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RESEARCH ARTICLE

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ENVIRONMENT RESEARCH

The potential ecological risk of veterinary pharmaceuticals from swine wastewater on freshwater aquatic environment

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Abstract

The impact of pharmaceutical residue transport in the aquatic ecosystem has become an increasing subject of environmental interest due to the inherent bioactivity of trace levels of antibiotics and the negative environmental and public health impact. In this study, three veterinary pharmaceuticals including tetracycline, ivermectin, and salicylic acid were investigated in a piggery effluent from Western Cape, South Africa. Three freshwater organisms' taxonomic groups (*Pseudokirchneriella subcapitata*, *Daphnia magna*, and *Tetrahymena thermophila*) were used to determine the ecological risk of different treated piggery effluent concentration range of 1%, 10%, and 20% and a cocktail mixture of veterinary pharmaceuticals of environmental concerns. The average concentration of veterinary pharmaceuticals was in the range of 47.35, 7.19, and 1.46 μg L^{-1} for salicylic acid, chloro-tetracycline, and ivermectin, respectively. *P. subcapitata* exposed to 20% piggery wastewater effluent at 24- and 48-h EC_{50} showed a toxicity value of 14.2% and 13.6% (v/v), respectively. The study established the ecological risk of the test compounds as low to medium risk for low-level dose and low concentrations of piggery effluent. The relative sensitivity ranking of the taxa drawn is microalgae > protozoa > Cladocera. The study results demonstrated that a high dose of piggery effluent and mixtures of veterinary pharmaceutical can pose a high risk in freshwater ecosystems.

Practitioner Points:

- Transport processes of veterinary antibiotics into the environment were investigated.
- Dilution effect of the veterinary pharmaceutical on the antibiotic levels exists.
- High dose of piggery effluent presented an ecological risk.

KEYWORDS
ecological risk, effluent, freshwater ecosystem, piggery, veterinary pharmaceuticals

INTRODUCTION

Veterinary pharmaceuticals have been extensively used in previous decades in pig farm production to increase productivity and improve animal health (Ramírez-Morales et al., 2021), although the pattern of consumption using these pharmaceuticals is varied across countries (Jones et al., 2010; Lekagul et al., 2019). Veterinary pharmaceuticals are biologically active substances used in the treatment, prevention, and control of various diseases of livestock animals (Chan et al., 2022). When the antibiotic metabolism is poor in the animal gut, a large proportion of the residual antibiotics are excreted unchanged to the environment via river runoff or stream from the pig farms (Chan et al., 2020). Although the residual concentration of antibiotics over 450 μg L^{-1} in surface water may not be deemed to be unsafe for human, but bioassay showed that some antibiotic in surface water at a concentration below 10 μg L^{-1} can exhibit high ecological risks to aquatic organisms (Danner et al., 2019). Therefore, the aquatic contamination by veterinary pharmaceuticals due to their increasing applications in livestock animals is becoming an increasing environmental concern to all life forms and the ecosystems (Fekadu et al., 2019).

Commercial pig farms account for about 74% of intensive administration of pharmaceutical compounds due to their beneficial effects on growth rate and feed conversion efficiency and for the prevention and treatment of disease, which has thus become a concern and potential risk (Diana et al., 2021). Studies have shown the increased presence of veterinary pharmaceuticals in different environmental compartments (Obimakinde et al., 2016) where these substances are found either as parent materials or as a range of transformed products in the aquatic environment (Hu & Cheng, 2016). The removal processes and transportation of veterinary pharmaceuticals antibiotics and the piggery farm effluent are relatively dependent on the physicochemical and dilution properties (Selvam et al., 2017). The rate of disappearance of antibiotics via dilution process is substantially correlated to the seasonal variation such as rainy and dry seasons, leading to various dilution factors on the effluents (Lei et al., 2019); however, the total removal processes of antibiotics in the piggery farm effluent are not clearly understood.

Studies have shown that veterinary pharmaceuticals are toxic to water species such as plants, algae, phytoplankton, marine bacteria, fish, and crustaceans (Bártíková et al., 2016). These freshwater organisms such as Cladoceras: *Daphnia magna*, algae: *Pseudokirchneriella subcapitata*, and protozoa: *Tetrahymena thermophila* are representatives of the trophic levels in the freshwater ecosystem (Pereao et al., 2021), and these organisms also form the basis of the freshwater food chain, linking the physicochemical quality of water with other higher trophic levels of freshwater ecosystems. These organisms have been developed and used in many bioassay studies and the primary producers among them manifest rapid reproduction rate, which is affected when exposed to toxicants (Boisson & Perrodin, 2006; Perrodin et al., 2013). Models are available to provide both simple and affordable strategies in working out the concentrations of the toxic substances, and the conceptual model used in this study was previously described by Perrodin et al. (2013).

Ecological risk assessment evaluates the risk associated with the discharge of pollutants in the environment and methods used in assessing ecological risk tend to link the risks with the chemicals used in the environment (Ankley et al., 2021). Risk quotient (RQ) values are often used to classify aquatic organism's ecological risk to antibiotics and are typically defined based on the actual antibiotics quantities present and the predicted no-effect concentration (PNEC) in the test toxicant (Cardini et al., 2021; Y. Zhang et al., 2020). The estimation of the actual risk is built on the ratio of predicted effect concentration (PEC) to the PNECs for the aquatic organisms exposed. However, PEC may be replaced by measured environmental concentration (MEC) of the test toxicant (Straub et al., 2019). Ferrari et al. (2003) used the MEC–PNEC ratio to estimate the ecological risk of carbamazepine, clofibric acid, and diclofenac in the aquatic ecosystem. Preliminary ecological risk of the discharge of pharmaceutical using the MEC–PNEC ratio on surface water in Bangladesh showed that carbamazepine had low risk whereas erythromycin, tylosin, and sulfamethoxazole indicated a medium risk to sensitive aquatic organisms (Hossain et al., 2018).

Livestock farming is a major industry contributing to the South African agricultural economy. The farms contribute approximately 85% of the required meat consumption and 48% of agricultural output in South Africa and employed about 3005 workers in about 132 commercial

farms, 19 research study farms, and 400 smallholder farms and, in the last decade, about 27 million pigs were processed, yielding more than 2 million tons of pork meat (Munzhelele et al., 2017). Agriculture is one of the most important mainstay occupations in the South Africa's Western Cape province, and livestock farms compose of both informal and industrial arrays of poultry, piggeries, diary, and feedlot cattle farms (Fatoki et al., 2018). Stellenbosch is a municipal city in the Western Cape province of South Africa and is characterized by big and small commercial livestock farm of various animal species such as pigs, cattle, sheep, poultry, and birds of different species (Udebuani et al., 2021), which accounts for the selection of the study area. However, the study of contamination by consortium of veterinary pharmaceuticals especially from piggery effluent and the ecological risk consequence associated with the discharge in the natural environments is very limited in South Africa.

This study intends to determine the occurrence of three veterinary pharmaceuticals in a piggery effluent and their hazard in a commercial livestock production area in the Western Cape, South Africa. Furthermore, ecotoxicological tests on three taxonomic organisms (*P. subcapitata*, *D. magna*, and *T. thermophila*) were performed to assess the environmental risk estimate on the effluent samples. The results were used to clarify the category of piggery effluents and mixtures of veterinary pharmaceutical contributing to the dilution concentration and toxicity of the piggery effluents. The study predicted the effect on freshwater organisms, which can cause ecological dysfunction and instability through the food web. This systematic study can provide a significant reference for veterinary pharmaceutical in piggery effluents for farmers as stakeholders, policymakers, and researchers.

MATERIALS AND METHODS

Study site

Piggery effluent was collected from a farm in Stellenbosch, Western Cape, South Africa, as shown in Figure 1. This piggery farm lies between 33°53′43.2″S latitude and 18°48′33.8″ longitude. The study area showed a population increase from 90,000 in 2010 to a population density of 196,036 persons in 2021 (Stellenbosch Municipality, 2021). The study location is an old commercial town in South Africa situated along the banks of Eerste River, in the Western Cape

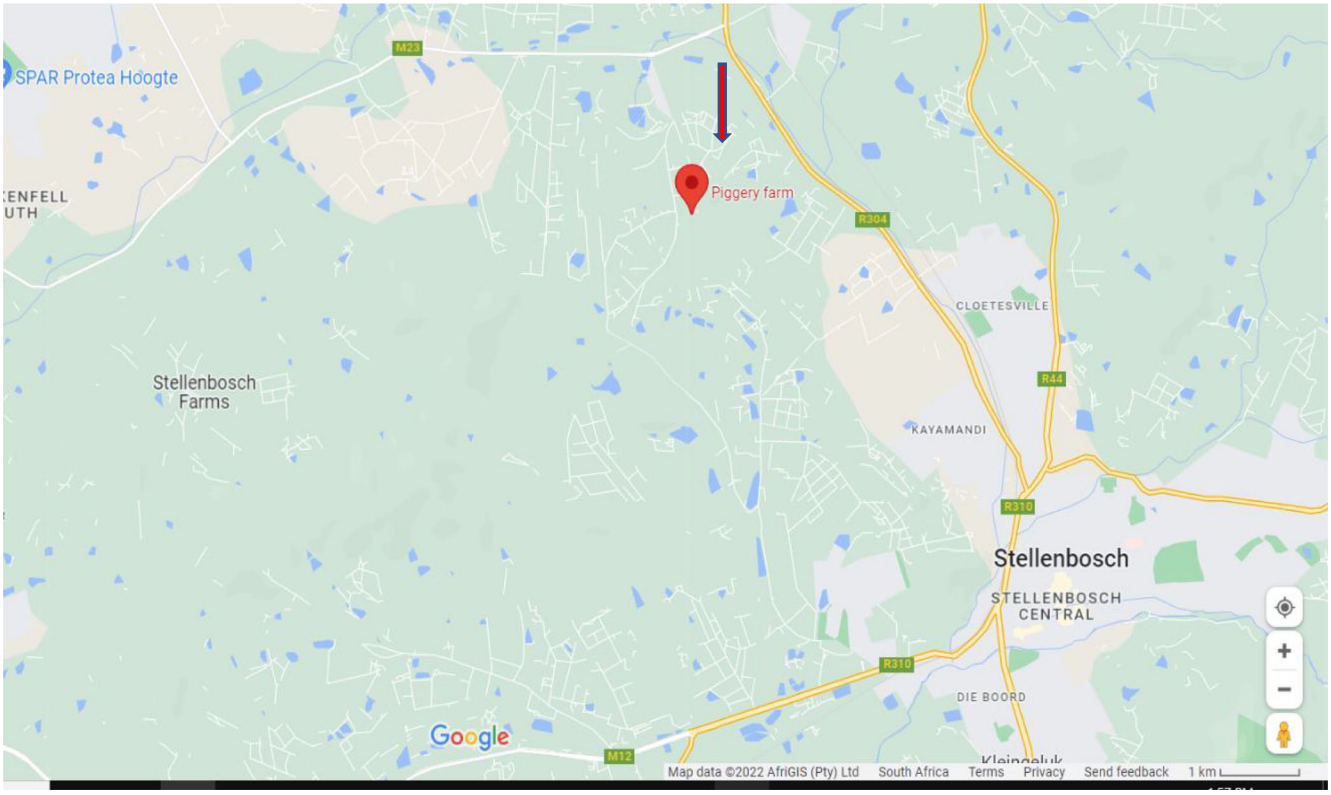

FIGURE 1 Map showing the sampling location in Stellenbosch, South Africa (*Source*: Google maps)

province of South Africa, and approximately 55 km from the Eastern Cape (Musakwa & Van Niekerk, 2013). The piggery wastes were pushed from the pig's pens through an underground channel and discharged into a retention pond (considered to be semi-treatment of the effluent wastewater). The raw piggery wastewater effluent was collected directly from three sampling points from the streams of the effluent wastewater. The effluent was initially semi-treated in a chamber that separated the solid waste from the liquid waste. The piggery effluent was collected from the wastewater surface flowing out of the chamber. The three sampling points were near to the point of discharge of the semi-treated effluent (considered as upstream), middle (mid-stream), and at the point of entry to the storage system (downstream).

Chemicals

The veterinary pharmaceuticals, salicylic acid (95%), ivermectin (95%), and tetracycline (99.9%), HPLC grade methanol (99.9%), and HPLC grade acetonitrile (>98%) used in the study were obtained from Sigma-Aldrich, South Africa, as pure standards. Oxygen (99.998%) was supplied by Air-Liquide, South Africa. Milli-Q water – 18 (Synergy Ultrapure Water System, Millipore, France) was used in all the experimental preparation.

Preparation of stock solution

A total of 100 mg L^{-1} stock solutions of the veterinary pharmaceuticals (ivermectin, tetracycline, and salicylic acid) were prepared from a weighted base of the drugs where 0.01-g crystal of each of the veterinary pharmaceutical used was dissolved in 100-ml Milli-Q water. The prepared stock solutions were kept in a refrigerator at a temperature of 4°C and were used within 48 h to minimize errors associated with possible analyte degradation. The lowest and highest concentrations of each veterinary pharmaceutical were mixed separately, as shown in Table 1, based on the concentration of the veterinary pharmaceutical medicines found in surface water around agricultural livestock farms by Fatoki et al. (2018). The outcome of this study was used as a guide to the concentrations of veterinary pharmaceuticals that already existed in the freshwater ecosystem. The mixtures of low concentration of the drug substances formed the low-level dose (LLD) and high-level dose (HLD) of veterinary substances used in this study.

TABLE 1 The low- and high-concentration doses of veterinary pharmaceuticals previously detected in agricultural farms as used in this study

Veterinary pharmaceuticals (μg L^{-1})	Low concentration detected in the field (μg L^{-1})	High concentration detected in the field (μg L^{-1})
Tetracycline	3.45	4.88
Ivermectin	1.74	1.97
Salicylic acid	1.37	19.50

Pharmaceuticals extraction and quantification

The solid-phase extraction (SPE) method was used for the extraction of the tested antibiotics from the piggery farm wastewater. The procedure developed by Fatoki et al. (2018) was adapted for this purpose. Briefly, the hydrophilic–lipophilic balance SPE cartridges (200 mg/6 ml) were supplied by Sigma-Aldrich and were conditioned using 3-ml methanol, 5-ml 30% methanol, and 5-ml Milli-Q water at 1 ml min^{-1}. A 500-ml sample previously filtered using a 0.45-μm filter was now loaded on the previously conditioned SPE column and eluted at 1 ml min^{-1} flow rate. Thereafter, 5 ml of Milli-Q water was passed through and left for 30 min on the vacuum manifold to dry (−70 kPa). A total of 3.5 ml of methanol and followed by 3.5 ml of n-hexane/acetone (50/50 v/v) were used to recover the retained analytes. This was blown to dryness under a gentle stream of nitrogen and reconstituted in a 1-ml methanol/water (50/50 v/v). The analysis was done using a Waters Chromatogram (2695) equipped with a binary HPLC pump (Waters 1525), an autosampler (Waters 2707), and a dual-wavelength absorbance detector (2487) and operated on a Breeze software. Automatically injected 10 μl of the extract was passed into an Ace 5 C18 column (150 × 4.6 mm; 5-μm particle size). The mobile phase for the gradient elution was made up of 0.1% formic acid in Milli-Q water (mobile phase A) and acetonitrile (mobile phase B). There was a gradual increase in the gradient to 100% of mobile phase B over 35 min (Table 2). The operational flow rate was 1 ml min^{-1}, with a wavelength of 254 nm set for the UV–vis detector.

Test organisms

The test freshwater organisms used in this study, *D. magna* (DAPHTOXKIT F MAGNA), *P. subcapitata*

(ALGALTOXKITF), and *T. thermophila* (PROTOXKITF), were purchased from the Microbiotest Inc., Belgium. The neonates of *D. magna* after hatching of the ephippia were used in the test. The observed effects on the test organisms include growth inhibitions (algae and protozoa) and mobility (Cladoceras), as shown in Figure 2.

Experimental exposures—Standard acute toxicity test using test organism

Ecotoxicity test using freshwater organisms standard operational procedure was carried out as reported by Udebuani et al. (2021) to investigate the veterinary pharmaceutical cocktail and piggery effluent. The algal toxicity test kit (algaltoxkit) included a green microalgae, *P. subcapitata*, and the test adhered to OECD guideline 201 (OECD, 2006) and ISO norm 8692 based on the American Society for Testing and Material (ASTM) recommendation. The Crustacean toxicity test kit (daphtoxkit) included crustaceans, *D. magna*, and ISO 6341 and OECD guideline 202 (OECD, 2004) test procedures were followed. The ciliate protozoan toxicity test kit (protoxkit F) included a protozoan, *T. thermophila*, and the test was a 24-h reproductive inhibition test and adhered to the procedure of OECD guideline 202 (OECD, 2017) as recommended by ASTM. The three different cultured media were reconstituted with water prepared with Milli-Q® water, 294 mg/L $CaCl_2{\cdot}2H_2O$, 123.25 mg/L $MgSO_4{\cdot}7H_2O$, 5.75 mg/L KCl, and 64.75 mg/L $NaHCO_3$ in accordance with OECD guideline 202, and all the test specifications for the freshwater acute tests are shown in Table 3.

TABLE 2 Chromatographic parameters for the quantification of pharmaceuticals

Chromatograph	Waters		
Detector	UV		
Column	Ace 5 C8 (15 × 4.6 mm)		
Injection volume	10 µl		
Mobile phase	A: 0.1% formic acid in Milli-Q water		
	B: Acetonitrile		
Flow rate	1 ml min^{-1}		
Gradient elution	Time (min)	%A	%B
	0	90	10
	35	0	100
	38	0	100
	45	90	10
Temperature	25°C		
Data collection	Breeze software Version 2		

Ecological risk assessment

The potential ability of pollutants to generate undesired environmental effects in aquatic organisms is measured by calculating the RQs, and the ecological risk caused by the three veterinary pharmaceuticals in piggery effluent from Western Cape, South Africa, was assessed using the deterministic quotient method (N. Liu et al., 2020). The chronic deterministic risk method was calculated by the division of the MEC by the PNEC, as presented in Equation (1).

$$RQ = {}^{MEC}/_{PNEC} \quad (1)$$

The ecological risk assessment was ranked as follows (Bu et al., 2013; N. Liu et al., 2020):

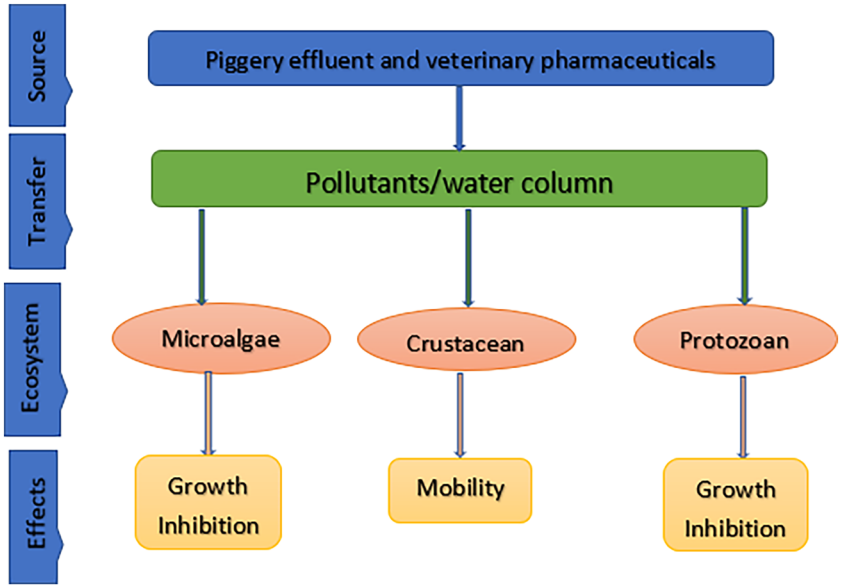

FIGURE 2 Conceptual model for freshwater organisms exposed to veterinary pharmaceuticals and wastewater effluent

TABLE 3 Freshwater acute toxicity test specification operational standards using algae, daphnid, and protozoa

	Algae	Daphnid	Protozoa
Species	*Pseudokirchneriella subcapitata*	*Daphnia magna*	*Tetrahymena thermophila*
Length of test (h)	72	48	24
Organism source	Laboratory cultured	Laboratory reared	Laboratory reared
Dilution medium	Algae assay medium	Standard freshwater	Protox freshwater
Temperature (°C)	24 ± 2	20 ± 1	22 ± 2
Photo period	24-h light	16-h light:8-h dark	-
Illumination (lux)	4300 ± 430	6000	-
Test vessel size	125-ml Erlenmeyer	100-ml flask	Ciliate inoculum tube
Test volume (ml)	50	100	50
Test chamber	Environmental chamber	Environmental chamber	Environmental chamber
Effect criteria	Cell counts, total cell volume	Mortality sublethal effect	Optical density/cell growth
Day observation	Cell count, cell volume	Temperature, pH, and dissolved oxygen	Temperature, pH, and dissolved oxygen

TABLE 4 Pharmaceutical concentrations in piggery effluent

Analyte	Retention Time (min)	Pharmaceutical concentrations (μg L^{-1})				
		Sample 1	Sample 2	Sample 3	Average	SD
Tetracycline	9.56	6.76	8.45	6.36	7.19	±1.11
Salicylic acid	11.4	45.35	46.05	50.65	47.35	±2.88
Ivermectin	35.5	1.59	1.44	1.36	1.46	±0.12

RQ < 0.1 = risks were insignificant,
0.1 ≤ RQ < 1 = low risk,
1 ≤ RQ < 10 = moderate risk, and
RQ ≥ 10 = high risk,

where MEC is the measured environmental concentration analyzed for the veterinary pharmaceutical in the sampling locations and PNEC is the predicted no-effect concentrations resulting from dividing the toxicity data by the assessment factors (AFs) on the test endpoints (Okonski et al., 2021; Wang et al., 2021). The measured environmental concentration (MEC_{SW}) used in this study was obtained from the analysis of concentrations of the selected veterinary pharmaceutical compound available in the veterinary pharmaceutical wastewater obtained from the piggery effluent collected from a farm in Stellenbosch, Western Cape. The averages of the concentration of the veterinary pharmaceuticals (ivermectin, salicylic acid, and tetracycline) in the surface water (Table 4) were used as our MEC_{SW}. The MEC can be used in place of the predicted environmental concentration (Gumbi et al., 2022). The no observed effect concentration (NOEC) data used in this study were generated using the ToxRat software for 24- to 72-h EC_{50} *P. subcapitata* and 24- to 48-h LC_{50} *D. magna*, but the NOEC for *T. thermophila* was not calculated from the ToxRat software. The AF is a default safety factor for chronic and acute toxicity in use to derive PNECs (Yan et al., 2013). The AF used for this study was obtained from previously reported work (N. Liu et al., 2020); the AF used for *P. subcapitata* in algae population was 10, the AF value for *D. magna* crustacean population was 10, and the AF used for *T. thermophila* protozoan population was 100. The PNEC is used to assess the actual ecological risk of discharged veterinary pharmaceutical and piggery effluent. The effect assessment was evaluated based on the estimation of PNEC and is the concentration of the toxicants in freshwater below which adverse effect is most unlikely to occur during short-term exposure. PNEC was calculated by dividing the lowest available NOEC by the AF, as shown in Equation (2). However, the NOEC of *T. thermophila* was not given from the ToxRat software, but the endpoint (EC_{50}) was divided by the AF for *T. thermophila*, which is 100, as shown in Equation (3).

$$PNEC = {}^{NOEC}/_{AF} \quad (2)$$

TABLE 5 Acute freshwater toxicity data for different percentages of unfiltered piggery effluents, low and high level dose of veterinary pharmaceutical cocktail on test organisms

Toxicant	Organisms	Endpoint	EF ($\mu g\ L^{-1}$)	Endpoint	EF ($\mu g\ L^{-1}$)	Endpoint	EF ($\mu g\ L^{-1}$)
1% unfiltered piggery effluent	*Pseudokirchneriella subcapitata*	24-h EC_{50}	nd	24-h EC_{20}	nd	24-h EC_{10}	nd
		48-h EC_{50}	nd	48-h EC_{20}	125.7 (77.1–201.2)	48-h EC_{10}	41.8 (28.6–60.1)
		72-h EC_{50}	nd	72-h EC_{20}	nd	72-h EC_{10}	nd
	Daphnia magna	24-h LC_{50}	23.2 (17.7–30.4)	24-h LC_{20}	11.4 (7.2–15.3)	24-h LC_{10}	nd
		48-h LC_{50}	nd	48-h LC_{20}	nd	48-h LC_{10}	nd
	Tetrahymena thermophila	24-h EC_{50}	6.59	24-h EC_{20}	4.86	24-h EC_{10}	4.39
10% unfiltered piggery effluent	*P. subcapitata*	24-h EC_{50}	25.6 (nd)	24-h EC_{20}	25.4 (nd)	24-h EC_{10}	nd
		48-h EC_{50}	49.3 (nd)	48-h EC_{20}	45.9 (nd)	48-h EC_{10}	nd
		72-h EC_{50}	nd	72-h EC_{20}	nd	72-h EC_{10}	nd
	D. magna	24-h LC_{50}	nd	24-h LC_{20}	nd	24-h LC_{10}	nd
		48-h LC_{50}	nd	48-h LC_{20}	nd	48-h LC_{10}	nd
	T. thermophila	24-h EC_{50}	4.81	24-h EC_{20}	3.9	24-h EC_{10}	3.64
20% unfiltered piggery effluent	*P. subcapitata*	24-h EC_{50}	14.2 (nd)	24-h EC_{20}	13.5 (nd)	24-h EC_{10}	13.2 (nd)
		48-h EC_{50}	13.6 (nd)	48-h EC_{20}	12.3 (nd)	48-h EC_{10}	12.4 (nd)
		72-h EC_{50}	nd	72-h EC_{20}	nd	72-h EC_{10}	nd
	D. magna	24-h LC_{50}	nd	24-h LC_{20}	nd	24-h LC_{10}	nd
		48-h LC_{50}	nd	48-h LC_{20}	nd	48-h LC_{10}	nd
	T. thermophila	24-h EC_{50}	52.4	24-h EC_{20}	55.0	24-h EC_{10}	55.9
HLD veterinary pharmaceutical	*P. subcapitata*	24-h EC_{50}	nd	24-h EC_{20}	nd	24-h EC_{10}	nd
		48-h EC_{50}	nd	48-h EC_{20}	nd	48-h EC_{10}	42.82 (9.34–196.3)
		72-h EC_{50}	nd	72-h EC_{20}	nd	72-h EC_{10}	nd
	D. magna	24-h LC_{50}	20.2 (16.4–24.8)	24-h LC_{20}	13.1 (9.4–16.1)	24-h LC_{10}	nd
		48-h LC_{50}	19.0 (15.0–23.0)	48-h LC_{20}	11.1 (7.6–14.1)	48-h LC_{10}	nd
	T. thermophila	24-h EC_{50}	0.014	24-h EC_{20}	0	24-h EC_{10}	0
LLD veterinary pharmaceutical	*P. subcapitata*	24-h EC_{50}	nd	24-h EC_{20}	11.55 (0.71–215.13)	24-h EC_{10}	1.0 (0.07–15.3)
		48-h EC_{50}	nd	48-h EC_{20}	10.5 (2.7–42.3)	48-h EC_{10}	1.97 (0.51–7.7)
		72-h EC_{50}	nd	72-h EC_{20}	15.98 (6.2–41.3)	72-h EC_{10}	4.4 (1.7–11.3)
	D. magna	24-h LC_{50}	101 (nd)	24-h LC_{20}	89.7 (nd)	24-h LC_{10}	nd
		48-h LC_{50}	79.8 (nd)	48-h LC_{20}	40.3 (nd)	48-h LC_{10}	nd
	T. thermophila	24-h EC_{50}	0.001	24-h EC_{20}	3.03	24-h EC_{20}	41.1

Abbreviations: (), low and upper 95% confidence limits; EF, effect concentration; HLD, high-level dose ; LLD, low-level dose; nd, not determined.

$$PNEC_{(Tetrahymena\ thermophila)} = EC_{50}/AF \quad (3)$$

Statistical analysis

Data collected from the study were analyzed using routine statistical tools as means and percentages. One-way analysis of variance (ANOVA) and Pearson's correlation coefficient were also used in all cases to see if the mean concentration of toxicants varied among the three different concentrations of the test samples monitored at 5% level of significance. EC values for effective concentrations were estimated by Probit analysis with linear maximum likelihood regression (Toxicity Response Analysis and Testing version TRPRO310; ToxRat Solutions GmbH, Alsdorf, Germany).

RESULTS

The quantification results for the pharmaceuticals present in the piggery effluent are presented in Table 4. The trend of the selected pharmaceuticals in the samples showed that salicylic acid, which is commonly used in anti-inflammatory, keratolytic, and dermatic products (Heberer, 2002) and in animal husbandry, recorded the highest concentration (45.35–50.65 μg L^{-1}). This was followed by chloro-tetracycline (6.36–8.45 μg L^{-1}) and ivermectin (1.36–1.59 μg L^{-1}), which were the lowest. A similar trend was previously reported by Fatoki et al. (2018) for surface water around livestock agricultural farms obtained in the Western Cape region of South Africa. The elution chromatogram at wavelength of 254 nm for the quantified pharmaceuticals is presented in Figure S1.

Freshwater organism's toxicity test for HLD and LLD of veterinary pharmaceutical cocktails was investigated where *D. magna*, *P. subcapitata*, and *T. thermophila* were exposed to high-level dose (HLD) of veterinary pharmaceutical cocktail at different endpoints. *D. magna* exposed to HLD of veterinary pharmaceutical cocktail showed the lowest toxicity values compared with the other toxicants, as shown in Table 5. *T. thermophila* exposed to 24-h EC_{50} HLD of veterinary pharmaceutical cocktail showed a much lower toxicity value (0.014 μg L^{-1}) compared with *D. magna*. The concentration of the HLD of veterinary pharmaceutical cocktail that affected 50% of *D. magna* at 24-h LC_{50} and 48-h LC_{50} was 20.2 (16.4–24.8 μg L^{-1}) and 19.0 (15.0–23.9 μg L^{-1}), respectively.

The values obtained from the concentration of *D. magna* exposed to HLD at 24-h LC_{20} and 48-h LC_{20} were 13.1 (9.4–16.1 μg L^{-1}) and 11.1 (7.6–14.1 μg L^{-1}), whereas the 24- to 48-h LC_{10} did not record any toxicity value and the effect concentrations obtained at these endpoints were almost in the same range. *D. magna* showed the same sensitivity to HLD even as exposure time progresses at different endpoints. The 24- to 72-h EC_{50} for *P. subcapitata* exposed to 1% unfiltered piggery wastewater effluent did not show any value. However, 48-h EC_{50} and EC_{10} *P. subcapitata* exposed to 1% unfiltered piggery effluent showed 125.7 (77.1–201.2 μg L^{-1}) and 41.8 (28.6–60.1 μg L^{-1}), respectively. *D. magna* exposed to 1% unfiltered piggery effluent at 24-h LC_{50} and LC_{20} exhibited an acute toxicity value of 23.2 (17.7–30.4 μg L^{-1}) and 11.4 (7.2–15.3 μg L^{-1}), respectively. The 24- to 48-h EC_{50} *P. subcapitata* exposed to 10% unfiltered piggery effluent showed a toxicity value of 25.6–49.3 μg L^{-1}. The values obtained for 24- to 48-h EC_{50} also gave similar values of 25.4–45.9 μg L^{-1}. Nothing was detected at 72-h EC_{50} and EC_{10}. *P. subcapitata* exposed to 20% unfiltered piggery effluent at 24-h EC_{50} and 48-h EC_{50} exhibited the toxicity of 14.2 and 13.6 μg L^{-1}. The 24-h EC_{50} and 48-h EC_{20} showed 13.5 and 12.3 μg L^{-1}, respectively.

The result showed that the effective concentration that can affect 50% of the population of *P. subcapitata* exposed to 20% unfiltered piggery wastewater effluent at 24- to 48-h EC_{50} was in the range of 13.6–14.2 μg L^{-1}. Similarly, *D. magna* did not exhibit any toxicity value in all the exposures. The *P. subcapitata* exposed to 20% unfiltered piggery wastewater effluent showed freshwater toxicity values of 24-h EC_{50}: 14.2 (not determined [nd]), EC_{20}: 13.5 (nd), and EC_{10}: 13.2 (nd), and the 48-h EC_{50}, EC_{20}, and EC_{10} showed a toxicity value of 13.6, 12.3, and 12.4 μg L^{-1}, respectively. The toxicity values obtained for different endpoints for 48-h EC_{50-10} were not significantly different from one another and the 72-h EC_{10-50} showed no toxicity value. The lowest observed effect concentration (LOEC) and NOEC values for the growth rate of *P. subcapitata* exposed at different concentrations of piggery effluent and veterinary pharmaceutical cocktail are shown in Table 6. The LOEC for *P. subcapitata* exposed to 1% unfiltered piggery effluent was at 48-h exposure, which was less than or equal to 6.25% concentration, and the highest LOEC was obtained at 72 h, which was greater than 100%.

The ecological risk assessment is crucial in estimating the associated ecological risk with pharmaceuticals present in piggery effluents in several environmental matrices. The harmful dose of pharmaceuticals existing in the piggery effluent to a given species in the adjoining aquatic ecosystem is estimated using the risk assessment.

TABLE 6 Risk characterization, and NOEC, MEC, PNEC, and RQ of piggery effluent and veterinary pharmaceuticals in freshwater ecosystem

Toxicant	Test organisms	Exposure duration	NOEC (μg L^{-1})	MEC (μg L^{-1})	PNEC (μg L^{-1})	Risk quotient
1% unfiltered piggery effluent	*Pseudokirchneriella subcapitata*	24-h EC_{50}	5.94	4.3	0.06	71.7
		48-h EC_{50}	3.09	4.3	0.03	143.3
		72-h EC_{50}	2.27	4.3	0.02	215
	Daphnia magna	24-h LC_{50}	25	4.3	2.5	1.72
		48-h LC_{50}	60	4.3	6	0.72
	Tetrahymena thermophila	24-h EC_{50}	6.59	4.3	0.07	61.4
10% unfiltered piggery effluent	*P. subcapitata*	24-h EC_{50}	6	4.3	0.06	71.7
		48-h EC_{50}	3.29	4.3	0.03	143.3
		72-h EC_{50}	0.38	4.3	0	4.3
	D. magna	24-h LC_{50}	100	4.3	10	0.43
		48-h LC_{50}	100	4.3	10	0.43
	T. thermophila	24-h EC_{50}	4.81	4.3	0.05	86
20% unfiltered piggery effluent	*P. subcapitata*	24-h EC_{50}	2.61	4.3	0.03	143.3
		48-h EC_{50}	1.31	4.3	0.01	430
		72-h EC_{50}	1.85	4.3	0.02	215
	D. magna	24-h LC_{50}	100	4.3	10	0.43
		48-h LC_{50}	100	4.3	10	0.43
	T. thermophila	24-h EC_{50}	52.4	4.3	0.52	8.27
HLD veterinary pharmaceutical	*P. subcapitata*	24-h EC_{50}	7.06	19.5	0.07	278.6
		48-h EC_{50}	4.15	19.5	0.04	487.5
		72-h EC_{50}	3.06	19.5	0.03	650
	D. magna	24-h LC_{50}	5	19.5	0.5	39
		48-h LC_{50}	20	19.5	2	9.75
	T. thermophila	24-h EC_{50}	0.014	19.5	0.00	19.5
LLD veterinary pharmaceutical	*P. subcapitata*	24-h EC_{50}	1.68	17.9	0.02	895
		48-h EC_{50}	4.55	17.9	0.05	358
		72-h EC_{50}	2.37	17.9	0.02	895
	D. magna	24-h LC_{50}	0	17.9	0	17.9
		48-h LC_{50}	5	17.9	0.5	35.8
	T. thermophila	24-h EC_{50}	0.001	17.9	0.00	17.9

Abbreviations: HLD, high-level dose; LLD, low-level dose; MEC, measured environmental concentration; NOEC, no observed effect concentration; PNEC, predicted no-effect concentration; RQ, risk quotient.

The hazard quotient (HQ) or RQ is the ratio of the piggery effluent's anticipated MEC or PEC to the PNEC (Ashfaq et al., 2017). In case of LLD veterinary pharmaceutical, the RQ values were found to be 895, 35.8, and 17.9 for *P. subcapitata*, *D. magna*, and *T. thermophila*, respectively, and these obtained results indicate high risk of LLD veterinary pharmaceutical to *P. subcapitata*, *D. magna*, and *T. thermophila*. The RQ values for HLD veterinary pharmaceutical were found to be 650, 9.75, and 19.5 against *P. subcapitata*, *D. magna*, and *T. thermophila*, and these resultant RQ values (>10) imply very high risk to these tested freshwater organisms except for *D. magna*, which exhibit moderate risk.

In the unfiltered piggery effluent (1%), the RQ values were found to be 215, 0.72, and 61.4 for *P. subcapitata*, *D. magna*, and *T. thermophila*. The *P. subcapitata* and

T. thermophila can cause high risk but *D. magna* RQ value indicates low risk (<1) to the freshwater organisms. The value of 10% unfiltered piggery effluent was assessed against *P. subcapitata*, *D. magna*, and *T. thermophila*, and the values were found to be 4.3, 0.43, and 86, respectively, for the tested aquatic species. The results imply that 10% unfiltered piggery effluent can pose moderate level of risk to *P. subcapitata* because the RQ values is <10 and the *D. magna* values present low risk whereas *T. thermophila* indicate very high risk. The RQ values of 20% unfiltered piggery effluent were calculated against *P. subcapitata*, *D. magna*, and *T. thermophila*, which were found to be 215, 0.43, and 8.27, respectively, and these result values indicate very high, low, and moderate risk to the tested organisms, respectively. RQ values for *P. subcapitata* were found to have very high risk in different effect concentrations time intervals of 24-h EC_{50}, 48-h EC_{50}, and 72-h EC_{50} for the effluent dilutions, which can possibly cause potential risk to freshwater species. The values for the RQ also increased with increasing dilutions except in 72-h EC_{50} for 10% unfiltered piggery effluent.

Higher ecological risk values were obtained in the exposure of *P. subcapitata* to 1% unfiltered piggery manure at 24-h EC_{50}, 48-h EC_{50}, and 72-h EC_{50} exposure durations (71.7, 143.3, and 215, respectively). *D. magna* to 1% unfiltered piggery effluent at 24-h EC_{50} and 48-h EC_{50} exposure durations showed the lowest risk values (1.72 and 0.72) compared with the higher values obtained from *P. subcapitata* and *T. thermophila* (61.43). At 10% unfiltered piggery waste exposure, higher ecological risk values were obtained with *P. subcapitata* exposed, this is followed by *T. thermophila* exposed to 24-h EC_{50} (86), and the least ecological risk values were obtained in *D. magna* exposed to 28- to 48-h LC_{50} at 0.43 and 0.43, respectively. The highest ecological risk samples were obtained at 48-h EC_{50} exposure of *P. subcapitata* to 20% unfiltered piggery effluent compared with the risk values obtained from other organisms used in this study. The result of ecological RQ obtained using the MEC obtained in HLD and LLD concentrations used in this study showed that the highest risk values were obtained from exposure of *P. subcapitata*, exposed to 24-h EC_{50}, 48-h EC_{50}, and 72-h EC_{50} (895, 358, and 895, respectively) of HLD of the veterinary pharmaceuticals used. Also, the risk values obtained in *P. subcapitata* exposed to 24-h EC_{50}, 48-h EC_{50}, and 72-h EC_{50} of LLD of the veterinary pharmaceuticals gave a similar trend result of 278.6, 487.5, and 650, respectively. However, lower values were obtained in *D. magna* and *T. thermophila* exposures, but the values were still significant to disrupt the structural function of freshwater ecosystem.

The NOEC for the growth rate of *P. subcapitata* was observed at 48 h, which was also less than at 6.25% concentration, and the NOEC for *P. subcapitata* exposed to 10% unfiltered piggery effluent was obtained at 72-h exposure whereas the LOEC and the NOEC for a growth rate of *P. subcapitata* at 24-h exposure were less than or equal to 6.25% concentration of the effluent. The LOEC and NOEC at 48 and 72 h were of the highest concentration of greater than or equal to 100% concentration of the effluent. The study has established that *P. subcapitata* and *D. magna* exposed to LLD of veterinary pharmaceutical showed high ecological risk. However, the result of risk characterization with the MEC from effluent wastewater shows that *P. subcapitata* and *T. thermophila* had the highest risk levels.

DISCUSSION

The result of pharmaceutical concentrations in piggery effluent in Table 4 showed that the tested piggery effluent associated with the veterinary pharmaceuticals may constitute contaminants in the effluent samples. The concentrations reported by Fatoki et al. (2018) for surface water around livestock agricultural farms were lower for salicylic acid (1.37–19.50 μg L^{-1}) and tetracycline (3.45–4.88 μg L^{-1}) and were closely related for ivermectin (1.74–1.97 μg L^{-1}) when compared with the concentrations reported in this study for tetracycline (7.19 μg L^{-1}), salicylic acid (47.35 μg L^{-1}), and ivermectin (1.46 μg L^{-1}), but the results showed a wide use of the three antibiotics in the surface water from the pig farm effluent. The variable concentrations in the effluent may be associated with peculiar drugs commonly administered to the piggery farms, and the occurrence of painkillers and antibiotics such as salicylic acid and tetracycline are frequently prescribed medications in animal husbandry whereas ivermectin will treat and control mites, lungworms, kidney lice, worms, and gastrointestinal roundworms in pigs (Ashraf & Prichard, 2016; Pasay et al., 2019). Tetracyclines are the most widely used antibiotics as veterinary medicines and more than 75% are excreted in an active form and released into the environment through animal urine and feces, which results in high levels of tetracyclines in the aquatic environment, causing adverse effects on the ecological system and human health (Xu et al., 2021). The study has shown that salicylic acid, which is used to treat inflammation and dermatological problems in livestock animals, recorded the highest values, compared with other veterinary pharmaceuticals used in this study. The low-level concentrations of salicylic acid, tetracycline, and ivermectin are indications of the prevalence of pharmaceutical residue in animal waste and as a major constituent and source of

contamination in water system with the possibilities of adverse environmental and public health effects.

The ecotoxicological effects of various residues of active pharmaceutical constituents and their metabolites on microbes, animals, and plants in the environment are not clearly understood because of the combined action of various pollution factors, but their chronic and acute consequences such as the potential indirect effects on wider ecosystems or endocrine disruptions on low levels of exposure to these substances have been reported (Fatoki et al., 2018). High residual concentration of tetracyclines in the pig farm antibiotic in wastewater (3.7–1000 μg L^{-1}) was reported (Chan et al., 2020; Lekagul et al., 2019), whereas other researchers reported the residual concentration of tetracyclines, sulfonamides, and quinolones at a low concentration below 1.1–360 μg L^{-1} detection in a river, which exhibited relatively high ecological risks to aquatic organisms (Y. Jiang et al., 2014; R. Zhang et al., 2012). Thus, the residual antibiotic in surface water, even at low concentration, could also exert selective pressure to the bacterial population to acquire antibiotic-resistant (Kümmerer, 2009a, 2009b), which is more harmful to human and animal because river water is widely used as a source of drinking water, irrigation, and recreational purposes (J. Liu et al., 2018; Xi et al., 2009). Therefore, the allergic response and resistant pathogens due to the likelihood of the presence of antibiotics are of most concern even in very low concentrations.

Environmental Hazard Assessment and Classification of European Community (Directive 67/548: EEC) reported that a chemical is classified as toxic to aquatic organisms when its EC_{50} is between 1 and 10 mg L^{-1} (Carlsson et al., 2006) and when the toxicants uptake into the body exceeds the combined rate of excretion and detoxification. Based on this classification, the toxicity values of *D. magna* exposed to different dilutions of unfiltered piggery wastewater 24- to 48-h EC_{50} were within the stipulated value. This study showed variations in the sensitivity of freshwater organisms (*P. subcapitata*, *D. magna*, and *T. thermophila*) to various concentrations of unfiltered piggery effluent and veterinary pharmaceuticals. The sensitivity of these test organisms to piggery effluent and mixture of veterinary pharmaceuticals is the measure of how responsive the organisms are to the test compounds.

The lower the toxicity values, the more sensitive the organisms are to the test compound. Much lower toxicity values were recorded for low- level dose (LLD) veterinary pharmaceuticals using *P. subcapitata* at 72-h EC_{20} (15.98 [6.20–41.3]), 72-h EC_{10} (4.39 [1.70–11.3]), 48-h EC_{20} (10.50 [2.66–42.3]), and 24-h EC_{10} (0.981 [0.068–15.3]), as shown in Table 5, and they are considered as relevant environmental concentrations, which can be used to measure the adverse effect of a toxicant in the environment (Beasley et al., 2015) and are also considered and treated as surrogate endpoints to the NOEC (Oris et al., 2012). High sensitivity to these toxicants observed in algae is attributed to its affinity to the active biocidal property of the compound, which disrupts the lipid synthesis destabilizing membrane (Franz et al., 2008) and which can uncouple oxidatively (Negrelo Newton et al., 2005). Studies have shown toxicity data from 72-h algal growth test to exhibit greater sensitivity than the results obtained from the acute lethal test of some test species (Dom et al., 2010), but this current study has established that *P. subcapitata* shows greater ecosystem sensitivity to piggery effluent and an LLD of veterinary pharmaceutical compared with other freshwater organisms used in this study. Additionally, 1% unfiltered piggery effluent and an HLD of veterinary pharmaceutical cocktail exerted acute toxicity effect on *D. magna* and *T. thermophila*. Also, *D. magna* were sensitive to an HLD of veterinary pharmaceutical at 24-h LC_{50} (20.2 [16.4–24.8] μg L^{-1}) and 48-h LC_{50} (19.0 [15.0–23.9] μg L^{-1}).

Similar toxicity values have been reported for *D. magna* exposed to various veterinary substances (Białk-Bielińska et al., 2011; De Liguoro et al., 2009), but *D. magna* exposed to antibiotics had higher toxicity values (74.3 [52.1–96.5] μg L^{-1}) when compared with the values obtained from this study. The relative sensitivity of the taxa drawn from this study was microalga (72-h growth tests) > *T. thermophila* (24-h growth inhibition test) > *D. magna* (48-h lethal test). The result obtained in this study established that the effluent wastewater even as it is semi-treated has very high risk on freshwater ecosystem. The study has shown that the worse affected organisms were the *P. subcapitata*, which showed the highest values, which is similar to the report of Dom et al. (2010). The potency of a toxicant is associated with the lower amount required to cause a response and supports that the test compounds used affected the freshwater organisms, which are the ecosystem primary producers. The lower the LC_{50}/EC_{50}, the higher the potency of the test compound (X. Jiang & Kopp-Schneider, 2014; Raj et al., 2013), and this study established that the piggery effluent and mixtures of veterinary pharmaceutical are potent on the test organisms used.

Thus, ecological risk assessment in Table 6 showed that there is an expected high level of harm and risk to the freshwater ecology because of the mixing and release of untreated or semi-treated and complex pharmaceutical effluent wastewater into the surrounding river and surface water. The results from the study have shown high ecological risk of release of veterinary pharmaceutical in freshwater ecosystem. The ecological risk

characterization values of the measured environmental concentration ($MEC_{wastewater}$) were detected from the surface water channel of semi-treated effluent water from piggery farm and the PNEC of the veterinary pharmaceutical. The result in both LLD and HLD of veterinary pharmaceuticals showed the highest values with *P. subcapitata* (358–895 μg/L for LLD and 278.6–650 μg/L for HLD). This is followed by values obtained with exposure of *T. thermophila*; however, *D. magna* showed the lowest values of risk characterization for 24- to 48-h exposure (17.9–35.8 μg/L for LLD and 9.75–39 μg/L for HLD). The risk value obtained from *P. subcapitata* exposed to LLD of veterinary pharmaceuticals at 24- to 72-h EC_{50} was 895, whereas *D. magna* exposed to LLD of veterinary pharmaceuticals at 24- to 48-h LC_{50} gave a risk value of 35.8.

Risk characterization of piggery effluence and veterinary pharmaceutical in freshwater ecosystem

Ecological risk assessment tends to identify the likelihood for effects, the extent and the uncertainty associated with chemicals, community, and an ecosystem. The potential ecological risk values for piggery effluent and mixtures of veterinary pharmaceutical impact on the freshwater organism from different trophic levels were summarized in Table 6. The study showed a moderate to negligible impact for freshwater organisms exposed to an LLD of veterinary pharmaceuticals and 1% unfiltered piggery wastewater effluent. The moderate impact that was recorded in this study signified that a portion of the organism population is affected, and this can cause a change in the abundance and distribution of the population. This will, in turn, threaten the integrity of the whole population in the freshwater ecosystem. The result of MEC:PNECs of HLD of veterinary pharmaceutical and 1%–20% unfiltered piggery effluent showed a major impact on the test toxicants. The ecological implication of the major impact signifies that the entire species used in this study were impacted in a significant magnitude to cause a decline in abundance and distribution of organisms in the freshwater ecosystem.

P. subcapitata, *D. magna*, and *T. thermophila* are the base of the aquatic food chain and they are the fundamental link of physicochemical parameters of the waterbody and the higher trophic levels. Based on the results obtained in this study, higher concentrations of piggery wastewater effluent and veterinary pharmaceutical cocktails are dangerous toxicants for the freshwater ecosystem with similar results reported by other researchers (Dahshan et al., 2015; Huang et al., 2014; Miller et al., 2018). The risk of high doses of piggery effluent and veterinary pharmaceutical from the surrounding environment should be given more attention and monitoring. The study has shown that the combined existence of these substances in the freshwater can produce a substantial toxicity effect on the freshwater organism and these organisms are the primary producers and consumers that are important in the functionality and sustainability of the freshwater ecosystem. Pollution from piggery effluent and veterinary pharmaceutical with long bioaccumulation history in surface water can alter the function of one or more organisms and their biological process. The discharge of piggery waste containing veterinary pharmaceutical residues to the surroundings especially those with proximity to surface water is a threat to human health and a strong ecological risk to the aquatic ecosystem.

CONCLUSION

In this study, the occurrence and ecological risks of veterinary pharmaceutical in piggery effluents were investigated due to the indiscriminate discharge of animal waste and pharmaceuticals in the environment and for proper regulation and control of veterinary substances in the environment. The study revealed the test compounds as significant toxicants capable of causing alterations of the trophic structures and functions of a freshwater ecosystem. The different toxicity values obtained uncovered the potency, sensitivity, and different effects of the test compounds on freshwater organisms. Much of the variability is attributed to species and physicochemical differences, which depicts the natural ecological niche the test organisms occupy. The average concentration levels of studied pharmaceuticals were in the range of 47.35, 7.19, and 1.46 μg L^{-1} for salicylic acid, tetracycline, and ivermectin, respectively. *P. subcapitata* exposed to 20% unfiltered piggery wastewater effluent at 24- and 48-h EC_{50} showed a toxicity value of 14.2% and 13.6% (v/v), respectively. The toxicity and the risk values have linked exposure, short-term accumulation with high-level biological dysfunction, and instability through effects on species of freshwater organisms and food web. The study, therefore, suggests that all measures to control and mitigate the indiscriminate discharge of piggery effluent and veterinary pharmaceuticals must be implemented. Further research is needed to understand the mode of action of these test compounds on freshwater organisms.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

Not applicable.

AUTHOR CONTRIBUTIONS

Material preparation, data collection and analysis, investigation, and writing the manuscript—original draft, first author: Angela C. Udebuani. *Material preparation, investigation, data collection and analysis, software, and writing the manuscript—review and editing*: Omoniyi Pereao. *Analysis and writing the manuscript*: Michael O. Akharame. *Supervision, study conception and design, and funding acquisition*: Olalekan S. Fatoki. *Supervision, study conception and design, software, and funding acquisition*: Beatrice O. Opeolu. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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