Environ Monit Assess (2021) 193:293
https://doi.org/10.1007/s10661-021-09085-z

Acute toxicity of piggery effluent and veterinary pharmaceutical cocktail on freshwater organisms

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Received: 25 September 2020 / Accepted: 15 April 2021

Abstract Intensive livestock farming has increased the use of veterinary pharmaceuticals in many developing countries, and this is considered a significant concern to the freshwater ecosystem. However, the information on the potential acute toxicity of piggery effluent waste and the veterinary pharmaceutical effluent discharged into the aquatic environment is limited. This study assessed the adverse effect of a piggery effluent and the cocktail mixtures of high- and low-level doses of three frequently occurring veterinary pharmaceuticals (tetracycline (TETR), ivermectin (IVER), and salicylic acid (SALA)) on freshwater organisms using three representative freshwater biotests organisms: *Pseudokirchneriella subcapitata* (*P. subcapitata*), *Daphnia magna* (*D. magna*), and *Tetrahymena thermophila* (*T. thermophila*). The freshwater organism test results showed that the 24-h and 48-h EC_{50} algal toxicity to *P. subcapitata* exposed to 10% unfiltered piggery effluent were 25.6 and 49.3% respectively while the 24-h LC_{50} value to Cladocera, *D. magna* exposed to unfiltered piggery effluent was 23.2 (17.7–30.4)%. The 24-h EC_{50} protozoan toxicity to *T. thermophila* exposed to 1% HLD veterinary pharmaceuticals was 0.014 µg/L. Thus, the study established the different sensitivities of freshwater organisms to various percentage levels of piggery effluent and high- and low-level doses of veterinary pharmaceutical. The piggery effluent and the pharmaceutical cocktail mixtures have potential toxicological effects on the freshwater ecosystem.

Keywords Piggery farm · Tetracycline · Ivermectin · Salicylic acid · *Daphnia magna* · South Africa

Introduction

In pig production, veterinary pharmaceuticals are mostly used to prevent, control, and treat various diseases of pigs. The drugs are formulated against the target causative agent such as anti-parasitic (against parasite), anti-microbial (against microorganisms), and anthelmintic (against helminths), while others are used as tranquillizers, feed additives, and growth enhancer (Bártíková et al., 2016), and in all the various veterinary drugs administered, antibiotics are the highest drugs (61%) used in treating livestock animals (Sjölund et al., 2016). Environmental pollution from

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Published online: 23 April 2021

livestock production, especially those released from veterinary pharmaceuticals used in combating diseases of livestock animals, is part of the underlying factors threatening human and the ecosystem (Fekadu et al., 2019; Pino-Otín et al., 2017). The piggery industry is gradually expanding, due to the demand for cheap sources of affordable protein. It is a major source of livelihood in many developing countries but the knowledge and information about pig production and its impact on the environment are limited in most regions of Africa (Penrith et al., 2013).

The antibiotics are used for metaphylactic purposes, in fattening pigs in the farms (Lekagul et al., 2019), but Kaczala and Blum (2016) reported intensive livestock practices as the reason for a consistent source of veterinary substances in the environment. Stevens et al. (2007) reported higher antibiotic use for indoor pig farming (64–74%) compared to traditional outdoor farming (60%), while urine and excreta remained the major entry route of these veterinary pharmaceuticals into the environment (Kaczala & Blum, 2016; Kołodziejska et al., 2013) which are eventually discharged into the environment as parent compounds (not metabolised) or as transformed products (not completely metabolised). Veterinary substances are discharged into the aquatic environment through stormwater runoffs and leaching, and some previous studies showed that the administered antibiotics are excreted as parent compound without being metabolised (Dahshan et al., 2015; Mehdi et al., 2018). Other disposal methods include improper dumping of unused/expired drugs, and drug containers as shown in Fig. 1 may also infiltrate into groundwaters and impact human and environmental health (Dahshan et al., 2015), and veterinary pharmaceuticals are now commonly detected in both minute and large quantities in the environment (Lim et al., 2013; Wang et al., 2014).

The use and identification of compounds by chemical analysis do not provide sufficient information of their effects on the environment, especially with regard to the possibility of mutual effects (antagonistic, synergistic, or additive) of the chemicals in complex mixtures such as macronutrients, organic and inorganic substances, and micropollutants (Bundschuh, 2014; Laquaz et al., 2018). Ecotoxicological bioassays are being incorporated to evaluate biological effects caused by the wastewater effluents discharge into the ecosystems (Mendonça et al., 2009; Rogowska et al., 2020). Aquatic ecotoxicity evaluation is becoming a dependable instrument for monitoring and detecting nonconformities in water quality which involves the use of micro biotest organism for evaluating the toxic impacts of polluted water, and these test items are selected based on their chemical, toxicological, ecological, and taxonomic exposure criteria (Celente, 2020; Chen et al., 2021). The detection of veterinary substances in the ecosystem indicates that our environment is contaminated and loaded with chemical substances. Some documented environmental effects of veterinary pharmaceuticals include inhibition of natural bacterial growth (Felis et al., 2020), conferment of antibacterial resistance to microbes (Lépesová et al., 2018;

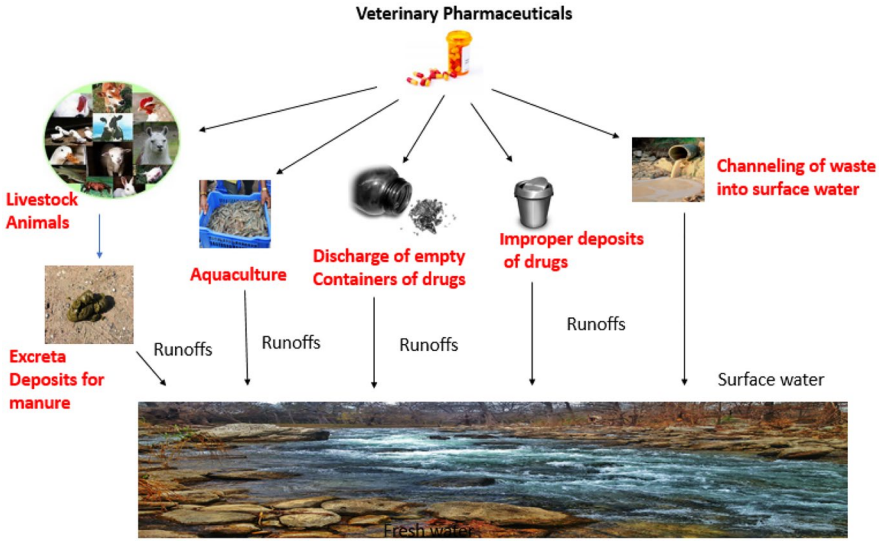

Fig. 1 Pathways of veterinary pharmaceuticals into a freshwater ecosystem

Varela et al., 2016), endocrine disruptions (Kaltungo & Musa, 2013; Peng et al., 2012), and phytotoxic effects (Janeczko et al., 2005).

Information on acute toxicity effects of mixtures of veterinary pharmaceuticals and piggery effluent on freshwater organisms are limited in literature. The present study evaluates the acute toxicity of piggery wastewater effluent as well as low- and high-level mixtures of veterinary pharmaceuticals previously detected in piggery effluents on some model biotest organisms in the freshwater ecosystem. The present study contributes to the information and research on the ecotoxicity of wastewater effluents.

Materials and methods

Study area

Stellenbosch is a metropolitan city centre in the Western Cape province of South Africa and the fourth largest province in South Africa with a geographical location lying approximately on latitude and longitude coordinates of 33° 56′ 12″ S, 18° 51′ 41″E. The farming community produce vegetables and other agricultural product and contribute a relatively high percentage of the South African total agricultural capacity. Stellenbosch is characterised by small and big commercial livestock farm of different animal species such as sheep, birds of different species, cattle, pig, birds, and poultry. The soil in the area ranges from dark alluvium to clay and a well-drained terrain. Summer in the study area starts in December and ends in February and is characterised by warm and dry to hot air with temperatures rising to over 40 °C, while winter begins in June and ends in August and comes with cool rain and wind with the temperature range at 16 °C. The study area (Fig. 2) has a population density of 77,476 people as of 2011 in 23,730 households, and by 2016, the municipal population density rose and was estimated to be 173,197 (*Community* Survey, 2016).

Sample collection

Piggery wastewater effluent samples used in this investigation were collected from a farm in Stellenbosch, Western Cape province of South Africa, at a location near the semi-treated wastewater effluent discharge point where waste from the piggery was passed through a chamber waiting channel which tends to separate the solid waste from the liquid waste

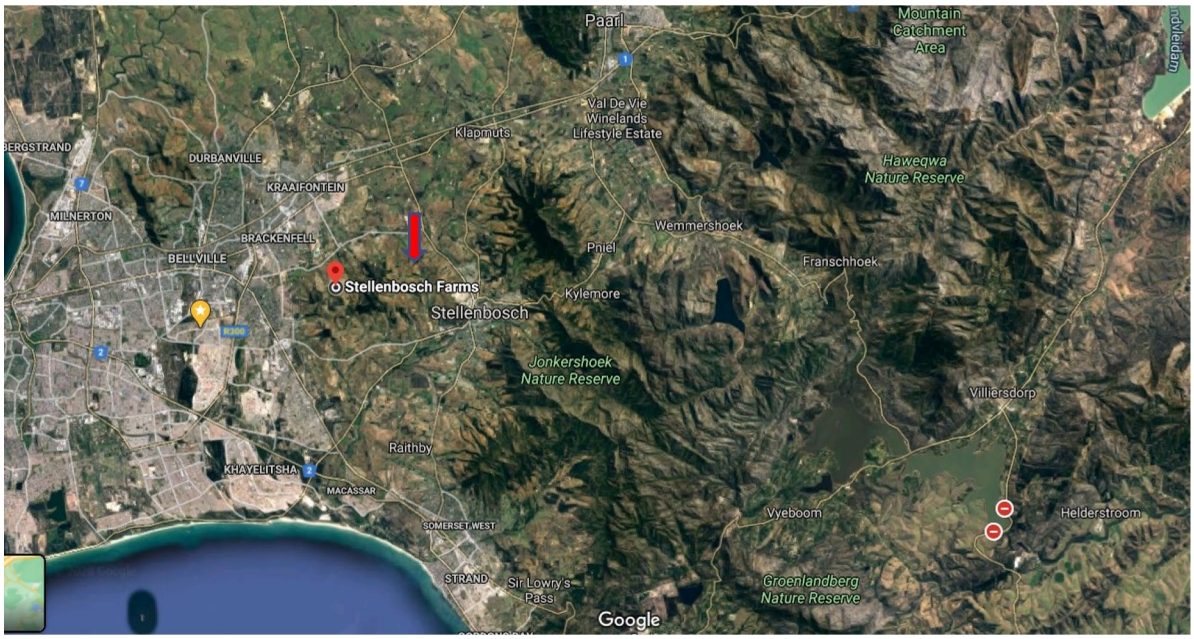

Fig. 2 Map of the study area (Stellenbosch Farm) (Source: Google maps)

before discharge into the river. The semi-treated effluent water samples were scooped out from the effluent flow and collected against the water current. The sampling was carried out in the early hours of the day in June 2019. All the triplicate samples were collected in an amber glass bottle and transported in a cooler box under controlled temperature conditions and stored in the refrigerator (− 4 °C) in the laboratory for further analysis.

Physicochemical analysis

Physicochemical factors such as pH, temperature, total dissolved solids (TDS), dissolved oxygen (DO), conductivity, and redox potential were measured instantly at the site using multi-parameter equipment (SensoDirect 150) Lovibond Tintometer between 10 and 11 a.m.

Chemicals

Tetracycline (99.9%), Ivermectin (95%), Salicylic acid (95%), and HPLC grade methanol (99.9%) were purchased from Sigma Aldrich, South Africa. Milli-Q water (Synergy Ultrapure Water System, Millipore, France) was used in all solution preparations.

Stock solution and preparation of working standards

Stock solutions (100 mg/L) of three pharmaceuticals of TETR, IVER, and SALA were prepared differently on a weight basis by dissolving 0.01 g crystals of each in 100 mL methanol. The stock solutions were stored in a refrigerator at 4 °C and used within 48 h to reduce errors related to potential degradation of the analyte. Thereafter, low-level dose (LLD) and high-level dose (HLD) concentrations of the standards were prepared from the initial stocks based on the previous study (Fatoki et al., 2018) on veterinary effluent. The working solutions of veterinary pharmaceuticals were prepared by mixing the cocktail of different low- and high-level doses of veterinary pharmaceutical (TETR—3.46 and 93.87 µg/L; IVER—1.74 and 2.6 µg/L; and SALA—1.37 and 15.29 µg/L).

Freshwater test

ALGALTOXKIT F, PROTOXKIT F, and DAPHTOXKIT F were purchased from microBio Tests, Kleimoer 15, 9030 Maria Kerke (Gent) Belgium. Acute toxicity biotest with the freshwater organisms exposed to veterinary pharmaceutical cocktail and piggery effluent used a standard operational procedure based on the recommendation of the American Society for Testing and Material (ASTM) were carried out as follows: the algal toxicity test with ALGALTOXKIT using green microalgae; *P. subcapitata* adhered to the procedure of ISO norm 8692 and an Organization for Economic Co-operation and Development (OECD) guideline 201. The Crustacean toxicity test with DAPHTOXKIT using crustaceans, *D. magna*, adhered to the procedure ISO 6341, and OECD guideline 202. The ciliate protozoan toxicity test with PROTOXKIT F using protozoa, *T. thermophila*, which was a 24-h reproductive inhibition test, adhered to OECD guideline 202. The three different cultured medium was reconstituted with water prepared with Milli-Q® water, and the chemical composition of *D. magna* freshwater medium was 64.75 mg/L $NaHCO_3$, 5.75 mg/L KCl, 123.25 mg/L $MgSO_4$-$7H_2O$, and 294 mg/L $CaCl_2 \cdot 2H_2O$, while the media composition for *P. subcapitata* includes a stock solution of macronutrients, trace elements, Fe-EDTA, and $NaHCO_3$, and the reconstitution medium for *T. thermophila* included $NaHCO_3$, $CaSO_4$, $MgSO_4$, and KCl. The test specification for freshwater acute tests is shown in Table 1.

Statistical analysis

EC values for effective concentrations were estimated by Probit analysis, and a Trimmed Spearman–Karber test evaluation was conducted.

Results

The result of the physicochemical analysis showed the temperature of the three samples ranged from 13.9 to 20.4 °C with a decreased temperature recorded for the piggery effluent. The pH ranged from 7.1 to 7.7 pH units which depicted basicity, and they are within the WHO permissible range of 6.5–8.5 pH units (WHO,

Table 1 Freshwater acute toxicity specifications tests with daphnia, algae, and protozoa

	Algae	Daphnid	Protozoa
Species	*Pseudokirchneriella subcapitata*	*Daphnia magna*	*Tetrahymena thermophila*
Exposure time (h)	72	48	24
Source of organism	Laboratory cultured	Laboratory reared	Laboratory reared
Dilution water	Algae assay medium	Standard freshwater	Protox freshwater
Incubation temperature (°C)	23 ± 2	20 ± 1	22 ± 2
Photoperiod	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 (LC/EC_{50})	Growth rate inhibition	Mortality/immobilization effect	Growth inhibition
Day Observation	Cell count and cell volume	Dissolved oxygen, pH, and temperature	Dissolved oxygen, pH, and temperature

2017). Conductivity values obtained from the different samples of piggery effluent and veterinary pharmaceuticals ranged from 6.88 to 40.1 μScm^{-1}. The LLD of veterinary pharmaceuticals showed the highest values of electrical conductivity, while the lowest value was obtained from the piggery effluent sample (Table 2).

The wastewater effluent was studied as a whole-mixture method based on the direct ecotoxicological evaluation of the effluent which allowed analysing the effluent samples as if it was a single chemical (Backhaus et al., 2010). The results of *P. subcapitata* exposed to 1, 10, and 20% of unfiltered piggery effluent are presented in Tables 3, 4, and 5. An increased number of cells were detected at 1% unfiltered piggery effluent (6.25–100%) from 24- to 72-h exposure. At 10% unfiltered piggery effluent, an increased number of cells were observed at low concentration (6.25–25.0%) after 24–48-h exposure compared to the control. The lower concentration of 20% unfiltered piggery effluent (6.25–12.5) showed an increase in the number of the cell from 24 to 72-h; however, no cell was recorded from 25.0 to 100% concentration of 20% piggery effluent. The growth rate of *P. subcapitata* in 1 and 10% concentrations of the test compounds decreased, while a zero-growth rate was observed for treatment exposures of 25–100 at 20% unfiltered piggery effluent. Higher concentrations of 1, 10 (50–100%), and 20% treatments inhibited the growth of *P. subcapitata*. The toxicity result of the *P. subcapitata* exposed to 1% unfiltered piggery effluent wastewater did not record any toxicity value for EC_{50} at 24–72-h. However, the EC_{50} toxicity values at 24–48-h for 10 and 20% unfiltered piggery effluent wastewater, recorded 25.6, 49.3, and 14.2, and 13.6% respectively. The result showed that *P. subcapitata*

Table 2 The physical and chemical properties of veterinary pharmaceutical and piggery effluent

Parameters	High-level dose of veterinary pharmaceutical cocktail	Low-level dose of veterinary pharmaceutical cocktail	Piggery effluent	WHO (2017)/ APHA (1998)
Colour	Yellowish	Colourless	Brownish	Colourless
Odour	Odourless	Odourless	Unpleasant	Odourless
Temperature (°C)	20.3	20.4	13.9	20–30
pH	7.1	7.3	7.7	6.5–9.0
Dissolved oxygen (mg/L)	24.9	7.3	06–07	> 5.0
Electrical conductivity μScm^{-1}	24.9	40.1	6.88	250
Redox potential (mV)	281	276	− 155	

Table 3 The number of cells, growth rate, and percentage inhibition of *P. subcapitata* exposed to 1% unfiltered piggery effluent

	Number of cells (cell/mL)			Growth rate			Percentage inhibition (%)		
Exposure time (h)	24	48	72	24	48	72	24	48	72
Control	50.3	180.8	358.6	6.31	3.8	2.8	0	0	0
6.25	72.2	186.7	398.6	6.4	3.7	2.7	-1.2	3.4	2
12.5	72.4	216.8	147.3	6.1	3.6	0.8	3.3	5.2	72
25	78.3	237.8	0.1	6.0	3.5	-0.2	5.2	6.6	108.3
50	102.4	222.4	435.6	6.0	3.4	2.5	5.4	11.2	10.3
100	165.7	212.7	396.2	5.9	3.1	2.3	5.8	18.5	17.5
EC_{50}	Nd	Nd	nd						

nd not detected

was more sensitive to 20% unfiltered piggery effluent wastewater compared to 10% unfiltered piggery effluent wastewater.

The acute freshwater test results for *D. magna* exposed to different concentrations (1, 10, and 20%) of piggery effluent, HLD, and LLD of veterinary pharmaceutical cocktail are shown in Table 6. The results showed that a higher concentration of 1% piggery effluent caused significant percentage immobility of *D. magna* after 24-h exposure. It was observed that even the lowest concentration of 1% unfiltered piggery effluent caused 50% immobility of the test organism. Total percentage immobility was recorded in *D. magna* exposed to concentrations 6.25–100% of 10–20% piggery effluent compared to the control which recorded no immobility of the test organism. The different concentrations of LLD of veterinary pharmaceuticals cocktail caused no percentage immobility of *D. magna*. However, at 48-h exposure, significant percentage immobility of the test organism was observed at the highest concentration of HLD (25.0–100%) after 24–48-h exposure. The toxicity result of the study showed that *D. magna* exposed to 1% unfiltered piggery effluent at 24-h LC_{50} recorded 23.2 (17.7–30.4)% and no value was obtained at 48-h LC_{50} and higher percentage levels unfiltered effluent wastewater. Also, the toxicity values at 24–48- h EC_{50} for HLD and LLD veterinary pharmaceutical cocktail recorded 20.2 (16.4–24.8), 19.0 (15.0–23.9) µg/L and 101.9, 79.8 µg/L respectively.

The result of an acute freshwater test for the ciliate, *T. thermophila*, is shown in Table 7. The LLD pharmaceutical cocktail showed no significant growth inhibition in the different concentrations exposed at 24-h exposure. The percentage inhibition values obtained in concentrations 12.5 and 100% treatment showed the values of 42.37 and 37.85% respectively. The HLD pharmaceutical cocktail showed significant percentage growth inhibition in the different treatment of the test compound (12.5–100%), compared to the control which showed no percentage inhibition. The toxicity result of *T. thermophila* exposed to a high and low level of veterinary pharmaceutical cocktail showed the value 0.014 and 0.001 µg/l respectively for the

Table 4 The number of cells, growth rate, and percentage inhibition of *P. subcapitata* exposed to 10% unfiltered piggery effluent

	Number of cells (cell/mL)			Growth rate			Percentage inhibition (%)		
Exposure time (h)	24	48	72	24	48	72	24	48	72
Control	42.5	174.2	428.3	6.2	3.8	2.8	0	0	0
6.25	124.9	222.3	0.1	6.0	3.3	−0.4	3.7	13.9	113.2
12.5	205.8	276.1	0.1	6.0	3.1	−0.5	3.7	17.7	119.1
25	357	343.9	419.8	6.0	3.0	2.1	3	21.4	27.3
50	0.1	292	404.2	−2.7	1.4	1.9	143.9	62.6	34.8
100	0.1	0.1	146.9	−2.9	−1.5	−0.0	147.2	138.5	101.7
EC_{50}	25.6	49.3							

Table 5 The number of cells, growth rate, and percentage inhibition of *P. subcapitata* exposed to 20% unfiltered piggery effluent

	Number of cells (cell/mL)			Growth rate			Percentage inhibition (%)		
Exposure time (h)	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	56.4	178.4	374.3	6.4	3.8	2.8	0	0	0
6.25	199.5	271.4	0.1	6.3	3.3	−0.4	1.4	12.4	116.1
12.5	357.3	337.5	358.7	6.2	3.1	2.1	2.1	17.9	24.6
25	0.1	0.1	351.7	−2.6	−1.3	1.8	141.1	134.7	32.6
50	0.1	0.1	0.1	0	0	0	- -	- -	- -
100	0.1	0.1	0.1	0	0	0	- -	- -	- -
EC_{50}	14.2	13.6							

levels of the toxicants used. The statistical analysis indicated a significant difference between the data from the pharmaceutical piggery effluent and the control ($p > 0.05$). Comparably, correlation analysis revealed no significant relationship between veterinary pharmaceutical and piggery effluent ($p > 0.05$).

The optical density and percentage inhibition of *Tetrahymena thermophila* exposed to 1, 10, and 20% of piggery effluent were studied, and the results are presented in Table 8.

The optical density and percentage inhibition increased with an increase in the concentration of piggery effluent and exposure time. *T. thermophila* proved more sensitive to 10% piggery effluent with an EC_{50} of 4.81%. This is followed by 1% piggery effluent, which gave EC_{50} 6.59%. The same organism was not very sensitive to 20% piggery effluent with an EC_{50} of 52.39%. The result of the different piggery effluent showed a significant increase in percentage inhibition and higher values in sensitivity compared to that of HLD and LLD. *Tetrahymena thermophila* proved more sensitive to 1% HLD veterinary pharmaceutical with a 24-h EC_{50} of 0.014 µg/L and that of 1% LLD veterinary pharmaceutical with a 24-h EC_{50} of 0.001 µg/L. The study showed a strong relationship between the percentage concentrations of toxicants and percentage growth inhibitions in *T. thermophila.*

Table 6 The Percentage of immobile *D. magna* from exposed piggery effluent and veterinary pharmaceuticals (HLD and LLD) at 24- h and 48- h

	Number of organisms exposed (cell/mL)	Percent immobile in 1% piggery effluent (%)		Percent immobile in 10% piggery effluent (%)		Percent immobile high-level dose of veterinary pharmaceutical cocktail (%)		Percent immobile low- level dose of veterinary pharmaceutical cocktail (%)	
Exposure time (h)		24	48	24	48	24	48	24	48
Control	20	00 (0)	00 (0)	0.0 (0)	00 (0)	00 (0)	00 (0)	00 (0)	5.0 (1)
6.25	20	5.0 (1)	50 (10)	100.0 (20)	100 (20)	5 (1)	10 (2)	00 (0)	5.0 (1)
12.5	20	25.0 (5)	60 (12)	100.0 (20)	100 (20)	5 (1)	20 (4)	00 (0)	00 (0)
25	20	60.0 (12)	90 (18)	100.0 (20)	100 (20)	75 (15)	55 (11)	00 (0)	5 (1)
50	20	70.0 (14)	85 (17)	100.0 (20)	100 (20)	95 (19)	100 (20)	00 (0)	00 (0)
100	20	100.0 (20)	100 (20)	100.0 (20)	100 (20)	100 (20)	100 (20)	45 (9)	85 (17)
LC_{50}		23.2 (17.7–30.4)	Nd	Nd	nd	20.2 (16.4–24.8)	19.0 (15.0–23.9)	101.9	79.8

nd not detected

Table 7 The optical density and percentage inhibition of *T. thermophila* exposed to high-level and low-level doses of veterinary pharmaceutical cocktail

	Optical density	Optical density	Percentage inhibition of high-level dose of veterinary pharmaceutical cocktail (%)	Optical density	Optical density	Percentage inhibition of low-level dose of veterinary pharmaceutical cocktail (%)
Exposure time (h)	0	24		0	24	
Control	0.8	0.7	0	0.8	0.7	0
6.25	0.8	0.8	4.5	0.8	0.7	−14.1
12.5	0.8	0.8	93.2	0.8	0.8	42.4
25	0.8	0.8	91.5	0.8	0.7	−12.4
50	0.8	0.8	137.9	0.8	0.7	−25.4
100	0.8	0.8	93.8	0.8	0.7	37.9
EC50 (µg/l)			0.014			0.001

Discussion

The low values of conductivity indicated that the effluent water was highly contaminated and was not mineralised (Salam et al. 2013). The results showed different percentage levels of piggery effluent affected the growth of *P. subcapitata* (Tables 3, 4, and 5). The study also showed the sensitivities of *P. subcapitata* to the different percentage levels of unfiltered piggery effluent. Unfiltered piggery effluent was used in the study because this is the form the effluent was discharged into the environment and filtering may sieve out the targeted veterinary substances in the effluent and to avoid high fatalities of the test organisms, different percentages of the piggery effluent such as 1, 10, and 20% were investigated using the prepared freshwater as dilution medium. Gomes et al. (2020) demonstrated in their study that *C. fluminea* can remove antibiotic from three pharmaceutical mixture of emerging concern through biofiltration activity. Swine wastewater effluent presented lower toxicity and decreased inhibition when biofiltration and Fenton's were used as an adjuvant process and this outcome was related to the removal of species such as antibiotic by biofiltration after 30 min of exposure (Domingues et al., 2021). Studies have shown the toxicity of chemicals to algae (Baran et al., 2006; Huang et al., 2014; Kolar et al., 2014), and environmental hazard assessment and classification of the European Community (Directive 67/548/EEC) stated that chemicals can be categorised as toxic to an aquatic organism when the EC_{50} of that chemical is between 1 and 10 mg/L (Carlsson et al., 2006). Lim et al., (2013) also reported chemicals that have

Table 8 The optical density and percentage inhibition of *Tetrahymena thermophila* exposed to 1, 10, and 20% piggery effluent

	1% Piggery Effluent			10% Piggery Effluent			20% Piggery Effluent		
	Optical	Density	Percentage inhibition	Optical	Density	Percentage inhibition	Optical	Density	Percentage inhibition
Exposure time (h)	0	24		0	24		0	24	
Control	0.84	0.8	0	0.95	0.9	0	0.55	0.51	0
6.25	0.95	0.88	−93.2	1.03	1.03	105.83	0.65	1.29	1928.57
12.5	0.93	0.94	131.08	0.99	1.04	196.12	0.68	0.98	944.29
25	0.87	0.89	144.59	0.96	1.05	275.73	0.72	0.85	468.57
50	0.98	1.04	267.57	1.06	1.2	379.61	0.78	0.79	108.57
100	0.99	1.07	317.57	1.09	1.29	493.2	0.87	0.72	−348.57
24 h EC50 (%)	6.59			4.81			52.39		

their LC/EC_{50} < or = 10 mg/L are toxic and those that have their LC/EC_{50} < or = 1 mg/L are very toxic. In this study, except for the value of EC_{50} for 1% piggery effluent, the toxicity value of 10% and 20% piggery effluent were far above these stipulated values.

The piggery effluents are complex matrices enriched in nutrients that could induce river eutrophication (algae bloom); algae has an essential function in the entire water environment as a primary producer. while the harmful consequences of antibiotics on algae may disturb higher trophic levels organisms (Felis et al., 2020). It was shown that trimethoprim and sulfamethoxazole mixture (molar ratio of 3:20) triggered synergistic growth inhibition for green algae and the LC_{50} value was 0.28 mg/L (Eguchi et al., 2004). The toxic effects of antibiotics on green algae are related to the inhibition and interference of chloroplast metabolisms such as interrelated protein synthesis and photosynthesis procedures because green algae are eukaryotic organisms and the chloroplast belongs to the semi-autonomous organelle, which disrupts the mode of action of the photosynthetic apparatus and consequently affects the cell growth (Fu et al., 2017; Kovalakova et al., 2020; Magdaleno et al., 2015). Piggery effluents most at times are not considered toxic and are sometimes used in drought areas of most developing countries to irrigate crop plant (Ungureanu et al., 2020), and in some areas, piggery effluent finds its way into surface water through stormwater runoffs (Liu et al., 2019). This study has revealed that piggery effluent can be toxic and could pose an ecological risk to organisms such as microalgae which play an important role in the freshwater ecosystem.

The 24- and 48-h LC_{50} values of the high-level dose of veterinary pharmaceutical for *D. magna* were 20.2 (16.4–24.8)% and 19.0 (15.0–23.9)% respectively. The percentage immobility for high dose level of the veterinary pharmaceutical cocktail was significantly higher in increased treatment concentrations (25.0, 50.0, and 100%). The 24-h and 48-h LC_{50} values for low-level dose (LLD) of veterinary pharmaceutical cocktail showed higher values (101.9 and 79.8 µg/L) respectively when compared to the high dose level. This shows that *D. magna* can be sensitive to a high dose level of veterinary pharmaceutical at a smaller value as seen in this study. However, the high LC_{50} values of the low-level dose of veterinary pharmaceutical toxicity to *D. magna* can manifest and upset the aquatic environment just as though there was no directly noticed acute toxicity and some studies have shown the LC_{50} values of some veterinary pharmaceutical in *D. magna* to be 221 mg/L (Wollenberger et al., 2000), 248–270 mg/L (De Liguoro et al., 2010; Kim et al., 2007), and 48 mg/L (Huang et al., 2014). Most of these values were far higher than the values obtained in this study as shown in Table 6 (*D. magna* exposed to 1% piggery effluent 24- h: 23.3 (17.7–30.4), HLD 24- h: 20.2 (16.4–24.8), 48- h: 19.0 (15.0–23.9), and LLD 24- h: 101.9, 48- h: 79.8), and this could be attributed to dose–response factor (Diaz-Sosa et al., 2020; Rozman et al., 2017); however, the lower the amount of chemical required, the greater the potency of that chemical (Lewandowski & Norman, 2015). The results obtained from the present study shows that the different percentage concentrations of piggery effluent and a high-level dose of mixtures of veterinary pharmaceuticals used in this study can cause acute toxicity to *D. magna*. The release of low concentrations of 1% and 10% unfiltered piggery effluent and high dose levels of veterinary pharmaceuticals can affect freshwater organism like daphnid by causing direct acute toxicity (Huang et al., 2014). Similar trends were reported compared to our study on the toxicity of veterinary antibiotic on *D. magna* when Zhang et al., (2021) reported acute toxicities of chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FLO) and their mixtures on *D. magna* under two representative temperatures (20 and 25 °C) of the aquatic environment and nearly all groups indicated a certain significant increased tendency to the inhibition rate with the rise of temperature, while Martins et al., (2013) reported that the veterinary antibiotic FLO decreased the somatic growth and impaired the reproductive output of *D. magna* (EC_{20} = 6.9 mg/L; EC_{50} = 7.6 mg/L) at 20 °C.

The ciliated protozoa *Tetrahymena* sp. has been in use for decades in toxicology as a useful model organism for environmental research and molecular and cellular biologists (Gutiérrez et al., 2003) as a useful eukaryotic model system for mechanistic studies, because it contains many genes conserved in several eukaryotes (including humans), and different from other widely used unicellular model organisms (Mortimer et al., 2010). Veterinary pharmaceutical effects on protozoa are important because they are vital members of the aquatic food chain and an essential part of the activated sludge biological consortium involved in wastewaters treatment. Carbajo et al. (2015) reported an industrial wastewater effluent to be highly toxic to *V. fischeri* and *T. thermophila* with

EC_{50} of 0.051 and 0.318% respectively. According to the environmental hazard assessment and classification of the European Community (Directive 67/548/EEC), a chemical can be classified as "very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment" when the EC_{50} of the chemical is ≤ 1 mg/ L (Carlsson et al., 2006); the toxicity of HLD veterinary pharmaceutical to *T. thermophila* was within this range. In as much as all the freshwater organisms used in this study showed some level of toxicity sensitivity to piggery effluent and veterinary pharmaceutical cocktail. The lowest toxicity value obtained in this study is from *T. thermophila* exposed to mixtures of veterinary pharmaceuticals (HLD and LLD) with 10% piggery effluent with an EC_{50} of 4.81% and 1% piggery effluent which gave EC_{50} 6.59%. This is followed by *P. subcapitata* exposed to 20% piggery effluent which gave EC_{50} of 13.6%. *D. magna* showed less sensitivity to a low-level dose of veterinary pharmaceutical cocktail compared with piggery effluent and high dose level of veterinary pharmaceutical used in this study. This shows that the 1% low-level dose of veterinary pharmaceutical was not enough to cause toxicity in *D. magna*.

The assessment concluded that *T. thermophilia* showed high sensitivity to mixtures of veterinary pharmaceuticals (HLD, LLD) and different concentrations of piggery effluent especially at 10 and 1% indicating that the organism showed more sensitivity to the mixture of veterinary pharmaceutical when compared with the piggery effluent and different dose level of veterinary pharmaceutical used in this study. This can be explained by the lack of cell walls which exposes *T. thermophilia* to a swift response to environmental stress (Doerder, 2014). In the reaction to environmental stress, there is an appearance of some structural changes in the cell of *T. thermophilia* which reflected the variation in the metabolic state of the cell of that organism (Maurya et al., 2019). After exposure to these toxicants, the increased optical density and percentage growth inhibition were established in this study and these measurements helped to provide the toxic impact of the toxicant specifically. However, there is a strong correlation between percentage inhibition and concentrations of piggery effluent which Maurya and Pandey (2020) reported as dose response-dependent in toxicological studies. This study has shown that the three freshwater organisms which make up the different trophic levels are linked up through energy flows between producers and consumers which indicated the sensitivity and toxicity to the chemical substances used. Therefore, the discharge of piggery effluent and the mixtures of this veterinary pharmaceutical cocktail are sources of pollution that can affect the health of the freshwater ecosystem. However, various types of veterinary substances exist which may exhibit a toxic effect on freshwater organisms and the knowledge of the behaviour of these piggeries and veterinary pharmaceutical which can induce toxicity in freshwater organisms is important.

Conclusion

This study assessed piggery effluents and a cocktail mixture of high- and low-level doses of three frequently occurring veterinary pharmaceuticals as a potential source of pollution of the freshwater ecosystem. It established the sensitivity and adverse effect of these toxicants on microalgae (*P. subcapitata*), Cladocera (*D. magna*), and protozoa (*T. thermophila*), and the freshwater organisms exposed to different concentrations of piggery effluent and, high and low levels of veterinary pharmaceuticals drastically inhibited the growth of these organisms. The freshwater organisms biotest results showed that the 24-h and 48-h EC_{50} algal toxicity to *P. subcapitata* exposed to 10% unfiltered piggery effluent were 25.6 and 49.3% respectively, while the 24-h LC_{50} value to Cladocera, *D. magna*, exposed to unfiltered piggery effluent was 23.2 (17.7–30.4)%, while the 24-h EC_{50} protozoan toxicity to *T. thermophila* exposed to 1% HLD veterinary pharmaceuticals was 0.014 µg/L. The high-level dose of veterinary pharmaceutical to *D. magna* showed that 24-h and 48-h LC_{50} were 20.2 (16.4–24.8) µg/L and 19.0 (15.0–23.9) µg/L. The study revealed that the piggery effluent and chemical compounds can have potential toxicological effects on the freshwater ecosystem. The study showed a relationship existed between exposure (dose) and the different concentrations of the chemicals discharged to the environment. The chemicals present in the piggery effluents significantly affected the population of the freshwater organisms through dose–response factors, and *T. thermophila* was the most sensitive organism when compared to other biotests for the chemical substances at 48-h EC_{50} exposure to HLD of veterinary pharmaceuticals.

The studies and information on the toxicological effect of piggery effluent and levels of veterinary pharmaceutical (in the environment) on the freshwater ecosystem are scarce, and more research are needed on the information on the ecological risk of toxic substance released to the freshwater environment.

Author contribution Material preparation, data collection and analysis, investigation, and writing the manuscript-original draft (Angela C. Udebuani).
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All authors read and approved the final manuscript.

Funding This study was supported by the Water Research Commission, South Africa (WRC K4/2500); Environmental Chemistry and Toxicology research group, Cape Peninsula University of Technology, and the Cape Peninsula University of Technology Postgraduate Research Scholarship.

Data availability All the data generated or analysed during this study are included in this published article.

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