Water Air Soil Pollut (2022) 233:405
https://doi.org/10.1007/s11270-022-05875-4

Occurrence and Risk Assessment of Antibiotic Residues in Sewage Sludge of Two Nigerian Hospital Wastewater Treatment Plants

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Received: 18 March 2022 / Accepted: 21 September 2022 / Published online: 1 October 2022

Abstract Antibiotic residues in sewage sludge can present detrimental environmental effects due to sewage sludge application onto soils for agricultural purposes. Reports on the occurrence of antibiotics in sewage sludge and risk assessment due to the application of sewage sludge onto soils are still limited in Africa. The occurrence of fourteen antibiotic residues in sewage sludge from two Nigerian hospital wastewater treatment plants was investigated. For the first time, the potential environmental risk of target antibiotics associated with the use of sewage sludge for application onto soils in Nigeria was assessed. Risk assessment was carried out using both terrestrial and aquatic toxicity data. All target antibiotics were detected in at least one sludge sample. Ciprofloxacin and ofloxacin (fluoroquinolones) had the highest concentrations, up to 674 ng g^{-1} dry weight for ciprofloxacin. All ten antibiotics evaluated for terrestrial ecological risk in sludge-amended soils presented low risk. Only three out of thirteen antibiotics assessed for aquatic ecological risk in sludge-amended soils posed medium risk, while the remaining antibiotics presented low risk. Antibiotic mixtures presented low risk in sludge-amended soils. A more holistic evaluation of the potential risks due to a mixture of a wider scope of chemicals in Nigerian sewage sludge is recommended prior to application onto soils as fertilizers for agricultural purposes.

Keywords Pharmaceuticals · Antibiotics · Sewage sludge · Sludge-amended soil · Risk assessment · Emerging contaminants

1 Introduction

Globally, antibiotics are consumed extensively in human medicine, veterinary medicine, and aquaculture for the purpose of preventing or treating microbial infections (Gworek et al., 2021; Kümmerer, 2009). It was reported that the global human consumption of antibiotics increased by 65% from 2000 to 2015 and may rise by 200% in 2030 if the same trend of consumption continues (Klein et al., 2018; Scaria et al., 2021). A major concern about the release of antibiotics into the environment is the selection of antimicrobial resistance, which may reduce the therapeutic potential of antibiotics against human and animal pathogens (Felis et al., 2020; Kümmerer, 2009). This concern is more worrisome

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-022-05875-4.

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in developing countries like Nigeria which usually have a combination of poor practice for prescription and unregulated over-the-counter sale of antibiotics (Oduyebo et al., 2017). Overprescription or overuse of antibiotics, among many other classes of drugs, is a problem in most Nigerian hospitals (Fowotade et al., 2020; Sodipo et al., 2017). The rates of antibiotics prescription in Nigeria have been found to considerably outweigh the estimated world average (Fowotade et al., 2020). Moreover, among the different therapeutic classes of pharmaceuticals (such as nonsteroidal anti-inflammatory drugs, anticancers, and antidiabetics), the primary risk to soil and aquatic ecosystems is from antibiotics (Bourdat-Deschamps et al., 2017). Therefore, antibiotics are our focus in the present study.

In keeping with the zero waste strategy aimed at minimizing the amount of generated waste and to promote the development of circular economy and bioeconomy, sewage sludge is increasingly being reused (Buta et al., 2021; Song et al., 2015). Thus, sewage sludge has found wide application as fertilizer for agricultural purposes, and one of the main sources of releasing antibiotics into the environment is sewage sludge introduced into soils for fertilizing purposes (Gworek et al., 2021). While the use of sewage sludge to add nutrients and organic matter could be beneficial for the soil, it also represents a risk due to the presence of contaminants like pharmaceuticals, other organic molecules, heavy metals, and pathogens (Lamastra et al., 2018). These antibiotics in sewage sludge may pose an environmental risk to terrestrial and aquatic ecosystems. Zhao et al. (2022) recently reported that in an agricultural land with long-term manure application of 10–80 t ha^{-1} yearly, multi-class antibiotics of total concentration between 15.45 ng g^{-1} and 327 ng g^{-1} in soil can reduce crop yields by modifying soil bacterial communities and earthworm populations. However, some other studies have found low exposure risk to soil fauna at environmentally relevant concentrations (Baguer et al., 2000; Pino et al., 2015). The effects induced by the mixture of antibiotics have been emphasized to be significantly greater than the effect of individual antibiotic compounds (additive or synergistic effects) (Felis et al., 2020; Thiele-Bruhn, 2019), although the mixture of antibiotics having different modes of action (bactericidal or bacteriostatic) has been found to have antagonistic effects (Ocampo et al., 2014; Thiele-Bruhn, 2019). It should be mentioned that the selective pressure is not only linked to the presence of antibiotics but also to the presence of trace metals, for example. It is therefore important to evaluate the potential environmental risk of individual antibiotics and antibiotic mixtures in sewage sludge and sludge-amended soils.

In wastewater treatment plants (WWTPs), large amounts of pharmaceuticals are sorbed to solids of sewage sludge. Consequently, antibiotics have been detected and quantified in sewage sludge worldwide (An et al., 2015; Arun et al., 2020; Aydin et al., 2022; Cheng et al., 2014; Dorival-García et al., 2015; Göbel et al., 2005; Lindberg et al., 2005; Muriuki et al., 2020; Thomaidi et al., 2016). Sorption of pharmaceuticals to sewage sludge is complex, involving factors such as hydrophobic, hydrogen-bonding, and charge-related interactions (Berthod et al., 2017). Some antibiotics, especially hydrophobic antibiotics with limited mobility and low biodegradation, are prone to adsorption onto sewage sludge and are more stable in sludge than in wastewater (Harrower et al., 2021). Hence, sewage sludge can serve as an important sink for antibiotics in wastewater treatment. The pollution levels of antibiotics can, therefore, be assessed by their concentration profiles in sewage sludge.

The issues associated with environmental contamination of antibiotics via sewage sludge from Nigerian WWTPs are important and urgently need to be addressed. One of the most common fates of sewage sludge in Nigeria is land application for agricultural purposes (Ajibola et al., 2021a). To date, scientific data regarding the contamination of sewage sludge by antibiotic compounds and associated environmental risks due to the disposal of sewage sludge in Africa, including Nigeria, are still limited. It is therefore crucial to determine the environmental levels of antibiotic compounds in sewage sludge from Nigerian WWTPs in order to estimate the potential environmental risks of sewage sludge through land application for agricultural purposes. Wastewater from hospital is widely known as a point source of antibiotics in the environment (Verlicchi et al., 2012; Ajibola et al. 2012b). Hence, residual sewage sludge in Nigerian hospital WWTPs is likely to contain these antibiotics.

The objectives of the present study were to investigate the occurrence of fourteen antibiotics (two tetracyclines, three fluoroquinolones, three macrolides,

four sulfonamides, trimethoprim, and thiamphenicol) in sewage sludge from two Nigerian hospital WWTPs and to assess the potential environmental risk associated with the use of sewage sludge for land application in agricultural practices in Nigeria. Sewage sludge samples were collected from two Nigerian hospital WWTPs and analyzed for fourteen antibiotics. A risk quotient approach using environmental and predicted no-effect concentrations was employed for assessing the potential environmental risk associated with the occurrence of individual antibiotics in sewage sludge. The potential threats of individual antibiotics and antibiotic mixtures in sludge-amended soils were also investigated. To our knowledge, this is the first study from Nigeria to evaluate the environmental risk of antibiotics in sewage sludge for land application onto soils for agricultural purposes.

2 Materials and Methods

2.1 Chemicals and Reagents

Analytical standards of all target antibiotics were of high purity and were purchased from Sigma Aldrich (Steinheim, Germany). Methanol, acetonitrile, water, and formic acid (all of LC–MS grade) were obtained from Fischer Chemical. Trisodium citrate dihydrate (Na_3Cit), disodium citrate sesquihydrate (Na_2Cit), and $Na_2EDTA.2H_2O$ were obtained from Sigma Aldrich (Steinheim, Germany). Sodium chloride, 99.5% for analysis, and anhydrous $MgSO_4$ were obtained from ACROS Organics™. Primary and secondary amine (PSA) bulk sorbent was purchased from Supelco (Bellefonte, USA). Syringe filters (0.2-mm PTFE Agilent) were obtained from Agilent. Stock standard solution (1000 mg L^{-1}) of individual target antibiotic was prepared in methanol except ciprofloxacin which was prepared in methanol and Milli-Q water (50:50). A working solution containing all target antibiotics was prepared in acetonitrile with a concentration of 50 mg L^{-1}. Stock and working solutions were stored in the freezer at − 20 °C.

2.2 Sample Collection and Pretreatment

Sewage sludge samples were collected from two hospital wastewater treatment plants (WWTPs) in Ibadan and Lagos, South-Western Nigeria: University College Hospital (UCH) WWTP in Ibadan and Ijaiye WWTP in Lagos. UCH is a university teaching hospital, and there are some staff residential quarters and students' hostels within the hospital which is an indication that domestic wastewater may contribute to the wastewater stream at UCH WWTP. Details about the characteristics and operational parameters of the wastewater treatment plants have been presented in previous papers (Ajibola et al., 2020, 2021b). Sewage sludge samples were collected in September 2017. Primary and secondary sludge samples were collected from UCH WWTP during five consecutive days (September 11, 2017, to September 15, 2017), while secondary sludge samples were collected from Ijaiye WWTP during four consecutive days (September 5, 2017, to September 8, 2017). An approximate 2 L of sludge sample which comprises three grab samples that were pooled together was collected daily from each WWTP. Amber glass bottles were used for the collection of sewage sludge samples. The sludge samples were transported into the laboratory in an insulated box (cooler) with ice and kept in the freezer at − 20 °C. The samples were air-dried, ground, sieved, wrapped with aluminum foil, and stored in the freezer before transportation to the Centre of Applied Geosciences, University of Tübingen, Germany, for analyses.

2.3 Extraction and Cleanup

Extraction of target antibiotics from sewage sludge was carried out using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction protocol according to Ajibola et al. (2020). Briefly, 1-g homogenized and dried sewage sludge sample was weighed into a 50-mL centrifuge tube. Two hundred µL of LC–MS grade water was added. The sample was stirred vigorously and placed in darkness overnight. Extracting solvents 10-mL 0.2-M Na_2EDTA (in water), 8-mL acetonitrile, and 2-mL methanol were added to the sample in succession. The sample was vortex-mixed for 15 s followed by the addition of citrate buffer salt (consisting of 4-g $MgSO_4$, 1.0-g NaCl, 1.0-g Na_3Cit, and 0.5-g Na_2Cit). The centrifuge tube was shaken immediately and vortex-mixed for 1 min. The sample was ultrasonicated for 10 min and centrifuged at 3500 rpm for 10 min.

The cleanup of QuEChERS extract was carried out by dispersive solid-phase extraction (d-SPE).

Five mL of supernatant organic phase was transferred into a 15-mL centrifuge tube containing 200-mg $MgSO_4$ and 150-mg PSA sorbent. The mixture was shaken manually, vortex-mixed for 1 min, and centrifuged at 1500 rpm for 5 min. The supernatant was decanted, filtered with 0.2-mm PTFE syringe filter (Agilent) into a glass vial, and evaporated to dryness under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted with 0.5 mL of a mixture of acetonitrile and water (0.1% formic acid), 5:95 (v/v), and transferred into an autosampler vial for LC–MS/MS analysis.

2.4 LC–MS/MS Analysis

Analysis of target antibiotics was performed by liquid chromatography-tandem mass spectrometry using the method of Ajibola et al. (2020). Briefly, the chromatographic analysis was performed using a 1290 Infinity HPLC system (Agilent Technologies, Germany) coupled to 6490 triple quadrupole mass spectrometer with Agilent Jet Stream Technology (Agilent Technologies, Germany). Chromatographic separation was achieved on a Poroshell EC-C18 column (100 mm × 2.1 mm, 2.7 µm, Agilent Technologies, Germany), protected by a Poroshell EC-C18 guard column (2.1 × 5 mm, 2.7 µm, Agilent Technologies, Germany). A gradient elution program using ultrapure water (containing 0.1% formic acid) and acetonitrile (containing 0.1% formic acid) was used. Using electrospray ionization source in both positive and negative modes by fast polarity switching, MS/MS analysis of target antibiotics was achieved in multiple reaction monitoring (MRM) mode.

2.5 Quality Assurance and Quality Control

Procedural blank samples were analyzed for every batch of 10 samples. Mobile phase solvent was also injected into the LC–MS/MS instrument to check for the presence of target antibiotics and cross contamination. Triplicate determinations were carried out for all sludge samples to avoid false positives or negatives. Recovery was carried out by comparing the peak area of each compound in sludge sample spiked before extraction to the peak area in sludge extract spiked after extraction. Quantification of target antibiotics was carried out by matrix-matched calibration (pre-extraction spikes). Details of analytical methodology and quality assurance have been reported in our previous paper (Ajibola et al., 2020). Recoveries at 500 ng g^{-1} spiking level ranged from 4 to 125%; method limits of detection (LODs) ranged from 0.003 to 120.39 ng g^{-1}, while method limits of quantification (LOQs) ranged from 0.01 to 364.81 ng g^{-1}.

2.6 Toxicity Data Collection

The potential ecological risk for land application of sewage sludge from two Nigerian hospital WWTPs onto soil for agricultural purposes was evaluated. For this purpose, toxicity data for terrestrial organisms including soil invertebrates (earthworms, springtail, wasps) were collected from literature, when available. Toxicity data of target antibiotics to plants (root elongation, seed germination), bird (mallard duck and northern bobwhite), and soil microbial respiration were also collected from literature. The terrestrial ecotoxicological data are presented in Table S1 (Supplementary material). However, there are scanty data on the toxicity of target antibiotics to terrestrial organisms in literature. To this end, we also searched for aquatic toxicity data for the target antibiotics. Aquatic toxicity data were collected for three groups of organisms (fish, daphnia, and algae) and are presented in Table S2 (Supplementary material). We used the lowest toxicity value out of the three values for each antibiotic to estimate the worst-case scenario.

2.7 Ecological Risk Assessment of Target Antibiotics in Sludge-Amended Soil

Predicted no-effect concentration (PNEC) of antibiotic based on terrestrial toxicity data ($PNEC_{soil\text{-}terrestrial}$) was calculated by dividing EC_{50}/ LC_{50}/ NOEC value (mg/Kg) by a suitable assessment factor as presented in Table S1 (Supplementary material). The assessment factors (AFs) are based on the recommendation of the European Medicine Agency (EMEA, 2006) as adopted by Bourdat-Deschamps et al. (2017): the AF is 10 for data from chronic toxicity tests, for NOEC from acute toxicity tests, or from EC_{50} from microbial

activity tests; AF is 50 for EC_{50} or NOEC from plant growth tests, and AF is 1000 if EC_{50}/ LC_{50} are available for one tested organism.

$PNEC_{\text{soil-terrestrial}}$ was derived using Eq. (1), and the values are provided in Table S1 (Supplementary material).

$$\text{PNEC(soil} - \text{terrestrial)} = \frac{\text{EC50 or LC50 or NOEC}}{\text{Assessment factor}} \quad (1)$$

Due to the scarcity of terrestrial toxicity data of pharmaceuticals, some authors also used the aquatic toxicity data to estimate $PNEC_{\text{soil}}$ through the equilibrium partition approach (Arun et al., 2020; Aydin et al., 2022; Martin et al., 2012a; Mejías et al, 2021; Thomaidi et al., 2016; Verlicchi & Zambello, 2015). Therefore, in this work, potential ecotoxicological risk of some target antibiotics was also evaluated based on aquatic toxicity data (*PNEC*water) for calculating ($PNEC_{\text{soil-aquatic}}$), using the equilibrium partition method (Eq. 2) according to the European guidance on information requirements and chemical safety assessment (ECHA, 2008, Chapter R.10):

$$\text{PNEC(soil} - \text{aquatic)} = \frac{\text{Ksoil.water}}{\text{RHOsoil}} \times \text{PNECwater} \times 1000 \quad (2)$$

where $K_{\text{soil.water}}$ is soil–water partition coefficient and RHO_{soil} is the bulk density of wet soil (1700 kg m^{-3}). $K_{\text{soil.water}}$ was calculated using Eq. (3) according to the European Chemical Agency (ECHA, 2016, R.16):

$$\text{Ksoil.water} = Fair_{\text{soil}} \times K_{\text{air-water}} + Fwater_{\text{soil}} + Fsolid_{\text{soil}} \times \frac{\text{Foc(soil)Koc}}{1000} \times RHO_{solid} \quad (3)$$

where $Fair_{\text{soil}}$ represents the volume fraction air in soil (0.2), $K_{\text{air-water}}$ is the air–water partition coefficient (zero for nonvolatile substances), $Fwater_{\text{soil}}$ represents the volume fraction water in soil (0.2), $Fsolid_{\text{soil}}$ is the fraction solid in soil (0.6), *Foc(soil)* represents the weight fraction organic carbon in soil (0.02), *Koc* is the organic water partition coefficient (collected from literature and presented in Table S3 (Supplementary material)), and RHO_{solid} is the density of solid phase (2500 kg m^{-3}). Substituting all the default values in brackets into Eq. (3), $K_{\text{soil.water}}$ can be calculated. If the calculated $K_{\text{soil.water}}$ is substituted for $K_{\text{soil.water}}$ in Eq. 2, the equilibrium partition equation can be simplified into Eq. (4):

$$\text{PNEC(soil} - \text{aquatic)} = (0.1176 + 0.01764 \text{ X Koc}) \text{ X PNECwater} \quad (4)$$

where *Koc* is the organic carbon partition coefficient of antibiotic (as L Kg^{-1}) collected from literature and presented in Table S3 (Supplementary material). *PNEC*soil-aquatic values for target antibiotics are provided in Table S4 (Supplementary material). However, the equilibrium partition method can result in overestimation or underestimation of toxicity and should only be considered for identifying substances requiring further testing on soil organisms.

For the assessment of sewage sludge for land application in agricultural purposes (sludge-amended soil), predicted environmental concentrations of target antibiotics in soil (PEC_{soil}) were used and were calculated by using the following equation (Eq. 5) according to Ghirardini et al. (2020) as recommended by the European Technical Guidance Document on risk assessment (EC-TGD, 2003):

$$\text{PEC(soil)} = \text{Co(soil)} + \frac{\text{MEC(sludge) X APP(sludge)}}{\text{DEPTH(soil) X RHO(soil)}} \quad (5)$$

where *Co(soil)* is the background concentration of antibiotic in the soil before application of sludge (was assumed to be zero in this study), *MEC(sludge)* represents the maximum measured concentration in secondary sludge (μg Kg^{-1}), *DEPTH(soil)* is the mixing depth of 0.2 m generally used for agricultural soil, *RHO(soil)* is the bulk density of wet soil (1700 kg m^{-3}), and *APP(sludge)* is the typical application rate of dry sludge onto soil which is 0.5 kg m^{-2} generally used for agricultural soil. We are not aware of any regulatory limit for the application rate of sewage sludge onto soils in Nigeria. But there are some recommendations from scientific research ranging from 0.2 to 2.0 kg m^{-2} (Kuti et al., 2018; Onweremadu et al., 2007). We used application rate of 0.5 kg m^{-2} in this study since it falls within the recommended range and it is the typical application rate used generally for agricultural soil in some other studies (Martin et al., 2012b; Mejías et al., 2021; Thomaidi et al., 2016; Verlicchi & Zambello, 2015). Calculated *PEC(soil)* values of

Table 1 Maximum measured environmental concentration (MEC) in sludge and predicted environmental concentration (PEC) of antibiotics in sludge-amended soil

Compound	*MEC* (ng g^{-1})			PEC_{soil} (ng g^{-1})		
	UCH primary sludge	UCH secondary sludge	Ijaiye secondary sludge	UCH primary sludge-amended soil	UCH secondary sludge-amended soil	Ijaiye secondary sludge-amended soil
Azithromycin	39.92	374.6	255.15	0.059	0.551	0.3752
Ciprofloxacin	64.9	399.5	674.04	0.095	0.588	0.9912
Clarithromycin	10.4	9	5.44	0.015	0.013	0.0080
Erythromycin	21.3	23.5	nd	0.031	0.035	nc
Norfloxacin	8.38	8.38	8.38	0.012	0.012	0.0123
Ofloxacin	165.7	350	387.97	0.244	0.515	0.5705
Oxytetracycline	364.81	364.81	364.81	0.536	0.536	0.5365
Sulfadimethoxine	0.09	0.09	22.26	0.00013	0.00013	0.0327
Sulfadoxine	3.6	2.3	nd	0.005	0.003	nc
Sulfamethazine	0.03	1.6	0.03	0.00004	0.002	0.00004
Sulfamethoxazole	0.04	0.04	0.04	0.00006	0.00006	0.00006
Tetracycline	29	287.2	310.19	0.043	0.422	0.4562
Thiamphenicol	nd	85.65	nd	nc	0.126	nc
Trimethoprim	17	8.8	0.01	0.025	0.013	0.00001

nd, not detected; nc, not calculated because compound was not detected in sludge

target antibiotics ranged from 0.00001 (for trimethoprim) to 0.9912 ng g^{-1} (for ciprofloxacin) and are provided in Table 1.

Risk quotient of target antibiotics in sludge-amended soil for terrestrial organisms ($RQ_{soil\text{-}terrestrial}$) was calculated as the ratio between the predicted environmental concentration in soil (PEC) and the predicted no-effect concentration (PNEC) derived from terrestrial toxicity data ($PNEC_{soil\text{-}terrestrial}$) according to Eq. (6) (Thomaidi et al., 2016).

$$\mathrm{RQ(soil-terrestrial)} = \frac{\mathrm{PEC(soil)}}{\mathrm{PNEC(soil-terrestrial)}} \quad (6)$$

Risk quotient (RQ) of target antibiotics in sludge-amended soil based on aquatic toxicity data ($RQ_{soil\text{-}aquatic}$) was calculated by using Eq. (7):

$$\mathrm{RQ(soil-aquatic)} = \frac{\mathrm{PEC(soil)}}{\mathrm{PNEC(soil-aquatic)}} \quad (7)$$

The criteria for evaluating the risk were based on those commonly used: a high risk if $RQ \geq 1$, medium risk if $0.1 < RQ < 1$, and low risk if $RQ \leq 0.1$ (Gros et al., 2019; Verlicchi & Zambello, 2015).

2.8 Risk Assessment of Antibiotic Mixtures in Sludge-Amended Soil

To evaluate the potential risk posed by antibiotic mixtures in soil, it was proposed to combine the lowest PNECs for single compounds and mixtures with the highest available MECs (Thiele-Bruhn, 2019). Because PNECs of mixtures are not available, Thiele-Bruhn (2019) proposed the calculation of a weighted PNEC for each specific mixture (*PNECmix*) that depends on the relative contribution of the individual compounds and their individual PNECs to the overall mixture (Eq. 8):

$$\mathrm{PNECmix} = \sum_{i=1}^{n} (\mathrm{PNECi} \times \frac{\mathrm{PECi}}{\sum \mathrm{PECi}}) \quad (8)$$

where *PECi* is the predicted environmental concentration of individual antibiotic, PNECi is corresponding predicted no-effect concentration of individual antibiotic (calculated from aquatic toxicity data in this study), *ΣPECi* is the sum of individual concentrations of all evaluated antibiotics, and *PNECmix* is the sum of predicted no-effect concentrations of target antibiotics, calculated from aquatic toxicity data ($PNEC_{soil\text{-}aquatic}$) (Table S4, Supplementary material).

Table 2 Concentration profiles (ng g^{-1}) of target antibiotics in sewage sludge[a]

Compound	MLOQ (ng g^{-1})	UCH WWTP								Ijaiye WWTP			
		Primary sludge				Secondary sludge				Secondary sludge			
		Mean	Min	Max	Freq (%)	Mean	Min	Max	Freq (%)	Mean	Min	Max	Freq (%)
Azithromycin	39.92	<LOQ	<LOQ	<LOQ	100	220.3	98.9	374.6	100	255.2	<LOQ	255.2	100
Ciprofloxacin	0.10	52.2	<LOQ	64.9	100	333.2	<LOQ	399.5	100	385.7	112.03	674.0	100
Clarithromycin	0.05	7.0	3.8	10.4	100	6.6	4.1	9.0	100	5.4	nd	5.4	33
Erythromycin	11.96	19.8	18.1	21.3	100	19.8	16.4	23.5	100	nd	nd	nd	0
Norfloxacin	8.38	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	100
Ofloxacin	0.05	95.2	<LOQ	165.7	100	168.3	59.0	350.0	100	276.9	152.68	388.0	100
Oxytetracycline	364.81	<LOQ	nd	<LOQ	50	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	100
Sulfadimethoxine	0.09	<LOQ	<LOQ	<LOQ	100	<LOQ	nd	<LOQ	75	21.3	19.73	22.3	100
Sulfadoxine	0.02	2.6	nd	3.6	75	2.2	nd	2.3	75	nd	nd	nd	0
Sulfamethazine	0.03	<LOQ	<LOQ	<LOQ	100	1.6	<LOQ	1.6	100	<LOQ	<LOQ	<LOQ	100
Sulfamethoxazole	0.04	<LOQ	<LOQ	<LOQ	100	<LOQ	nd	<LOQ	75	<LOQ	<LOQ	<LOQ	100
Tetracycline	13.78	25.7	nd	29.0	50	114.6	5.9	287.2	100	230.1	179.58	310.2	100
Thiamphenicol	85.65	nd	nd	nd	0	<LOQ	nd	<LOQ	25	nd	nd	nd	0
Trimethoprim	0.01	14.8	<LOQ	17.0	100	3.5	0.8	8.8	100	<LOQ	<LOQ	<LOQ	100

[a]Data for the first day of sampling for both WWTPs have been reported in Ajibola et al. (2020) and therefore were not included in the results presented herein

Freq, frequency of detection; LOQ, method limit of quantification; nd, not detected

Corresponding RQ of the mixture (*RQmix*) was then calculated using Eq. (9) according to Thiele-Bruhn (2019):

$$\mathrm{RQmix} = \sum_{i=1}^{n} \left(\frac{\mathrm{PECi}}{\mathrm{PNECmix}}\right) \quad (9)$$

where PEC*i* is the predicted environmental concentration of individual antibiotic and *PNECmix* is the sum of predicted no-effect concentrations of target antibiotics, calculated from aquatic toxicity data ($PNEC_{\text{soil-aquatic}}$) (Table S4, Supplementary material).

3 Results and Discussion

3.1 Occurrence of Antibiotics in Sewage Sludge

The concentration profiles of target antibiotics in sewage sludge from two Nigerian hospital WWTPs (UCH and Ijaiye) are presented in Table 2. Data for the first day of sampling for both WWTPs have been reported in our previously published paper on analytical methodology (Ajibola et al., 2020) and therefore were not included in the results presented herein (Table 2).

3.1.1 Macrolides

All three macrolides (azithromycin, clarithromycin, and erythromycin) were detected in sewage sludge from the two investigated hospital WWTPs. Frequency of detection was 100% for azithromycin in all sewage sludge samples and also 100% for clarithromycin and erythromycin in UCH sludge samples (primary and secondary), but erythromycin was not detected in Ijaiye sludge samples. Azithromycin concentrations were <LOQ in UCH primary sludge, whereas mean concentrations of 220.3 ng g^{-1} and 255.2 ng g^{-1} were measured for azithromycin in UCH secondary sludge and Ijaiye sludge, respectively. Erythromycin was quantified up to 21.3 ng g^{-1} and 23.5 ng g^{-1} in UCH primary sludge and UCH secondary sludge, respectively. Erythromycin was not detected in Ijaiye sludge. Clarithromycin concentrations ranged from 3.8–10.4 ng g^{-1} in UCH primary sludge and 4.1–9.0 ng g^{-1} in UCH secondary sludge, whereas a concentration of 5.4 ng g^{-1} was determined in Ijaiye sludge. The measured high concentrations of azithromycin compared to clarithromycin and erythromycin in secondary sludge indicate that the prescription rate of azithromycin was

probably higher than that of clarithromycin or erythromycin at both Ijaiye and UCH hospitals during the period of sampling. Moreover, among the macrolides, azithromycin has higher potential for sorption onto sludge than either clarithromycin or erythromycin based on their Log K_{ow} and Log K_d values (Table S3 (Supplementary material); Verlicchi & Zambello, 2015). Similar concentration levels of azithromycin to the present study were reported in sludge samples from WWTPs in Greece (Gago-Ferrero et al., 2015; Thomaidi et al., 2016) and higher levels of azithromycin in sludge from Switzerland (Göbel et al., 2005), while clarithromycin was reported in higher amounts than in the present study (Aydin et al., 2022; Gago-Ferrero et al., 2015; Göbel et al., 2005; Thomaidi et al., 2016).

3.1.2 Fluoroquinolones

All target fluoroquinolones (ciprofloxacin, norfloxacin, and ofloxacin) were detected in all sewage sludge samples from both hospital WWTPs. The frequency of detection was 100% for all three fluoroquinolones, indicating their frequent usage at the two hospitals. The highest concentrations of ciprofloxacin (674 ng g^{-1}) and ofloxacin (388 ng g^{-1}) were found in Ijaiye sludge. Norfloxacin was detected at <LOQ in UCH (primary and secondary) and Ijaiye sludge samples, implying probably less application or usage of norfloxacin at the two hospitals in comparison with the other two fluoroquinolones. Even higher levels of these fluoroquinolones were detected in sewage sludge in Switzerland (Golet et al., 2002), Sweden (Lindberg et al., 2005), China (Cheng et al., 2014; Huang et al., 2013), India (Arun et al., 2020), and Kenya (Muriuki et al., 2020). Similar or lower concentrations of target fluoroquinolones were measured by other researchers (Aydin et al., 2022; Dorival-García et al., 2015; Gago-Ferrero et al., 2015; Khadra et al., 2019; Thomaidi et al., 2016; Yan et al., 2014). Adsorption of fluoroquinolones on the sludge has been observed to be the main pathway of their elimination during wastewater treatment processes (Khadra et al., 2019). This might be responsible for their detection in all sewage sludge samples in this study (100% frequency of detection). Moreover, compounds with high K_d and low K_{ow} values such as fluoroquinolones (Harrower et al., 2021) have been found to interact electrostatically during sorption to sludge (Dubey et al., 2021; Ternes et al., 2004).

3.1.3 Tetracyclines

Oxytetracycline and tetracycline were detected in sludge samples from both hospital WWTPs. The frequency of detection of both tetracycline antibiotics was 100% in secondary sludge samples from both WWTPs. However, the frequency of detection in UCH primary sludge was 50% for both oxytetracycline and tetracycline. The highest concentration of 310.2 ng g^{-1} was determined for tetracycline (Ijaiye sludge), while oxytetracycline was measured at <LOQ in sludge samples from both WWTPs. Tetracycline was among antibiotics with high concentrations, especially in UCH secondary sludge and Ijaiye sludge. In China, higher concentrations of target tetracyclines were reported in sewage sludge samples, up to 36650 ng g^{-1} and 2943 ng g^{-1} for oxytetracycline and tetracycline, respectively (An et al., 2015; Cheng et al., 2014). Lower or similar concentrations of these tetracyclines, however, were reported in some other studies (Aydin et al., 2022; Gago-Ferrero et al., 2015; Thomaidi et al., 2016). Removal of tetracyclines in pharmaceutical wastewater treatment systems is primarily through sorption (Scaria et al., 2021). Pharmaceuticals such as tetracyclines, which are generally neutral or positively charged at pH 7, are highly sorbed to primary and secondary sludge (Mejías et al., 2021). During WWTP process, complex formation of tetracyclines with cations like Mg^{2+}, Ca^{2+}, and Cu^{2+} present in sludge can also occur (Mejías et al., 2021) which can contribute to increased tetracycline concentrations in sewage sludge.

3.1.4 Sulfonamides

Sulfadimethoxine, sulfamethazine, and sulfamethoxazole were detected in UCH primary sludge samples at concentrations below the LOQs. Sulfadimethoxine was quantified in Ijaiye sludge up to 22.3 ng $g^{-1,}$ while sulfamethoxazole and sulfamethazine were detected below LOQ. Sulfadoxine was not detected in Ijaiye sludge, and its concentration in UCH primary sludge and UCH secondary sludge ranged from not detected (3.6 ng g^{-1}) and not detected (2.3 ng g^{-1}), respectively. Generally, lower concentrations were measured for the sulfonamides in comparison to ciprofloxacin, ofloxacin, azithromycin, and tetracycline. Cheng et al. (2014) also measured lower concentrations of sulfonamides than tetracyclines and

fluoroquinolones. The occurrence of sulfonamides at lower concentrations may be explained by lower sludge-water partitioning coefficients (K_d) of sulfonamides in comparison to the K_d values of fluoroquinolones and tetracyclines (Table S3, supplementary material; Verlicchi & Zambello, 2015) and also lower concentrations of sulfonamides in wastewater influents due to lower consumption and prescription rates (Mejías et al., 2021; Dubey et. al., 2021). Similar concentration levels of sulfonamides were reported in Europe and China (Cheng et al., 2014; García-Galán et al., 2013; Huang et al., 2013; Lillenberg et al., 2009). However, much higher concentrations of sulfamethoxazole were measured in sewage sludge from Switzerland up to 68,000 ng g^{-1} (Göbel et al., 2005), from Spain up to 2743 ng g^{-1} in secondary sludge (Martin et al., 2012b), and from Kenya up to 500 ng g^{-1} (Muriuki et al., 2020).

3.1.5 Other Antibiotics

Thiamphenicol was detected only in UCH secondary sludge at concentrations below LOQ and in only one sample. The absence of thiamphenicol in most sewage sludge samples may be explained by its main use as veterinary antibiotic. Thiamphenicol has low octanol-water partition coefficient (Log K_{ow} of −0.27) (Gago-Ferrero et al., 2015) and is therefore expected to have low sorption on sewage sludge according to the rules of thumb in predicting sorption behavior (Verlicchi & Zambello, 2015). There is generally scarce data on the occurrence of thiamphenicol in sewage sludge. Gago-Ferrero et al. (2015) included thiamphenicol among the pharmaceuticals analyzed in their study; thiamphenicol was however not detected. Trimethoprim was detected in all sludge samples but below LOQ in Ijaiye sludge. The highest amounts of trimethoprim were quantified in UCH sludge, up to 17 ng g^{-1} in UCH primary sludge. Trimethoprim was not detected in Greece by Gago-Ferrero et al. (2015) and in Spain by Martin et al. (2012b). However, in Kenya, much higher concentrations of trimethoprim were reported up to 510 ng g^{-1} (Muriuki et al., 2020) and in Switzerland at 41,000 ng g^{-1} (Göbel et al., 2005).

It should be stated that different treatment processes such as thermal, liming, composting, and digestion may have impact on the concentrations of organic compounds in sewage sludge (Verlicchi & Zambello, 2015). However, the sewage sludges in this study were untreated, and this may have contributed to the elevated concentrations measured for some of the antibiotics, especially azithromycin, ciprofloxacin, ofloxacin, and tetracycline. There are currently limited studies in Africa on the occurrence of antibiotics in sewage sludge. Therefore, there is scarce data from Africa to compare with our results. Only one study has quantified some antibiotics in sewage sludge from Nigeria to date (Olarinmoye et al., 2016). Azithromycin was measured in higher amounts in the present study than in the study by Olarinmoye and coworkers (Olarinmoye et al., 2016). However, lower levels of clarithromycin, erythromycin, sulfamethoxazole, and trimethoprim were determined in this study. Table 3 shows the reported concentrations of target antibiotics in sewage sludge from different countries in comparison with the values measured in this study.

3.2 Risk Assessment of Individual Antibiotics in Sludge-Amended Soil Based on Terrestrial Toxicity Data

Ecotoxicological data of pharmaceuticals including antibiotics for terrestrial organisms are scarce in literature (Bourdat-Deschamps et al., 2017). Ecotoxicological data of target antibiotics for earthworms, springtail, wasp, mallard duck, and northern bobwhite were obtained from literature (Table S1, supplementary material). When available, we also collected toxicity data for soil microbial respiration, nitrification, or plants (seedling height or root length). Risk quotients ($RQ_{soil\text{-}terrestrial}$) were calculated for 10 antibiotics for which terrestrial toxicity data were available in the literature. The risk quotients ($RQ_{soil\text{-}terrestrial}$) of target antibiotics for terrestrial organisms are provided in Table 4. Ciprofloxacin had the highest $RQ_{soil\text{-}terrestrial}$ value of 0.092 for the root elongation of lettuce. The $RQ_{soil\text{-}terrestrial}$ values for all 10 antibiotics were less than 1. The low $RQ_{soil\text{-}terrestrial}$ values were not only influenced by high $PNEC_{soil\text{-}terrestrial}$ values but also low PEC_{soil} (Table 1 and Table 4). This implies that the target antibiotics posed low risk to terrestrial organisms. Moreover, large quantities of a compound will have a low impact if it is rapidly degraded. So the impact depends also on the behavior and fate of the antibiotics in soil like sorption and degradation. Tetracycline, oxytetracyline, and ofloxacin were reported to pose low risk to terrestrial organisms in previous studies

Table 3 Reported concentrations of target antibiotics in sewage sludge from different countries in comparison with this study

Country	Antibiotic concentrations (ng g^{-1})	References
Switzerland	AZM: 64,000, CLA: 67,000, SMX: 68,000, TRM: 41,000	Göbel et al., 2005
	CFX: up to 2420, NFX: up to 2370	Golet et al., 2002
Sweden	CFX: up to 4800, OFX: up to 2000, NFX: up to 4200	Lindberg et al., 2005
Spain	CFX: up to 836, OFX: up to 719.2, NFX: up to 131	Dorival-Garcial et al., 2015
	SMX: up to 4.95, SMZ: up to 139.24, SDM: up to 1.48, SFD: nd	García-Galán et al., 2013
	TRM: nd	Martin et al. (2012b)
Greece	AZM: 60.8–267, CLA: < LOQ–41.1, CFX: 34.1–115, NFX: 36.6–76.6, OFX: 15–42, TC: < LOQ–191, OTC: 8.9–159, THIAMP: nd	Gago-Ferrero et al., 2015
	AZM: 91.4–204, CLA: 42.4–122, CFX: 80.3–107, NFX: 149–242, OFX: 119–159, TC: 18.6–37.2, OTC: 20–40.3	Thomaidi et al., 2016
Turkey	AZM: nd–1494, CLA: nd–1496, ERY: 0.03–13, CFX: nd–503, OTC: 2.25–36.3, SMX: nd–25.7, SMZ: nd–6.63, TRM: nd–4.34	Aydin et al., 2022
Morocco	CLA: 2, CFX: 4.2, OFX: 2.9, TC: 0.2, TRM: 0.2	Khadra et al., 2019
Kenya	CFX: up to 3500, NFX: up to 2700, SMX: up to 600, TRM: up to 600	Muriuki et al., 2020
Nigeria	AZM: < 10, CLA: up to 43, ERY: up to 147, SMX: up to 11, TRM: up to 38	Olarinmoye et al., 2016
China	AZM: 60–550, ERY: 5–10, NFX: 10–105, OFX: 20–200, SMX: 0.47–21.14, SMZ: 0.36–1.93	Yan et al., 2014
	CFX: 140–4720, NFX: 107–15,675, OFX: 305–7950, TC: 101–2943, OTC: 316–36,650, SMX: up to 51.8, SMZ: up to 45, SDM: up to 15	Cheng et al., 2014
	CFX: 1390.5–2442.5, NFX: 815–3552.5, OFX: 2052.5–5975, TC: 100.5–600, OTC: 315.8–3262.5, SMX: MDL–30.4, SMZ: MDL–32.7, SDM: MDL–11.4	Huang et al., 2013
	TC: 297–2175, OTC: 174–7370, SMX: 11–665, SMZ: 0.73–27.14	An et al., 2015
India	CFX: 4600, NFX: 2560, OFX: 2790	Arun et al., 2020
Nigeria	AZM: < LOQ–374.6, CLA: nd–10.4, ERY: nd–23.5, CFX: < LOQ–674, NFX: < LOQ, OFX: < LOQ–388, TC: nd–310.2, OTC: < LOQ, SMX: < LOQ, SDM: up to 22.3, SMZ: up to 1.6, SFD: up to 3.6, TRM: up to 17, THIAMP: < LOQ	This study

AZM, azithromycin; CLA, clarithromycin; ERY, erythromycin; CFX, ciprofloxacin; NFX, norfloxacin; OFX, ofloxacin; TC, tetracycline; OTC, oxytetracycline; SMX, sulfamethoxazole; SMZ, sulfamethazine; SDM, sulfadimethoxine; SFD, sulfadoxine; TRM, trimethoprim; THIAMP, thiamphenicol; nd, not detected; < LOQ, below method limit of quantification; MDL, method detection limit

(Bourdat-Deschamps et al., 2017; Gros et al., 2019; Thomaidi et al., 2016). Ciprofloxacin and tetracycline can affect microbial nitrification and denitrification processes in soil at concentrations greater than 1000 ng g^{-1} (Bourdat-Deschamps et al., 2017; Roose-Amsaleg & Laverman, 2016). In this work, the predicted environmental concentrations of ciprofloxacin in soil (PEC_{soil}) for UCH sludge-amended soil and Ijaiye sludge-amended soil were 0.558 ng g^{-1} and 0.9912 ng g^{-1}, respectively, while the PEC_{soil} values of tetracycline for UCH and Ijaiye were 0.422 ng g^{-1} and 0.4562 ng g^{-1}, respectively. These PEC_{soil} values are very much lower than 1000 ng g^{-1}, and this implies that ciprofloxacin and tetracycline may not affect microbial nitrification and denitrification processes. Moreover, to the best of our knowledge, target antibiotics have not been measured in Nigerian soil till date, including the present study. The predicted concentrations in soil (PEC_{soil}) in the present study were largely lower than the measured concentrations in soil (MEC_{soil}) in some other studies: Yang et al. (2016) in Kenya, Arun et al. (2020) in India, and Gu et al. (2019) in China. So, there is a chance to underestimate RQs for these antibiotics in Nigerian soil.

3.3 Risk Assessment of Individual Antibiotics in Sludge-Amended Soil Based on Aquatic Toxicity Data

Risk quotients of individual antibiotics in sludge-amended soil based on aquatic toxicity data ($RQ_{soil\text{-}aquatic}$) were calculated for all target antibiotics except thiamphenicol due to unavailability of *Koc* and toxicity data in literature. Risk quotients ($RQ_{soil\text{-}aquatic}$) for target antibiotics in sludge-amended

Table 4 Risk quotients of antibiotics for terrestrial organisms ($RQ_{\text{soil-terrestrial}}$) in sewage sludge-amended soil

Antibiotic	Ecotoxicological end points	$PNEC_{\text{soil-terrestrial}}$ (ng g^{-1})	Risk quotient ($RQ_{\text{soil-terrestrial}}$)	
			UCH	Ijaiye
Tetracycline	*Eisenia fetida (earthworm)*	2735	1.5×10^{-4}	1.7×10^{-4}
	Folsomia candida (springtail)	2560	1.7×10^{-4}	1.8×10^{-4}
	Rice and cucumber (seedling height)	30,000	1.4×10^{-5}	1.5×10^{-5}
	Rice and cucumber (root length)	30,000	1.4×10^{-5}	1.5×10^{-5}
Oxytetracycline	*Eisenia fetida (earthworm)*	1000	5.4×10^{-4}	5.4×10^{-4}
	A.*calignosa (earthworm)*	5000	1.1×10^{-4}	1.1×10^{-4}
	Folsomia fimetaria (springtail)	5000	1.1×10^{-4}	1.1×10^{-4}
	E. crypticus (wasp)	2701	2.0×10^{-4}	2.0×10^{-4}
	Mallard duck and northern bobwhite	5620	9.5×10^{-5}	9.5×10^{-5}
	Soil microbial respiration	1000	5.4×10^{-4}	5.4×10^{-4}
	Toxicity to plants	10,000	5.4×10^{-5}	5.4×10^{-5}
Sulfamethoxazole	*Eisenia fetida (earthworm)*	4000	1.5×10^{-8}	1.5×10^{-8}
	Rice (seedling height)	38	1.5×10^{-6}	1.5×10^{-6}
	Rice (root length)	13	4.5×10^{-6}	4.5×10^{-6}
	Cucumber (seedling height)	300	2.0×10^{-7}	2.0×10^{-7}
	Cucumber (root length)	300	2.0×10^{-7}	2.0×10^{-7}
Sulfamethazine	Rice (seedling height)	220	1.1×10^{-5}	1.0×10^{-7}
	Rice (root length)	43	5.5×10^{-5}	5.1×10^{-7}
	Cucumber (seedling height)	300	7.8×10^{-6}	7.4×10^{-8}
	Cucumber (root length)	300	7.8×10^{-6}	7.4×10^{-8}
	Rice (root elongation)	20	1.2×10^{-4}	1.1×10^{-6}
Sulfadimethoxine	*Eisenia fetida (earthworm)*	2000	6.6×10^{-8}	1.6×10^{-5}
	Rice (seedling height)	300	4.4×10^{-7}	1.1×10^{-4}
	Rice (root length)	300	4.4×10^{-7}	1.1×10^{-4}
	Cucumber (seedling height)	300	4.4×10^{-7}	1.1×10^{-4}
	Cucumber (root length)	300	4.4×10^{-7}	1.1×10^{-4}

Table 4 (continued)

Antibiotic	Ecotoxicological end points	$PNEC_{soil\text{-}terrestrial}$ (ng g^{-1})	Risk quotient ($RQ_{soil\text{-}terrestrial}$)	
			UCH	Ijaiye
Norfloxacin	Lettuce (root elongation)	86	1.4×10^{-4}	1.4×10^{-4}
	Carrot (root elongation)	190	6.5×10^{-5}	6.5×10^{-5}
	Cucumber (root elongation)	131	9.4×10^{-5}	9.4×10^{-5}
	Tomato (root elongation)	56	2.2×10^{-4}	2.2×10^{-4}
Ofloxacin	Soil microorganism	779	6.4×10^{-4}	7.3×10^{-4}
Ciprofloxacin	Lettuce (root elongation)	10.8	5.4×10^{-2}	9.2×10^{-2}
Erythromycin	Lettuce (root elongation)	696	5.0×10^{-5}	nc
	Carrot (root elongation)	3036	1.1×10^{-5}	nc
	Cucumber (root elongation)	3036	1.1×10^{-5}	nc
	Tomato (root elongation)	3036	1.1×10^{-5}	nc
Trimethoprim	*Eisenia fetida (earthworm)*	2000	6.5×10^{-6}	5.0×10^{-9}
	Rice/cucumber (seedling height)	300	4×10^{-5}	3.3×10^{-8}
	Rice/cucumber (root length)	300	4×10^{-5}	3.3×10^{-8}

nc, not calculated because compound was not detected in sludge

soils are presented in Table S5, and the corresponding logarithms (to base 10) of the $RQ_{soil\text{-}aquatic}$ values are shown in Fig. 1.

The risk quotient values ranged from 1.0×10^{-8} to 0.402 in sludge-amended soils ($RQ_{soil\text{-}aquatic}$). Clarithromycin and tetracycline in both UCH and Ijaiye sludge-amended soils presented medium risk to aquatic organisms. Erythromycin in only UCH sludge-amended soil also posed medium risk. All remaining antibiotics presented low risk in sludge-amended soils. Aydin et al. (2022) also reported that clarithromycin and oxytetracycline in digested sludge-amended soil posed medium risk to the environment. In a study by Thomaidi et al. (2016), ciprofloxacin, norfloxacin, and oxytetracycline also caused low risks in sludge-amended soils; however, ofloxacin and tetracycline presented high risks in their study (Thomaidi et al., 2016). Low PNEC values (0.014 and 0.1) rather than MEC_{sludge} or PEC_{soil} were responsible for the high RQ values observed for ofloxacin and tetracycline in Thomaidi et al. (2016). Due to low PNEC values assessed from water-soil partitioning, high risks of ofloxacin and azithromycin were also observed by Verlicchi and Zambello (2015) and Aydin et al. (2022), respectively. Similarly, $PNEC_{soil\text{-}aquatic}$ is an influencing parameter for medium or low risks ($RQ_{soil\text{-}aquatic}$) of target antibiotics in this study.

It should be emphasized that toxicity data for aquatic organisms cannot replace data for soil-dwelling organisms because the effects on aquatic species can only be considered as effects on soil organisms that are exposed exclusively to the soil pore water of the soil (ECHA, 2008, R.10). If $RQ_{soil\text{-}aquatic}$ calculated from equilibrium partition method is greater than one (> 1), tests with soil organisms should be considered an essential

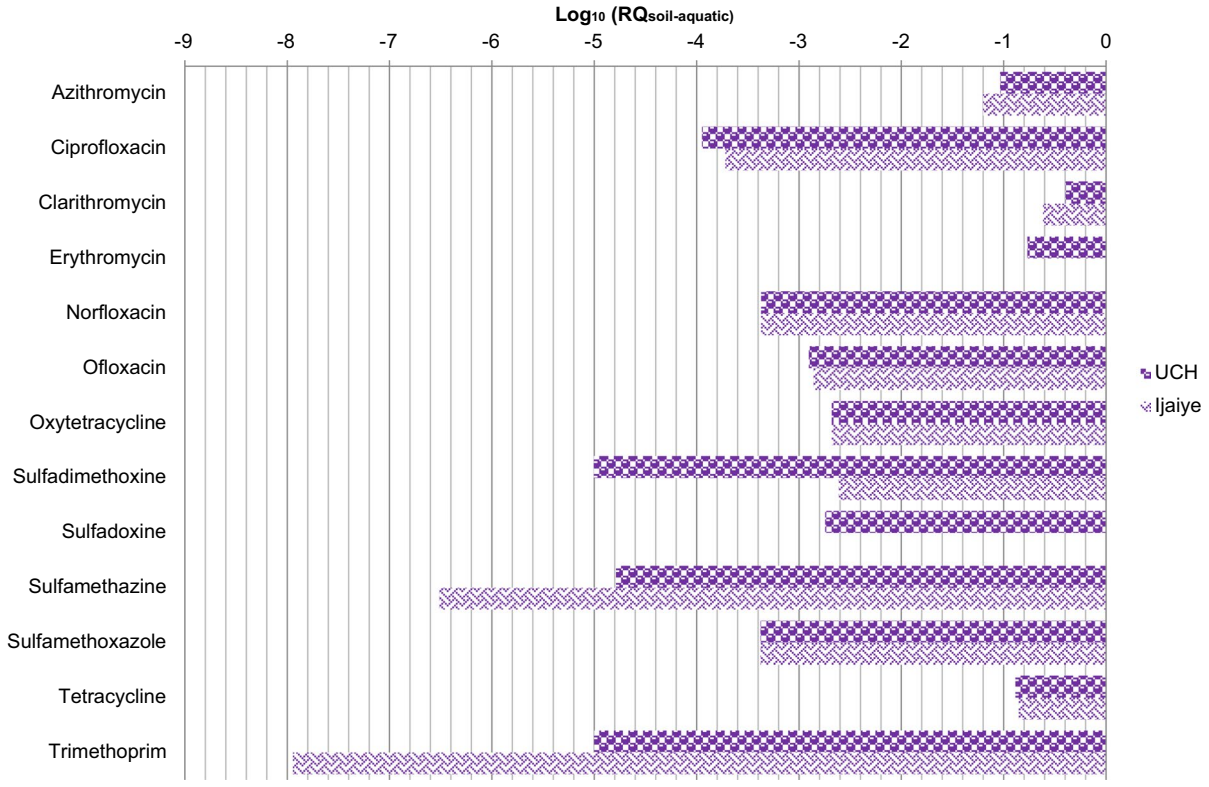

Fig. 1 Risk assessment of antibiotics in sludge-amended soil based on aquatic toxicity data

requirement for a refined hazard assessment. In this study, none of the evaluated antibiotics had $RQ_{\text{soil-aquatic}}$ greater than one.

3.4 Risk Assessment of Antibiotic Mixtures in Sludge-Amended Soil

Antibiotics are generally present in the aquatic and terrestrial environment as a mixture; the resultant ecotoxicological effect should therefore be much greater than the effect of an individual antibiotic (Felis et al., 2020). We evaluated the risk quotients associated with target antibiotic mixtures (*RQmix*) in sludge-amended soils following the approach proposed by Thiele-Bruhn (2019) as described above under the "Materials and Methods" section. The calculated risk quotients of antibiotic mixtures (*RQmix*) in sludge-amended soil are provided in Table S6 (Supplementary material). The calculated combined risk quotients (*RQmix*) of the antibiotic mixtures were 0.0021 and 0.0016 in UCH sludge-amended soil and Ijaiye sludge-amended soil, respectively. This indicates that the antibiotic mixtures in the sludge-amended soils posed no serious risk to the aquatic organisms.

Although pharmaceuticals having the same mode of action are assumed to cause additive toxic effects when they are combined, antibiotics belonging to the same class have been found to also exhibit synergistic effects (combined effects more potent than combined potencies of each antibiotic) (Thiele-Bruhn, 2019; Vasquez et al., 2014). Antibiotics evaluated for risk assessment in this study belong to five different classes (macrolides, fluoroquinolones, tetracyclines, sulfonamides, and trimethoprim). While fluoroquinolones' mode of action is bactericidal, the mode of action of other classes of antibiotics in this work is bacteriostatic (Thiele-Bruhn, 2019). The interactions between bactericidal antibiotics and bacteriostatic antibiotics are largely antagonistic (Ocampo et al., 2014; Thiele-Bruhn, 2019). This implies that combined effect of antibiotic mixtures will be less than combined potencies of individual antibiotic. Moreover, antagonistic effects occur more often at low concentrations of mixed chemicals (Thiele-Bruhn, 2019; Vasquez

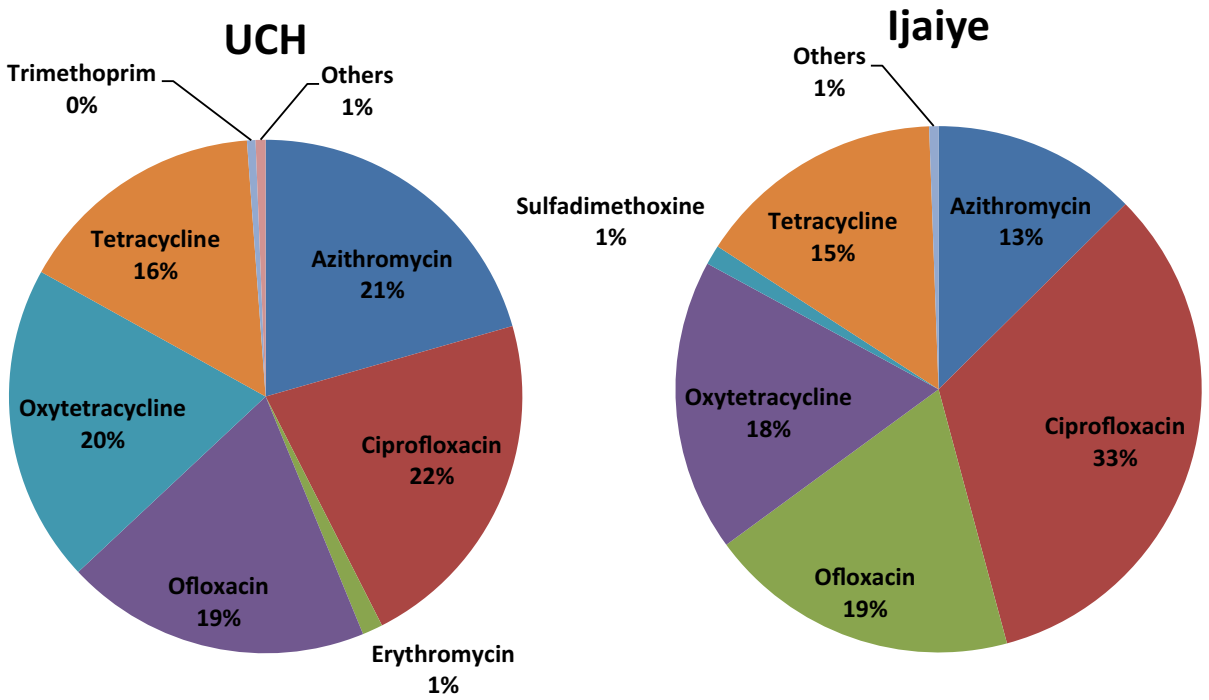

Fig. 2 Percentage contribution of individual antibiotics to the risk of antibiotic mixtures in sludge-amended soil

et al., 2014), like the concentrations measured in this study. This antagonistic interaction was observed in this work in which the combined risk quotients (*RQmix*) of the antibiotic mixtures (0.0021 and 0.0016 for UCH sludge-amended soil and Ijaiye sludge-amended soil, respectively) were lower than the respective sum of the risk quotients of the individual antibiotics (0.801 and 0.453 for UCH sludge-amended soil and Ijaiye sludge-amended soil, respectively) (Table S5 and Table S6, Supplementary material). Overall, the combined risk quotients (*RQmix*) of the antibiotic mixtures in sludge-amended soils in this study posed no serious risk to the environment.

The percentage contributions of individual antibiotics to the combined risk quotients in sludge-amended soils were also calculated and are presented in Fig. 2 and Table S6.

Ciprofloxacin had the highest contributions to the mixture toxicity in the sludge-amended soils, accounting for a total of 22% and 33% in UCH sludge-amended soil and Ijaiye sludge-amended soil, respectively. Other antibiotics which contributed significantly to the mixture toxicity were ofloxacin, oxytetracycline, tetracycline, and azithromycin.

Despite the low risk quotients of the antibiotic mixtures obtained for the sludge-amended soils in the present study, caution should be taken in considering the application of sewage sludge as fertilizers for agricultural purposes. This is because sewage sludge usually may contain heavy metals and a myriad of other organic chemicals and pharmaceuticals other than antibiotics evaluated in the present study. Therefore, a more holistic evaluation of the potential risks due to a mixture of wider scope of emerging organic chemicals in Nigerian sewage sludge is recommended prior to application as fertilizers for agricultural purposes.

4 Conclusions

The occurrence of fourteen antibiotic compounds was investigated in sewage sludge from two Nigerian hospital WWTPs (UCH and Ijaiye WWTPs). Potential ecotoxicological risks of target antibiotics in sewage sludge for application onto agricultural soils (sludge-amended soil) were evaluated. All target antibiotics were detected at varying

frequencies across the two WWTPs. The highest concentration was determined for ciprofloxacin (up to 674 ng g^{-1} in Alausa sludge). All evaluated antibiotics for terrestrial ecological risk presented low risk in sludge-amended soils to terrestrial organisms. Out of 13 antibiotics assessed for aquatic ecological risk, only clarithromycin, erythromycin, and tetracycline posed medium risk to aquatic organisms, with the remaining antibiotics presenting low risk. The antibiotic mixtures presented low risk in sludge-amended soils. Ciprofloxacin had the highest contribution to the toxicity of the evaluated antibiotic mixtures in the sludge-amended soils. To our knowledge, this work presents the first study on ecotoxicological risk assessment of antibiotics in sewage sludge and sludge-amended soils in Nigeria. A more holistic evaluation of potential risks should include heavy metals and a wider scope of organic chemicals in Nigerian sewage sludge and sludge-amended soils.

Acknowledgements The authors gratefully acknowledge the German Research Foundation (DFG) and The World Academy of Sciences (TWAS) for the award of TWAS-DFG cooperation visits fellowship to Akinranti Ajibola. Selina Tisler is appreciated for technical support in instrumental analysis. Personnel at the investigated wastewater treatment plants are acknowledged for assistance in the collection of sludge samples.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data Availability The datasets generated and analyzed during the current study are partly included in the supplementary information file and available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

Glossary

EC_{50}	Effective concentration leading to 50% of the effect, compared to a nonexposed control
LC_{50}	Exposure concentration lethal to half of the test animals
NOEC	No observed effect concentration
PNEC	Predicted no-effect concentration
MEC	Measured environmental concentration
PEC	Predicted environmental concentration
RQ	Risk quotient
AF	Assessment factor

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