Science of the Total Environment 622–623 (2018) 293–305

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence and risk assessment of antibiotics in water and lettuce in Ghana

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HIGHLIGHTS

- Antibiotics occur in wastewater and lettuce samples in Ghana.
- All water bodies studied were significantly contaminated with antibiotics.
- Ciprofloxacin showed medium toxicity risk to algae.
- High risk for antibiotics resistance development in the environment could occur for ciprofloxacin, erythromycin, sulphamethoxazole and trimethoprim.
- Antibiotics loads were significantly reduced by WSPs up to 96%.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 30 June 2017
Received in revised form 24 November 2017
Accepted 24 November 2017
Available online 13 December 2017

Editor: D. Barcelo

Keywords:
Antibiotic uptake
Wastewater stabilization pond
Irrigation water
Hospital wastewater
Human exposure
Antibiotics resistance

ABSTRACT

Hospital wastewater and effluents from waste stabilization ponds in Kumasi, Ghana, are directly discharged as low quality water into nearby streams which are eventually used to irrigate vegetables. The presence of 12 commonly used antibiotics in Ghana (metronidazole, ciprofloxacin, erythromycin, trimethoprim, ampicillin, cefuroxime, sulfamethoxazole, amoxicillin, tetracycline, oxytetracycline, chlortetracycline and doxycycline) were investigated in water and lettuce samples collected in three different areas in Kumasi, Ghana. The water samples were from hospital wastewater, wastewater stabilization ponds, rivers and irrigation water, while the lettuce samples were from vegetable farms and market vendors. Antibiotics in water samples were extracted using SPE while antibiotics in lettuce samples were extracted using accelerated solvent extraction followed by SPE. All extracted antibiotics samples were analyzed by HPLC-MS/MS. All studied compounds were detected in concentrations significantly higher ($p = 0.01$) in hospital wastewater than in the other water sources. The highest concentration found in the present study was 15 μg/L for ciprofloxacin in hospital wastewater. Irrigation water samples analyzed had concentrations of antibiotics up to 0.2 μg/L. Wastewater stabilization ponds are low technology but effective means of removing antibiotics with removal efficiency up to 95% recorded in this study. However, some chemicals are still found in levels indicating medium to high risk of antibiotics resistance development in the environment. The total concentrations of antibiotics detected in edible lettuce tissues from vegetable farms and vegetable sellers at the markets were in the range of 12.0–104 and 11.0–41.4 ng/kg (fresh weight) respectively. The antibiotics found with high concentrations in all the samples were sulfamethoxazole, erythromycin, ciprofloxacin, cefuroxime and trimethoprim. Furthermore, our study confirms the presence of seven antibiotics in lettuce from irrigation farms and markets, suggesting an indirect exposure of humans to

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https://doi.org/10.1016/j.scitotenv.2017.11.287

antibiotics through vegetable consumption and drinking water in Ghana. However, estimated daily intake for a standard 60 kg woman was 0.3 ng/day, indicating low risk for human health.

1. Introduction

Antibiotics are among contaminants of emerging concern. The main mode of entry of antibiotics into the aquatic environment is through wastewater (Karthikeyan and Meyer, 2006; Szekeres et al., 2017) and effluent from landfills (Eggen et al., 2010; Simon et al., 2005). Also, antibiotics are not completely removed in sewage treatment plants (Chang et al., 2010) and a wide range of antibiotics have been detected in wastewater stabilization ponds (WSPs) (Møller et al. 2016), municipal sewage (Castiglioni et al., 2006), hospital sewage (Duong et al., 2008), surface water (Alder et al., 2004; Arikan et al., 2008; Carmona et al., 2014; Kemper, 2008; Kolpin et al., 2002), and groundwater (Fram and Belitz, 2011; Hirsch et al., 1999; Hu et al., 2010; Lapworth et al., 2012).

The inherit effects of antibiotics include hypersensitive reactions, protracted toxic effects due to exposure to low-level antibiotics for a long time (Sarmah et al., 2006), antibiotic-resistant bacteria development and spread (van den Bogaard, 2000; Gullberg et al., 2011) and abnormalities in digestive system functioning (Bedford, 2000; Deitch, 1990; Schuijt et al., 2013). The public has easy accessibility to antibiotics in low and middle-income countries (LMICs), from a variety of sources, including drugstores, over-the-counter chemical shops, hospitals, and roadside stalls (Lerbech et al., 2014; Senah, 1997; Wolf-Gould et al., 1991). Antibiotics can sometimes be bought at pharmacies and drugstores in LMICs without prescription, despite prohibiting legislations to stop selling (Bekoe et al., 2014; Lerbech et al., 2014). Extensive accessibility to antibiotics in developing countries like Ghana could lead to continuous exposure to antibiotics via food and water. Studies have reported that 34–70% Ghanaians have their urine contaminated with antibiotics suggesting that the Ghanaian public may unintentionally be exposed to antibiotics (Lerbech et al., 2014). This is concerning, since studies indicate that safe levels of exposure may be extremely low, as low as 0.1 μg/L, due to the development of resistance at sub-minimum inhibitory concentration (sub-MIC) levels (Gullberg et al., 2011). Thus, studies are needed to identify antibiotics in the environment and potential human exposure via food and water.

The use of wastewater for irrigation is common in LMICs, including Ghana (Cornish and Aidoo, 2000; Drechsel and Keraita, 2014; Keraita et al., 2008). Usually, untreated and/or partially treated wastewater from urban areas of LMICs is discharged into drains, smaller streams and other tributaries of larger water bodies, where it is mixed with storm and freshwater (diluted wastewater) before it is used by farmers. This surface water is referred to as low quality water (Raschid-Sally and Jayakody, 2008).

Recent studies have demonstrated uptake of antibiotics by crop plants like lettuce and other vegetables under real or simulated field conditions (Ahmed et al., 2015; Azanu et al., 2016; Boxall et al., 2006; Chitescu et al., 2013; Christou et al., 2017; Dolliver et al., 2007; Riemenschneider et al., 2016) and their subsequent entrance to the food web via food consumption have been predicted (Azanu et al., 2016).

In Ghana, studies on the occurrence of hormones (Asem-Hiablie et al., 2013), pesticides (Adu-Kumi et al., 2010) and metals (Anim-Gyampo et al., 2013) in environmental samples have been extensively done but no research so far on antibiotics in the environment and no risk assessment on antibiotics in the environment have been conducted. Additionally, although antibiotics have been found in surface waters in some developing counties with similar wastewater management challenges like Ghana and uses low quality water for irrigation of vegetables, no study has recorded levels of antibiotics in vegetables irrigated with low quality water contaminated with antibiotics.

The purposes of this work were to study the occurrence and distribution of 12 antibiotics in hospital wastewater, rivers, WSPs wastewater, and low quality water used for vegetable irrigation. Furthermore, we performed a risk assessment on the antibiotics found in the water samples and estimated the risk quotients (RQs) for antibiotic resistance in various water bodies and estimated human exposure from lettuce irrigated with low quality water. Lettuce was chosen because it is cultivated all year round in Ghana, eaten with less or without cooking, and uptake studies performed using lettuce show that it could take up antibiotics (Azanu et al., 2016).

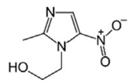
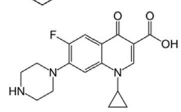
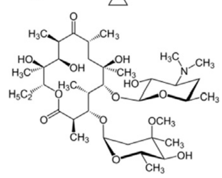
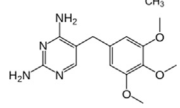
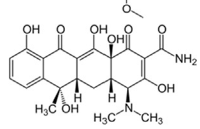
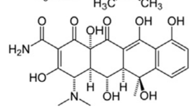
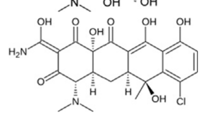
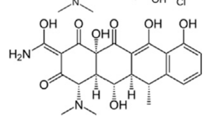
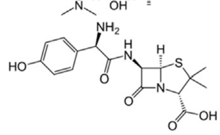
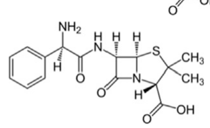
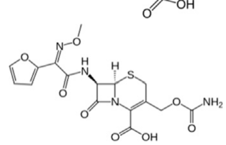
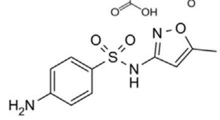
2. Materials and methods

2.1. Chemicals

A total of 12 antibiotics, all on the Ghana Essential Medicines List and the 2011 National Health Insurance Drug List (Ministry of Health, 2010; NHIA, 2011), were studied. These were ciprofloxacin (quinolone), erythromycin (macrolide), trimethoprim and sulfamethoxazole (sulfonamides), amoxicillin, ampicillin and cefuroxime (β-lactams), metronidazole (nitroimidazole) and doxycycline, tetracycline, chlorotetracycline and oxytetracycline (tetracyclines). The chemical structures and physicochemical properties are shown in Table 1. Selection of antibiotics was based on four factors: 1) frequency of prescribed usage for human-use in Ghana (Ministry of Health, 2004), 2) known or suspected environmental and species impact (Ash et al., 1999), 3) persistence in aquatic environments and previous detections in wastewater and surface waters (Kolpin et al., 2002), and 4) inclusion in previous studies of antibiotics in urine samples from outpatients in Ghana (Lerbech et al., 2014).

Amoxicillin (CAS #: 267-87-780, 98% pure) was obtained from Duchefa (Haarlem, Holland). Ampicillin (CAS #: 69-53-4, 97% pure), metronidazole (CAS #: 443-48-1, 98% pure), cefuroxime (CAS #: 55268-75-2, 97% pure), ciprofloxacin (CAS #: 85721-33-1, 96% pure), erythromycin (CAS #: 114-07-8, 97% pure), trimethoprim (CAS #: 738-70-5, 98% pure), and sulfamethoxazole (CAS #: 723-46-6, 97% pure) were purchased from Fluka (Brøndby, Denmark). Tetracycline hydrochloride (CAS #: 60-54-8, >96% pure), oxytetracycline (CAS #: 79-57-2, 97% pure) and chlorotetracycline (CAS #: 64-72-2, 97% pure) were purchased from Sigma-Aldrich (Steinheim, Germany), and doxycycline hydrochloride (CAS #: 564-25-0, 98% pure) was obtained from Takeda Pharma (Roskilde, Denmark). The internal standard (IS) d_4-sulfamethoxazole was purchased from Toronto Research Chemicals (Toronto, Canada), and the ISs d_8-ciprofloxacin and d_3-trimethoprim from QMX Laboratories (Thaxted, UK). Formic acid (98–100% pure, Ph Eur) was purchased from Merck KGaA (Darmstadt, Germany). Methanol (HPLC grade) was purchased from Lab-Scan (Gliwice, Poland). Antibiotics standard solutions were prepared by dissolving the solids in methanol and kept in freezer at −18 °C. Internal standard solution mixture and standard antibiotics-mix were with a concentration of 2.5 mg/mL and 5 mg/L respectively. These solutions were prepared by taken known amount of each stock solution and mixing with methanol in a 5 mL volumetric flask. The standard antibiotics-mix and internal standard solution-mix were kept in brown bottles to protect them from light and stored at −18 °C. The working standards for calibration were prepared few hours before analysis.

Table 1
Chemical structures and relevant physicochemical properties for the investigated antibiotics.

Antibiotic CAS-nr	ID	Structure	Molecular formula	MW	Log Kow	pKa	Solubility (g/L)
Metronidazole 443-48-1	MET		$C_6H_9N_3O_3$	171.2	−0.02	15.44	9.5
Ciprofloxacin 85721-33-1	CIP		$C_{17}H_{18}FN_3O_3$	331.3	0.28	6.09	30
Erythromycin 114-07-8	ERY		$C_{37}H_{67}NO_{13}$	734.0	3.06	8.9	0.0042
Trimethoprim 738-70-5	TRIM		$C_{14}H_{18}N_4O_3$	290.3	0.91	7.12	0.4
Tetracycline 60-54-8	TC		$C_{22}H_{24}N_2O_8$	444.4	−1.18	3.3, 7.68, 9.69	0.231
Oxytetracycline 79-57-2	OTC		$C_{22}H_{24}N_2O_9$	460.4	−0.90	3.27, 7.32, 9.11	0.31
Chlorotetracycline 64-72-2	CTC		$C_{22}H_{23}ClN_2O_8$	478.8	−3.6	3.3, 7.4, 9.3	1000
Doxycycline 564-25-0	DC		$C_{22}H_{24}N_2O_8$	444.4	−0.02	2.2, 7.75	0.63
Amoxicillin 267-87-780	AMX		$C_{16}H_{19}N_3O_5S$	365.4	0.87	2.8, 7.2	4.0
Ampicillin 69-53-4	AMP		$C_{16}H_{19}N_3O_4S$	349.4	1.35	3.24	7.5
Cefuroxime 55268-75-2	CEF		$C_{16}H_{16}N_4O_8S$	424.4	−0.16	3.15	0.145
Sulfamethoxazole 723-46-6	SUL		$C_{10}H_{11}N_3O_3S$	253.3	0.89	6.16	0.61

2.2. Study area

The study was conducted in Kumasi (Fig. 1) which is located in central part of Ghana with population of about 2 million (Ghana Statistical Service, 2012). In urban Kumasi, most land where farming occurs belongs to government institutions like the university KNUST and to private developers. The total area used for open space farming in the city (including tubers and cereals) was about 70 ha in 2005 with 41 ha under irrigated vegetable farming (Obuobie et al., 2006). The total land area used for open space vegetable farming in Kumasi was recently estimated to be 59 ha in the dry season and 48 ha in the rainy season (Drechsel and Keraita, 2014) indicating an increase in the total area used for vegetable farming. According to Agodzo et al. (2003) irrigation water requirements for most vegetables grown in Ghana vary between 300 and 700 mL/day depending on the climatic conditions and the crop species. The estimated water productivity of lettuce in Kumasi, vary between 10 and 15 kg m^{-3} depending on cropping density, irrigation method, season and soil (Drechsel and Keraita, 2014). The cropping density used in the various farms in Ghana range from 13 to 15 lettuce plants m^{-2} (Drechsel and Keraita, 2014). The irrigation rate used in the farms sampled varies depending on soil, crop and weather, with 5–7 L/m^2/day estimated for the dry season. Irrigation is also done in the rainy season on days without rain as especially the exotic lettuce responds quickly to water shortage, however irrigation rates could not

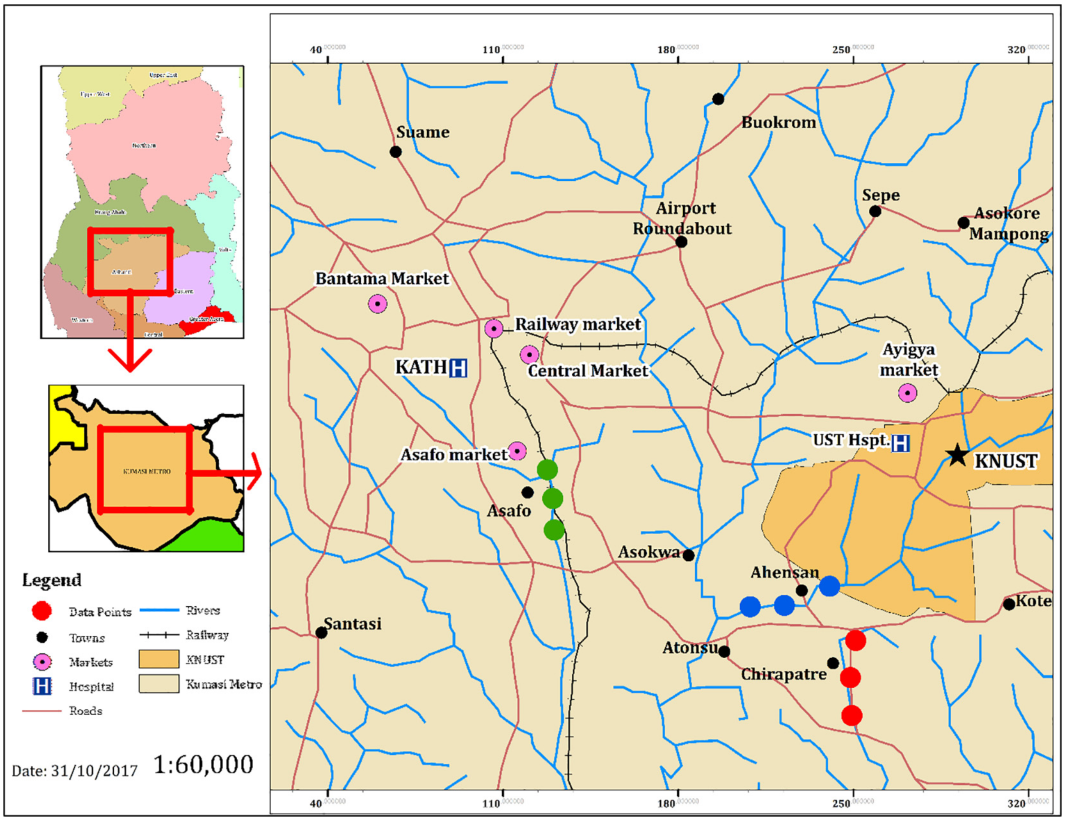


Fig. 1. Map of Ghana showing Kumasi, Ahensan sampling points as blue, Chirapatre sampling points as red and Asafo sampling points as green. KNUST: Kwame Nkrumah University of Science and Technology; KATH: Komfo Anokye Teaching Hospital; USTH: University Hospital.

be estimated. Three irrigation methods are implemented under actual field conditions during the dry and wet seasons on irrigated urban vegetable farms in Kumasi: furrow, low-head drip kits and the conventional watering cans (Keraita et al., 2008). Conventional watering cans were the one used in all the sampling sites studied. Generally, across Ghana, most farmers irrigate in the mornings (5–8 am) and evenings (5–7 pm), because it is the periods of low evapotranspiration rates (Drechsel and Keraita, 2014).

2.2.1. Water and lettuce sampling study area

For easy comparison of data, the study city, Kumasi, was divided into three sampling areas. These are Ahensan sampling area, Chirapatre sampling area and Asafo sampling area (Fig. 1). Water samples from WSPs, rivers and irrigation farm sites were collected from all three sampling areas.

Ahensan sampling area is located in Asokwa sub-metro of Kumasi with a WSP serving about 200 houses with population estimated to be 1500. The WSP descriptive characteristics are reported in supplementary data (Table S1) and performance of the WSPs in Table S2. The area is drained by Wiwi and Sisa River. The effluent after WSP treatment is discharged into the Wiwi river through a nearby stream which is used for vegetable irrigation downstream.

At Ahensan sampling area, influent and effluent from Ahensan WSP were sampled and three vegetable farms were also sampled. The first farm (Ahensan gate) with estimated land size of 0.49 ha is about 0.5 km upstream to where the Ahensan WSP effluent enters the stream. The second farm (Bomsu farm) with estimated land size of 0.4 ha is 0.7 km downstream from the entry of the effluent of Ahensan WSP to the stream. The third farm (Gyenyase farm) with estimated land size of 4.9 ha is about 3 km downstream from where the Ahensan WSP effluent enters the stream (Fig. 1).

Chirapatre sampling area is also in the Asokwa sub-metro of Kumasi, located on a hill sloping towards Oda River, with a network of sewer lines connected from 300 households with an estimated population of 1800 people to the Chirapatre WSP. At Chirapatre sampling area, the influent and effluent from Chirapatre WSP were sampled. The three vegetable farms sampled in the area were: 1) Karikari farms with estimated land size of 0.4 ha and about 1 km upstream from Chirapatre WSP effluent. 2) Chirapatre farms, estimated to have a land size of 2.0 ha and about 1 km downstream from the entry point of the Chirapatre WSP effluent and 3) Ramseyer farms which have estimated land size of 1.2 ha and is located about 2.5 km downstream from the point of entry of the Chirapatre WSP effluent.

Asafo sampling area is in Bantama sub-metro of Kumasi, which includes Asafo WSP system, serving approximately 20,000 people. Influent and effluent from Asafo WSPs joins the Subin River, which runs through the commercial centre of Kumasi and merges with the River Oda downstream at Asago, which is the site of a rural farming community.

Additionally, wastewater effluents from two hospitals in Kumasi namely Komfo Anokye Teaching Hospital (KATH) and University Hospital (USTH) were selected. The KATH sampling site is the only teaching hospital in Kumasi and the second largest hospital in Ghana with 1200 beds. University Hospital is a referral hospital with 120 beds for the Kwame Nkrumah University of Science and Technology community and patronized by the surrounding sub-metro population in Kumasi. These sampling sites were selected based on their potential to have high levels of antibiotic residues and their wastewater drains entering directly into the nearby streams.

Lettuce samples (above ground parts) were collected from farms where irrigation water and river samples were collected as described above. Lettuce samples were also collected from markets. In Kumasi, there are three large market (Central Market, Railway Market and

Asafo Market) and 18 neighborhood markets. About 20 wholesalers and 160 permanent retailers of lettuce have been estimated in these markets (Henseler et al., 2005). Sampling was done in the three large markets and two neighborhood markets (Bantama market and Ayigya market) (Fig. 1). The Kumasi Central Market (also known as Kejetia Market) is the largest single open air market in West Africa with over 10,000 stores and stalls.

2.3. Sampling plan

2.3.1. Water sampling

The inlet and outlet of three WSPs were sampled. The effluents from the two hospitals described above were also sampled. Furthermore, the river that passes along the farm, which is either diverted to the farm for irrigation or fetched directly for watering vegetables, was sampled. Lastly, at each farm, the main surface water used by farmers was sampled.

At each sampling point, two replicate composite water samples were collected. A total volume of 1 L (pooling 200 ml aliquots for 5 times) was collected from the same site within 30 minute interval into 1.5 L brown HDPE bottles.

Sampling was conducted by sampling one area on each day; For the first sampling period, Ahensan sampling area was sampled on 12th March, followed by Chirapatre sampling area on the 13th March and finally, Asafo sampling area on the 14th March 2014. The second period of sampling was done from 2 to 4th April 2014, following the same order of Ahensan, Chirapatre and Asafo sampling area. The third sampling period fell on 23–25th April 2014 with Ahensan sampled first followed by Chirapatre and then Asafo sampling area. A total of 81 samples were collected comprising, hospitals; 6 (3 × 2); WSP: 18 (3 × 6); rivers: 39 (3 × 13) and irrigation: 18 (3 × 6) samples.

2.3.2. Lettuce sampling

For farm site sampling, 5–6 whole plants were removed from each plot and combined as one single sample. Soil samples were collected randomly at each plot and composited in single sample for the determination of soil characteristics (Table S3). Lettuce samples were collected from five markets. Three permanent retailers were randomly selected at each market, 5–6 whole lettuce were purchased from each retailer and combined as one single sample. Overall, 45 sampling points consisting of 30 farm beds and 15 market retailers were sampled in two sampling period; 18th–22nd August 2015 (first), 22nd–26th September 2015 (second). A total of 90 lettuce samples were collected and transported to the laboratory within 3 h where they were processed.

2.4. Sample preparation and antibiotics extraction

2.4.1. Water samples

Water samples collected were transported to the organic laboratory at Department of Chemistry, KNUST, Ghana. The samples were filtered twice. The first filtration was through a grade 5 filter paper (Munktell Filter AB, Falun, Sweden) with particle retention of 20 μm. The second filtration was through a grade 120 H filter paper (Munktell Filter AB, Falun, Sweden) with particle retention of 1–2 μm. After filtration, the pH was measured using universal pH indicator strips and adjusted to 7 ± 0.3 with 2 M H_2SO_4 (Sigma-Aldrich) or 2 M NaOH (Merck). The filtered samples were divided to 2 × 100 mL into brown HDPE bottles and spiked with 100 μL aliquot of 2.5 μg/mL internal standard mixture containing ciprofloxacin-d_8 (d-Cip), trimethoprim-d_3 (d-Trim) and sulfamethoxazole-d_4 (d-Sul).

The water samples were loaded on hydrophilic-lipophilic balance (HLB) solid-phase extraction (SPE) cartridges (200 mg sorbent, 30 μm, 6 cm^3) purchased from Waters Oasis (Massachusetts, USA). The SPE cartridges were conditioned with 2 mL MeOH followed by 2 mL 0.01 M citrate buffer and lastly with 2 mL Milli-Q water. A 100 mL portion of water samples were loaded onto SPE cartridges at a flow rate of 1.5 mL/min and allowed to dry for 2 h. The dried SPE cartridges were then packed and refrigerated at −4 °C before transport to Toxicology Laboratory, University of Copenhagen, Denmark, for elution and analysis.

In Denmark, the washing of the dried SPE cartridges was performed with 3 mL of 5% MeOH in water and then allowed to dry under vacuum for about 10 min. The sorbents were eluted with 3 mL MeOH acidified with 0.1% formic acid. The elution was done at a flow rate of 1 mL/min. Evaporation of the eluates to dryness was done under nitrogen gas at a temperature of 30 °C. Reconstitution of dried eluates were done with 1000 μL 1% MeOH and transferred into brown flat-cap HPLC-vials for analysis.

2.4.2. Lettuce samples

Lettuce leaf samples collected were washed with distilled water and air dried on a tissue paper for 60 min to allow some of the water on the leaf to dry according to Dolliver et al. (2007). Leaves of lettuce samples were then chopped and three replicates composite samples for each sampling point were packaged in a zipped polyethene bags and stored at 4 °C.

The extraction of 12 antibiotics from lettuce samples was done using PLE technique followed by a clean-up with HLB SPE cartridges. A 0.1 g aliquot of sample was blended gently with 5 g Ottawa sand (20–30 mesh, AppliChem, Darmstadt, Germany) used for the PLE extraction. The eluates were kept in flat-cap HPLC-vials for analysis. The full method used for extraction is reported in a previous paper (Azanu et al., 2016).

2.5. Chemical analysis

Antibiotics in samples were determined with Agilent 1290 Infinity Binary System LC-MS/MS from Agilent Technologies Inc. (CA, USA). The instrument conditions and binary gradient used are reported in a previous paper (Azanu et al., 2016).

Mass spectrometer used was AB SCIEX QTRAP 4500 System equipped with an electrospray ionization (ESI) source (Turbo Ionspray) and with continuous electron multiplier detector (Applied Biosystems, Foster City, CA, USA). For MS detection, positive mode (ESI+) was performed for metronidazole, ciprofloxacin, erythromycin, trimethoprim, tetracycline, oxytetracycline, chlorotetracycline, doxycycline, amoxicillin, ciprofloxacin-d_8 and trimethoprim-d_3. The negative mode (ESI-) was performed for ampicillin, cefuroxime, sulfamethoxazole and sulfamethoxazole-d_4. The MS interface temperature was 300 °C, collision gas flow was 6 L/min, and the curtain gas flow was 12 L/min. The nebulizer gas flow was 8 L/min and Ion spray voltage was 5000 V. The MS system optimized multiple reaction monitoring (MRM) mode parameters are listed in Table S4. Analyst v. 1.4.2 software (Applied Biosystems) on a Windows XP platform-based data-processing system was used to collect and treat the data with a Savitzky-Golay smoothing factor of 3.

2.6. Quality assurance

Quality validation of the method was conducted in accordance to standard guidelines (European Medical Agency, 2012; U.S. Food and Drug Adminstration, 2001). For each antibiotic, a 9-point calibration curve with solution concentrations ranging from 0.1–1000 ng/mL was used to investigate the linearity of the method. Precision of the method was established by injecting 1 ng/mL standard antibiotics-mixture for 8 times. The regression coefficient (r^2) for all the 12 antibiotics ranged from 0.987 to 0.999 and precision in terms of coefficient of variation ranged from 3 to 6%. Limit of quantification (LOQ) and limit of detection (LOD) was determined from the standard deviation (σ) of the response from 0.1 ng/mL antibiotics standard solution. The standard solution was injected 6 times and the slope (S) of the calibration curve determined. The LOQ and LOD were calculated from10σ/S and 3.3σ/S respectively (U.S. Food and Drug Adminstration, 2001). For water samples, the LOD calculated for the antibiotics ranged from 0.80 to 2.75 ng/L, while the LOQ ranged from 2.42 to 8.33 ng/L whereas LOD for all antibiotics ranged from 2 to 8 ng/L, and LOQ ranged from 7 to 23 ng/L for the lettuces samples.

Absolute and relative recoveries were investigated with water from Søstein Lake (55.701316N, 12.565211E), Copenhagen. This lake was chosen because it is clean and antibiotics test performed on samples from this lake showed levels below detection limits. Four L water samples were collected into 5 L brown HDPE bottles and transported to the laboratory. Samples were then filtered and pH adjusted as described above. The filtered water was divided to 12 × 100 mL into brown bottles. The first four bottles were pre-spiked (prior to SPE) with 20 μL of 5 μg/mL antibiotics mixture and post-spiked with 100 μl of 2.5 μg/mL IS-mixture before LC determination. The subsequent four samples were post-spiked (after SPE) with 20 μL of 5 μg/mL antibiotics mixture and 100 μl of 2.5 μg/mL IS-mixture. The last four samples were pre-spiked with 100 μl of 2.5 μg/mL IS-mixture and post-spiked with 20 μL of 5 μg/mL antibiotics mixture before the LC determination. The concentration of antibiotics in the samples was determined on calibration curves constructed for each individual analyte. The absolute recovery (%) ranged from 62.6 to 101.0% and the relative recovery ranged from 82.4 to 105.2%. They were all above the minimum acceptable target value of 60% (Venn, 2008).

Absolute and relative recovery for antibiotic extraction from lettuce was investigated using organic Iceberg lettuce (Lot. Number: NL-310-01, bought at Fotex supermarket in Copenhagen, Denmark). Initially, the lettuce was washed and freeze dried overnight. The organic lettuce used for the spike-recovery experiment recorded below detection for all the antibiotics studied. A 0.5 g sample was weighed and mixed with 5 g of Ottawa sand. The samples were packed in PLE extraction cells. The first four samples were spiked (prior to PLE) with 20 μL of 5 μg/mL antibiotics-mix, and spiked with 20 μL of 2.5 μg/mL IS-mixture before LC determination. The subsequent four samples were post-spiked (before the LC determination) with 20 μL of 5 μg/mL antibiotics-mix and 20 μL of 2.5 μg/mL IS-mix. The last four samples were pre-spiked with 20 μL of 2.5 μg/mL IS-mix before the PLE and finally with 20 μL of 5 μg/mL antibiotic-mix before the LC determination. Absolute recovery (%) ranged from 61.0 to 83.5% and the relative recovery ranged from 64.8 to 112.1%.

2.7. Statistical analysis

The data obtained were statistically evaluated for mean, standard deviation (SD) and coefficient of variation (CV %) using Microsoft Office Excel 2013 (Version 15, Microsoft, USA). One-way ANOVA was performed to reveal the differences in various sources of water using GraphPad Prism version 5.01 for Windows (GraphPad Software Inc., USA). The confidence level used for these analysis was 95%, hence p-value <0.05 was considered statistically significant.

Principal component analysis (PCA) based on antibiotics concentrations in samples was done, to determine the distribution pattern of antibiotics in the sampling area and in water sources, using JMP statistical software v. 10 (SAS Institute). The principal components were extracted with eigenvalues >1.

2.8. Estimation of removal efficiency of antibiotics from WSPs

Concentrations of antibiotics in WSP influents and effluents were multiplied by the flow rates, to obtain loads for each antibiotics of the 3 WSPs. The total load (sum of the loads of the 12 antibiotics substances) was determined and used to calculate the removal efficiency by comparing the total loads of antibiotics in influents and effluents in each WSP.

2.9. Risk assessment

2.9.1. Environmental risk assessment

The potential risk posed by each antibiotics on environmental species was assessed by calculating the risk quotient (RQ) as the ratio between its maximum measured environmental concentration (MEC) and its predicted no-effect concentration (PNEC), as suggested by EMEA (2006). To cover most parts of the river food chain, RQ was calculated at three different trophic levels of the ecosystem, algae (*Desmodesmus subspicatus*), daphnid (*Daphnia magna*) and fish (*Oncorhynchus mykiss*). PNEC values assumed for this risk analysis correspond to the lowest ecotoxicological PNEC values found in the literature or calculated from the ecological structure activity relationships (ECOSAR) model (US-EPA, 2012). PNEC was calculated by dividing the toxicity data by an assessment factor (AF). The AF is an arbitrary factor to consider the inherent uncertainty in the obtained laboratory toxicity data. In this study, AF of 100 was used to derive PNECs (Leung et al., 2012). A commonly used risk ranking criterion; RQ < 0.1, minimal risk to aquatic organisms, 0.1 ≤ RQ ≥ 1, median risk; RQ ≥ 1, high risk was applied (Hernando et al., 2006). Measured environmental concentrations (MEC) of antibiotics in low quality water samples analyzed were used with PNEC to calculate RQ for algae (*Desmodesmus subspicatus*), daphnid (*Daphnia magna*) and fish (*Oncorhynchus mykiss*).

2.9.2. Antibiotics resistance risk assessment

Estimation of antibiotics resistance risk was conducted based on MIC values and AF of 1 was used. Gullberg et al. (2011) reported MIC values of 0.1 μg/L for CIP, 15 μg/L for TC, and 1000 μg/L for streptomycin. Therefore, in this study MICs assumed to cause antibiotics resistance used were, 0.1 μg/L for CIP, 1000 μg/L for ERY based on streptomycin since both antibiotics belong to the macrolide class, 15 μg/L for TC, OTC, CTC and DC, and 0.1 μg/L for the remaining antibiotics based on a worst case scenario.

2.10. Estimation of daily intake of antibiotics in lettuce samples

The antibiotics daily intake through consumption of lettuce was estimated from the concentration of antibiotics in lettuce and the quantity of lettuce consumed. Lettuce consumption rates and formula used are reported in previous paper (Azanu et al., 2016). The standard body weight of 60 kg for an adult woman was used (Akoto et al., 2013; Bortey-Sam et al., 2015).

3. Results

3.1. Occurrence of antibiotics in water sources

3.1.1. Antibiotics in hospital wastewater

In general, the antibiotics concentrations in hospital samples (Table 2) were significantly higher ($p = 0.003$) than in other water bodies (i.e. WSP inlet and outlet, rivers and irrigation water). Comparison of the two hospitals wastewater studied revealed significantly higher ($p = 0.02$) concentrations of antibiotics in KATH to USTH. In general, the antibiotics with the highest concentrations were: ciprofloxacin (11,352 to 15,733 ng/L) and erythromycin (7944 to 10,613 ng/L). The antibiotics found in the lowest concentrations were the tetracyclines.

3.1.2. Antibiotics in WSP influent and effluent wastewater

Sulfamethoxazole recorded the highest concentration (7194 ng/L) in all the influent WSP samples followed by ciprofloxacin (2371 ng/L) (Table 2). The antibiotic with the lowest concentration in the influent was AMX (2.0–6.0 ng/L). The antibiotics concentration in all the influent WSPs samples followed an increasing order as: AMX < MET < CTC < DC < TC < OTC < AMP < CEF < TRIM < ERY < CIP < SUL.

The antibiotic with the highest concentration was erythromycin whereas amoxicillin recorded the lowest levels. There was a significant ($p = 0.01$) decrease in antibiotics concentration in effluent samples to that in influent samples from the WSPs studied. There was no significant differences ($p = 0.13$) in the rate of reduction between the 3 WSPs effluent studied. The total load for the influent and effluents of the 3 WSPs was in the range of 93 to 1354 mg/day and 10 to

Table 2
Antibiotic concentrations (ng/L) in various water sources investigated in Kumasi. Where N is the number of composite samples collected.

Antibiotics	Hospitals	WSP influent	WSP effluent	Rivers	Irrigation water
	N = 6	N = 9	N = 9	N = 39	N = 18
MET	247–420	3.5[a]–24	3.0[a]–19	<LOD–363	3.0[a]–33
CIP	11,352–15,733	47–2371	27–262	25–1168	47–146
ERY	7944–10,613	96–1931	47–882	7.0–1149	6.7–136
TRIM	94–4826	35–1668	31–255	17–820	19–98
TC	58–116	13–199	11–24	11–30	11–16
OTC	75–252	4.3[a]–233	2.4[a]–24	3.0–26	2.2[a]–9.2
CTC	16–24	9.2–83	6.0–19	5.3[a]–44	4.3[a]–14
DC	24–120	21–153	14–49	8.3–68	9.4–25
AMX	2.0–6.0	n.d.–5.9	n.d.–1.3[a]	n.d.–2.7	n.d.–1.3[a]
AMP	107–324	82–556	51–97	21–184	30–74
CEF	1052–1557	109–1277	58–345	32–868	21–65
SUL	2315–3590	242–7194	103–320	13–2861	11–56

LOD ≤ a ≤ LOQ, n.d. means not detected.

71 mg/day respectively. The removal efficiency, however, of the 3 WSP studied ranged from 89% to 95% (Fig. 2) being highest for Asafo WSP.

The antibiotics were still present in WSPs effluents, with concentrations ranging from 1.3 to 882 ng/L. The levels found in the WSPs effluent were significantly higher ($p = 0.02$) than concentration obtained in rivers samples analyzed and were also significantly higher ($p = 0.001$) than irrigation water samples studied.

3.1.3. Occurrence of antibiotics in rivers

Sulfamethoxazole (2861 ng/L) recorded the highest concentration in the river samples analyzed (Table 2), followed by CIP and ERY. The concentration of amoxicillin in river samples was least represented with maximum concentration being 2.7 ng/L (Table 2). One-way ANOVA indicated statistically differences ($p = 0.001$) among communities. This is as a result of higher antibiotics concentrations found in Asafo than Ahensan and Chirapatre. The Turkey HSD test revealed statistical significant difference among samples from Asafo and Ahensan ($p = 0.02$) and Asafo and Chirapatre ($p = 0.03$). There was no significant difference between antibiotic concentration in samples from Chirapatre and Ahensan ($p = 0.7$). The antibiotic concentrations recorded in upstream samples collected before the entry of the effluent from WSPs, were lower than the concentrations in midstream and downstream samples (Fig. S1a–c).

3.1.4. Occurrence of antibiotics in irrigation water

In general, the concentrations of antibiotics in water used for irrigation of vegetables were lower than the water collected from the river, ranging from <LOD to 146 ng/L (ciprofloxacin) (Table 2). It is worth noting, that the differences between the antibiotics concentrations in irrigation water and river water source was statistically significant for tetracycline ($p = 0.04$) and cefuroxime ($p = 0.01$) but not significant for ciprofloxacin, metronidazole, erythromycin, trimethoprim, oxytetracycline, chlorotetracycline, doxycycline, amoxicillin, ampicillin, and sulfamethoxazole.

The PCA results of the distribution pattern of antibiotics in effluent samples showed a significant separation between antibiotics in various sampling areas (Fig. S2). Ahensan effluent water (H), was grouped in the bottom left corner, Chirapatre effluent water (G), clustered on the top left quadrant, and finally Asafo effluent water (D). Interestingly, metronidazole correlated strongly with Chirapatre effluent water and Asafo effluent samples strongly correlated with erythromycin, tetracycline, sulfamethoxazole, ciprofloxacin and trimethoprim. Ahensan effluent water, however did not have any antibiotics correlating with it. The distribution pattern of antibiotics in river (Fig. S3) and irrigation water (Fig. S4) were not well characterised by PCA.

3.2. Occurrence of antibiotics in lettuce

Out of the 12 antibiotics analyzed, five antibiotics (metronidazole, ciprofloxacin, erythromycin, cefuroxime and sulfamethoxazole) were detected in market samples (Fig. 3a) while trimethoprim and ampicillin in addition to the five found in the market samples were detected in the farm samples (Fig. 3b). Considering the mean concentration, the antibiotics in the lettuce samples could be arranged in the following increasing order: SUL < AMP < CEF < MET < CIP < ERY < TRIM. There was significant difference ($p = 0.001$) between the total concentrations of antibiotics found in all the lettuces samples. Trimethoprim was found in lettuce samples at two beds at the upstream farm site (Karikari farm site) along the Oti River during the first sampling and in lettuce at one bed during the second sampling section. The trimethoprim concentrations were in the range 32.7–40.5 ng/kg. However, the midstream farm site recorded a higher trimethoprim level of 104 ng/kg at Ramseyer farm downstream at concentration 59.8 ng/kg (Table S5). The average concentration of all antibiotics showed an increasing order of Karikari < Chirapatre < Ramseyer but there was no significant difference (Fig. 3c). Antibiotics levels were generally higher in Chirapatre farms than Ahensan farms and showed a significant

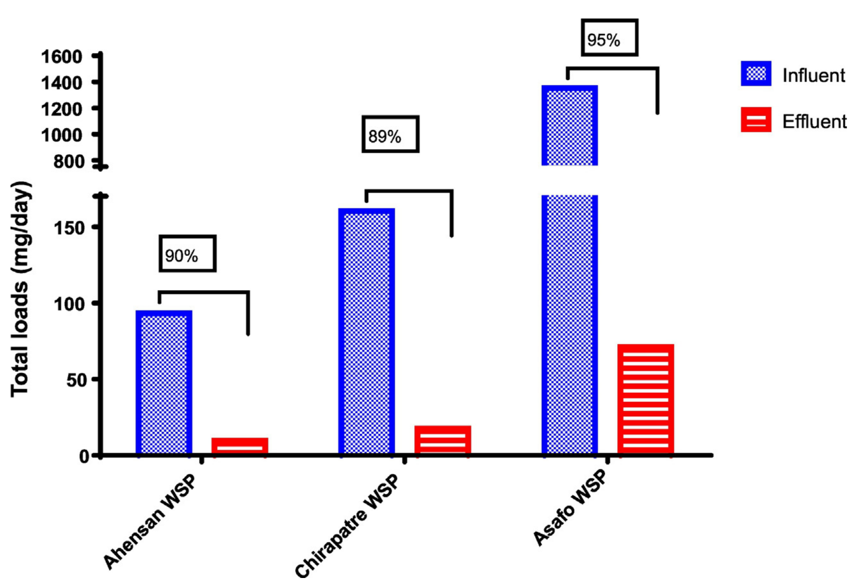


Fig. 2. Removal rates in WSPs obtained from total loads of antibiotics in influent and effluent.

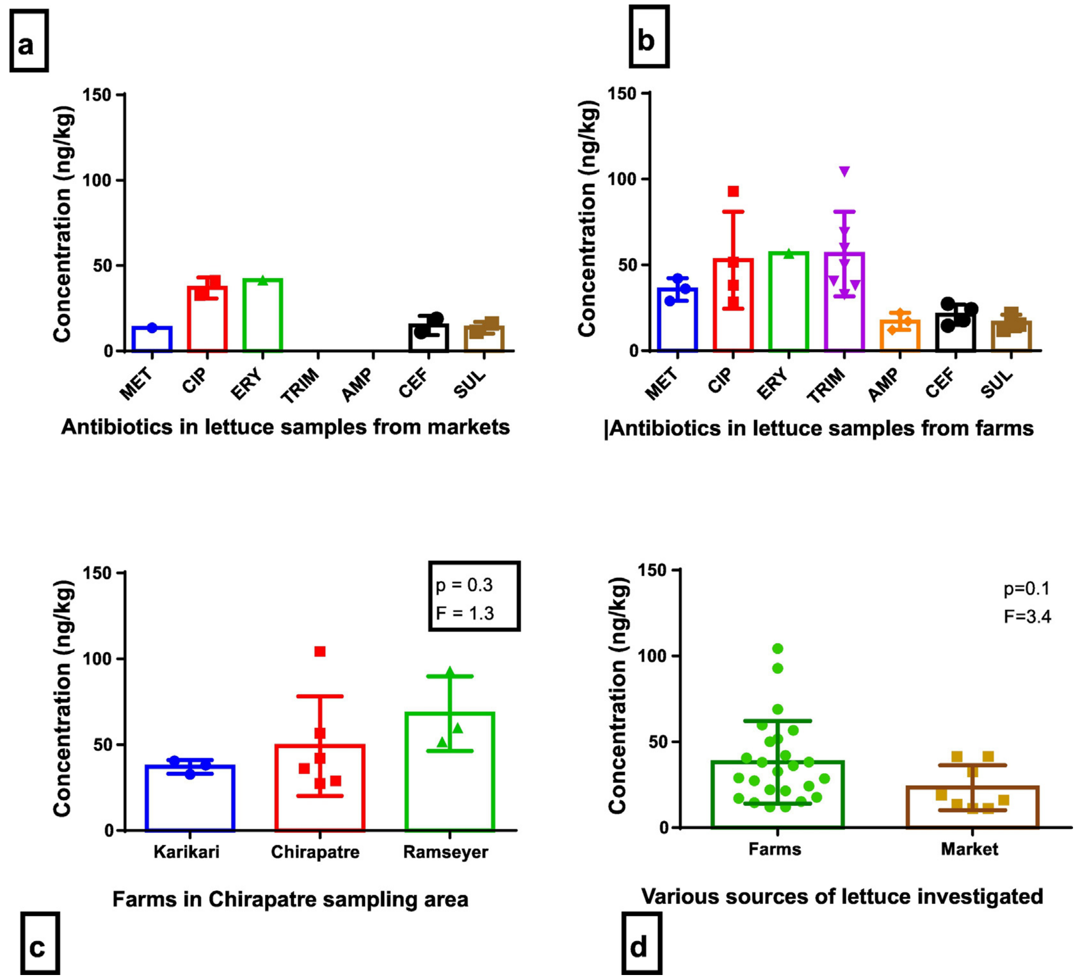


Fig. 3. Mean concentrations of antibiotics in lettuce sorted by (a) antibiotics in market samples (b) antibiotics in farm samples (c) Chirapatre area (d) various sources sampling sites.

difference ($p = 0.02$) between the concentrations of antibiotics in lettuce from the various vegetable farming communities. The average concentrations of all antibiotics recorded in lettuce samples were 38.1 ± 24.0 and 23.3 ± 13.1 ng/kg for all farm sites and all markets respectively (Fig. 3d). Although there were differences between the mean concentrations of total antibiotics in various sources of lettuce, the difference was not statistically significant ($p = 0.1$). At least one antibiotic was determined at four out of five markets. However, central market recorded the highest number of antibiotics (4) detected (Table S5). The PCA distribution pattern of antibiotics in lettuce samples from the farm and market samples (Fig. S5) did not indicate any clear patterns.

3.3. Risk assessment

3.3.1. Estimation of daily intake of antibiotics through lettuce consumption

The estimated daily intake (EDI) of antibiotics was dependent on both the antibiotics concentration in vegetables and the amount of consumption of the respective food. The lettuce consumption rate in Ghana was estimated to be 13 mg/kg body weight/day (Amoah et al., 2007), corresponding to a total estimated daily intake (EDI) of approximately 0.3 ng/day for the seven antibiotics detected in the lettuce, for a standard 60 kg adult woman (Table 3). The EDI for individual antibiotics ranged from 0.08 ng/day for TRIM to 0.02 ng/day for AMP and SUL.

3.3.2. Environmental risk assessment

A realistic evaluation of risks in low quality water collected from Kumasi, Ghana was performed using long-term toxicity data, with both standard and non-standard organisms. PNEC values and RQs for each antibiotic are shown in Table 4.

The RQ for CIP was 1.3×10^{-1} for exposure to algae, indicating a medium toxicity risk. The remaining 11 antibiotics showed low toxicity risk with RQs below 1.0×10^{-1} (lowest being 4.6×10^{-9}). The RQs for the antibiotics studied ranged from 4.1×10^{-10} to 1.5×10^{-3} and 7.6×10^{-11} to 3.3×10^{-4} for daphnids and fishes respectively. These RQs are far below 1.0×10^{-1} hence indicating a low risk of toxicity (Table 4). In general, RQs decreased in the following order: hospitals > WSPs effluent > river > irrigation water.

3.3.3. Risk assessment for antibiotic resistance

The estimated RQs for antibiotics resistance development based on Gullberg et al. (2011) ranged from 0.013 to 157.3 (Fig. 4). The RQ values

Table 3
Antibiotic frequency and concentrations (ng/kg) in lettuce and maximum estimated daily intake (EDI) (ng/day) in a standard 60 kg woman.

Antibiotic	Frequency (%)	Average concentration in lettuce (ng/kg)	Range (ng/kg)	Max EDI (ng/day)
MET	4.4	32.5	13.5–44.0	0.034
CIP	6.7	39.8	28.5–92.8	0.072
ERY	2.2	49.1	41.4–56.7	0.044
TRIM	7.8	50.1	32.7–104	0.081
AMP	3.3	17.1	12.0–22.0	0.017
CEF	6.7	18.3	11.0–27.3	0.021
SUL	5.6	15.2	11.2–21.4	0.017
ΣAntibiotics				0.287

Table 4
Risk assessment of antibiotics in low quality water of Kumasi, Ghana.

Antibiotics	MEC (μg/L)	PNEC algae (μg/L)	RQ algae	PNEC daphnid (μg/L)	RQ daphnid	PNEC fishes (μg/L)	RQ fishes
MET	3.0E– 01	2.9E+04[b]	1.0E −05	3.2E+04[b]	9.4E −06	8.8E+04[b]	3.4E −06
CIP	8.2E −01	6.1E+00[a]	1.3E −01	9.9E+03[a]	8.3E −05	2.5E+06[a]	3.3E −07
ERY	7.5E −01	1.2E+03[b]	6.2E −04	1.3E+03[b]	5.7E −04	2.2E+03[b]	3.3E −04
TRIM	8.0E −01	1.1E+02[a]	7.3E −03	5.5E+02[a]	1.5E −03	1.0E+03[a]	8.0E −04
TC	2.6E −02	9.1E+05[b]	2.9E −08	1.0E+06[b]	2.6E −08	3.6E+06[b]	7.3E −09
OTC	2.1E −02	1.7E+07[b]	1.3E −09	1.9E+07[b]	1.1E −09	8.9E+07[b]	2.4E −10
CTC	3.0E −02	6.5E+07[b]	4.6E −10	7.2E+07[b]	4.1E −10	3.9E+08[b]	7.6E −11
DC	5.1E −02	9.8E+05[b]	5.2E −08	1.1E+06[b]	4.8E −08	3.8E+06[b]	1.3E −08
AMX	1.7E −03	1.0E+04[b]	1.7E −07	1.1E+04[b]	1.6E −07	2.5E+04[b]	6.8E −08
AMP	1.8E −01	3.9E+03[b]	4.6E −05	4.2E+03[b]	4.3E −05	8.9E+03[b]	2.0E −05
CEF	8.2E −01	4.2E+04[b]	1.9E −05	4.6E+04[b]	1.8E −05	1.2E+05[b]	6.8E −06
SUL	2.4E+00	1.7E+04[b]	1.4E −04	1.9E+04[b]	1.3E −04	4.8E+04[b]	5.0E −05

Medium risk indicated by $0.1 \leq RQ \geq 1$ is highlighted in red.
[a]Growth inhibition endpoint.
[b]PNEC predicted with ECOSAR.

of 6 out of 12 antibiotics were above 1 for hospital wastewater samples while 4 out of 12 antibiotics studied had RQs higher than 1 for WSP effluents. In the case of river samples, 5 out of 12 antibiotics found had RQs above 1. However, for irrigation water samples, the RQs of antibiotics above 1 were 4 out of 12 antibiotics studied. The RQs of CIP ranged from 1.5–157.3 and were above 1 for all sources of water while tetracycline's and amoxicillin were below 1 for all water sources.

4. Discussion

In this study, all water bodies were significantly contaminated with antibiotics. The concentrations of antibiotics in rivers impacted by wastewater discharges were up to 3 μg/L whereas concentrations in irrigation water were usually below 0.2 μg/L. These concentrations may affect the environment and potentially also human health since surface water in developing countries like Ghana is used for drinking and food preparation (Obiri-Danso and Jones, 2005). The maximum ciprofloxacin concentrations found in hospital wastewater and rivers were 15 μg/L and 1.2 μg/L, respectively. This is 150 and 12 times higher than the 0.1 μg/L observed to promote antimicrobial resistance (Gullberg et al., 2011). Therefore, consumption of these surface waters investigated could expose people to sub-MIC concentrations, which could lead to antibiotics resistance development in the human gut. This important aspect needs further investigation.

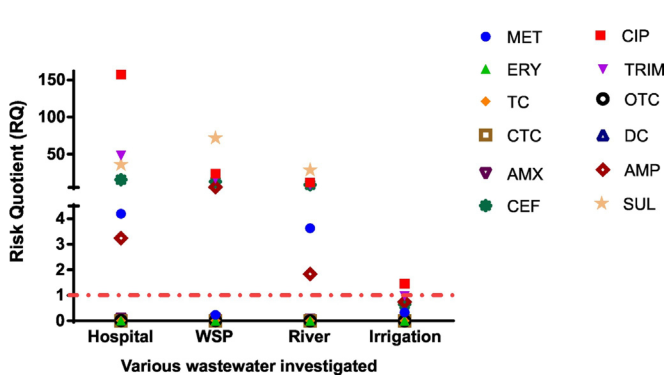


Fig. 4. Estimated risk quotient (RQ) for antibiotic resistance via various water bodies.

The high prevalence of ciprofloxacin found in hospital samples in this study could be due to the high rate of usage at the hospitals. In Ghana, ciprofloxacin is recommended in the national treatment (Kamberi et al., 1999). Due to its availability in oral and intravenous formulations, favorable bioavailability and pharmacokinetics which allows twice–daily treatment it is the most frequently prescribed fluoroquinolone for urinary tract infections (UTIs) (Tagoe and Attah, 2010). In Ghana, the UTI prevalence has been estimated to be in the range 16-57% (Afriyie et al., 2015; Boye et al., 2012; Lutterodt et al., 2014). However, antibiotics like ciprofloxacin, erythromycin and tetracyclines have also been detected in urine samples from individuals in Ghana not treated with antibiotics (Lerbech et al., 2014) suggesting the possibility of unintentional exposure to antibiotics through the environment. Other studies have revealed the misuse of antibiotics in Ghana and mismanagement of antibiotics waste disposal, suggesting the possibility of antibiotics being present in Ghanaian environment (Sasu et al., 2012).

The concentrations of all antibiotics in the downstream river samples were higher than those of upstream and midstream samples. This could be due to untreated sewage sludge being discharged into the stream joining the sampling point downstream. Additionally, there were no significant differences found between the concentrations of 10 out of 12 antibiotics in irrigation water. Actually, the vegetable farms are <40 m from the rivers. In fact, canals have been channeled in most farms to use the river water for irrigation. Interestingly, during the dry season the farms are moved closer to the rivers for proximity to water and when rain set in the whole area is flooded. Consequently, there could be contamination of surface water used for vegetable production.

In this study, ciprofloxacin concentrations in hospital effluent samples were generally lower than concentrations reported in European hospitals such as in Switzerland 0.3–29 μg/L (Alder et al., 2004), in Sweden 3.6–101 μg/L (Lindberg et al., 2004) and in Germany 0.7–125 μg/L (Hartmann et al., 1999; Ohlsen et al., 2003), indicating that the development of antimicrobial resistance may not only be a local issue. A study showed that quinolone antibiotics like ciprofloxacin, may exert genotoxic effects for the genetically modified bacterial strain, *Salmonella enterica* serotype *Typhimurium*, at concentrations as low as 25 μg/L

(Hartmann et al., 1998), further indicating little or no safety margins for this antibiotic.

The β-lactam class of antibiotics is the most frequently prescribed class of antimicrobials in Ghana, particularly amoxicillin, followed by ampicillin and cefuroxime (Tagoe and Attah, 2010). The lowest PNEC level of amoxicillin for *Shigella dysenteriae* derived from the lowest available acute concentration of 50% lethality (LC50, 48 h) has been determined to be 3.7 ng/L (Kümmerer and Henninger, 2003). In this study, amoxicillin was detected in all three WSPs examined, with levels up to 8.5 ng/L and 18 out of 102 WSP samples recorded amoxicillin concentrations that were about two times higher than the amoxicillin PNEC level. These findings suggest that there could be an adverse effect on pathogenic bacteria in the WSPs in Kumasi like *Aeromonas veronii*, *Aeromonas hydrophila*, and *Clostridium perfringens*. There is a need for further investigation to assess these effects.

Applying the risk ranking scale (Hernando et al., 2006), on RQs of all antibiotics calculated for algae, showed ciprofloxacin with RQ of 1.3×10^{-1} could cause median risk to algae in aquatic environment. The rest of the antibiotics would cause minimal risk to the algae, daphnids and fish in the aquatic environment. Comparatively a study focused on the occurrence of pharmaceuticals in water from Pego-Oliva Marsh, Spain, found ciprofloxacin RQ to be 6.9 in algae (Vazquez-Roig et al., 2012). Other studies have reported high RQs in surface water due to the presence of antibiotics in high concentrations including sulfamethoxazole (García-Galán et al., 2011), amoxicillin and oxytetracycline (Jones et al., 2002). This risk evaluation has its limitations, such as the lack of long-term toxicological studies, but on the other hand additive and/or synergistic effects could be expected (Gros et al., 2010), making the real hazard greater than that calculated one for individual antibiotics, since mixture of compounds with the same pharmacological mechanism is present in the water. For example, assuming similar mechanisms and concentration addition for the 4 tetracyclines investigated in the water bodies sampled, the RQs for this group would be 1.4×10^{-7} for algae, 1.3×10^{-7} for daphnids, 3.6×10^{-8} for fish, demonstrating low risks for toxic effects.

Antibiotics resistance due to microbial exposure at sub-MIC concentrations could occur in the study area since Gullberg et al. (2011) found minimum selective concentration (MSC) for antibiotic resistant mutant to be 100 ng/L for ciprofloxacin and 15 μg/L for tetracycline suggesting that there is medium to high risk of antibiotics resistance development in the environment. The estimated RQs for ciprofloxacin were above 1 for all sources of water and demonstrate that ciprofloxacin resistance development, not toxic effects, should be the major concern with antibiotics in aquatic environments. This finding may be due to the fact that ciprofloxacin is the most prescribed UTI drug in Ghana (Lutterodt et al., 2014).

The fact that antibiotic levels several hundred-fold below the MIC of the susceptible strains can select resistant bacteria means that the sub-MIC selective window is much larger than the traditional selective window (Andersson and Hughes, 2014). Further work would be required to determine the extent of exposure and the risk associated. Since these point sources of antibiotics in Ghana are discharged into streams and nearby rivers used for farming, there is a need to investigate the possibility of contamination of food thereby exposing vegetable consumers to antibiotics.

Result revealed that, even simple technology such as WSPs significantly reduces antibiotic loads with up to 96%. This result suggests sewage sludge as the main reservoir of antibiotics, due to their strong sorption properties (Boxall et al., 2002; Kümmerer, 2009; Tolls, 2001), and indicates the importance of sludge management strategies to avoid most of the human-excreted antibiotics from entering the environment through sludge disposal. Various studies have reported the occurrence and concentration of antibiotics in sewage sludges (Alder et al., 2004; Gao et al., 2012; Giger et al., 2003). The potential hazards of sewage sludge with such high levels of antibiotics include bacterial toxicity in sewage sludge and soil (Halling-Sørensen et al., 2002), and formation of resistance in microorganisms (Al-Ahmad et al., 1999) promoted by exposure of the microbes to sub-MIC doses of antibiotics (Gavalchin and Katz, 1994). The antibiotics removal processes which have been reported in WSPs include settling (sorption), biodegradation, hydrolysis and photolysis (Bendz et al., 2005; Castiglioni et al., 2006; Gao et al., 2012; Møller et al., 2015; Verlicchi et al., 2012). However, the major removal processes identified in WSPs in Tanzania was settling (Møller et al., 2015) which could possibly be the major removal processes occurring in the WSPs considered in this study. This could be due to the similar WSPs design and climatic conditions found in both countries. Hospital effluent was the main point source of impact. In fact, the relative contribution to antibiotics discharged into the environment in the present study was 94% discharged through the hospital effluents whereas the WSP effluents only contributed with 6%. As antibiotics are often associated with hospital wastewater (Kümmerer et al., 2000; Watkinson et al., 2009), it was not surprising that higher concentrations of all the 12 antibiotics studied were reported in KATH wastewater. The wastewater from KATH flows directly into surface water, and is therefore a major source of introduction of antibiotics to the environment. Clearly, local point sources such as hospitals should not be built without also building at least a WSP system to go with it, not forgetting advance types of wastewater treatment works which could provide better removal efficiencies.

The levels of antibiotics in lettuce reported in this study were in the ng/kg fresh weight range. These low levels could be anticipated due to low environmental concentrations determined in the irrigation water. Also, the low uptake of antibiotics by plants would affect the levels found in plants. The uptake of antibiotics by plants mostly depends on soil pore water properties, soil properties, the concentration of antibiotics in wastewater applied for irrigation and physicochemical properties of the antibiotics in question (Christou et al., 2017; Wu et al., 2014; Zhang et al., 2016). Earlier studies have shown that plants take up <2% of the pharmaceuticals applied to soil (Dolliver et al., 2007; Kumar et al., 2005). In a tetracycline and amoxicillin uptake study carried out in Ghana, the lettuce and carrot plants took up approximately 0.02% of the antibiotics applied (Azanu et al., 2016). Boxall et al. (2006) found that the total accumulation of sulfamethazine in plant tissue after 45 days of growth was <0.1% of the amount applied to soil via manure. Antibiotics concentrations in plants reported so far in literature are uptake studies with spiked concentrations mostly higher than what have been reported in the environment (Dolliver et al., 2007). Antibiotics in lettuce plant were mostly found in Chirapatre sampling area, which also showed high concentration of most antibiotics in irrigation water.

Tetracyclines and amoxicillin were not detected in any of the lettuce samples. Similar finding was observed when uptake of TCs into pinto beans and coconut trees was studied during direct application (Batchelder, 1982; McCoy, 1976). Furthermore, Boxall et al. (2006), detected no amoxicillin, when uptake studies with 1 mg/kg amoxicillin in manure was performed. In contrast, greenhouse uptake studies of antibiotics by plants revealed that up to 37 ng/g tetracycline was taken up by lettuce and carrot when irrigated with water spiked with tetracycline at levels up to 15 mg/L for 30 days (Azanu et al., 2016). However, these exposure levels were higher than the levels found in actual irrigation water used by farmer in the study area sampled (16 ng/L) in this present study. This may be the major reason for not detecting tetracycline in the lettuce samples. Additionally, tetracyclines are strongly adsorbed to soil due to their high sorption coefficient (Boxall et al., 2006; Tolls, 2001), hence less bioavailable for plant uptake.

The mean concentration of all antibiotics found in the market samples was 23 ± 13 ng/kg, and the average intake of all antibiotics studies for a standard 60 kg woman would then be around 0.3 ng/day (Table 3). Prosser and Sibley (2015), calculated ADI values for toxicity based on the lowest daily therapeutic dose and a safety factor of 1000 for ERY to be 4.4 μg kg^{-1} body weight day^{-1} and SUL to be 5.7 μg kg^{-1} body weight day^{-1}. The EDIs for these antibiotics during lettuce consumption are consequently much lower than their respective ADIs. However, antibiotic resistance development, not toxicity is the major concern related to antibiotics.

It has been shown that antibiotics concentrations far below the MIC can select for antibiotic resistance (Gullberg et al., 2011; Sandegren, 2014). This study indicates a low risk for antibiotics resistance development, from lettuce consumption. The possible reasons could be due to the low occurrence of antibiotics in lettuce and due to the extremely low antibiotic intake via lettuce on a daily basis. Consequently, the occurrence of antibiotics in urine in persons in Ghana that have not taken any antibiotics (Lerbech et al., 2014) can most likely not be explained from lettuce consumption or vegetables consumption in general. This study therefore proffers that other food sources may be responsible for the unintentional exposure of Ghanaians to antibiotics. In particular meat such as chicken and beef, since large amounts of antibiotics is well known to be used in livestock for meat production (Adzitey, 2013; Landers et al., 2012; Mubito et al., 2014; Nonga et al., 2009; Nonga and Muhairwa, 2010). Also, herbal medicines, widely used in LMICs, may be a source to unintentional exposure to antibiotics, due to adulteration of herbal medicines with antibiotics (Ernst, 2002). The present study revealed that there are substantial levels of antibiotics in wastewater of the WSPs studied and it is possible that fish produced by aquaculture could accumulate or become contaminated with these antibiotics. Since these WSPs are used for aquaculture (Darko et al., 2016; Tenkorang et al., 2012), these fish could also be an important contributor to antibiotics residues ingestion.

5. Conclusion

In the present study, the 12 antibiotics investigated were found at levels above their respective LOD's in all water bodies indicating substantial pollution of freshwater resources in Ghana with antibiotics. Sulfamethoxazole, erythromycin, ciprofloxacin, cefuroxime and trimethoprim were the most frequently detected antibiotics in all water samples, hence could also be considered as or add to the list of potential critical compounds from an environmental risk point of view in Ghana. Based on classical risk assessment, ciprofloxacin may cause median risk whereas the rest of the antibiotics would cause minimal risk to aquatic organisms. However, antibiotics concentrations found in water bodies in this study could cause medium to high risk of antibiotics resistance development in the environment and perhaps also in humans if surface water is used as drinking water. However, simple technology such as WSPs could significantly reduce antibiotic loads in wastewater. Low quality water used for vegetable irrigation was found to contain antibiotics up to 0.2 μg/L, and the present study demonstrates consumer antibiotic exposure through food of plant origin, but the proportion of antibiotics taken up by plans such as lettuce is low. Thus, the daily exposure to antibiotics via lettuce, and perhaps vegetables in general, is low and the associated risks may therefore also be low. For food safety reasons, it needs to be investigated in further research, whether low levels of antibiotics in food plants can contribute to development of bacterial resistance in the human gut.

Acknowledgement

The project was funded by Danida, the Ministry of Foreign Affairs of Denmark under the Safe Water for Food (SaWaFo) program, grant number; 11-058 DHI. We gratefully acknowledge late Professor Emeritus Sven Erik Jørgensen for providing valuable input to the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.11.287.

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