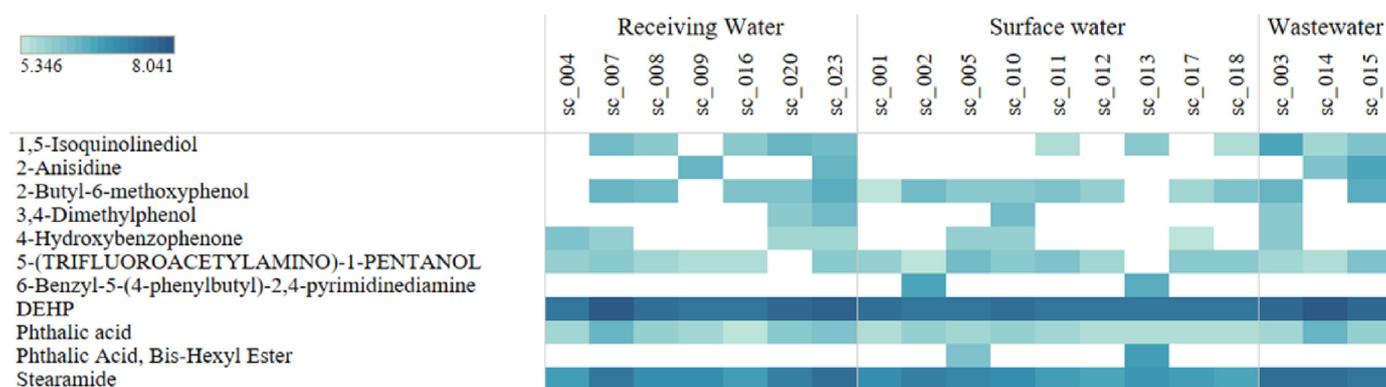

Fig. 8. Heat map showing spatial distribution of industrial chemicals and intermediate molecules in the various samples. Colour indicates the peak area (log10 transformed) as defined in the legend.

known to lead to harmful effects such as endocrine disruption and carcinogenicity [48].

This category of CECs includes industrial chemicals from manufacturing industries including compounds such as plasticizers, emulsifiers, lubricants, dyes as well as intermediate compounds used in the synthesis of other products. These chemicals have a broad range of uses and are often ingredients in a range of products produced industrially. As a result, many of them are often classified into other categories such as plastics or personal care products. One such example is phthalic acid which is used in

the manufacture of plastics and cosmetics. Three chemicals (stearamide, pthalic acid, and di(2-ethylhexyl) phthalate) were detected in all of the aquatic samples. Both stearamide and di(2-ethylhexyl) phthalate (DEHP) are used in the manufacture of plastics such as polyvinyl chloride (PVC). DEHP has been shown to carry a range of adverse effects including possible carcinogenic responses from humans [49], and was detected in a study in Botswana which was conducted in 2008 [16]. It is highly persistent and has been detected in both southern Africa and Europe [50,51].

3.5. Personal care products

Five unique CECs were identified as personal care products in the samples collected, with all five of these being detected in both RW and WW. Four such compounds were detected in SW. Fig. 9 presents the distribution of personal care products (PCPs) as a function of the sampling sites.

The number of detections per sample varied from 1(SC_017) to 5 (SC_003, SC_007, SC_016 and SC_020). Diazolidinyl urea, an anti-microbial preservative found in cosmetics was the most commonly detected PCP followed by lauryl sulfate which is a surfactant used in cosmetics. Out of the PCP compounds identified, only guanine was detected in our previous study.

We note that a smaller number of personal care products were detected in this work compared to previous non-targeted analysis studies of aquatic samples in the Southern African Development Community. As an example, a recent NTA study by Abafe et al. of CECs in Southern African surface and wastewater detected a significantly larger number of personal care products [20], including common CECs such as oleic acid, dodecyl sulfate and linear alkylbenzene sulfates. Although different methods of analysis have been employed in this study compared to that of Abafe et al., the lower levels of such compounds detected here may reflect the relatively low human population density of Botswana compared to the regions studied in South Africa [20].

4. Further discussion

This work expands on the very limited previous measurements of chemical pollution in Botswana [14–17], applying non-targeted screening of aquatic samples acquired from a range of locations across Botswana. With the exception of eight chemicals (allopurinol, eugenol, gamolenic acid, guanine, griseofulvin, pregabalin, triethanolamine, and DEHP) that were reported in environmental samples taken in Botswana previously, this work has revealed the presence of a further 106 chemicals of emerging concern at Level 2b confidence that have not been previously reported in Botswana. Compounds detected included pharmaceuticals, pesticides, psychoactive compounds, industrial chemicals and personal care products. These were detected in decreasing order pharmaceuticals > pesticides > psychoactive substances >

industrial chemicals > personal care products. Our receiving water samples contained the highest number of chemicals while surface water samples contained the least, suggesting the existence of pathways of environmental pollution such as agricultural run-off, leachate and direct discharge of sewage that should be explored in future work. Many of the chemicals found in this study have been reported previously in other parts of the world, but the prevalence of psychoactive substances is notable, and warrants further investigation of the presence and also the societal use of psychoactive compounds across Botswana. Overall, the study illustrates that the aquatic systems of Botswana are subject to extensive pollution, despite WWTP processing. While this is in line with the known persistence of certain contaminants following WWTP processing in other related geographical areas, including South Africa [52,53], it is especially concerning in Botswana given its ecological sensitivity and reliance on surface waters as drinking water sources.

The overview study presented here provides information to guide prioritization efforts for second-stage monitoring campaigns. Quantitative targeted analysis is an acknowledged and widely applied next step following studies such as the one presented here. Indeed, quantification is important in providing a fuller understanding of mass load and ecotoxicological risk of the CECs detected to both humans and aquatic biota [24]. While such studies have traditionally employed available analytical standards, we note that important progress is being made in developing methods that quantify contaminants without analytical standards through the application of machine learning algorithms that estimate instrument responses for particular compounds. The recent work of Johnson and Abrahamsson provides an elegant example of such work [54]. Artificial intelligence methods will be very valuable in driving much wider monitoring of CECs in countries such as Botswana, given their lower cost base, and the reduced requirement for analytical standards that quantitative analysis demands.

5. Summary

Water samples collected from 13 geographically disperse locations around Botswana have not been analysed using high resolution mass spectrometry with non-target and suspect screening methods. A total of 114 contaminants of emerging concern (CECs) were identified including 106 compounds which had not been detected previously in Botswana. Moreover, the prevalence of different CECs in three distinct water environments (wastewater, receiving water and surface water) will facilitate better informed choices for sampling locations in future work. This overview of the nature and frequency of individual CECs found across Botswana provides the first holistic picture of the levels of contamination across the country. Importantly, detection frequencies are reported to guide future prioritization efforts, and enable the rational selection of chemicals for analysis by quantitative methods. Such
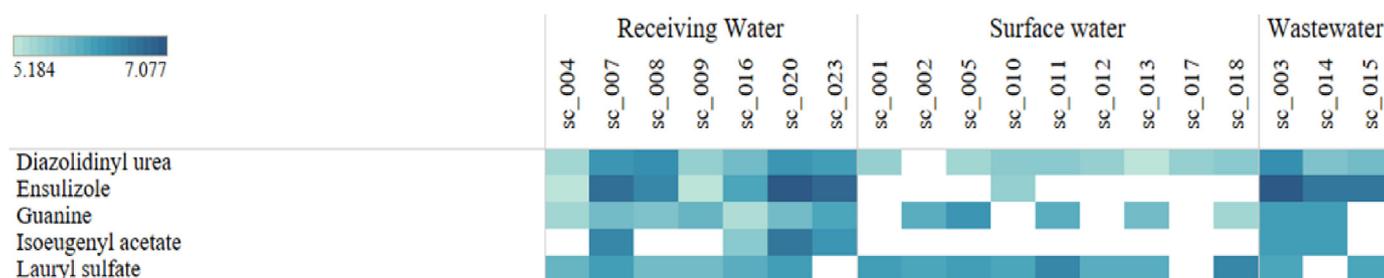

Fig. 9. Heat map showing spatial distribution of personal care products in the various samples. Colour indicates the peak area (log10 transformed) as defined in the legend.

work is essential for gaining a proper understanding of the total mass load of CECs present, and critical for assessing toxicological risk in fragile Botswanan ecosystems.

Data availability statement

Raw HRMS data files used in this study are openly available online on the Zenodo data repository (DOI: http://10.5281/zenodo. 10419291).

Conflicts of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Kgato P. Selwe: Writing − review & editing, Writing − original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Charlotte R. Head:** Writing − review & editing, Validation, Investigation, Formal analysis. **Gothatamang N. Phokedi:** Writing − review & editing, Resources, Investigation. **Jens E.T. Andersen:** Writing − review & editing, Resources, Investigation. **J. Brett Sallach:** Writing − review & editing, Supervision, Conceptualization. **Caroline E.H. Dessent:** Writing − review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare there are no conflicts of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2024.100377.

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