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Non-targeted screening of emerging contaminants in South African surface and wastewater

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ABSTRACT

Targeted analysis of contaminants in water are often focused on a narrow range of chemicals, falling short of the true pollution status of water bodies. Non targeted screening presents several advantages in identifying less prioritized pollutants, entirely unknown compounds and transformation products. In this study we employed a non-targeted screening workflow established on a high-resolution quadrupole time-of-flight mass spectrometer coupled to an ultrahigh performance liquid chromatograph (UHPLC-QTOF-MS) to identify known and unknown pollutants in South African waste and surface waters. Level two confidence identification of 315 compounds was achieved based on mass accuracy, isotope patterns and MS/MS spectra match. Pharmaceuticals, drugs, and metabolites made up 40% of the detected compounds, biological compounds and industrial chemicals along with their metabolites constituted 24 and 18% respectively, while personal care products, pesticides and food additives made up approximately 5, 4 and 4% respectively. Several antiretroviral drugs were confirmed with level one confidence using isotope labelled standards. A wide range of "new" pharmaceuticals, pesticides and metabolites were documented in South African waters for the first time. Seventeen (17) pharmaceuticals were reported for the first time in South African waters, of which four are reported for the first time ever in surface water.

The result of this exploratory study highlights the presence of several contaminants of public health concern that have hitherto received little to no attention in previous wastewater-based epidemiological studies. We provide a detailed list of priority contaminants for future studies on targeted-analysis.

1. Introduction

Pollution crises have continued to worsen over the world. Industrial revolution led to extensive production and use of chemicals, ushering diverse classes of chemical pollutants into the environment. Households alone, are estimated to consume between 30, 000–70, 000 chemicals comprising mainly of surfactants, pharmaceuticals and biocides [1]. There are 191 million compounds in the registry of the Chemical Abstracts Service (CAS), comprising of organic, inorganic, minerals, polymers, coordination compounds among many others, and this list continue to grow daily [2]. Many of these chemicals end up in the aquatic system with potential harm to aquatic and human life.

Surface and ground waters are the primary sources of potable drinking water in many countries. However, surface water often is a receptacle for domestic and industrial wastewater. Conventional water treatment processes are designed to tackle known inorganic and organic pollutants. The treatment of water to potable standards have become increasingly difficult with the increasing list of known pollutants. The analysis of these pollutants in water is usually achieved via targeted analysis approach, using established and

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validated liquid chromatography and gas chromatography coupled with mass spectrometry detection techniques [3]. However, these established techniques only cover a fraction of known pollutants, leaving many legacy and emerging compounds undetected and unstudied. For instance, the European Water Framework Directive focuses on 76 priority substances and 17 watchlist compounds for monitoring in the integrated river basin management for Europe, while the United States Clean Water Act controls 126 chemical substances in surface waters [4]. Target analysis alone does not present the actual pollution status of the water systems.

Non target analysis has been used as a forensic tool to explore and broaden the scope of investigated organic pollutants in water with the aid of spectral and structural information from known libraries [5].

Early advancements in gas chromatography coupled with electron ionization mass spectrometry (GC-EI-MS) made the structural identification of a wide range of compounds possible [6]. Data on legacy and persistent pollutants such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, dioxins, flame retardants, surfactants, organochlorine pesticides among others have been generated over the years. Similarly, advancements in electrospray ionization (ESI) technology made liquid chromatography coupled mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) additional tools for identification and structural elucidation of a large array of polar and less volatile organic compounds [7]. Spectral information of compounds such as phenolics, pharmaceuticals, illicit and drugs of abuse, pesticides, industrial chemicals, food additives and many others were incorporated into established databases such as the National Institute of Standards and Technology (NIST) mass spectral library [5]. This information is used to identify non targeted compounds in water with a level of certainty [5]. High resolution mass spectrometry (HRMS) has evolved into a great tool for the detection and identification of thousands of organic compounds within the short intervals typical of chromatographic separation [4].

Incorporating non-target screening workflow for surface and wastewater provide a real time insights of emerging and recurrent pollutants of concern and interest, with a view to constantly update the list of targeted pollutants and to get an authentic picture of the potential environmental and human health risk.

Many reports exist on the occurrence of different classes of pollutants in South African water streams. Most of this study are often focused on isolated class of “known” contaminants of concern, hence does not provide the true pollution status of the water systems. However, two studies recently reported the screening of polar [8] and semi volatile [9] emerging contaminants in South African wastewater treatment plants. Little is known on the full spectrum of contaminants of concern in South African surface and drinking water sources. Consequently, the aim of the current study was to identify polar organic pollutants present in South African surface and wastewaters by non-target screening (NTS) using a high resolution time-of-flight mass spectrometry with tandem ultrahigh performance liquid chromatograph (UHPLC-QTOF/MS).

2. Materials and methods

2.1. Chemicals and reagents

Ultra-pure water (>18 MΩ @ 25 °C), acetonitrile (>99.9%) and methanol (>99.9%) were products of ROMIL-SpS™ obtained from Microsep (Pty) Ltd, Johannesburg, South Africa, LC-MS grade (>99.99%) ethyl acetate was purchased from Honeywell, South Africa. Liquid chromatography grade formic acid and ammonium formate were supplied by Merck, South Africa. Strata X polymeric reversed phase cartridges (200 mg, 6 mL) were supplied by Anatech Instruments (Pty) Ltd, South Africa. Formic acid (99%) was obtained from Merck (South Africa) and 0.22 μm polytetrafluoroethylene (PTFE) syringe filters were supplied by Microsep (Pty) Ltd, Johannesburg, South Africa. The internal (surrogate) standards sulfamethoxazole-(phenyl-$^{13}C_6$) (SULF-$^{13}C_6$), enrofloxacin-d_5 hydrochloride (ENR-d_5), and flubendazole-d_3 (FLU-d_3) and nevirapine-d_4 (NVP-d_4) were purchased from Sigma Aldrich, Johannesburg, South Africa.

2.2. Sample collection and preparation

A total of twenty-three samples comprising of four borehole, four river and fifteen wastewater samples were collected from Limpopo (19) and KwaZulu-Natal (4) Provinces in South Africa. The samples were denoted as S1 − S23. S1 − S4 were borehole samples, S5, S6, S22 and S23 were river samples, while S8 − S21 were wastewater samples. S1 − S19 were samples collected from Limpopo and S21 − S24 were samples collected from KwaZulu-Natal province. All samples were collected in pre-cleaned high density polyethylene bottles and refrigerated at 4 ± 3 °C until analysis. All samples were analysed within six months of sampling. Full description of sampling sites is presented in supplementary information.

Sample preparation and extractions for the analysis of polar organic compounds in water followed our previously validated method [10] with slight modifications. Briefly, aliquots of 500 mL each of the borehole and river samples and 100 mL of the wastewater samples were filtered through a glass membrane filter. The filtered sample were then spiked with the mixture of internal standards (SULF-$^{13}C_6$, ENR-d_5, FLU-d_3 and NVP-d_4) at a concentration of 500 ng mL^{-1} for influent and effluent samples, and 50 ng mL^{-1} for the surface water samples. The samples were loaded onto preconditioned Strata X polymeric reversed phase cartridge and eluted with methanol (5 mL) and ethyl acetate (3 mL). The eluates were concentrated to incipient dryness in a Zymark Turbo Vap LV evaporation workstation and reconstituted with 1 mL of 85:15 v/v mixture of 0.1% formic acid in methanol and 0.1% formic acid in water. This 1 mL extract was then filtered through a 0.22 μm syringe filter and 5 μL was injected into the UHPLC-QTOF/MS.

2.3. Instrumentation

Non-target analysis was performed with an ABSciex 6600 series TripleTOF coupled with an ABSciex ExionLC™ ultrahigh performance liquid chromatography system (AB Sciex, Framingham, USA). The QTOF was operated in positive polarity electrospray ionization mode. Data acquisition was performed by information dependent acquisition (IDA) using a TOF-MS survey scan 100−1000 Da (100 ms) and up to 10 dependent TOFMS/MS scans 50−1000 Da (100 ms). The collision energy (CE) was 35 V with collision energy spread (CES) of ±15 V. Chromatographic separation was achieved on a SCIEX ExionLC™ AD Series using a Luna® Omega polar C18 column (2.1 × 100 mm, 3 μm). Gradient elution was employed for the separation with 0.1% formic acid and 2 mM ammonium formate in water (A) and 0.1% formic acid and 2 mM ammonium formate in a 1:1 mixture of acetonitrile and methanol (B) at a constant flow rate of 0.5 mL min^{-1}. The column oven temperature was set at 40 °C, and the injection volume was 5 μL. The LC-QTOF system was operated with an ABSciex OS® software. Library searches were performed with SCIEX All-in-one HR-MS/MS library version 2.0 and the NIST library 2017 version (SCIEX format) containing spectral for 3900 and over 13800 compounds, respectively. The optimized MS parameter and LC gradient are summarised in Tables 1a & 1b, respectively.

Table 1a
Optimized TOF/MS parameter.

Parameter	Value
TOF/MS (Da)	Min. = 100 Max. = 2000
Scan Type	TOF/MS
Accumulation Time (seconds)	0.250
Ion spray voltage (V)	5500
Temperature (°C)	550
Curtain gas (psi)	25
Ion Source Gas 1 (psi)	40
Ion Source Gas 2 (psi)	50
Collision Energy (eV)	10
Declustering potential (eV)	80

Table 1b
Binary gradient programme.

Time/min	A Concentration/%	B Concentration/%
0.00	95	5
1.00	95	5
16.00	5	95
20.0	5	95
20.10	95	5
26.00	95	5

2.4. Quality control

For level 1 identification of the antiretroviral drugs, isotope-labelled standards were employed, flubendazole-d_3 was used as the internal standard for tenofovir and zidovudine, nevirapine-d_4 was the internal standard for nevirapine, abacavir, efavirenz, lopinavir, saquinavir mesylate and ritonavir, while sulfamethoxazole-(phenyl-$^{13}C_6$) was used as the internal standard for lamivudine, emtricitabine, stavudine and didanosine. Solvent and method blanks were employed, and no contamination during the extraction process was observed. Mass spectral were background corrected by subtracting the spectral of blank sample (deionised water) in each analysis batch using a dynamic background subtraction™ algorithm. This improves the identification of low-abundance compounds by minimising the collection of MS/MS spectral on background ions. The recovery of internal (surrogate) standard was greater 70% for all standards.

2.5. Non target screening

A full MS scan of the protonated and deprotonated molecular ions and adducts as well as fragment ions was carried out. The MS data were processed using Sciex OS™ (PeakView™ and MultiQuant™) softwares. PeakView was used to calculate parameters such as characteristic adducts, mass error, isotopic difference, and purity score. Data reduction was achieved by applying screening criteria such as blank background correction, signal to noise ratio of $\geq$3, peak intensity of >1000 cps, mass error of <5 ppm, isotopic difference of $\leq$20% [11,12], and MS/MS spectra match with library and formular finder score of $\geq$60. The workflow is shown in Fig. 1. The confidence of identification system of Schymanski et al. was adopted [13].

2.6. Statistical analysis

Visual representation of data such as charts and heat maps were performed with Microsoft Excel® 2016 and XLSTAT Version 2021.3.1.

3. Results and discussion

3.1. Detected organic pollutants

A total of 315 unique organic contaminants of concern were detected and identified in the water samples with level two (L2) confidence. Three antiretroviral drugs; abacavir, tenofovir and efavirenz were confirmed with level one (L1) confidence using stable isotope labelled standards. The distribution of the detected compounds varied greatly with the location of the sampling site (province wise). Approximately 74% (232) of the identified compounds were exclusively detected in samples from Limpopo province, while 22% (68) were exclusively detected in samples from KwaZulu-Natal province, the remaining 4.8% (15) of the compounds were detected in both provinces.

Approximately 69% (218) of the identified compounds were exclusively detected in wastewater samples, while 10.5% (33) and 2.5% (8) were exclusively detected in river and borehole samples respectively (Fig. 2).

The classes of compounds detected ranged from pharmaceuticals, drugs and related metabolites to biological compounds such as amino acids, peptides, hormones, etc and their metabolites. Personal care products and their metabolites, pesticides, herbicides, food additives and industrial chemicals were also detected with level 2 identification. Of this, 126 (40%) of the detected compounds were pharmaceuticals, drugs, and their metabolites, while 77 (24%) were biological compounds, 56 (18%) were industrial chemicals. Personal care product, pesticides and food additives made up 5 (17), 4 (12) and 4% (12) respectively (Fig. 3).

The total pollutant load per sample is shown in Fig. 4. Wastewater samples (S8 − S21) presents the highest number of detected compounds making up 82% of the total detected compounds. Borehole and river samples contributed 4.7 and 13% respectively. Sample S12 had the highest detected number of compounds (95) while sample 4 had the least detected number (5) of compounds.

3.2. Distribution of the detected organic compounds in water

3.2.1. Pharmaceuticals, antibiotics, opioids, drugs, and related metabolites

Prescription drugs, illicit drugs and drugs of abuse and their transformation products were most detected in the surface and wastewater samples. The distribution of pharmaceuticals and metabolites is presented in Supplementary Fig. S1. Most of the drugs and their metabolites were detected in wastewater and river samples, with the exception of 13-cis-retinoic acid used for acne treatment [14] and z-lingustilide (a Chinese herbal drug used as an anti-inflammatory agent [15]), aspirin and acetaminophen that were detected in borehole water samples.

The opioid -tramadol, a prescription drug and its major metabolite *o-desmethyl-cis-tramadol* were the most detected in the samples, present in 68% of samples across river and wastewater samples. Other commonly detected opioids were codeine, levorphanol, dextrorphan, morphine, hydromorphone and meperidine (and its metabolite normeperidine). Propofol, a non-barbiturate sedative was also commonly detected in the river and wastewater samples. About 53% of the samples contained metoprolol acid − a metabolite of the antihypertensive drug atenolol which was detected in 42% of the river and wastewater samples. Similarly, varsatan, an angiotensin 11 receptor blocker was also commonly detected in the river and wastewater samples. Two antipsychotic drugs sulpiride and amisulpride were detected in 47 and 37% of the samples. Other commonly detected drugs in the wastewater samples include the vasoconstrictor prostaglandin F2 alpha metabolites, 17-trifluoromethylphenyltrinorprostaglandin F2 and 15(R)-

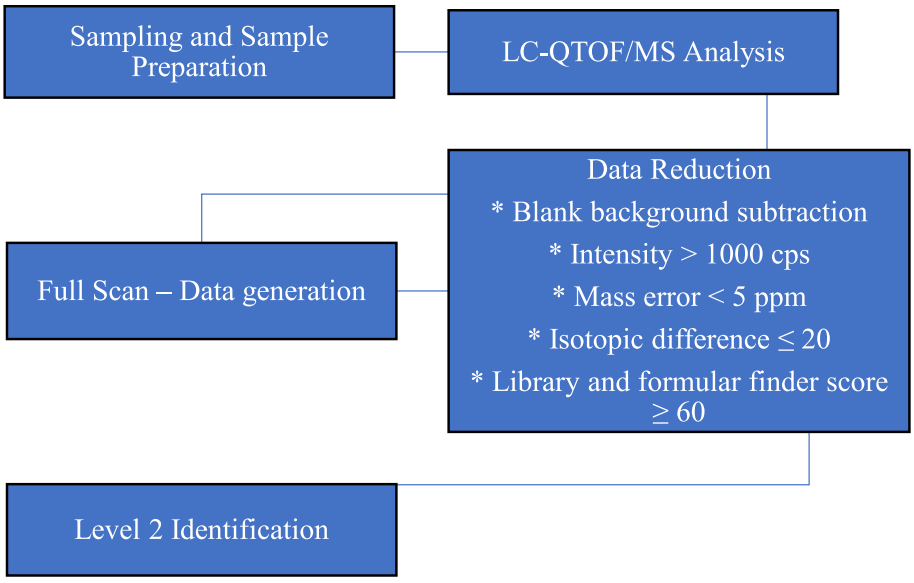

Fig. 1. Workflow for non target screening of water samples with LC-QTOF-MS.

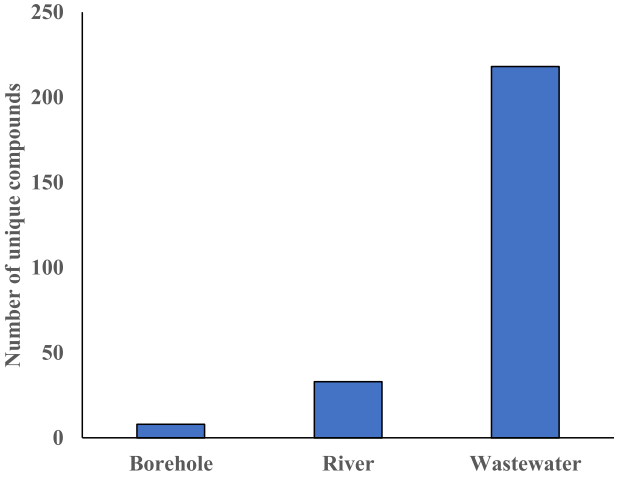

Fig. 2. Distribution of unique compounds according to sample type.

15-Methylprostaglandin F2 alpha methyl ester were widespread (about 37% of samples).

Trimethoprim was the most abundant antibiotics detected in 58% of the samples. This was followed by sulfamethoxazole, levofloxacin, ciprofloxacin, fluconazole, and azithromycin in the river and wastewater samples. Two antiretroviral drugs – abacavir and tenofovir were commonly detected in the wastewater and river water samples. Antidepressant drug venlafaxine and antidiabetic drug metformin were detected in all the wastewater and river samples. Other pharmaceuticals detected include nicotinamide, niacinamide, flucytosine, gentiopricoside, antimalaria drugs artemisinin and mefloquine, anthraquinone, dexpanthenol, steroids beclomethasone and tibolone, anticonvulsants carbamazepine and lamotrigine, anticancer drug triciribine and several veterinary drug such as tiaprost, thiabendazole, stachydrine among others.

Although some of these contaminants for example antiretrovirals [10], atenolol [16], caffeine [17], carbamazepine [18], levorphanol, codeine, tramadol [19], ciprofloxacin [20], flucytosine [21] lamotrigine [22], ibuprofen [20], sulfamethoxazole [23],

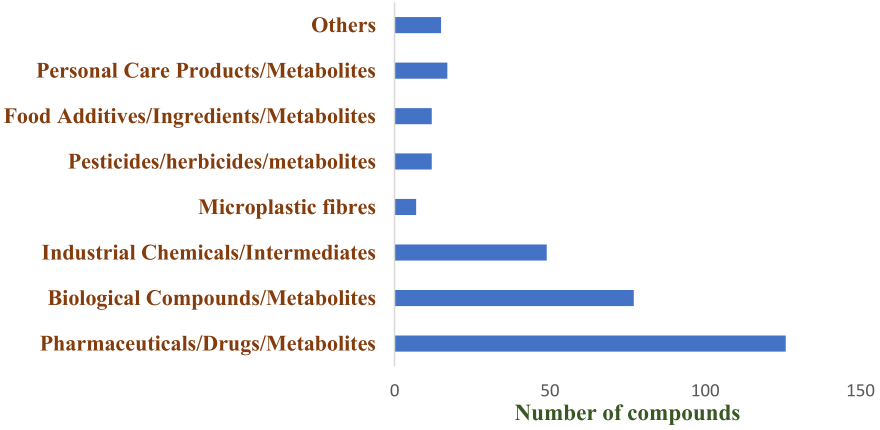

Fig. 3. The distribution of the classes of organic compounds in the water samples.

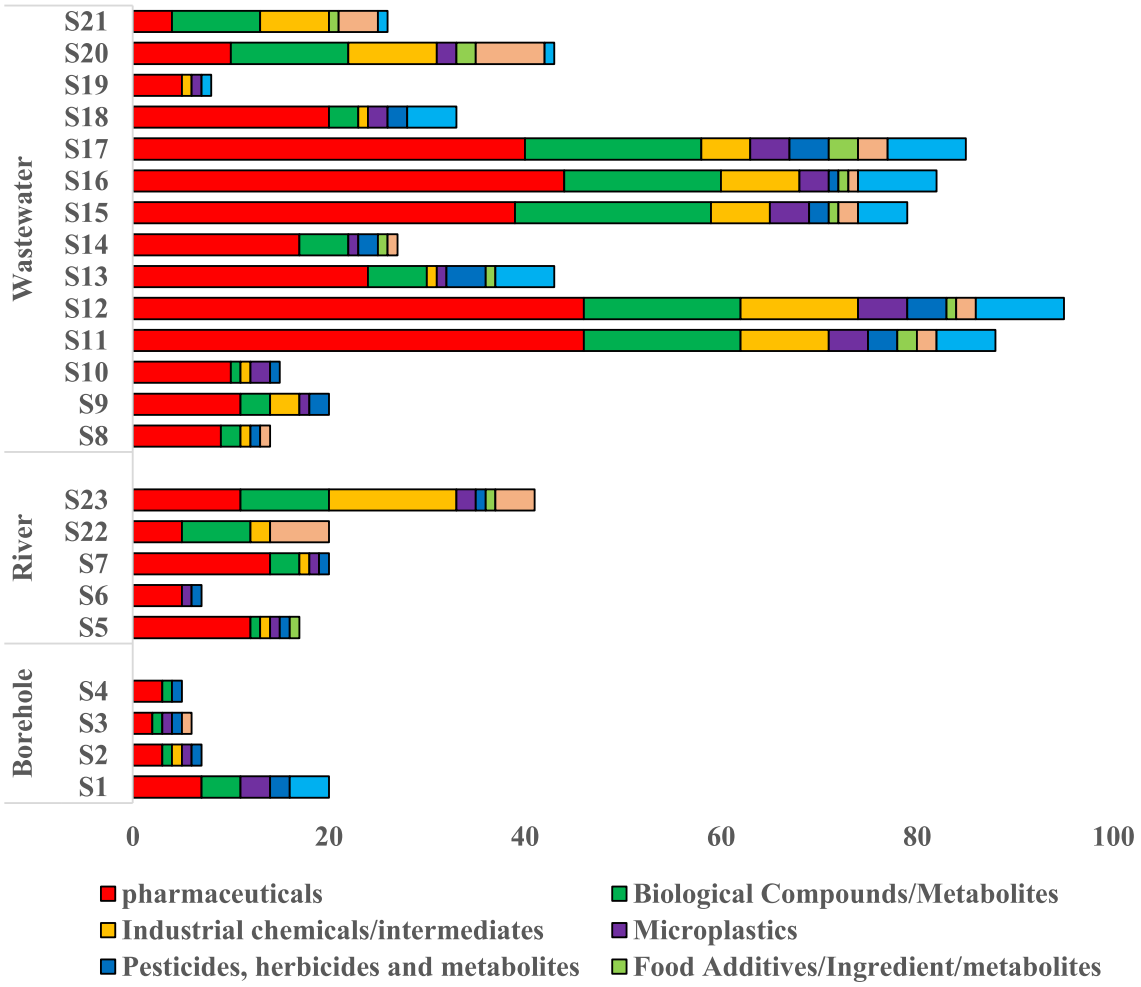

Fig. 4. Total pollutant load in each sample matrix.

acetominophen, azithromycin, metformin [20], trimethoprim [16], paraxanthine, fluconazole, valsartan, metropolol [24], and venlafaxine [25] have been reported previously in South African surface and wastewater, to the best of our knowledge others such as alverine, acecarbromal, artemisinin, beclomethasone, cimetidine, cotinine, griseofulvin, lumichrome, mefloquine, moxisylyte, oxypendyl, perazine, thiabendazole, tibolone, triciribine, nitroxinil and propofol are reported for the first time in South African waters. Similarly, we report for the first time the occurrence of residues of moxisylyte, oxypendyl, perazine and triciribine in surface waters. The presence of these compounds in the surface water stream may present ecotoxicological risks to aquatic biota with an attendant public health implications ranging from multi-drug resistance, synergistic effects as well as persistence and pollution in drinking waters for human and livestocks.

3.2.2. *Biological compounds and metabolites*

The distribution of biological compounds and metabolites in the samples was consistent with the distribution of pharmaceuticals and metabolites, most of the pollutants from this class were also detected in wastewater samples (Supplementary Fig. S2). The decreasing order of detection in the samples was wastewater > river water > borehole water. The compounds in this category comprised of hormones, plant growth regulators, fatty acids, peptides, amino acids and derivatives, phytochemicals, and many others. Plant growth hormones such as jasmonic acid, trigonelline, and *trans*-zeatin were identified in the samples. Other recurrent plant growth hormones also detected include 1H-indole-3-propanoic acid, 3-indoleacetic acid, 3-indoleacrylic acid and *trans*-trans-10,11-epoxyfarnesenic acid methyl ester. However, the plant hormones were largely detected in the wastewater samples. Xanthines, alkaloids, primidine and derivatives were some of the common phytochemicals detected. The samples were equally rich in other plant phytochemicals such as harpagoside, cnicin, indirubin, magnolin, piperitone, polygodial, ricinin, rosarin, strychnine, hexaethylene glycol, cyanidin-3-O-rhamnoside cation, sempevirin cation, 7,8-dihydro-α-ionone etc. Long chain hydrocarbons of plant and animal origin such as fatty acids were prevalent in the samples. Fatty acids detected include among others 7-keto-8-aminopelargonic acid, 1,11-undecanedicarboxylic acid, 16-hydroxyhexadecanoic acid, 3-Furancarboxylic acid, tetrahydro-4-methylene-2-octyl-5-oxo-, (2R,3S), 3-hydroxyoctadecanoic acid, heptadecanoic acid, tridecanoic acid. Cell based materials for instance cytosine and phospholipids like phosphocoline and glycerophosphocoline were also identified.

A large array of amino acids and peptides were detected in the samples. These are typical forms of dissolve organic nitrogen (DON) in water, and they make up between 15 and 35% of dissolved organic nitrogen in drinking water sources [26]. Free amino acids

and peptides in water are precursors for potentially harmful nitrogenous disinfection (for example chlorination) bye-products (N-DBPs) such as haloacetic acids (HAAs), trihalomethanes (THMs), haloactonitriles (HANs) amongst others [27]. Many of the formed N-DPBs are unregulated and have been reported to exhibit carcinogenic behaviours [28]. Some of the detected amino acids and peptides in the water samples include o-tyrosine, arginino-succinic acid, dipeptides such as tyrosine-threonine, histidine-threonine, glutamine-histidine, glutamic acid-isoleucine, asparagin lysine, tryptophan-arginine and proline-phenylalanine. Amino acids are important precursors for the formation of toxic disinfection by-products such as trihalomethanes which are potentially genotoxic and mutagenic [29].

Other oligopeptides detected include thyrosine-cystine-arginine, methionine-glycine-arginine, lysine-trptophan-arginine, valine-tyrosine-arginine, glutamine-trptophan-arginine, cystine-aspartic acid-arginine, pyroglutamic acid-glycine-arginine, and benzoyl-phenylalanine-alanine-arginine. Amino acid metabolites such as L-carnitine, N-benzyl-L-methylcathinone, isatin, and kynurenic acid were also present in the samples. Secondary metabolites such as 5-(2- hydroxyethyl)-4-methylthiazole were also recurrent in the samples.

3.2.3. Industrial chemicals and intermediates

Industrial chemicals and intermediates detected with level 2 confidence identification in samples are presented in Supplementary Fig. S3. These classes of compounds often exclude compounds that are agricultural, veterinary, or pharmaceutic chemical products. This class also excludes all chemicals used in food production, as ingredients or flavours as well as compounds for therapeutic purpose [30]. However, many chemicals find applications in different kinds of product and can be vaguely moved from one class to another.

N, N-dimethylaniline, an important intermediate in the manufacture of many dyes, pigments and other substances was detected in the water samples. Another important derivative of aniline; 2,4,5-trimethylaniline used in dye manufacture was detected. Isophorone, an important solvent in the production of printing inks, paints and adhesives was also widely detected in the samples. Triethanolamine used in the production of epoxy resins and as thickeners in cosmetics [29] was detected in the water samples. Triethyl phosphate, a catalyst used in the production of resin modifiers and plasticizers was among the detected compounds.

Benzoic acid and some of its derivatives such as 3-aminobenzoic acid, methy-3-hydroxybenzoate, 3-hydroxybenzoic acid, 3-hydroxybenzaldehyde, N-methylbenzaldehyde, p-acetaminobenzaldehyde were positively identified in the samples. Other compounds detected include isonicotinamide, dodecanoic acid, 3,4-dichlorophenol, 2-methoxybenzyl alcohol, 2,4-diaminotoluene and many others.

The toxicity of the detected chemicals are diverse, ranging from mild irritation to humans for instance triethanolamine [31], and isophorone [32], to mutagenic compounds such as 2,4,5-trimethylaniline a derivative of aniline [33].

3.2.4. Plastic additives

The full list of microplastic fibres and additives detected in the samples are presented in Supplementary Fig. S4. Low molecular weight polyethylene glycols were recurrent in the samples. Tetraethylene glycol (TEG) with vast applications in industrial and domestic consumer products was the most detected in the samples from all sources. The high frequency of detection of TEG in the water samples is consistent with the wide range of industrial and consumer product containing TEG. TEG is a plasticizer used in different polymers and have application as a solvent in inks, toners, colorants and dye products [34]. TEG is also used in lubricants, plating and surface treating agents and as a component of adhesives and synthetic resins [35]. Pentaethylene glycol, hexaethylene glycol and decaethylene glycol were also detected in the samples. Pentaethylene glycol is a component of common auto fluids and additives such as brake fluids, power steering fluids, fuel injector cleaners. Decaethylene glycol is a precursor for the synthesis of numerous important chemicals such as proteolysis targeting chimera (PROTAC) and surfactants. Phthalic anhydride was detected in river and wastewater samples. It is commonly used in solvents, polystyrene resins, cellulose and rubber [36]. Of particular interest is the detection of several highly toxic plastic additive chemicals e.g. triethyl citrate and the probable human carcinogen - diethylhexyl phthalate (DEPH) [34], commonly used as plasticizers in various polymers such as polyvinyl chloride.

3.2.5. Pesticides, herbicides, and metabolites

Several classes of agricultural chemicals were detected in the samples (Fig. 5). Notably, Fenpropidin was the most abundant, detected in 74% of all samples. Fenpropidin is a piperidine fungicide applied to mostly cereals to control diseases [37] and have been reported to be persistent in soil and water and highly toxicity to algae. In addition, the antifungal agrochemical lauryl guanidine was also detected. Lauryl guanidine is used to control leaf spot disease in fruits like cherries and apples among others [38]. The insect repellent diethyltoluamide (DEET) was detected in the samples (8.7%). DEET is extensively used as an active ingredient in insect repellent products such as aerosols, creams, lotions, soaps etc. DEET is among the most detected trace organic pollutants in water bodies [39]. Strychnine pesticide was detected in approximately 35% of samples. Strychnine are highly poisonous to human and animals, hence banned under Commission Directive 91/414/EEC of the European Commission [40].

The broad-spectrum phenyl urea herbicide terbuthiuron was also detected in the samples. Terbuthiuron has been reported in surface runoff water [41], the persistence and high leaching potential of terbuthiuron in soil [42] makes possible the transfer of the

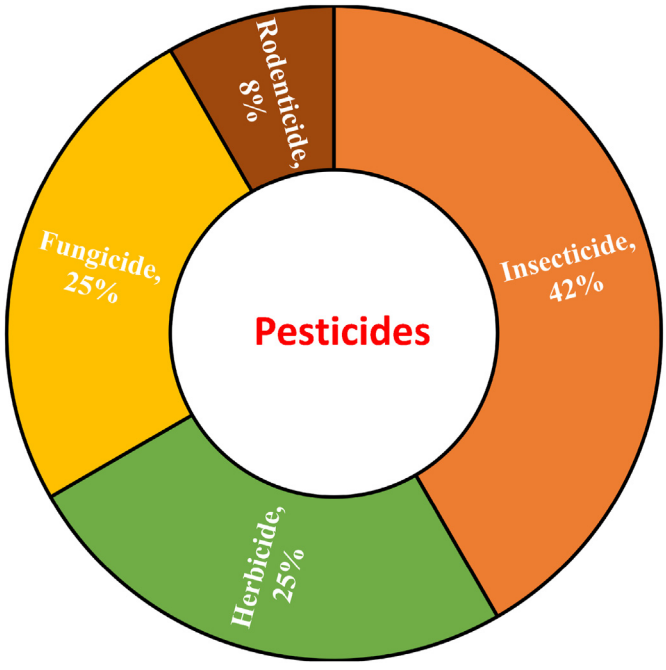

Fig. 5. The classes of pesticides detected in the samples.

herbicide to aquatic bodies. Another member of the phenyl urea herbicides detected was flumeturon used largely in cotton and sugar plantations. It is persistent and very mobile in soil and easily sips into ground water [43]. Furthermore, 2-hydroxyatrazine, a major metabolite of the herbicide atrazine was detected [44]. Atrazine and its metabolites are frequently detected in drinking water [45], agricultural runoff and surface water [46] as well as underground well water [47]. Atrazine herbicide is produced and widely used in South Africa [48]. Carbendazim, a broad spectrum benzimidazole fungicide used extensively in agriculture to protect crops such as vegetables, cereals and fruits was detected [49]. Carbendazim is a potential endocrine disruptor and has been broadly reported in drinking water, surface water [50] and wastewater [51]. Further detected in the samples was the insect growth regulator dicyclanil used to prevent flystrike in sheep and lambs [52]. South Africa along with Australia and New Zealand are the major users of dicyclanil [53]. Esfenvalerate, a widely used pyrethroid pesticide was detected. It is hydrophobic limiting the risk of contamination of water bodies through runoffs, however, high toxic effects have been reported on aquatic organisms such as fishes at very low concentration ($\leq 1\ \mu g\ L^{-1}$) [54]. The full list of the different classes of pesticides detected in this study is presented in Supplementary Material Fig. S5.

Of all the pesticides detected in this study, only tebuthiuron [55] has been previously reported in South African waters to the best of our knowledge. Even though atrazine has been previously reported [55], its metabolite, 2-hydroxyatrazine is being reported for the first time. Fenvalerate an isomer of esfenvalerate has been detected previously [56].

The presence of these long list of "new chemicals" with previously reported aquatic toxicity highlights the urgent need for researchers and regulatory bodies to extend the spectrum of pesticides and other agricultural chemicals monitored in surface water streams in South Africa in order to protect public health.

3.2.6. Food additives

This class of compounds detected in the water samples include flavouring agents and many others (Supplementary Fig. S6). Sabinene is a vital component of plant based essential oils, it is used in the food industry as a flavouring agent [57]. For instance, it is responsible for the spiciness of black pepper and present in other common spices such as nutmeg and turmeric [58]. It is also used in perfumes and pharmaceuticals [57]. Another flavouring compound detected was ethyl isobutyrate. Ethyl isobutyrate is a natural flavouring found in fruits like banana, grape, cashew, strawberry and apple [59]. Sucralose an artificial sweetener was detected in the samples. It is found in a wide variety of food which include beverages, breakfast cereals, dairy products, confectionaries and so on [60]. About 92–97% of consumed sucralose in humans is excreted via urine and faeces [60], thus, unmetabolized sucralose has been detected in domestic wastewater, surface water [61], underground water and drinking water [62]. Citric acid is commonly detected in surface and wastewater, about 70% of commercially produced citric acid is utilized in food production [63]. 4-hydroxybenzoic acid, a common metabolite of flavonoids and hydroxycinnamic acids was present in the samples. The flavouring ingredient 2-methoxycinnamaldehyde also known as cassiastearoptene was present in the samples. 2-methoxycinnamaldehyde is a member of the cinnamaldehydes, usually found in some herbs, spices, cinnamon [64] and can be used as a biomarker. Other food flavouring ingredients detected include 2,3,5-trimethylpyrazine, β-resorcyclic acid among others.

3.2.7. Personal care products and metabolites

Several chemicals used in a wide range of personal care products for example shampoo, fragrances, and detergents were detected across the wastewater and surface water samples. Piperitone, a ketone found in some essential oils and used in fragrances was detected. Azelaic acid, a skincare cosmetic ingredient naturally occurring in some plants (such as whole grain cereals) was present in the samples. A major component of soaps, oleic acid was also detected. Oleic acid is used as an emulsifying agent in soaps, it is also commonly added to creams as a softener and moisturizer. Another important cosmetic ingredient detected was stearic acid. Stearic acid is used in many personal care products as an emulsifier and lubricant in cosmetics.

Surfactants such as dodecyl sulphate, dodecyl benzene sulfonic acid and dodecyl phosphate were detected in the samples. Sodium dodecyl sulphate is the most common salt of dodecyl sulphate used in household detergents, tooth pastes, shampoos and cosmetics as surfactant [65], fat emulsifier, and wetting agent. Linear alkylbenzene sulfonates (LAS) are synthetic surfactants most widely used in household detergents and industrial cleaning agents [66]. Surfactants are known to cause foaming in rivers and wastewater systems [67]. Acute toxicity reports of LAS to aquatic organisms have been published [68]. The toxic antimicrobial agent used in cosmetics [69], 4-hydroxybenzoic acid propyl ester (propyl paraben) was detected in the river and wastewater samples. Parabens have been associated with the development of breast cancer [70], decreased secretion and function of testosterone [71] and endocrine disrupting activities [72]. Verbenone, a natural component of some essential oils used in perfumes was also detected. Other personal care product ingredients/metabolites detected included perillaldehyde, 3-methylindole among others (Supplementary Fig. S6).

3.2.8. Other kinds of chemicals

Several adducts ions including PEG-7mer, 8mer, 9mer, 10mer ammonium adducts, tetrabutylammonium cation and 4,9-dihydro-1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(2-pyrazinylmethyl)-1H-naphth2,3-dimidazolium cation were detected in the river and wastewater samples. Similarly, other compounds detected in the water samples included 1,3,4,6,7,8-Hexahydro-1-methyl-2H-pyrimido1,2-apyrimidine an organic compound used in several organic transformations, and 5-(2-Hydroxyethyl)-4-methylthiazole a substrate in thiamine biosynthesis, among others. (Supplementary Table 1).

3.3. Removal of organic contaminants of concern by wastewater treatment plants

The removal of organic contaminants of concern from wastewater by treatment plants was assessed by considering one WWTP each from Limpopo and KwaZulu-Natal provinces in South Africa, the removal efficiency of Lebowakgomo WWTP in Limpopo (denoted as LWWTP), and Northern WWTP in KwaZulu-Natal (denoted as NWWTP) were evaluated. LWWTP receives domestic, commercial, and industrial wastewater and runs an activated sludge system [73]. NWWTP receives wastewater with about 20% industrial component from surrounding industries such as petrochemical, construction, cosmetics and personal care product and textiles [10]. Treatment processes in the plant includes activated sludge system, anaerobic digestion and maturation ponds, the final effluent is chlorinated before discharge.

Remarkable differences were observed in the number of pollutants detected in the influent and effluent of the two WWTPs considered. As illustrated in Fig. 4, about 70% of the compounds found in the influent stream of the LWWTP (sample 12) were eliminated in the effluent (sample 14), while about 40% of the compounds detected in the influent stream (sample 20) of the Northern WWTP were eliminated in the effluent stream (sample 21). Compounds removed by the treatment processes in the plants

ranged from pharmaceuticals, personal care products, biological compounds, food additives to microplastics fibres. Pharmaceuticals such as antiretroviral drugs abacavir, tenofovir, and abuse drugs codeine, morphine, and dextrorphan were eliminated from the influent from the LWWTP. Other pharmaceuticals also eliminated include acetaminophen, dexpantherol, metformin, sulfamethoxazole, trimethoprim, and O-desmethyl-cis-tramadol. Reports have shown the removal of some pharmaceuticals by some WWTPs in South Africa to very high degrees (90–100%) [19,74]. Similarly, biological compounds eliminated from the influent of the LWWTP include peptides such as Glu-Ile, His-Thr, Thr-Cys-Arg, cytosine among others. Fibres decaethylene glycol and pentaethylene glycol were also eliminated. Secondary wastewater treatment processes have been shown to effectively remove some pharmaceuticals and personal care products from wastewater [75]. However, notable pollutants not eliminated by the treatment processes in the LWWTP include pharmaceuticals such as tramadol and sulpiride, fibres such as hexaethylene glycol and phthalic anhydride; pesticides fenpropidin and strychnine, food additives 2-methoxycinnamaldehyde among others. Furthermore, a few other compounds that were not detected in the influent from LWWTP were detected in the effluent. These include atenolol, triaprost and fluconazole, perillaldehyde and biological compounds such as magnolin. Increased concentration or emergence of some pollutants in wastewater effluent streams compared to source influent have been attributed to probable transformation of conjugated forms to free forms and biotransformation [76–78].

4. Conclusion

Non-target screening of South African waters revealed a large array of organic contaminants of concern. Compounds detected varied from pharmaceuticals, pesticides, personal care products, biological compounds, food additives, industrial chemicals to microplastics additives. The decreasing order of the frequency of detection was pharmaceuticals > biological compounds > industrial chemicals > personal care products > pesticides > food additives > microplastic additives. Wastewater presented the highest number of pollutants while borehole water samples had the least number of pollutants. With respect to the sampled regions, samples from Limpopo province presented more contaminants than samples from KwaZulu-Natal province.

Many of the detected compounds have been reported previously, some however, are reported for the first time in this study. Seventeen (17) pharmaceuticals were detected for the first time in South African waters and 4 out of which are also being reported for the first time ever in surface water.

The large number of compounds detected further paints a gloomy picture of the state of pollution of South African waters. However, the results also show the efficiency of elimination of some of the contaminants from WWTPs, while underscoring the persistence of some of the contaminants as seen across the sources of water sample.

The study highlights the need to broaden the scope of chemicals investigated in surface and wastewater sources as many potentially toxic compounds are going undetected and finding their way to drinking water sources. Future studies focussing on semi-to full quantitation of the large array of chemicals detected in these samples are required to provide insights into the mass load and ecotoxicological risk of these compounds to aquatic biota and human.

Declaration of competing interest

Authors declare that we do not have any conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2023.100246.

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