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5  
6 **Characterization of the Nairobi River catchment impact zone and**  
7 **occurrence of pharmaceuticals: implications for an impact zone inclusive**  
8 **environmental risk assessment**

9  
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18 **ABSTRACT**

19 The largely uncontrolled release of active pharmaceutical ingredients (APIs) within untreated  
20 wastewater discharged to waterbodies, associated with many rapidly urbanising centres is of  
21 growing concern owing to potential antimicrobial resistance, endocrine disruption and  
22 potential toxicity. A sampling campaign has been undertaken to assess the source, occurrence,  
23 magnitude and risk associated with APIs and other chemicals within the Nairobi/Athi river  
24 basin, in Kenya, East Africa. The catchment showed an extensive downstream impact zone  
25 estimated to extend 75 km, mostly, but not exclusively, derived from the direct discharge of  
26 untreated wastewater from the urban centre of Nairobi city. The exact extent of the downstream  
27 boundary of the Nairobi city impact zone was unclear owing to the inputs of untreated  
28 wastewater sources from the continuous urbanized areas along the river, which counteracted  
29 the natural attenuation caused by dilution and degradation. The most frequently detected APIs  
30 and chemicals were caffeine, carbamazepine, trimethoprim, nicotine, and sulfamethoxazole.  
31 Paracetamol, caffeine, sulfamethoxazole, and trimethoprim alone contributed 86% of the total  
32 amount of APIs determined along the Nairobi/Athi catchment. In addition to direct discharge  
33 of untreated domestic wastewater attributed to the informal settlements within the conurbation,  
34 other sources were linked to the industrial area in Nairobi City where drug formulation is  
35 known to occur, the Dandora landfill and veterinary medicines from upstream agriculture. It  
36 was shown that there was a possible environmental risk of API ecotoxicological effects beyond  
37 the end of the traditional impact zone defined by elevated biochemical oxygen demand  
38 concentrations; with metronidazole and sulfamethoxazole exhibiting the highest risk.

39 **Key words:** Pharmaceuticals; Nairobi; water quality; wastewater; Kenya; risk assessment

## 40 **1. Introduction**

41 The management of water quality is of utmost importance to guarantee the safeguard of  
42 environmental and human health and ensure sustainable development. The direct discharge of  
43 untreated wastewater (DDUW) is a significant source of water pollution constituting  
44 approximately 80% of the wastewater discharged globally (Koncagul et al., 2017). This is an  
45 obvious concern not only from major pollutants such as ammonia, biochemical oxygen demand  
46 (BOD), metals and persistent organic pollutants but also from the presence of emerging  
47 contaminants, such as active pharmaceutical ingredients (APIs) which have implications for  
48 environmental as well as human health. Any discharge of a chemical to a receiving water results  
49 in its dilution within a mixing zone downstream. For chemicals discharged at toxic levels, then  
50 there will be a zone downstream where significant ecological harm would be expected, prior  
51 to sufficient dilution occurring to reduce levels to below ecotoxicological thresholds. This  
52 “impact zone” is well established for BOD and ammonia, however, for chemicals which may  
53 be more toxic and persistent, their ecological impact may extend beyond the impact zone for  
54 BOD and ammonia (Bagnis et al., 2019, 2018). Little attention has been devoted to the  
55 environmental risk assessment of APIs and other ‘down the drain’ chemicals in areas of poor  
56 wastewater treatment in order to assess the extent and significance of impact zones within  
57 heavily polluted catchments.

58 In the past decade there has been a global increase of production and consumption of APIs in  
59 low and low-middle income countries (LLMICs) where the DDUW is prevalent (Kookana et  
60 al., 2014). In particular, recent investigations have highlighted the widespread occurrence of  
61 high concentrations of APIs in pan-African rivers, unequivocally ascribed to the poor African  
62 wastewater treatment coverage and efficiency (Agunbiade and Moodley, 2014; K'oreje et al.,  
63 2016, 2012; Madikizela et al., 2017; Matongo et al., 2015; Ngumba et al., 2016; Schoeman et  
64 al., 2015; Wang et al., 2014; Wood et al., 2015). A relatively well studied example of such  
65 contaminated areas in Africa is the Nairobi River catchment, flowing through the capital city  
66 of Kenya, Nairobi (K'oreje et al., 2012; Mbui et al., 2016; Ngumba et al., 2016). Nairobi was  
67 established in the early 1990's with a population of 250,000 and was reputed as a city with  
68 high environmental standards and was labelled accordingly as “the green city in the sun”.  
69 However, due to rapid urbanization and population growth (3,149,000 officially, but  
70 potentially double this in reality) its reputation has changed, and owing to inadequate waste  
71 management, the water bodies comprising the Nairobi catchment are severely polluted (Mbui  
72 et al., 2016; Mobegi et al., 2016). The wastewater generated in the city's informal settlements

73 and from the centre is mostly directly discharged in the Nairobi River basin without treatment,  
74 leading to an extensive impact zone characterized by the occurrence of high concentrations of  
75 pollutants such as ammonia, BOD combined with low dissolved oxygen and the potential  
76 presence of trace metals and APIs (K'oreje et al., 2016, 2012; Ngumba et al., 2016) together  
77 with other emerging and traditional organic contaminants (Kithiia, 2007; Kithiia and  
78 Ongwenyi, 1997; Mbui et al., 2016; Mobegi et al., 2016; Njuguna, 1979). The water within the  
79 catchment is a critical resource, for irrigation, industry, potable water after treatment and in  
80 some cases untreated drinking water.

81 Unlike the other previous studies of this catchment, this work within the Nairobi/Athi  
82 catchment investigated a wider variety of APIs and used a risk assessment to determine the  
83 extent of the potential impact zone for APIs and whether it may extend beyond that of pollutants  
84 such as BOD and ammonia. Furthermore a source apportionment exercise was carried out using  
85 a principle component analysis to combine available chemical data and knowledge gained  
86 during a spatially extensive monitoring programme, to identify other potential sources of APIs  
87 in addition to domestically derived DDUW.

## 88 **2. Materials and methods**

### 89 **2.1. Study area and sampling**

90 The sampling area was located in the Nairobi capital province (1,661 m altitude and 696 km<sup>2</sup>  
91 of urban area) which is located in the Nairobi/Athi River catchment (

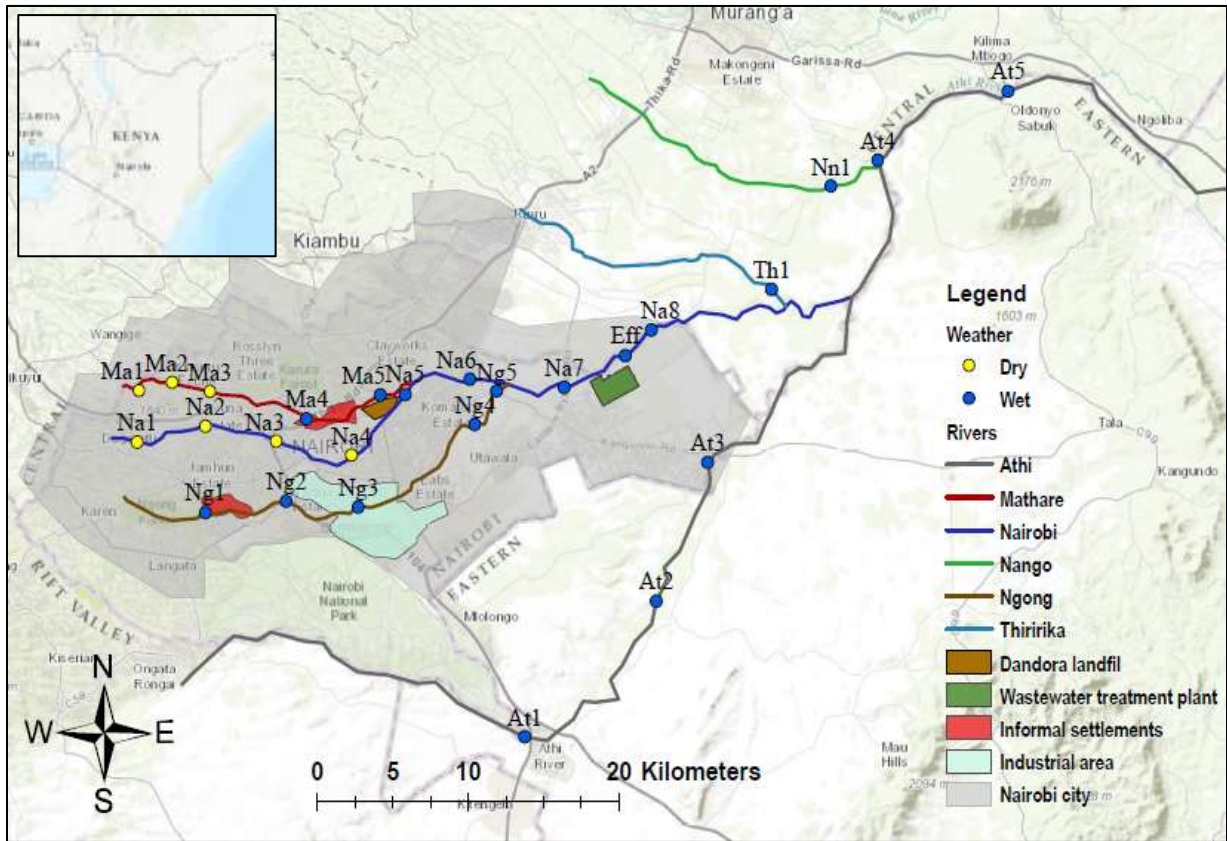
92

93 Figure 1). The Athi River is the second largest river basin in Kenya, after the Tana River. The  
94 catchment flows from the flanks of the Rift valley, the Aberdare ranges and the Ngong hills.  
95 Downstream of Nairobi the river flows through arid areas of Kenya to the Indian Ocean at  
96 Malindi. The Nairobi River is a main tributary, which itself has two main tributaries, the  
97 Mathare and Ngong Rivers, which drain Nairobi city centre and the surrounding urbanized  
98 zones, including informal settlements, industrial areas and agricultural lands (Figure 1)  
99 (Kithiia, 2007).

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**Figure 1 The Nairobi River catchment, the sampling points and the main sites of interest. The water flow is eastwards. “Eff” is the wastewater treatment plant effluent discharge point.**

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The Mathare and Ngong flow through large informal settlements areas (Mathare and Kibera), the latter also through the city’s industrial area. At the confluence of the Nairobi and Ngong lies the extensive Dandora landfill site (Muhonja et al., 2018). Dandora wastewater treatment stabilization ponds (WWTP) treat wastewater from approximately 27% of the city’s population and discharges to the Nairobi River downstream in the east of the city. Afterwards, the Nairobi River discharges into the Athi River which, after the Fourteen Falls, proceeds to the Indian Ocean. Also, two other minor tributaries of the Athi River were sampled before their confluence, namely Thiririka and Nango Rivers (

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Figure 1).

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Despite the sampling exercise being planned to occur during the dry season, owing to an unusual weather pattern most of the samples (19 out of 26) were collected during the start of the wet season in mid-March (

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129 Figure 1). The sampling campaign comprised a total of 26 sampling points: five along the  
130 Mathare River, five along the Ngong River, eight along the Nairobi River, one from the effluent  
131 of WWTP, five along the Athi River, and one each from the Thiririka and Nango Rivers at the  
132 confluence with the Athi River. The last sample was collected 75 kilometres downstream of  
133 the city, measured from the first upstream sample collected at the Nairobi River (Figure 1).

134 DDUW was identified as diffuse point sources, such as leaching of wastewater or overflowing  
135 pit latrines from informal settlements (marked in red in Figure 1). There were also expected to  
136 be significant discharges of APIs deriving from the industrial area (marked in light blue in  
137 Figure 1) (Ngumba et al., 2016). The samples were collected in 500 ml amber glass bottles and  
138 stored on ice and then overnight at 4 °C, with sample preparation completed within 24h.

## 139 **2.2. Chemicals**

140 The subset of 55 target APIs were selected from the list of APIs validated in the methodology  
141 of Furlong et al., (2014) and analysed at the Department of Environmental Sciences, University  
142 of York, York, United Kingdom.

## 143 **2.3. Analytical methodology**

### 144 *2.3.1. HPLC-MS direct injection methodology*

145 The determination of APIs in filtered water was achieved by a “direct aqueous injection - high  
146 performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) system  
147 methodology”, developed and validated by the United States Geological Survey (USGS)  
148 agency (Furlong et al., 2014).

149 Briefly, the method is validated for the determination of the 55 human-use APIs analysed in  
150 this work using “a direct-injection” of 100 µL volume of the pre-filtered (0.7 µm mesh glass  
151 filter) sample in an HPLC-MS/MS using an electrospray ionization source set in the positive  
152 mode. An inline stain-less filter was applied before the column (4.6 mm, 0.2 µm). The APIs  
153 were separated using a reversed phase column (Zorbax Eclipse plus-C18 HPLC column, 1.8  
154 µm particle size, 3.0 inner diameter and 100 mm of length) with a gradient of water modified  
155 with formic acid/ammonium formate and methanol.

156 The use of multiple reaction monitoring (MRM) was adopted to enhance the sensitivity and  
157 specificity of electrospray HPLC/MS/MS for the qualitative determination of the compounds

158 in the matrix. An internal standard method using stable isotope dilution standards (IDS) of  
159 target pharmaceuticals and the pesticide atrazine was used for quantification (Furlong et al.,  
160 2014). The goal of the methodology development was to provide a routine method for the  
161 determination of APIs at limit of detection (LOD) and quantification (LOQ) below 50 ng L<sup>-1</sup>  
162 (see Table S2 for the full list of individual API LOQ and LOD). Transformation products for  
163 many chemicals may also be of environmental concern, however, the absence of chemical  
164 calibration standards and the complexity of the mass spectra analysis prevented them being  
165 included in this suite of analysis. Full details of the methodology, analytical quality control and  
166 method validation are provided elsewhere (Wilkinson et al., 2019).

### 167 2.3.2. *Fluorescence spectrometry and total organic carbon analyses*

168 The peaks of fluorescence in the excitation/emission matrix (EEM) which correspond to  
169 tryptophan (230/350) and tyrosine (230/290) were used as a proxy of sewage contamination  
170 according to the method proposed by Bagnis et al., (2019), fluorescence spectrometry of the  
171 dissolved organic matter (DOM) can be used to characterize the extent of the impact zone. The  
172 samples were diluted to a TOC level of 5 mg L<sup>-1</sup> or less to allow quantification and to minimize  
173 the filter effects. The analyses were performed in triplicates using 1 ml of pre-filtered sample  
174 in a Hitachi F-4500 fluorescence spectrophotometer. A 3-D scan was performed at a range  
175 from 200 to 500 nm for both excitation and emission at a sampling interval of 10 nm and 2400  
176 nm/min of scan speed. A blank of ultra-high purity water (UHP) was subtracted from the  
177 samples to eliminate the signal noise from the actual sample. An external calibration curve  
178 from tryptophan (Acros Organic) and tyrosine (Sigma) standards was used to quantify the  
179 amount of both the tryptophan and tyrosine-like DOM. The concentrations of these two  
180 surrogates were summed to to generate an estimate of protein-like DOM (PL-DOM).

181 The total organic carbon (TOC) analyses were performed using high-temperature catalytic  
182 oxidation (TOC-5000A - Shimadzu) according to the method of Badr et al., (2003).

## 183 2.4. **Calculations**

184 The 5-d biochemical oxygen demand (BOD<sub>5</sub>) was estimated from the correlation of a data set  
185 of TOC and BOD<sub>5</sub> (Comber et al., 2018) and calculated as follow (Kwak et al., 2013):

$$BOD = \frac{(TOC + 9.9851)}{0.2876} \quad 1$$

## 186 **2.5. Source apportionment of APIs**

187 The Principal Component Analysis (PCA) statistical procedure, validated by Larsen and Baker  
188 (2003), was adopted to estimate the source apportionment of the APIs relative to the sampling  
189 points along the main stream of the Nairobi/Athi River (Na1 to Na8 and At4 to At5) as  
190 representative for the whole catchment, and the effluent from the wastewater treatment plant  
191 (Eff) as a source for comparison (

192

193 Figure 1).

194 Briefly, with the aim of explaining the variability of the APIs in a minimum number of factors,  
195 the data were reduced to Principal Components (PCs) through a factor analysis performed by  
196 means of SPSS Statistics 24 (IBM). The analysis was performed with Kaiser normalization and  
197 a varimax rotation to simplify the interpretation of the factors.

198 All the factors originated through the computation are orthogonal to each other reducing the  
199 covariance. The first PC corresponds to the component loadings (CL) relative to the linear  
200 combination of the original concentration values, and it accounts for the greatest variability.  
201 All the other components are in decreasing order of variability. All the components with eigen  
202 values less than 1 were excluded by default. Based on the sampling protocol 4 potential sources  
203 of APIs were selected for analysis, the influence of untreated wastewater entering the river  
204 from informal settlements, industrial discharges from the commercial area, effluent discharged  
205 from the wastewater treatment ponds and possible upstream agricultural inputs. The source  
206 emission of each API is indicated by the CL which express the relationship between the PC  
207 and the chemical (Dai et al., 2016; Larsen and Baker, 2003).

208 The most loaded factor scores ( $> 0.5$ ) for each API were originally considered amongst PCs,  
209 with some exceptions in a second analysis comparing the PC with the original concentrations.  
210 Thus, the pattern of each PC was critically analysed against literature information to determine  
211 the source apportionment.

## 212 **2.6. Environmental risk assessment**

213 A simplified environmental risk assessment (ERA) was performed using the measured  
214 concentrations at the furthest downstream sampling point along the impact zone. The



215 assessment was performed through the risk quotient (2) which is a unitless ratio of the measured  
216 environmental concentrations (MEC) of the APIs detected to the predicted no effect  
217 concentrations (PNEC), retrieved from recent published studies available in the literature.

$$RISK = \frac{MEC}{PNEC} \quad 2$$

218 The risk was evaluated based on the guidelines from the European Medicine Agency (EMA,  
219 2006). It should be noted, however, that ecotoxicological data for APIs is lacking and rarely  
220 are there full datasets for either chronic, sub-lethal endpoints or for all significant trophic levels.  
221 Taking this into account, for this appraisal the lowest PNEC available from reliable literature  
222 sources was used to compare with the MEC.

### 223 **3. Results and discussion**

#### 224 **3.1. Impact zone characterization**

225 The rivers physico-chemical parameters at each sampling point are shown in Table 1. The  
226 distance of each sampling point was measured relative to the first upstream sampling point for  
227 each river. A concise description of the sampling area and the respective elevation is also  
228 provided. The catchment mean of the physico-chemical parameters were: pH 8.5, conductivity  
229  $570.2 \mu\text{s cm}^{-1}$ , TDS 245.9 ppm, and temperature  $23.7^\circ\text{C}$ . The temperature varied accordingly  
230 to the time of sampling, the lowest in the early morning and increasing along the day until the  
231 afternoon ( $21\text{-}29^\circ\text{C}$ ). The altitude difference from the highest point of sample collection (Na1)  
232 to the lowest (At5) was of 416 m.

233 The estimated  $\text{BOD}_5$  recorded at the sample points along the Nairobi/Athi catchment allowed  
234 a prediction of the extent of the impact zone generated by the DDUW in the Nairobi and Athi  
235 River catchments (Table 1). Such estimates were based on the definition of impact zone as the  
236 area between the discharge point of untreated wastewater and the downstream point at which  
237 the concentration of  $\text{BOD}_5$  returns to the expected environmental range of typically less than 8  
238  $\text{mg L}^{-1}$  for unpolluted rivers (Bagnis et al., 2019, 2018).

239 The sampling points on the Nairobi and Mathare Rivers upstream the city centre showed very  
240 high  $\text{BOD}_5$   $1136 \text{ mg L}^{-1}$  and  $1349 \text{ mg L}^{-1}$  respectively, and concentrations of PL-DOM of  $0.3$   
241  $\text{mg L}^{-1}$  and  $2.0 \text{ mg L}^{-1}$  respectively, which suggest a higher contribution from sewage inputs to  
242 the Mathare River (Table 1). The range of predicted  $\text{BOD}_5$  values recorded at these sampling  
243 points were nearly three times above typical values for high strength crude sewage

244 (Tchobanoglous et al., 2003), but are in the observed range for industrial effluents (e.g. dyes  
245 and pharmaceutical factories) (Lokhande et al., 2011; Pittwell, 1988). This suggests the  
246 presence of industrial sources of pollution upstream the Nairobi city centre, however,  
247 additional studies would be necessary to ascertain their presence and nature.

**Table 1. A short description of each sampling point with accompanying physico-chemical parameters.**

Sampling point	River	Area	Distance (km)	Elevation (m)	pH	Conductivity ( $\mu\text{s cm}^{-1}$ )	TDS (PPM)	Temperature ( $^{\circ}\text{C}$ )	BOD <sub>5</sub> (mg L <sup>-1</sup> )	PL-DOM (mg L <sup>-1</sup> )
<b>At1</b>	Athi	Downstream Nairobi national park	0	1507	8.1	195	129	25.7	389.4	0.9
<b>At2</b>	Athi	Upstream a tanning plant WWD	13.1	1472	8.8	198	130	28.3	267.8	0.7
<b>At3</b>	Athi	Downstream a tanning plant WWD	23.2	1472	9.6	920	609	26.3	624.2	8.9
<b>At4</b>	Athi	Upstream Fourteen Falls	48.3	1428	8.1	302	199	22	160.3	1.2
<b>At5</b>	Athi	Fourteen Falls	59.3	1392	8	342	224	23.5	292.2	0.9
<b>Eff</b>	WWTP	Effluent WWTP	/	1480	8.8	1099	723	26	520.8	2.8
<b>Ma1</b>	Mathare	Upstream city centre	0	1781	7.4	530	359	21.2	1349	2.0
<b>Ma2</b>	Mathare	Dam	2.2	1770	8.3	420	276	24.7	197.4	0.6
<b>Ma3</b>	Mathare	Downstream dam	4.8	1734	7.3	153	101	22.1	901.8	0.6
<b>Ma4</b>	Mathare	Middle of Mathare slum	11.6	1627	8.4	486	320	21.3	297.2	3.7
<b>Ma5</b>	Mathare	Confluence with Nairobi River	19.5	1563	7.8	624	412	20.7	292.7	3.8
<b>Na1</b>	Nairobi	Upstream city centre	0	1808	7.6	362	239	20.1	1136	0.3
<b>Na2</b>	Nairobi	Upstream city centre	5.0	1728	8	1050	728	21	490.6	5.4
<b>Na3</b>	Nairobi	City centre	9.9	1680	7.9	768	508	26.2	454.2	4.0
<b>Na4</b>	Nairobi	Between city centre and Mathare River	15.5	1628	7.3	928	616	28.9	638.2	13.2
<b>Na5</b>	Nairobi	Confluence with Mathare River	21.0	1568	8.2	618	409	22.3	292.0	3.4
<b>Na6</b>	Nairobi	Confluence with Ngong River	25.7	1500	8	597	394	23.1	1421	2.8
<b>Na7</b>	Nairobi	Upstream WWTP	32.4	1491	8.5	788	522	27	515.2	0.6
<b>Na8</b>	Nairobi	Downstream WWTP	39.38	1459	8.6	935	615	25	503.5	2.9
<b>Ng1</b>	Ngong	Upstream Kibera Slum	0	1714	7.3	94	62.3	19.5	191.9	0.6
<b>Ng2</b>	Ngong	Middle Kibera Slum	5.7	1702	7.6	551	364	22	1115	4.5
<b>Ng3</b>	Ngong	Industrial area	11.2	1632	7.8	769	508	25.3	822.3	5.3
<b>Ng4</b>	Ngong	Quarry area	21.2	1547	7.8	817	539	25.2	508.5	3.6
<b>Ng5</b>	Ngong	Confluence with Nairobi River	24.0	1500	8.2	796	526	24	373.5	4.1
<b>Nn1</b>	Nango	Confluence with Athi River	/	1432	7.7	313	205	22.7	249.9	0.3
<b>Th1</b>	Thiririka	Confluence with Nairobi River	/	1430	7.7	171	112	22.5	206.2	0.5

250 The highest level of PL-DOM ( $13.2 \text{ mg L}^{-1}$ ) was observed along the Nairobi River at sampling  
251 point Na4 (Table 1), located between the city centre and the confluence to the Mathare River (  
252

253 Figure 1), which highlighted high inputs of sewage contamination from the densely populated  
254 city centre. Afterwards, the PL-DOM concentration steadily decreased owing to dilution and  
255 degradation until sampling point Na8 located after the effluent discharge point from the  
256 WWTP, where a slight increase was observed, consistent with the WWTP effluent discharge.  
257 The estimated BOD<sub>5</sub> transect along the Nairobi River showed a trend similar to the PL-DOM,  
258 with an exception of the sampling point before the confluence with the Ngong River (Na6),  
259 where the highest concentration was recorded ( $1421 \text{ mg L}^{-1}$ ).

260 The Mathare River below the dam, flows through the city's informal settlements and exhibited  
261 an increasing concentration of PL-DOM associated with wastewater. PL-DOM concentrations  
262 within the Mathare informal settlement areas were of a comparable magnitude to those  
263 measured in the Ngong river passing through similar settlements in Kibera (Table 1). The first  
264 upstream sampling point collected along the Ngong River (Ng1) showed a relatively low  
265 predicted BOD<sub>5</sub> ( $192 \text{ mg L}^{-1}$ ), whilst the highest predicted BOD<sub>5</sub> concentration was recorded  
266 just after the informal settlement of Kibera (Ng2) ( $1115 \text{ mg L}^{-1}$ ). The predicted BOD<sub>5</sub>  
267 concentration steadily decreased until the confluence with the Nairobi River most likely as an  
268 effect of dilution (Table 1). The PL-DOM showed an increase to a maximum at Kibera, then  
269 kept steady along the length of the river, suggesting continuous input of sewage all along the  
270 river which counteracted any dilution or attenuation. Another important contribution to this  
271 impact zone is the extensive industrial area located on the north side of the Ngong River (  
272

273 Figure 1).

274 The Athi River water quality showed an abrupt increase of PL-DOM at sampling point At3,  
275 most likely caused by the contribution of an upstream wastewater discharge point from a  
276 tannery. Thereafter, the PL-DOM concentrations gradually decreased to  $0.9 \text{ mg L}^{-1}$  at the last  
277 sampling point (At5) downstream from the confluence with the Nairobi River. Away from the  
278 tannery discharge, the predicted BOD<sub>5</sub> concentrations within the Athi River were relatively  
279 low compared with the rest of the catchment (Table 1). Also, the two smaller tributaries, joining  
280 the main river downstream of the Nairobi conurbation, the Thirika and Nango Rivers, recorded

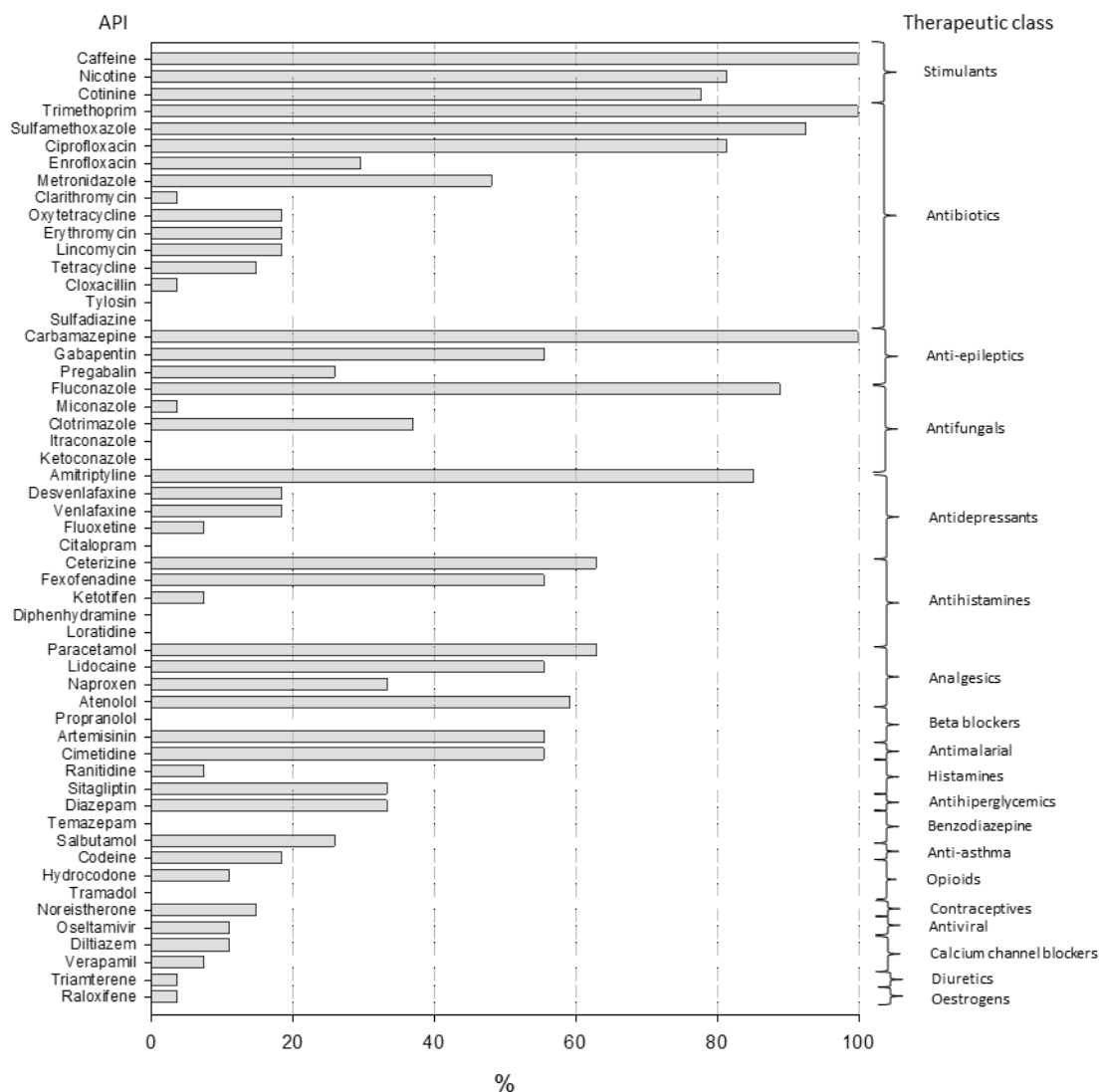
281 some of the lowest BOD<sub>5</sub> and PL-DOM concentrations within the whole catchment, reflecting  
282 their sub-catchments being away from high population densities (Figure 1).

283 Overall, a relatively rapid increase of PL-DOM and predicted BOD<sub>5</sub> was observed along the  
284 transect upstream and within the urban centre followed by a decrease thereafter. Besides the  
285 influence of industrial and domestic DDUW, this observation was at least partly caused by the  
286 change in hydrological conditions within the river during the sampling campaign. The start of  
287 the wet season coincided with sampling the downstream sites and so besides natural attenuation  
288 factors, such as (bio) degradation, greater dilution from rainwater runoff would have affected  
289 these sites (see Figure 1). The whole sampling area along the Nairobi/Athi catchment was  
290 heavily impacted by BOD<sub>5</sub> from numerous industrial and landfill sources as well as diffuse  
291 sewage pollution. There is no a clear end of such impact zone as the concentration of BOD<sub>5</sub> at  
292 the last downstream sampling point (At5; 75km from the first upstream site) was still greater  
293 than 8 mg L<sup>-1</sup>, considered as the threshold for the “severely polluted” categorization of water  
294 affected by wastewater pollution (Koncagul et al., 2017). However, it could be considered an  
295 overestimation if there was a significant proportion of recalcitrant DOM in solution, leading to  
296 a positive bias in predicted BOD<sub>5</sub>, as suggested by the modelling approach of Bagnis et al.,  
297 (2018). On the basis of this possible assumption, combined with the lack of any BOD data from  
298 further downstream, then for the purpose of undertaking a risk assessment, site At5 was  
299 assumed to be the end of the impact zone.

### 300 **3.2. Frequency of APIs detection**

301 The samples were collected in 27 locations along the catchment and analysed for the  
302 occurrence of 55 APIs belonging to 19 therapeutic categories (Figure 2). A full dataset of  
303 measured API concentrations is provided in Table S2 of the Electronic Supplementary  
304 Information. Forty-five out of the fifty-five compounds under scrutiny were detected in at least  
305 one sampling location, and at least one representative for each of the nineteen therapeutic  
306 classes was detected along the entire catchment.

307



308

309 **Figure 2 Frequency of detection of the 55 active pharmaceutical ingredients (APIs) at**  
 310 **the 27 sampling points (100%) grouped per therapeutic class.**

311

312 The APIs with the highest frequency of detection (>90%) were caffeine, carbamazepine, and  
 313 trimethoprim, detected in 100% of the sampling points, followed by sulfamethoxazole (93%),  
 314 fluconazole (89%), amitriptyline (85%), ciprofloxacin (81.5) and nicotine (81.5%) (Figure 2).  
 315 Unfortunately this data cannot be compared with consumption data for APIs in Kenya as there  
 316 are not accurate records, owing to a combination of drug company data being confidential and  
 317 high levels of over-the-counter medicines being sold by unregistered pharmacies.

318 Caffeine is a useful marker for DDUW contamination (Dai et al., 2016; Verlicchi et al., 2012),  
 319 because it is extensively removed during conventional wastewater treatment and so only low  
 320 levels would be expected in catchments with a developed wastewater system (Sui et al., 2010).

321 Its detection in all of the sampling points suggested extensive human-impacted contamination  
322 by untreated sewage throughout the catchment. Also, other human derived stimulants such as  
323 nicotine and its main metabolite cotinine were also frequently detected (81.5% and 78%  
324 respectively). However, it must also be recognized that the large areas allocated to coffee crops  
325 cultivation distributed throughout the Nairobi region, and the presence of tobacco factories in  
326 the industrial area of Nairobi, might contribute to the occurrence of these compounds in surface  
327 waters (Barjolle et al., 2017).

328 In a similar fashion, the antiepileptic drug carbamazepine is also used as a marker for sewage  
329 contamination, because of its persistence and high solubility, and it was consequently detected  
330 at all of the sampling points (100%), further suggesting the influence of domestic wastewater  
331 on the catchment (Durán-Álvarez et al., 2015; Gasser et al., 2011; Kruglova et al., 2014). In  
332 the same therapeutic class were the less frequently detected gabapentin (56%) and pregabalin  
333 (26%).

334 The antibiotics trimethoprim and sulfamethoxazole were detected with high frequency and high  
335 concentrations as has been the case for other African countries (aus der Beek et al., 2016).  
336 Three out of the thirteen antibiotics investigated in this work, namely trimethoprim,  
337 sulfamethoxazole, and ciprofloxacin were detected in a frequency higher than fifty percent;  
338 and seven, namely metronidazole, clarithromycin, lincomycin, erythromycin, oxytetracycline,  
339 tetracycline, and enrofloxacin in between 10 and 50 % of samples; but cloxacillin was detected  
340 in less than 10% of the samples collected and tylosin and sulfadiazine not detected at all (Figure  
341 2).

342 The antifungal fluconazole was detected at a frequency of 89% of the sampling points, followed  
343 by clotrimazole (37%) and miconazole (3.7%) belonging to the same therapeutic class and  
344 which are predominantly for human use, but are also effective for horses, cats and dogs. The  
345 remaining two antifungals itraconazole and ketoconazole were not detected.

346 The API amitriptyline was the most frequently detected in the antidepressant therapeutic class  
347 (85%), whilst desvenlafaxine and venlafaxine were detected at five sampling points each (19%)  
348 and fluoxetine at two locations (7.4%). The antidepressant citalopram was not detected.

349 Six APIs belonging to the class of antihistamine were investigated. The APIs cetirizine (63%)  
350 and fexofenadine (55.6%) were detected at a similar frequency; ketotifen was detected at only  
351 two sampling locations (7%); whilst the antihistamines diphenhydramine and loratidine were  
352 not detected at all.

353 Analgesics often predominate in API monitoring studies and paracetamol (also known as  
354 acetaminophen) was found in seventeen out of the 27 sampling locations (63%) followed by  
355 lidocaine (56%) and naproxen (33%). Paracetamol has been recognized as the most frequently  
356 detected API globally (Barra Caracciolo et al., 2015), even though it is quickly catabolized by  
357 microorganisms and consistently removed from water and wastewater (Baena-Nogueras et al.,  
358 2017; Lin et al., 2010; Yamamoto et al., 2009), and therefore typically absent in samples  
359 collected away from any source. Naproxen has a similar environmental behaviour and is  
360 quickly eliminated from the aqueous environment (Grenni et al., 2018).

361 The beta-blocker atenolol was detected at 60% of the sampling points, whilst propranolol was  
362 not detected. The antimalarial artemisinin was detected in fifteen sites out of the 27 (56%).

363 All the other compounds not listed so far fell below the detection frequency of 50%. A total of  
364 11 compounds out of the total 55 were not detected, namely tylosin, sulfadiazine, citalopram,  
365 itraconazole, ketoconazole, diphenhydramine, propranolol, miconazole, loratidine,  
366 temazepam, and tramadol; 3, namely cloxacillin, triamterene, raloxifene were detected only at  
367 one site; 3, namely fluoxetine, ketotifen, verapamil, in only 2 sites; and 3, namely oseltamivir,  
368 diltiazem, hydrocodone were detected only at three 3 sampling points. Of these compounds  
369 only four are on the WHO essential medicines list and possibly more importantly, only one,  
370 fluoxetine, is listed in the Kenyan list of essential medicines (*Kenya Essential Medicines List*,  
371 2016), which might explain their low frequencies of detection.

372 Compounds not detected or detected in less than 3 sampling points at concentrations  $<10 \text{ ng L}^{-1}$   
373 <sup>1</sup> in the impact zone were excluded from further statistical analyses as the risk is considered  
374 irrelevant in the EU ERA protocol (EMA, 2018) (S.3).

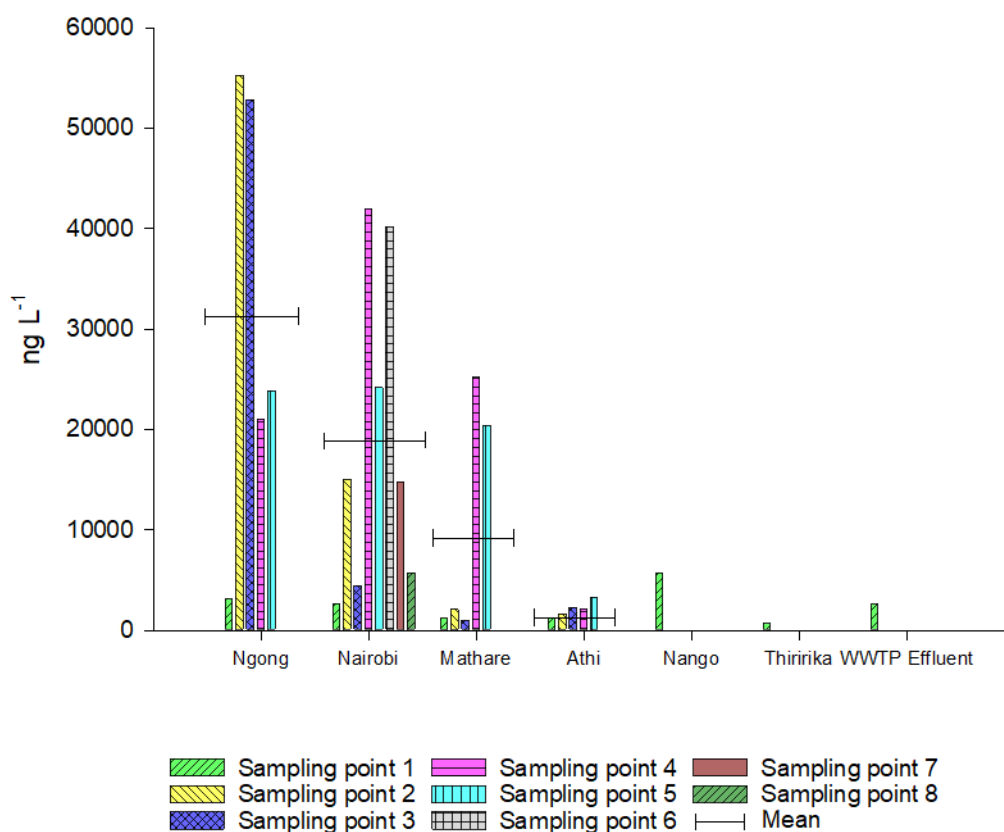
### 375 **3.3. Catchment APIs distribution**

376 It is not possible to represent 55 APIs across 26 sites in a graphical manner. Consequently, by  
377 way of summarising the data generated for all of the sites monitored the 55 API concentrations  
378 were summed in order to provide an overall burden on the catchment and to allow comparison  
379 with previous data (Table 2). Site Ng2 along the Ngong River exhibited the highest mean and  
380 maximum total API concentrations of 31,160 and 55,193  $\text{ng L}^{-1}$  respectively for all of the sites  
381 monitored (

382

383 Figure 13).





384

385 **Figure 3 Sum and mean of the 55 APIs at each river sampling point (e.g. Sampling point**  
 386 **1 of Ngong = Ng1; Sampling point 1 of Nairobi = Na1, etc.). The wastewater**  
 387 **treatment plant effluent concentration is to the far right for comparative**  
 388 **purposes.**

389 The total concentration of APIs increased from 3052 ng L<sup>-1</sup> at the sampling point Ng1,  
 390 upstream the informal settlement of Kibera, to the max total concentration of 55,193 ng L<sup>-1</sup> at  
 391 5.6 km downstream the slum (Ng2). The subsequent sample (Ng3) was collected in the middle  
 392 of the industrial area and other informal settlements and showed the second highest total  
 393 concentration of APIs along this river (52,792 ng L<sup>-1</sup>). The total concentrations at the last two  
 394 samples, Ng4 and Ng5, decreased to less than half the maximum concentration, respectively  
 395 21,000 and 23,776 ng L<sup>-1</sup>. Such a decrease is very likely due to a combination of reduced input  
 396 and dilution caused by recent rainfall runoff at the start of the wet season which arrived early.  
 397 It is also likely that biodegradation played a role on the decrease of concentrations of the  
 398 compounds more rapidly catabolised by microorganisms.

399 The average total APIs concentrations of the Nairobi was of 18,560 ng L<sup>-1</sup> and its maximum  
 400 concentration was of 41,954 ng L<sup>-1</sup> recorded at the sampling point Na4 located after the city  
 401 centre and before the confluence with the Mathare River (Figure 3). The samples collected

402 upstream (Na1, Na2) and in the city centre (Na3) showed a significantly lower concentration  
403 with respect to the samples collected downstream (Na4, Na5, Na6). The last two sampling  
404 locations recorded total concentrations similar to the upstream ones (Na7, Na8), showing a  
405 natural recovery of the river water quality with regard to APIs.

406 The Mathare River exhibited relatively less API contamination at locations upstream of the  
407 city (Ma1, Ma2, Ma3), but was the third highest river for average total amount of APIs (9913  
408 ng L<sup>-1</sup>) owing to two highly polluted sites (25,156 ng L<sup>-1</sup> at Ma4, and 20,343 ng L<sup>-1</sup> at Ma5).  
409 These last two sampling points are located in proximity of the Mathare informal settlement and  
410 the Dandora landfill (

411

412 Figure 1) which might explain the sudden increase in API levels.

413 The Thiririka River was sampled before the confluence with the Nairobi as it could potentially  
414 be contaminated by APIs from the upstream urban centre of Githurai located adjacent to  
415 Nairobi and therefore contributes to the impact zone. However the results showed a relatively  
416 low total concentration of pharmaceuticals (653 ng L<sup>-1</sup>).

417 The Athi River showed an average total APIs concentration of 2064 ng L<sup>-1</sup> and a max total  
418 APIs concentration of 3255 ng L<sup>-1</sup>. The first three sampling locations have no influence from  
419 the sources of APIs within the city centre. After the sampling point At3 the water quality is  
420 influenced by the confluence with the Nairobi River. However, the max total APIs  
421 concentration is detected at the sampling location At5 (3255 ng L<sup>-1</sup>), which is likely influenced  
422 by the waters coming from the Nango River (5674 ng L<sup>-1</sup>).

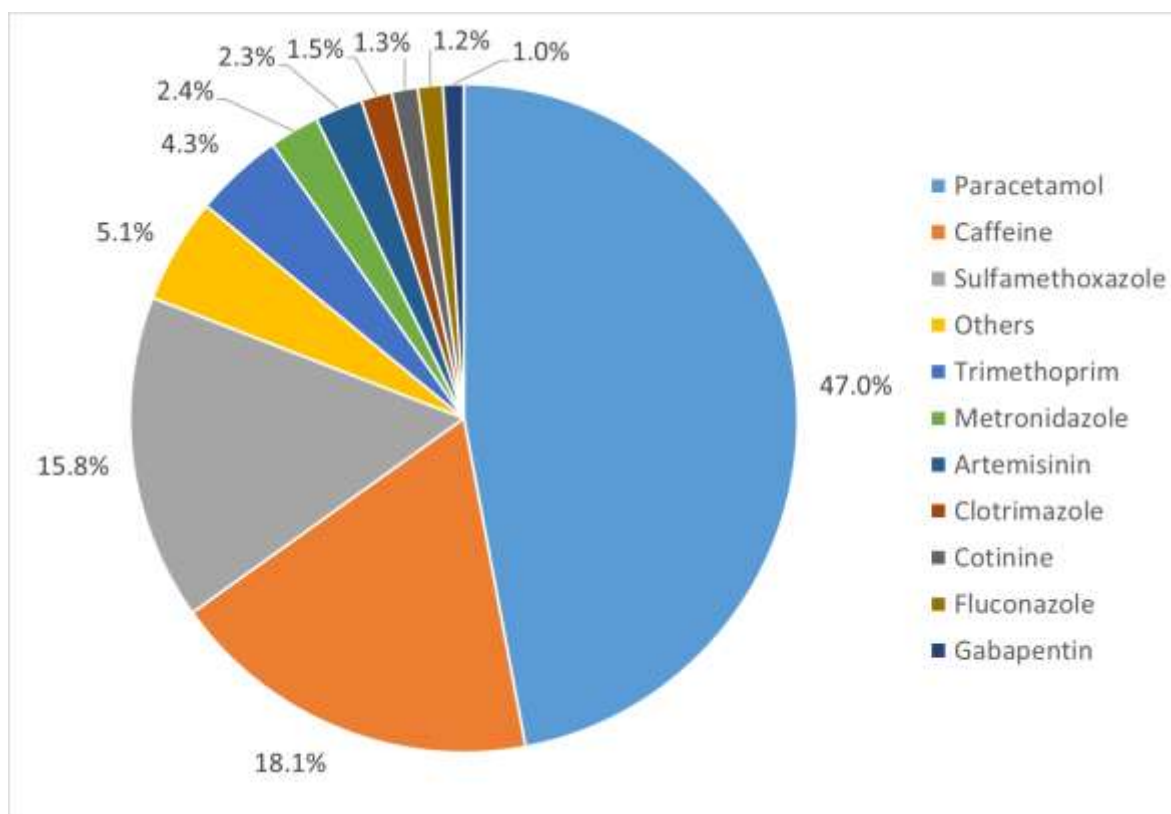
423 The sample collected from the effluent of the Dandora WWTP showed a total API  
424 concentration much lower (2586 ng L<sup>-1</sup>) than the averages observed in the Nairobi city rivers  
425 (Ngong, Mathare and Nairobi) which confirms the importance of the wastewater treatment in  
426 reducing the environmental occurrence of APIs within urban developments (Comber et al.,  
427 2018). Given the extremely high levels of parent APIs measured within the catchment, then  
428 there will obviously be concern regarding any potentially toxic transformation products.  
429 Although not determined as part of this programme of work, they should be considered in  
430 future monitoring, using a combination of occurrence data in studies such as this and  
431 transformation product ecotoxicological data, to identify high risk chemicals for further study.

#### 432 **3.4. APIs individual contribution and occurrence patterns**

433 The analgesic paracetamol was the compound with the highest contribution to the  
434 contamination by APIs (47.4%) (Figure 4), and the API occurring in the largest concentration  
435 in the Ngong River (max 31,003 ng L<sup>-1</sup>), the Nairobi River (max 24,541 ng L<sup>-1</sup>), and the  
436 Mathare River (max 14,180 ng L<sup>-1</sup>). These high concentrations were in contrast to its relative  
437 low frequency of detection (Figure 2) highlighting its well-known rapid biodegradation  
438 (Baena-Nogueras et al., 2017; Bagnis et al., 2019; Lin et al., 2010; Yamamoto et al., 2009),  
439 which together with dilution, significantly contributed to its decrease of occurrence in the  
440 environment and absence at sampling locations away from the source. Paracetamol was  
441 detected in other waterbodies of the African continent at concentrations in the same order of  
442 magnitude as recorded in this study (Table 2).

443 Sulfamethoxazole was the second most abundant API detected in the catchment (15.8%)  
444 (Figure 4), occurring as the most abundant API in the Athi River (max 1530 ng L<sup>-1</sup>, At5), and  
445 exhibited the second highest individual concentration in the River Ngong (11250 ng L<sup>-1</sup>, Ng2).  
446 This compound is used in large amounts globally and widely detected in water compartments,  
447 and according to the ERA performed by Straub (2015) the Nairobi/Athi catchment reported the  
448 highest global MEC (21,000 ng L<sup>-1</sup>) in a previous study (K'oreje et al., 2012) (Table 2). The  
449 widespread detection of sulfamethoxazole is owed partly to the highly variable removal rate,  
450 caused by the transformation of its metabolites Na-sulfamethoxazole and Glu-  
451 sulfamethoxazole back to sulfamethoxazole in WWTP's, which often results in a net negative  
452 removal (Göbel et al., 2004). The increase of concentration at the last sampling point with  
453 respect to the previous might be caused by a combination of transformation and the  
454 contribution of non-identified point sources. Regardless, once in the environment the main  
455 mechanism of removal is biodegradation, whilst photodegradation is significant only on a  
456 surface shallow layer (Straub, 2016).

457 The stimulant caffeine showed the third highest contribution (18.1%) and max concentration  
458 in the River Ngong (10891 ng L<sup>-1</sup>). This compound was detected in South African water bodies  
459 in comparable concentrations (Agunbiade and Moodley, 2016; Matongo et al., 2015) (Table  
460 2).



461

462 **Figure 4 Percentage contribution of each API (sum of each sampling point**  
 463 **concentration) to the total concentration of APIs detected along the**  
 464 **Nairobi/Athi catchment. The slice “others” contains the compounds occurring**  
 465 **for less than 1%, for ease of analyses.**

466 The antibiotic trimethoprim also showed an important environmental input relative to the total  
 467 APIs (4.3%) and it was detected at all sampling sites. The maximum concentration (3345 ng  
 468 L<sup>-1</sup>) was recorded downstream of the Dandora landfill, suggesting a leachate contribution from  
 469 this potential secondary environmental source of APIs, as previously observed in other studies  
 470 (Clarke et al., 2015; Masoner et al., 2014). This antibiotic was previously detected by other  
 471 studies concerning African water bodies showing concentrations consistent with this study  
 472 (Table 2). Trimethoprim is often prescribed with sulfamethoxazole and correlating the data for  
 473 this study shows a significant relationship between concentrations of the two antibiotics ( $r^2 =$   
 474 0.51).

475 These four compounds alone contributed 85.6% of the total amount of APIs detected along the  
 476 Nairobi/Athi catchment. Two of these are antibiotics, which might therefore be of concern  
 477 regarding antibiotic resistance within riverine systems as reported in other studies (Subirats et  
 478 al., 2017).

479 The other compounds with a contribution higher than 1% are the antibiotic metronidazole  
 480 (2.4%), the antifungals clotrimazole (1.5%) and fluconazole (1.2%), the stimulant cotinine  
 481 (1.3%), and the antimalarial artemisinin (2.3%). All the APIs contributing to the less than the  
 482 1% were grouped in one category that contributes to the 5.1% of the total (Figure 4).

483 In Table 2 are reported the concentrations of a list of APIs detected both in this study and in  
 484 other studies on the African continent (Table 2). For all of them the concentrations of reported  
 485 APIs are generally similar. Carbamazepine, however, shows concentrations much larger in the  
 486 study of K'oreje et al., (2016) than this study, both performed in the same catchment. This is  
 487 probably because of the different sampling periods, in fact the latter study was performed  
 488 during a dry season, whilst much of this work was performed during a wet season, which results  
 489 in significant dilution from rainfall runoff. Also ciprofloxacin was detected in much higher  
 490 concentrations in the study of Agunbiade and Moodley, (2014), though referring to a different  
 491 water body in South Africa.

492 **Table 2 APIs detected at the highest concentration (ng L<sup>-1</sup>) in comparison with previous**  
 493 **studies on the African continent (n.a: not available).**

API (ng L <sup>-1</sup> )	This study	K'Oreje et al. 2012	Agunbiade et al. 2014	Matongo et al. 2015	Ngumba et al. 2016
Carbamazepine	172	4000	n.a.	n.a.	n.a.
Caffeine	10890	n.a.	10000	33200	n.a.
Trimethoprim	3346	6000	n.a.	290	2650
Nicotine	872	n.a.	n.a.	n.a.	n.a.
Sulfamethoxazole	11250	21000	8000	5320	13765
Paracetamol	31003	16500	16060	1740	n.a.
Amitriptyline	54	n.a.	n.a.	n.a.	n.a.
Ciprofloxacin	168	n.a.	4000	n.a.	509

494

### 495 3.5. APIs source apportionment

496 The PCA analysis was performed with the purpose to reduce the complexity of the APIs dataset  
 497 along the Nairobi/Athi River and to allow an easier estimate of the sources of APIs; it resulted  
 498 in four PCs listed in Table 3. Using a combination of the location of the sampling sites within

499 the catchment, the PCA grouping using the API concentrations and estimates of the protein-  
 500 like dissolved organic material and the known physico-chemical attributes of the APIs such as  
 501 their persistence within sewage treatment, it is possible to split the dataset into 4 reasonably  
 502 clear potential sources namely; untreated wastewater, treated wastewater, point sources such  
 503 as landfill leachate and agriculture.

504 **Table 3** The four principal components (PC1, PC2, PC3, PC4) with the active  
 505 pharmaceutical ingredients (APIs) and the protein-like DOM (PL-DOM) as  
 506 variables along the Nairobi/Athi River sampling points. Also, the table  
 507 includes the estimate source and relative variance.

	PC 1	PC 2	PC 3	PC4
Amitriptyline	<b>0.876</b>	-0.090	-0.226	0.000
Artemisinin	-0.347	-0.246	-0.067	-0.495
Atenolol	<b>0.729</b>	<b>0.583</b>	0.202	-0.121
Caffeine	<b>0.538</b>	<b>0.774</b>	0.199	-0.079
Carbamazepine	<b>0.397</b>	0.160	<b>0.840</b>	-0.028
Cetirizine	0.067	0.347	<b>0.847</b>	-0.180
Cimetidine	<b>0.797</b>	0.320	0.319	0.069
Ciprofloxacin	-0.142	-0.127	-0.136	<b>0.902</b>
Clotrimazole	<b>0.544</b>	<b>0.497</b>	0.435	0.136
Cotinine	<b>0.956</b>	0.173	0.098	-0.093
Enrofloxacin	-0.090	-0.249	-0.215	<b>0.909</b>
Fluconazole	0.066	0.111	<b>0.915</b>	-0.228
Gabapentin	<b>0.500</b>	-0.340	<b>0.574</b>	-0.367
Metronidazole	-0.098	<b>0.974</b>	0.065	-0.074
Naproxen	<b>0.750</b>	-0.381	0.436	-0.055
Nicotine	<b>0.959</b>	0.082	0.211	-0.010
Paracetamol	<b>0.735</b>	<b>0.553</b>	0.238	0.051
Sulfamethoxazole	<b>0.948</b>	0.116	0.109	-0.176
Tetracycline	-0.140	0.077	-0.143	<b>0.972</b>
Trimethoprim	0.003	<b>0.980</b>	0.136	-0.02
PL-DOM	<b>0.876</b>	-0.157	<b>0.377</b>	-0.126
Estimated source	<b>Untreated</b>	<b>Point</b>	<b>Wastewater</b>	<b>Farming</b>
Variance (%)	46	17	16	9

508

509 **The first principal component (PC1)** showed the highest variance (46%) and was interpreted  
 510 as the diffuse discharge of untreated wastewater. This is because the PC was highly weighted  
 511 by APIs and protein-like DOM (PL-DOM) (Table 3). In fact, PC1 was heavily weighted by  
 512 caffeine, nicotine, and paracetamol which are typically detected in untreated wastewater but  
 513 completely or highly removed in wastewater treatment plants (Comber et al., 2018; Rosal et  
 514 al., 2010; Sui et al., 2010). Similarly owing to its persistence atenolol was also detected in  
 515 untreated wastewater (Castiglioni et al., 2006; Comber et al., 2018; Rajab et al., 2013; Rosal et

516 al., 2010) but was absent in the WWTP effluent, strengthening the assumption that direct  
517 discharge of untreated wastewater was a significant source to the river. Despite the information  
518 available in the literature about the degradability of the antidepressant amitriptyline is scarce,  
519 there is evidence of high persistence (Baena-Nogueras et al., 2017; Bagnis et al., 2019; Li et  
520 al., 2013). But, similar to atenolol, its detection in the river waters and the absence in the  
521 effluent of the Dandora wastewater treatment plant and the high PC weight (0.88) suggested a  
522 contribution of its occurrence from DDUW. Also clotrimazole, cotinine, naproxen, cimetidine  
523 and gabapentin showed high weighting. These compounds are in high concentration in the  
524 sampling area between Na4 and Na6 which correspond to the area between the city centre and  
525 the suburban area downstream. Since only around 28% of Nairobi is on mains sewerage, it is  
526 very likely that this area corresponds to the downstream boundary of the service (Ngumba et  
527 al., 2016). Also, leachate runoff from the Dandora landfill might contribute to this load (Na6).

528 **The second principal component (PC2)** contributed 17% of the total variance. This profile  
529 was highly weighted by the APIs atenolol, caffeine, clotrimazole, metronidazole, paracetamol,  
530 and trimethoprim. Because of the little significance of PL-DOM to this PC, it was assumed the  
531 source of these APIs was linked to poorly defined point sources along the river. The maximum  
532 concentration of trimethoprim was recorded at a downstream sampling point (Na6) with respect  
533 to the highest concentration of PL-DOM (Na4). The Na6 sampling area corresponded with the  
534 tract of river flowing next to the Dandora Landfill, whose leachate runoff might be deemed as  
535 a point source of trimethoprim (Clarke et al., 2015; Masoner et al., 2014). However, also the  
536 presence of other sources was considered likely, such as hospitals or veterinary clinics.

537 **The third principal component (PC3)** contributed 16% of the total variance. This PC  
538 represented the effluent from the WWTP as it is weighted by only the APIs that were detected  
539 in the effluent sample namely carbamazepine, cetirizine, fluconazole, and gabapentin, and  
540 moderately weighted by PL-DOM as well, typical of WWTP effluents.

541 **The fourth principal component (PC4)** contributed 9% of the total variance. This component  
542 was weighted only by the antibiotics also known to be used for veterinary purposes such as  
543 ciprofloxacin, tetracycline and enrofloxacin, which were detected at the sampling point Na1 in  
544 relatively high concentrations. These APIs are thought to represent sources from agriculturally  
545 dominated land use upstream of the city. Since these APIs are used for veterinary purposes as  
546 well as in human medicines, without further, more intensive sampling it was assumed they

547 were from agricultural sources (Alexandrino et al., 2017; Granados-chinchilla and Rodríguez,  
548 2017; Peng et al., 2016).

549 There would obviously be overlap between these potential sources within a catchment and with  
550 only limited data the outputs are tentative. However, they do suggest some important points (i)  
551 that there are multiple sources of APIs to the catchment, (ii) that the untreated wastewater  
552 inputs are of high significance (iii) other sources such as landfills need further study and (iv)  
553 as in other countries, agriculture is also likely to be a source of APIs.

#### 554 **Surface water ERA beyond the end of the impact zone**

555 The data provided a broad and detailed assessment of the extent of the contamination by the  
556 direct discharge of untreated wastewater in the Nairobi/Athi catchment and the occurrence of  
557 APIs at a point far from the source (At5). This last sampling point, even though still showing  
558 elevated levels of predicted BOD<sub>5</sub> (292 mg L<sup>-1</sup>) was taken as the end of the impact zone on the  
559 basis of allowing a risk assessment to be applied using the protocol for environmental risk  
560 assessment for medicinal active compounds (Bagnis et al., 2019; EMA, 2006). For APIs  
561 detected above 10 ng L<sup>-1</sup> and with a log K<sub>ow</sub> of less than 4.5 the calculated risk quotient was  
562 calculated as set out by the EMA (2006). The risk was labelled in severity as follows: RQ <0.01  
563 is insignificant; < 0.1 low risk; 0.1 ≤ RQ ≤ 1 medium risk; RQ > 1 high risk (Chen and Ying,  
564 2015).

565 **Table 4 Environmental risk assessment (ERA) for APIs at sampling point At5**

<b>ERA</b>					
<b>API*</b>	<b>MEC (ng L<sup>-1</sup>)</b>	<b>PNEC (ng L<sup>-1</sup>)</b>	<b>RQ</b>	<b>RISK</b>	<b>REFERENCE</b>
<b>SFX</b>	1529	560	<b>2.7</b>	High	AMR Industry Alliance, 2018;
<b>MTR</b>	182	130	<b>1.4</b>	High	Bengtsson-Palme and Larsson,
<b>FLC</b>	112	250	0.45	Moderate	AMR Industry Alliance, 2018
<b>TRM</b>	64.6	500	0.13	Moderate	Straub, 2013
<b>CTN</b>	87.5	1000	0.09	Low	Gosset et al. 2017
<b>PAR</b>	45.8	814	0.06	Low	Minguez et al. 2015
<b>AMI</b>	12.8	720	0.02	Low	Minguez et al. 2015
<b>ART</b>	466	19000	0.02	Low	Jessing et al. 2009
<b>CFF</b>	634	8700000	<0.01	Insignificant	ECHA
<b>CBZ</b>	55.2	100000	<0.01	Insignificant	Minguez et al. 2015
<b>GAB</b>	54.6	100000	<0.01	Insignificant	Minguez et al. 2015

566 \* AMI, amitriptyline; ART. artemisinin; CFF, caffeine; CRB, carbamazepine; CTN, cotinine; FLC, fluconazole;  
567 GAB, gabapentin; MTR, metronidazole; PAR, paracetamol; SFX, sulfamethoxazole; TRM, trimethoprim.

568



569 The lowest reported PNEC were selected or calculated from the available literature including  
570 tests of cyanobacteria, invertebrates, algae, fish and clinically relevant bacteria (AMR Industry  
571 Alliance, 2018; Bengtsson-Palme and Larsson, 2016; Chen and Ying, 2015; Gosset et al., 2017;  
572 Jessing et al., 2009; LePage et al., 2017; Minguéz et al., 2014; Straub, 2016, 2013; Tell et al.,  
573 2018). Fexofenadine, nicotine, lidocaine were detected at concentrations below 10 ng L<sup>-1</sup> and,  
574 according to the protocol of ERA for medicines of the EMA (2006), are unlikely to represent  
575 a risk for the environment. The log K<sub>ow</sub> for these APIs is also below 4.5, respectively 2.8, 1.2,  
576 2.3 (Drugbank, 2018), and therefore there is no need for an additional ERA involving the  
577 assessment of persistence, bio-accumulation and toxicity (EMA, 2006). Sulfamethoxazole and  
578 metronidazole were determined to be the highest risk driven largely by their low PNECs.  
579 Fluconazole and trimethoprim were the only other APIs deemed to be of concern with  
580 moderate RQs (0.45 and 0.13 respectively).

581 Therefore, despite the natural attenuation of APIs occurring along the impact zone it was shown  
582 there may still be concern regarding their effects at or near its boundary. It should be noted that  
583 for some APIs there are a lack of sub-lethal, chronic ecotoxicological data across trophic levels  
584 and so further work is required in order to generate PNEC data that may be used with a high  
585 degree of confidence. Furthermore, the sample collected at this point in the river (Site At5) was  
586 taken after the wet season rains had arrived and therefore potentially diluting the sources of  
587 APIs from the identified sources including DDUW. During the dry season with lower flows in  
588 the river and therefore less dilution, the risk from sources independent of rainfall (e.g. industrial  
589 and municipal sewage (treated or untreated) would be expected to be higher. This highlights  
590 the need for more detailed and seasonal surveys, extending further downstream than the last  
591 sample collected during this survey, to accurately assess the risk.

#### 592 **4. Conclusions**

593 Based on the data reported above the following conclusions may be drawn regarding the  
594 occurrence and potential impacts of APIs within the Nairobi catchment:

- 595 • The Nairobi/Athi catchment showed an extensive downstream impact zone mostly derived  
596 from the DDUW from the urban centre of Nairobi city.
- 597 • The impact zone extended downstream to a distance of about 75 km far from the city.  
598 However, its downstream boundary was unclear owing to the inputs of untreated

599 wastewater sources from the continuous urbanized areas along the river, which counteract  
600 the natural attenuation caused by dilution and degradation.

601 • The most frequently detected APIs and chemicals were caffeine, carbamazepine,  
602 trimethoprim, nicotine, and sulfamethoxazole. Paracetamol, caffeine, sulfamethoxazole,  
603 and trimethoprim alone contributed 86% of the total amount of APIs detected along the  
604 Nairobi/Athi catchment.

605 • The main API sources were attributed to the informal settlements and the industrial area  
606 in Nairobi City, as well as the Dandora landfill. Also, farming or agricultural sites upstream  
607 of the city were likely sources of veterinary APIs.

608 • It is shown that there is a potential environmental risk of API ecotoxicological impacts  
609 beyond the end of the impact zone, and a high risk for metronidazole and  
610 sulfamethoxazole. Given that these are both antibiotics, then their potential impact on  
611 antimicrobial resistance within the catchment also bares further investigation. However,  
612 any assessment would benefit from greater coverage of the catchment including sampling  
613 further downstream in order to better establish the extent of the mixing zone as well as a  
614 more systematic monitoring of wet and dry seasons, accompanied by hydrological data in  
615 order to be able to calculate loads to the catchment. Further, more detailed source  
616 apportionment as well as access to sales and consumption data would also assist in refining  
617 risk models.

618

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