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

Akinranti S. Ajibola · Christian Zwiener

Received: 18 March 2022 / Accepted: 21 September 2022 / Published online: 1 October 2022 © The Author(s) 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...
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 overdose 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-Garcia 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 (Na3Cit), disodium citrate sesquihydrate (Na2Cit), and Na2EDTA.2H2O were obtained from Sigma Aldrich (Steinheim, Germany). Sodium chloride, 99.5% for analysis, and anhydrous MgSO4 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 Na2EDTA (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 MgSO4, 1.0-g NaCl, 1.0-g Na3Cit, and 0.5-g Na2Cit). 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 MgSO₄ 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-terrestrial) was calculated by dividing EC₅₀/ LC₅₀/ 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 EC₅₀ from microbial...
activity tests; AF is 50 for EC50 or NOEC from plant growth tests, and AF is 1000 if EC50/ LC50 are available for one tested organism.

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

\[ PNEC(\text{soil} - \text{terrestrial}) = \frac{EC50 \text{ or } LC50 \text{ or NOEC}}{\text{Assessment factor}} \]  

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 (\( PNEC_{\text{water}} \)) 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):

\[ PNEC(\text{soil} - \text{aquatic}) = \frac{K_{\text{soil-water}}}{\text{RHO}_{\text{soil}}} \times \text{PNEC}_{\text{water}} \times 1000 \]  

where \( K_{\text{soil-water}} \) is soil–water partition coefficient and \( \text{RHO}_{\text{soil}} \) 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):

\[ K_{\text{soil-water}} = \frac{F_{\text{air}}_{\text{soil}} \times K_{\text{air-water}} + F_{\text{water}}_{\text{soil}}}{1000} \times \text{RHO}_{\text{solid}} \]  

where \( F_{\text{air}}_{\text{soil}} \) represents the volume fraction air in soil (0.2), \( K_{\text{air-water}} \) is the air–water partition coefficient (zero for nonvolatile substances), \( F_{\text{water}}_{\text{soil}} \) represents the volume fraction water in soil (0.2), \( F_{\text{solid}}_{\text{soil}} \) is the fraction solid in soil (0.6), \( F_{\text{oc}}_{\text{soil}} \) 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 \( \text{RHO}_{\text{solid}} \) 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):

\[ \text{PNEC}_{\text{soil}} = \text{PNEC}_{\text{soil-aquatic}} \times 1000 \]  

where \( Koc \) is the organic carbon partition coefficient of antibiotic (as L Kg\(^{-1}\)) collected from literature and presented in Table S3 (Supplementary material). \( PNEC_{\text{soil-aquatic}} \) 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 (\( PEC_{\text{soil}} \)) 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):

\[ PEC_{\text{soil}} = \frac{\text{MEC}(\text{sludge}) \times \text{APP}(\text{sludge})}{\text{DEPTH}(\text{soil}) \times \text{RHO}(\text{soil})} \]  

where \( Co(\text{soil}) \) is the background concentration of antibiotic in the soil before application of sludge (assumed to be zero in this study). \( \text{MEC}(\text{sludge}) \) represents the maximum measured concentration in secondary sludge (µg Kg\(^{-1}\)), \( \text{DEPTH}(\text{soil}) \) is the mixing depth of 0.2 m generally used for agricultural soil, \( \text{RHO}(\text{soil}) \) is the bulk density of wet soil (1700 kg m\(^{-3}\)), and \( \text{APP}(\text{sludge}) \) 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; Onwere-emadu 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 \( PEC(\text{soil}) \) values of...
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 (\(RQ_{\text{soil-terrestrial}}\)) 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 (\(PNEC_{\text{soil-terrestrial}}\)) according to Eq. (6) (Thomaidi et al., 2016).

\[
RQ(\text{soil} - \text{terrestrial}) = \frac{\text{PEC}(\text{soil})}{PNEC(\text{soil} - \text{terrestrial})} \tag{6}
\]

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

\[
RQ(\text{soil} - \text{aquatic}) = \frac{\text{PEC}(\text{soil})}{PNEC(\text{soil} - \text{aquatic})} \tag{7}
\]

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 (\(PNEC_{\text{mix}}\)) that depends on the relative contribution of the individual compounds and their individual PNECs to the overall mixture (Eq. 8):

\[
PNEC_{\text{mix}} = \sum_{i=1}^{n} \left( \frac{\text{PEC}_i \times \text{PNEC}_i}{\sum \text{PEC}_i} \right) \tag{8}
\]

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

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

| Compound          | MEC (ng g\(^{-1}\)) | \(\text{PEC}_{\text{soil}}\) (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                       |

\(\text{PEC}_{\text{soil}}\), not detected; nc, not calculated because compound was not detected in sludge.
Corresponding RQ of the mixture ($RQ_{mix}$) was then calculated using Eq. (9) according to Thiele-Bruhn (2019):

$$RQ_{mix} = \sum_{i=1}^{n} \left( \frac{PEC_i}{PNEC_{mix}} \right)$$

where $PEC_i$ is the predicted environmental concentration of individual antibiotic and $PNEC_{mix}$ is the sum of predicted no-effect concentrations of target antibiotics, calculated from aquatic toxicity data ($PNEC_{soil-aquatic}$) (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

### Table 2 Concentration profiles (ng $g^{-1}$) of target antibiotics in sewage sludge

| Compound            | MLOQ $(\text{ng } g^{-1})$ | UCH WWTP |                  |                  | Ijaiye WWTP |                  |                  |
|---------------------|-----------------------------|----------|-------------------|-------------------|-------------|-------------------|-------------------|
|                     |                             | Primary sludge | Secondary sludge | Secondary sludge | 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 | <LOQ  | <LOQ | <LOQ | 100 | <LOQ  | <LOQ | <LOQ | 100     |
| Sulfamethoxazole    | 0.04                        | <LOQ | <LOQ | <LOQ | 100 | <LOQ  | nd  | <LOQ | 75 | 230.1 | 179.58 | 310.2 | 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     |

*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.
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; 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 (Göbel 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 (Harower 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 mg g$^{-1}$ (Göbel et al., 2005), from Spain up to 2743 mg g$^{-1}$ in secondary sludge (Martin et al., 2012b), and from Kenya up to 500 mg 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 mg 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 mg g$^{-1}$ (Muriuki et al., 2020) and in Switzerland at 41,000 mg 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 co-workers (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 (Bourdais-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 ($RQ_{soil-terrestrial}$) were calculated for 10 antibiotics for which terrestrial toxicity data were available in the literature. The risk quotients ($RQ_{soil-terrestrial}$) of target antibiotics for terrestrial organisms are provided in Table 4. Ciprofloxacin had the highest $RQ_{soil-terrestrial}$ value of 0.092 for the root elongation of lettuce. The $RQ_{soil-terrestrial}$ values for all 10 antibiotics were less than 1. The low $RQ_{soil-terrestrial}$ values were not only influenced by high $PNEC_{soil-terrestrial}$ 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, oxytetracycline, and ofloxacin were reported to pose low risk to terrestrial organisms in previous studies.
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\(_{\text{soil}}\)) 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\(_{\text{soil}}\) values of tetracycline for UCH and Ijaiye were 0.422 ng g\(^{-1}\) and 0.4562 ng g\(^{-1}\), respectively. These PEC\(_{\text{soil}}\) 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\(_{\text{soil}}\)) in the present study were largely lower than the measured concentrations in soil (MEC\(_{\text{soil}}\)) 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\(_{\text{soil-aquatic}}\)) were calculated for all target antibiotics except thiamphenicol due to unavailability of Koc and toxicity data in literature. Risk quotients (RQ\(_{\text{soil-aquatic}}\)) for target antibiotics in sludge-amended soil can be calculated as follows:

\[
RQ_{\text{soil-aquatic}} = \frac{\text{PEC}_{\text{soil}}}{\text{EC}_{\text{aquatic}}}
\]

Where PEC\(_{\text{soil}}\) is the predicted environmental concentration of the antibiotic in soil and EC\(_{\text{aquatic}}\) is the effective concentration of the antibiotic in aquatic systems that causes a specific effect. The risk assessment can be further refined by incorporating the toxicity of the antibiotic in soil (EC\(_{\text{soil}}\)) and the partitioning coefficient of the antibiotic (Koc).

### 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, OFX: 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, OFX: <LOQ–191, SMX: 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–6,3, 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, OFX: 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 | Olarimoye 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, OFX: 107–15,675, OTC: 305–7950, TC: 101–2943, OTC: 316–36,650, SMX: up to 51.8, SMZ: up to 45, SDM: up to 15 | Cheng, 2014 |
|            | CFX: 1390.5–2442.5, OFX: 815–3552.5, OTC: 2052.5–5975, TC: 100.5–600, OTC: 315.8–3262.5, SMX: 60–550, SMZ: 38–254, SDM: 60–550 | Huang et al., 2013 |
| India      | CFX: 1390.5–2442.5, OFX: 815–3552.5, OTC: 2052.5–5975, TC: 100.5–600, OTC: 315.8–3262.5, SMX: 60–550, SMZ: 38–254, SDM: 60–550 | An et al., 2015 |
| Nigeria    | AZM: <LOQ–374.6, CLA: nd–10.4, ERY: nd–23.5, CFX: <LOQ–674, NFX: <LOQ–674, OFX: <LOQ–388, TC: nd–310.2, OTC: <LOQ–674, SMX: <LOQ–674, SDM: up to 22.3, SMZ: up to 1.6, SFD: up to 3.6, TRM: up to 17, THIAMP: <LOQ | Arun et al., 2020 |
|            | This study                               | Arun et al., 2020 |

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.
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| \textit{Eisenia fetida} (earthworm) | 2735 | \(1.5 \times 10^{-4}\) | \(1.7 \times 10^{-4}\) |
|             | \textit{Folsomia candida} (springtail) | 2560 | \(1.7 \times 10^{-4}\) | \(1.8 \times 10^{-4}\) |
|             | Rice and cucumber (seedling height) | 30,000 | \(1.4 \times 10^{-5}\) | \(1.5 \times 10^{-5}\) |
|             | Rice and cucumber (root length) | 30,000 | \(1.4 \times 10^{-5}\) | \(1.5 \times 10^{-5}\) |
| Oxytetracycline | \textit{Eisenia fetida} (earthworm) | 1000 | \(5.4 \times 10^{-4}\) | \(5.4 \times 10^{-4}\) |
|             | \textit{Acalignosa} (earthworm) | 5000 | \(1.1 \times 10^{-4}\) | \(1.1 \times 10^{-4}\) |
|             | \textit{Folsomia fimetaria} (springtail) | 5000 | \(1.1 \times 10^{-4}\) | \(1.1 \times 10^{-4}\) |
|             | \textit{E. crypticus} (wasp) | 2701 | \(2.0 \times 10^{-4}\) | \(2.0 \times 10^{-4}\) |
|             | Mallard duck and northern bobwhite | 5620 | \(9.5 \times 10^{-5}\) | \(9.5 \times 10^{-5}\) |
|             | Soil microbial respiration | 1000 | \(5.4 \times 10^{-4}\) | \(5.4 \times 10^{-4}\) |
|             | Toxicity to plants | 10,000 | \(5.4 \times 10^{-5}\) | \(5.4 \times 10^{-5}\) |
| Sulfamethoxazole | \textit{Eisenia fetida} (earthworm) | 4000 | \(1.5 \times 10^{-8}\) | \(1.5 \times 10^{-8}\) |
|             | Rice (seedling height) | 38 | \(1.5 \times 10^{-6}\) | \(1.5 \times 10^{-6}\) |
|             | Rice (root length) | 13 | \(4.5 \times 10^{-6}\) | \(4.5 \times 10^{-6}\) |
|             | Cucumber (seedling height) | 300 | \(2.0 \times 10^{-7}\) | \(2.0 \times 10^{-7}\) |
|             | Cucumber (root length) | 300 | \(2.0 \times 10^{-7}\) | \(2.0 \times 10^{-7}\) |
| Sulfamethazine | Rice (seedling height) | 220 | \(1.1 \times 10^{-5}\) | \(1.0 \times 10^{-7}\) |
|             | Rice (root length) | 43 | \(5.5 \times 10^{-5}\) | \(5.1 \times 10^{-7}\) |
|             | Cucumber (seedling height) | 300 | \(7.8 \times 10^{-6}\) | \(7.4 \times 10^{-8}\) |
|             | Cucumber (root length) | 300 | \(7.8 \times 10^{-6}\) | \(7.4 \times 10^{-8}\) |
|             | Rice (root elongation) | 20 | \(1.2 \times 10^{-4}\) | \(1.1 \times 10^{-6}\) |
| Sulfadimethoxine | \textit{Eisenia fetida} (earthworm) | 2000 | \(6.6 \times 10^{-8}\) | \(1.6 \times 10^{-5}\) |
|             | Rice (seedling height) | 300 | \(4.4 \times 10^{-7}\) | \(1.1 \times 10^{-4}\) |
|             | Rice (root length) | 300 | \(4.4 \times 10^{-7}\) | \(1.1 \times 10^{-4}\) |
|             | Cucumber (seedling height) | 300 | \(4.4 \times 10^{-7}\) | \(1.1 \times 10^{-4}\) |
|             | Cucumber (root length) | 300 | \(4.4 \times 10^{-7}\) | \(1.1 \times 10^{-4}\) |
soils are presented in Table S5, and the corresponding logarithms (to base 10) of the $RQ_{\text{soil-aquatic}}$ values are shown in Fig. 1. The risk quotient values ranged from $1.0 \times 10^{-8}$ to 0.402 in sludge-amended soils. 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 $MEC_{\text{sludge}}$ or $PEC_{\text{soil}}$ 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, $PNEC_{\text{soil-aquatic}}$ is an influencing parameter for medium or low risks ($RQ_{\text{soil-aquatic}}$) 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 $RQ_{\text{soil-aquatic}}$ calculated from equilibrium partition method is greater than one (>1), tests with soil organisms should be considered an essential

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

nc, not calculated because compound was not detected in sludge
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 ($RQ_{\text{mix}}$) 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 ($RQ_{\text{mix}}$) in sludge-amended soil are provided in Table S6 (Supplementary material). The calculated combined risk quotients ($RQ_{\text{mix}}$) 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...
et al., 2014), like the concentrations measured in this study. This antagonistic interaction was observed in this work in which the combined risk quotients ($R_{Q_{mix}}$) 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 ($R_{Q_{mix}}$) 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

| Abbreviation | Definition |
|--------------|------------|
| EC₅₀         | Effective concentration leading to 50% of the effect, compared to a nonexposed control |
| LC₅₀         | Exposure concentration lethal to half of the test animals |
| NOEC         | No observed effect concentration |
| PEC          | Predicted environmental concentration |
| MEC          | Measured environmental concentration |
| RQ           | Risk quotient |
| AF           | Assessment factor |

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Ajibola, A. S., Tisler, S., & Zwiener, C. (2020). Simultaneous determination of multiclass antibiotics in sewage sludge based on QuEChERS extraction and liquid chromatography-tandem mass spectrometry. *Analytical Methods, 12*, 576–586. https://doi.org/10.1039/c9ay02188d

Ajibola, A. S., Fawole, S. T., Ajibola, F. O., & Adeleye, G. O. (2021). Diclofenac and ibuprofen determination in sewage sludge using a QuEChERS approach: Occurrence and ecological risk assessment in three Nigerian wastewater treatment plants. *Bulletin of Environmental Contamination and Toxicology, 106*, 690–699. https://doi.org/10.1007/s00128-021-03139-1

Ajibola, A. S., Amoniyiyan, O. A., Ekoja, F. O., & Ajibola, F. O. (2021). QuEChERS approach for the analysis of three fluoroquinolone antibiotics in wastewater: Concentration profiles and ecological risk in two Nigerian hospital wastewater treatment plants. *Archives of Environmental Contamination and Toxicology, 80*, 389–401. https://doi.org/10.1007/s00244-020-00789-w

An, J., Chen, H., Wei, S., & Gu, J. (2015). Antibiotic contamination in animal manure, soil, and sewage sludge in Shenyang, northeast China. *Environmental Earth Sciences, 74*, 5077–5086. https://doi.org/10.1007/s12665-015-4528-y

Arumugam, R. M., Raj, J., Mukhopadhyay, M., Ilango, K., & Chakrabort, P. (2020). Occurrence, source and risk assessment of fluoroquinolones in dumpsite soil and sewage sludge from Chennai. *India. Environmental Toxicology and Pharmacology, 79*, 103410. https://doi.org/10.1016/j.etap.2020.103410

Aydin, S., Ulvi, A., Bedük, F., & Aydin, M. E. (2022). Pharmaceutical residues in digested sewage sludge: Occurrence, seasonal variation and risk assessment for soil. *Science of the Total Environment, 817*, 152864. https://doi.org/10.1016/j.scitotenv.2021.152864

Baguer, A. J., Jensen, J., & Krogh, P. H. (2000). Effects of the antibiotics oxytetracycline and tylosin on soil fauna. *Chemosphere, 40*, 751–757.
Berthod, L., Whitley, D. C., Roberts, G., Sharpe, A., Greenwood, R., & Mills, G. A. (2017). Quantitative structure-property relationships for predicting sorption of pharmaceuticals to sewage sludge during waste water treatment processes. *Science of the Total Environment*, 579, 1512–1520. https://doi.org/10.1016/j.scitotenv.2016.11.156

Bourdat-Deschamps, M., Ferhi, S., Bernet, N., Fedler, F., Crouzet, O., Patureau, D., Montenach, D., Moussard, G. D., Mercier, V., Benoit, P., & Houot, S. (2017). Fate and impacts of pharmaceuticals and personal care products after repeated applications of organic waste products in long-term field experiments. *Science of the Total Environment*, 607–608, 271–280. https://doi.org/10.1016/j.scitotenv.2017.06.240

Buta, M., Hubeny, J., Zehlínský, W., Harnisz, M., & Korzeniewska, E. (2021). Sewage sludge in agriculture – The effects of selected chemical pollutants and emerging genetic resistance determinants on the quality of soil and crops – A review. *Ecotoxicology and Environmental Safety*, 214, 112070. https://doi.org/10.1016/j.ecosafe.2021.112070

Cheng, M., Wu, L., Huang, Y., Luo, Y., & Christie, P. (2014). Total concentrations of heavy metals and occurrence of antibiotics in sewage sludges from cities throughout China. *Journal of Soils and Sediments*, 14, 1123–1135. https://doi.org/10.1007/s11368-014-0850-3

Dorival-García, N., Labajo-Recio, C., Zafra-Gómez, A., Juárez-Jiménez, B., & Vilchez, J. L. (2015). Improved sample treatment for the determination of 17 strong sorbed quinolone antibiotics from compost by ultra high performance liquid chromatography tandem mass spectrometry. *Talanta*, 138, 247–257. https://doi.org/10.1016/j.talanta.2015.03.011

Dubey, M., Mohapatra, S., Tyagi, V. K., Suthar, S., & Kazmi, A. A. (2021). Occurrence, fate, and persistence of emerging micropollutants in sewage sludge treatment. *Environmental Pollution*, 273, 116515. https://doi.org/10.1016/j.envpol.2021.116515

EC-TGD. (2003). *Technical guidance document on risk assessment in support of commission directive 93/67/EC on risk assessment for new notified substances*, commission regulation (EC) No 1488/94 on risk assessment for existing substances and directive 98/8/EC of the European parliament and of the council concerning the placing of biocidal products on the market, Parts I, II and IV of European Communities, EUR 20418 EN/1.

ECHA. (2008). *Guidance on information requirements and chemical safety assessment chapter R.10: Characterization of dose [concentration] - response for environment* (pp. 65). European Chemicals Agency, Helsinki, Finland.

ECHA. (2016). *Guidance on information requirements and chemical safety assessment chapter R.16: Environmental exposure assessment*. ECHA-16-G-03-EN (pp.178). European Chemicals Agency, Helsinki, Finland.

European Medicine Agency (EMEA). (2006). *Guideline on the environmental risk assessment of medicinal products for human use*. Committee for medicinal products for human use (CHMP). EMEA/CHMP/SWP/44447/00 corr 2. (pp. 12). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessment-medicinal-products-human-use-first-version_en.pdf. Accessed 29 Sept 2022

Felix, E., Kalka, J., Sochacki, A., Kowalska, K., Bajkacz, S., Harnisz, M., & Korzeniewska, E. (2020). Antimicrobial pharmaceuticals in the aquatic environment: Occurrence and environmental implications. *European Journal of Pharmacology*, 866, 172813. https://doi.org/10.1016/j.ejphar.2019.172813

Fowotade, A., Fasuyi, T., Aigbovo, O., Versporten, A., Adekanmbi, O., Akinyemi, O., Goossens, H., Kehinde, A., & Oduyebo, O. (2020). Point prevalence survey of antimicrobial prescribing in a Nigerian hospital: Findings and implications of antimicrobial resistance. *West African Journal of Medicine*, 37(3), 216–220.

Gago-Ferrero, P., Borova, V., Dasenaki, M. E., & Thomaidis, N. S. (2015). Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 407, 4287–4297. https://doi.org/10.1007/s00216-015-8540-6

García-Galán, M. J., Díaz-Cruz, S., & Barceló, D. (2013). Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography–electrospray-quadrupole linear ion trap mass spectrometry. *Journal of Chromatography A*, 1275, 32–40.

Ghirardini, A., Grillini, V., & Verlicchi, P. (2020). A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure – Environmental risk due to antibiotics after application to soil. *Science of the Total Environment*, 707, 136118. https://doi.org/10.1016/j.scitotenv.2019.136118

Göbel, A., Thomsen, A., Mcardell, C. S., Joss, A., & Giger, W. (2005). Occurrence and sorption behavior of sulfonamides, macrolides and trimethoprim in activated sludge treatment. *Environmental Science & Technology*, 39, 3981–3989. https://doi.org/10.1021/es048550a

Golet, E. M., Strehler, A., Alder, A. C., & Giger, W. (2002). Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. *Analytical Chemistry*, 74, 5455–5462. https://doi.org/10.1021/ac025762m

Gros, M., Mas-Pla, J., Boy-Roura, M., Geli, I., Domingo, F., & Petrović, M. (2019). Veterinary pharmaceuticals and antibiotics in manure and slurry and their fate in amended agricultural soils: Findings from an experimental field site (Baix Empordà, NE Catalonia). *Science of the Total Environment*, 654, 1337–1349. https://doi.org/10.1016/j.scitotenv.2018.11.061

Gu, D., Feng, Q., Guo, C., Hou, S., Lv, J., Zhang, Y., Sheng Yuan, S., & Zhao, X. (2019). Occurrence and risk assessment of antibiotics in manure, soil, wastewater, groundwater from livestock and poultry farms in Xuzhou, China. *Bulletin of Environmental Contamination and Toxicology*, 103(4), 590–596. https://doi.org/10.1007/s00128-019-02692-0

Gworek, B., Kijewska, M., Wrozek, J., & Graniewska, M. (2021). Pharmaceuticals in the soil and plant environment: A review. *Water, Air & Soil Pollution*, 232, 145. https://doi.org/10.1007/s11270-020-04954-8

Harrower, J., McNaughtan, M., Hunter, C., Hough, R., Zhang, Z., & Helwig, K. (2021). Chemical fate and partitioning behavior of antibiotics in the aquatic environment - A review. *Environmental Toxicology and Chemistry*, 40(12), 3275–3298. https://doi.org/10.1002/etc.5191
Huang, Y. J., Cheng, M. M., Li, W. H., Wu, L. H., Chen, Y. S., Luo, Y. M., Christie, P., & Zhang, H. B. (2013). Simultaneous extraction of four classes of antibiotics in soil, manure and sewage sludge and analysis by liquid chromatography-tandem mass spectrometry with the isotope labelled internal standard method. Analytical Methods, 5, 3721–3731. https://doi.org/10.1039/c3ay40220g

Khadera, A., Ezzaaria, A., Merhina, G., Capdeville, M., Budzinski, H., Hamdi, H., Pinelli, E., & Hafidi, M. (2019). Fate of antibiotics in present in a primary sludge of WWTP during their co-composting with palm wastes. Waste Management, 84, 13–19. https://doi.org/10.1016/j.wasman.2018.11.009

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U. s. a., 115, E3463–E3470. https://doi.org/10.1073/pnas.1717295115

Kümmerer, K. (2009). Antibiotics in the aquatic environment—A review—Part I. Chemosphere, 75, 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Kuti, A.I., Musa, J.J., Adeoye, P. A., Adabembe, B. A., Ani, O.C., Pius, O., & Aish, A. (2019). Effect of treated sewage sludge on the quality of okra fruit. AgricEngl: CIGR Journal, 20(1), 16–24

Lamastra, L., Suciu, N. A., & Trevisan, M. (2018). Sewage sludge for sustainable agriculture: Contaminants’ contents and potential use as fertilizer. Chemical and Biological Technologies in Agriculture, 5, 10–15. https://doi.org/10.1186/s40538-018-0122-3

Lillemberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, H., Amro, M., & Abbah, V. (2018). Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. Journal of Chromatography A, 1216, 5949–5954.

Lindberg, R., Wennberg, P., Johansson, M. I., Tysklind, M., & Andersson, B. A. V. (2005). Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environmental Science & Technology, 39, 3421–3429. https://doi.org/10.1021/es04413z

Martin, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., & Alonso, E. (2012). Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. Journal of Hazardous Materials, 239–240, 40–47. https://doi.org/10.1016/j.jhazmat.2012.04.068

Martin, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., & Alonso, E. (2012). Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. Journal of Environmental Management, 102, 18–25. https://doi.org/10.1016/j.jenvman.2012.02.020

Mejías, C., Martin, J., Santos, J. L., Aparicio, I., & Alonso, E. (2021). Occurrence of pharmaceuticals and their metabolites in sewage sludge and soil: A review on their distribution and environmental risk assessment. Trends in Environmental Analytical Chemistry, 30, e00125. https://doi.org/10.1016/j.teac.2021.e00125

Muriuki, C., Kairigo, P., Home, P., Ngumba, E., Raude, J., Gachanja, A., & Tuhkanen, T. (2020). Mass loading, distribution, and removal of antibiotics and antiretroviral drugs in selected wastewater treatment plants in Kenya. Science of the Total Environment, 743, 140655. https://doi.org/10.1016/j.scitenv.2020.140655

Ocampo, P. S., Lazár, V., Papp, B., Arnoldini, M., Wiesch, P. A., Busa-Fekete, G., Pal, C., Ackermann, M., & Bonhoeffer, S. (2014). Antagonism between bacteriostatic and bactericidal antibiotics is prevalent. Antimicrobial Agents and Chemistry, 58, 4573–4582. https://doi.org/10.1128/AAC.02463-14

Oduyebo, O. O., Olayinka, A. T., Iregbu, K. C., Versporten, A., Goossens, H., Nwajobi-Princewill, P. I., Jimoh, O., Ige, T. O., Aigbe, A. I., Ola-Bello, O. I., Aboderin, A. O., & Ogunsofa, F. T. (2017). A point prevalence survey of antimicrobial prescribing in four Nigerian tertiary hospitals. Annals of Tropical Pathology, 42–46. https://doi.org/10.4103/atp.atp_38_17

Olarimoye, O., Bakare, A., Ugwumba, O., & Hein, A. (2016). Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. Journal of Environmental Chemistry and Ecotoxicology, 8, 14–24. https://doi.org/10.5897/JECE2015.0364

Onweremadu, E. U., Asawalam, D. O., & Ibe, A. E. (2007). Changes in soil properties following application of composted sludge on an Isohygrotrophic Kandiudult. Research Journal of Environmental Toxicology, 1(2), 62–70.

Pino, M. R., Val, J., Mainar, A., Spaniolo, C., & Langa, E. (2015). Acute toxicological effects on the earthworm Eisenia fetida of 18 common pharmaceuticals in artificial soil. Science of the Total Environment, 518–519, 225–237.

Roose-Amsaleg, C., & Laverman, A. M. (2016). Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. Environmental Science and Pollution Research, 23, 4000–4012. https://doi.org/10.1007/s11356-015-4943-3

Scaria, J., Anupama, K. V., & Nidheesh, P. V. (2021). Tetracyclines in the environment: An overview on the occurrence, fate, toxicity, detection, removal methods, and sludge management. Science of the Total Environment, 771, 145291. https://doi.org/10.1016/j.scitenv.2021.145291

Sodipo, O. O., Adedokun, A., & Adejumo, O. (2017). Drug prescription pattern in a Nigerian teaching hospital. Nigeria Journal of Family Practice, 8(4), 29–33.

Song, Q., Li, J., & Zeng, X. (2015). Minimizing the increasing solid waste through zero waste strategy. Journal of Cleaner Production., 104, 199–210. https://doi.org/10.1016/j.jclepro.2014.08.027

Ternes, T. A., Herrmann, N., Bonerz, M., Ackermann, M., & Siegrist, T. (2014). Minimizing the increase in pharmaceuticals and musk fragrances in sewage sludge. Water Science of the Total Environment, 5897/518–519.

Thiele-Bruhn, S. (2019). Environmental risks from mixtures of antibiotic pharmaceuticals in soils — a literature review. In S. Korandi & I. Vogel (Eds.), (Texte 32/2019, Report...
No. (UBA-FB) FB000071, pp. 119). Umweltbundesamt, Dessau-Roßlau, Germany.

Thomaidi, V. S., Stasinakis, A. S., Borova, V. L., & Thomaidis, N. S. (2016). Assessing the risk associated with the presence of emerging organic contaminants in sludge-amended soil: A country-level analysis. *Science of the Total Environment, 548–549*, 280–288. https://doi.org/10.1016/j.scitotenv.2016.01.043

Vasquez, M. I., Lambrianides, A., Schneider, M., Kümmerer, K., & Fatta-Kassinos, D. (2014). Environmental side effects of pharmaceutical cocktails: What we know and what we should know. *Journal of Hazardous Materials, 279*, 169–189. https://doi.org/10.1016/j.hazmat.2014.06.069

Verlicchi, P., Al Aukidy, M., Galletti, A., Petrovic, M., & Barceló, D. (2012). Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of the Total Environment, 430*, 109–118. https://doi.org/10.1016/j.scitotenv.2012.04.055

Verlicchi, P., & Zambello, E. (2015). Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. *Science of the Total Environment, 538*, 750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108

Yan, Q., Gao, X., Chen, Y. P., Peng, X. Y., Zhang, Y. X., Gan, X. M., Zi, C. F., Guo, J. S., & &,. (2014). Occurrence, fate and ecotoxicological assessment of pharmaceutically active compounds in wastewater and sludge from wastewater treatment plants in Chongqing, the Three Gorges Reservoir Area. *Science of the Total Environment, 470–471*, 618–630. https://doi.org/10.1016/j.scitotenv.2013.09.032

Yang, Y., Owino, A. A., Gao, Y., Yan, X., Xu, C., & Wang, J. (2016). Occurrence, composition and risk assessment of antibiotics in soils from Kenya Africa. *Ecotoxicology, 25*(6), 1194–1201. https://doi.org/10.1007/s10646-016-1673-3

Zhao, F., Yang, L., Li, G., Fang, L., Yu, X., Tang, Y., Li, M., & Chen, L. (2022). Veterinary antibiotics can reduce crop yields by modifying soil bacterial community and earthworm population in agro-ecosystems. *Science of the Total Environment, 808*, 152056. https://doi.org/10.1016/j.scitotenv.2021.152056

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.