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Aquatic bioaccessibility of tetracycline antibiotics to higher fauna: Prediction based on the water-column/sediment partition coefficient

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ABSTRACT

Tetracycline drugs, extensively used as human and veterinary broad spectrum antibiotics, enter surface and drinking water sources through runoff and seepage following application of manure in agriculture, and direct discharge in pharmaceutical plant and hospital effluent. The aim of the present work was to study the bio-accessibility of tetracycline drugs in the aquatic environment to higher fauna. The study was achieved by first proposing an aquatic bio-accessibility coefficient towards higher fauna, defined as the fraction of the substance in solution in the water column over the mean concentration of the substance in a given aquatic system, and calculated assuming that non-settling adsorbed speciation forms are desorbed upon contact with the gastrointestinal fluid. The definition further assumes that organic substances residing within the sediment are not bioaccessible to higher fauna, but become bioaccessible following sediment-to-water-column equilibrium transfer. The bioaccessibility coefficients of tetracycline antibiotics in a typical tropical river ecosystem were studied by determining the distribution of the antibiotics between the water column and the sediment over a period of 90 days using microcosm experiments. Data are presented showing that, based on theoretical values calculated taking into account the different rates of degradation of different speciation forms of the antibiotic in both water and sediment phases of the aquatic ecosystem, oxytetracycline and chlortetracycline exhibit medium high aquatic bioaccessibility coefficients (1.0 – 1.5), while doxycycline exhibits a high aquatic bioaccessibility coefficient (1.5 – 2.0). The differences observed in the theoretical bio-accessibility coefficients of TCs are attributed to differences in the strengths of the adsorption bonds of different tetracyclines to colloidal particles. Data are further presented showing that experimental values of the aquatic bio-accessibility coefficient and water-clumn/sediment partition coefficient may also deviated considerably from their corresponding theoretical values, as a result of errors arising from re-suspension of antibiotic adsorbed to settling colloidal particles during sampling.

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Introduction

Tetracyclines (TCs) are widely used as human and veterinary broad spectrum antibacterial agents [1,2]. They are also added to animal feeds where they act as growth promoters [3]. About 70% of the antibiotic administered to domestic animals is excreted as the parent compound in faeces and urine [4,5], and find its way into surface and drinking water sources through runoff or seepage, and through direct application of manure in fields and vegetable gardens [6]. Thus tetracycline drugs have been detected in surface waters by a number of researchers. Chlortetracycline (CTC) has been detected in water at 0.03 μg/L [7], 0.16 μg/L [8] and 0.69 μg/L [9], and in sediments at 2 μg/g and 10 μg/g DW [7]. CTC has also been detected in surface and ground water samples in the USA [10]. Concentration levels ranging from ng L^{-1} to mg L^{-1} have been reported in Europe, America and Asia [11–13]. Depending on the aquatic bioaccessibility and bioavailability of the antibiotics, the presence of these antibiotics in the aquatic environment can lead to adverse effects to fish [14,15], and other higher fauna that can ingest the water, such as birds, crocodiles, as well as domestic animals. Of particular concern is the possible development by domestic animals of resistance to tetracycline drugs [16]. There is therefore need to establish the bioaccessibility of tetracycline drugs in the aquatic environment to higher fauna.

Bioaccessibilty in ecotoxicology is defined as the quantity or fraction of contaminant which is released from the sample matrix in the gastrointestinal (GI) tract and becomes available for absorption [17]. The methods available for determining bioaccessibility include in vitro methods that simulate GI digestion, ex vitro methods that employ GI organs in laboratory experiments, in situ assays employing intestinal perfusion in animals, and in vivo methods involving animal or human studies. For all these methods bioaccessibily (F_B) is given by

$$F_B = \left(\frac{Contaminant\ determined\ in\ the\ chyme}{Contaminant\ in\ thesample\ before\ digestion} \right) 100\% \tag{1}$$

Many studies of bioaccessibility have been conducted on the basis of Eq. (1); the studies cover both organics and inorganics, and several reviews have been published [17–21]. Comparisons between different methods for determining bioaccessibility are however difficult as conditions differ between them and only in vivo studies provide accurate values [22].

Organic compounds introduced into the aquatic environment distribute themselves between the water column and the sediment phase. In addition, it has been shown that organic compounds introduced into the aquatic environment undergo adsorption to both non-settling and settling colloidal particles, i.e., at least three speciation forms may be expected thus: dissolved, sobbed to non-settling particles and sobbed to settling particles [23]. Recently Zaranyika and Zomba [24] demonstrated through microcosm experiments that the dissipation of CTC in the aquatic environment follows a triphasic linear rate law both in the water phase and sediment phase. Similar results were reported previously for other members of tetracycline antibiotics, oxytetracycline and doxycycline [25]. The initial fast linear rate of dissipation was attributed to the dissipation of free molecules of the antibiotic in solution, i.e., dissolved molecular speciation form, while the subsequent slow linear rates of dissipation were attributed to the dissipation of the adsorbed speciation forms. Bioaccessibility to higher fauna of the antibiotic in sediment phase differs from that of antibiotic in solution in the water phase. Similarly the bio-accessibility of free molecules of antibiotic in solution differs from that of antibiotic adsorbed to non-settling and settling colloidal particles.

It is apparent from this discussion that the definition of bioaccessibility given above is not adequate to describe the bioavailability of organic substances in the aquatic environment to higher fauna. There is need to come up with a definition of aquatic bioaccessibility that takes into account the distribution of the substance under study between the water column and the sediment, as well as any speciation undergone by the substances. Because of the extremely large number of pollutants reaching the aquatic environment, it is also desirable that the definition proposed for bioaccessibility should be based on physico-chemical properties of the pollutant, without the need for simulated GI digestion. The aims of the present study were therefore (a) to propose a definition of the bioaccessibility of aquatic pollutants to higher fauna that takes into account patitioning of the substance between the water column and the sediment, as well as any speciation undergone by he substance, (b) to apply the proposed definition to study the bioaccessibility of tetracycline drugs in the aquatic environment to higher fauna, and (c) to assess the effect of speciation on the aquatic bioaccessibility of tetracyclines to higher fauna.

Theoretical

Bioaccessibility of organic substances in the aquatic environment to higher fauna

The bioavailability of organic substances in the aquatic and soil environments depends on the target organism, as well as the nature of the substance and its speciation form [26,27]. The term bioavailability derives from pharmacology, where it is defined as the fraction of administered dose of unchanged drug (i.e., parent compound) that reaches the systematic circulation [26]. The US National Research Council (NRC) [28], with reference to its usage in ecotoxicology, defined bioavailability as the amount of chemical that is actually taken up from the environment and is available to cause a biological response. Semple et al. [27], defined “a bioavailable substance as one that is freely available to cross an organism’s cellular membrane from the medium the organism inhabits at a given time”, and “a bioaccessible substance as one that is available to cross an organism’s cellular membrane from the environment, if the organism has access to the chemical”. More recently, bioaccessibility has been defined as the fraction of a chemical that is in solution in the gastrointestinal tract and is therefore available for absorption [19]. Bioaccessibility has also been defined as the fraction of a compound available for absorption,

and is defined by the total desorbed fraction [29]. It is apparent from these definitions that bioaccessibility depends on the target organism, as well as the nature of the substance and its speciation form [26].

The definition of bioaccessibility of organic substances to fish, birds and other higher animals must take into account the fact that these species actually drink the water, but should exclude the fraction of antibiotic adsorbed to non-settling colloidal particles. The substance adsorbed to non-settling colloidal particles in the water phase, however, becomes bioaccessible should it undergo desorption from the colloidal particle on contact with the intestinal fluid in the gastrointestinal tract. In addition, organic compounds introduced into the aquatic environment escape into the air above the water column, and become bioaccessible to higher fauna through inhalation. Thus the water-phase bioaccessibility to fish, birds and other animals, $B_{ac(wp)}$, can be defined as the total amount of the free substance in solution in the water phase, $C_{fs(w)}$, plus the concentration of the colloidal=particle-adsorbed substance in the water column, $C_{c(w)}(\phi)$, that is desorbed from the colloidal-particle-substance complex upon introduction into the intestinal fluid ($C_{c(w)}$ = concentration of colloidal-particle-adsorbed substance; ϕ = fraction desorbed upon contact with the intestinal fluid), plus the concentration of the substance in the air column above the water surface, C_{air}, i.e.,

$$\begin{aligned} B_{ac(wp)} &= C_{fs(w)} + \sum C_{c(w)}(\phi) + C_{air} \\ &= \alpha C_{o(w)} + \sum x_i C_{0(w)} \phi_i + C_{air} \\ &= C_{o(w)}(\alpha + \sum x_i \phi_i) \ + C_{air} = C_{wp} + C_{air} \end{aligned} \tag{1}$$

Where $C_{o(w)}$ = total concentration of substance in solution in the water phase (w), α = free molecular speciation coefficient or fraction of free molecules of pollutant in solution, χ_i = colloidal-particle-adsorbed speciation coefficient or the fraction of pollutant adsorbed to colloidal particles type i, Σ sums over all adsorbed speciation forms of the substance in solution in the water phase, and ϕ is the fraction of the adsorbed substance that is desorbed upon contact with the intestinal fluid, and $\phi = 0 - 1$; the subscripts *o, w, fs, c* and *i* denote initial concentration, water phase, free antibiotic molecules in solution, colloidal particle adsorbed antibiotic, and colloidal particle type i respectively. C_{wp} = total bioaccessible concentration in the water phase.

Water-phase aquatic bioaccessibility coefficient, $B_{acc(wp)}$

If we define the water phase bioaccessibility coefficient, $B_{acc(wp)}$, as the ratio of the water phase bioaccessibilty over the mean concentration of the substance in a given aquatic system, i.e.,

$$B_{acc(wp)} = \frac{B_{ac(wp)}}{\left(C_{o(w)} + C_{o(s)} + C_{(air)}\right)/3} = \frac{C_{wp} \ + C_{air}}{\left(C_{o(w)} + C_{o(s)} + C_{(air)}\right)/3} \tag{2}$$

where $C_{o(w)}$, and $C_{o(s)}$ are the total concentrations of the substance in the water phase and the sediment phase respectively, and $C_{(air)}$ is the vapour phase concentration of the substance in the air column above the water surface. The concentration of the substance lost via evaporation is often quite small, and quickly disperses into the surrounding atmosphere. Hence we can safely assume that $C_{wp} \gg C_{air}$ and $C_{o(w)} + C_{o(s)} \gg C_{T(air)}$. If, in addition, we assume that the organic substance residing within the sediment is not bioaccessible to higher fauna, but becomes bioaccessible following sediment-to-water column equilibrium partitioning, then Eq. (2) becomes

$$B_{ACC(wp)} = \frac{C_{wp}}{\left(C_{o(w)} + C_{o(s)}\right)/2} \tag{3}$$

Substituting from Eq. (1), Eq. (3) becomes

$$B_{ACC(wp)} = \frac{C_{o(w)}(\alpha + \sum x_i \phi_i)}{\left(C_{o(w)} + C_{o(s)}\right)/2} = \left(\frac{2K_p}{K_p + 1}\right)\left(\alpha + \sum x_i \phi_i\right) \tag{4}$$

Where

$$K_p = C_{o(w)}/C_{o(s)} \tag{5}$$

i.e. the partition coefficient (K_p) [23] of the substance between the water phase and the sediment phase.

The maximum value of $B_{ACC(wp)}$ occurs when α =1 and $\chi_i = 0$, so that

$$B_{ACC(wp)(max)} = \left(\frac{2\mathrm{Kp}}{\mathrm{Kp} + 1}\right) \tag{6}$$

Assuming none of the colloidal particle adsorbed pollutant in the water phase is desorbed on contact with the intestinal fluid, i.e., $\phi_I = 0$, then Eq. (4) becomes

$$B_{ACC(wp)(min)} = \left(\frac{2\mathrm{Kp}}{\mathrm{Kp} + 1}\right)\alpha \tag{7}$$

Eq. (7) then gives the minimum value of $B_{ACC(wp)}$ that can be expected in any aquatic ecosystem.

The definition of water phase bioaccessibility coefficient, $B_{ACC(wp)}$, in terms of Eqs. 4 provides a basis for classifying aquatic environmental pollutants into low and high bioaccessibility pollutants. For example according to Eq. (4), when the

pollutant is wholly in solution in the water phase (i.e., $\alpha = 1$ and $\chi_I = 0$), $B_{ACC(wp)} = 2$, and when $C_{o(w)} = C_{o(s)}$, i.e., when the substance is evenly distributed between the water column and the sediment phase and $\alpha = 1$ and $\chi_I = 0$, $B_{ACC(wp)} = 1$. When the pollutant is wholly in the sediment phase (i.e., α =0) and $\phi_i = 0$, $B_{ACC(wp)} = 0$. Thus aquatic environmental pollutants can be classified into low and high bioaccessibility aquatic pollutants for which $B_{ACC(wp)} = 0 - 1$ and $B_{ACC(wp)} = 1 - 2$ respectively. $B_{ACC(wp)}$ is calculated in terms of Eq. (7) if the value of ϕ_i is not known or in terms of Eq. (6) if the values of both α and ϕ are not known. The ability to classify organic aquatic pollutants on the basis of their water column/sediment partition coefficient into low, medium low, medium high, and high bio-accessibility pollutants is a significant contribution as currently pollutants are only classifiable on the basis of their biological effects (e.g., carcinogenic substances, endocrine disrupters, etc.), and on their persistence (e.g., persistent organic pollutants, POPs).

Materials and methods

Chemicals and reagents

Oxytetracycline (OTC), Doxycycline (DC) and Chlortetracycline (CTC). (95–98% purity), methanol and acetonitrile (HPLC grade solvents), primary and secondary amine (PSA) adsorbent (57,738-U-SUPELCO super clean) and MILLPORE 0.45-µm disposable filter units, were obtained from Sigma Aldrich, Germany. All other chemicals were analytical grade, obtained from SKYLABS, Gauteng, South Africa, River water (6 × 80 L) and sediment (6 × 2 kg) were collected between May and July from Wayerera river, Bindura, Zimbabwe (19° 19' 52'' South, 42° 21'52'' East). The physicochemical and microbiological properties of the river sediment, determined using standard methods [30,31], are shown in Table S1 Supplementary Material.

Microcosm experiments

The microcosm experiments were conducted in white plastic containers following a method reported previously [24], and designed to resemble as close as possible the actual environmental ecosystem in terms of chemical and microbiological composition. River water (1 × 80 L for each antibiotic OTC, DC and CTC) were transferred into separate 100 L white plastic tanks (Mega Pak Pvt Ltd, Harare, Zimbabwe). Two kilograms of sediment were added into the reactor and the level marked. All the tanks were then spiked with 1 mL of standard antibiotic solution containing 80 mg mL^{-1} antibiotic. The tanks were then stirred thoroughly to distribute the antibiotic, allowed to settle and then samples taken immediately to give day zero concentration levels for each antibiotic. The tanks were covered with perforated transparent polythene and left outside in a safe place close to the Department of Chemistry, Bindura University of Science Education. Thereafter, about 350 mL water samples and about 10 g sediment samples were collected in triplicate over a period of 90 days at 2 days intervals for the first week, 3 days intervals for the next 3 weeks, weekly intervals for the next 2 weeks, and finally at fortnightly intervals, each time compensating for evaporation by adding distilled water 24 hrs. prior to collecting samples. The new water level in the experimental tank was also marked after each sampling session. A stainless scoop was used to collect sediment samples from the bottom of the tanks, each time ensuring minimum agitation. Following sampling, samples were freeze dried in amber vials until required for analysis. The concentration of OTC, DC and CTC in the water phase and sediment phase were monitored as a function of time. The mean temperatures of 27 ± 5, 26 ± 4 and 27 ± 3°C, and mean pHs of 7.2 ± 0.4, 7.2 ± 0.4 and 7.1 ± 0.6, were recorded for OTC, DC and CTC experimental tanks respectively.

Preparation of standard solutions

Working standard solutions of OTC, DC and CTC were prepared by dissolving each standard in methanol to give an 80 mg mL^{-1} solution.

Sample extraction, clean up, concentration and analysis

Oxytetracycline, doxycycline and chlortetracycline were extracted and determined using a previously optimized ultrasonic assisted dispersive solid phase extraction method [25], and a previously optimized HPLC method based on UV-Visible detection [24]. The residual concentration data generated (Table S2) are plotted as a function of time in Figs 1. OTC, DC and CTC were not detected when blank samples of river water and sediment were analysed by the method. All peak assignments were confirmed by spiking with authentic standards. Peak purity, as assessed using the Varian Star chromatography software, was greater than or equal to 99% for all the peaks. $B_{ACC(max)}$, $B_{ACC(min)}$, and day zero K_p values were calculated for each antibiotic, see Table 1. In addition the value of $B_{ACC(max)}$ and K_p were calculated and plotted as a function of time in Figs. 2 and 3 respectively.

Results and discussion

Material balance calculations

The microcosm experiments were set up so as to achieve a concentration of 1 µg.mL^{-1} (or 0.08 g in 80 L) of each antibiotic in the water phase prior to partitioning into the sediment phase. Thus, on the basis of day zero analysis results of

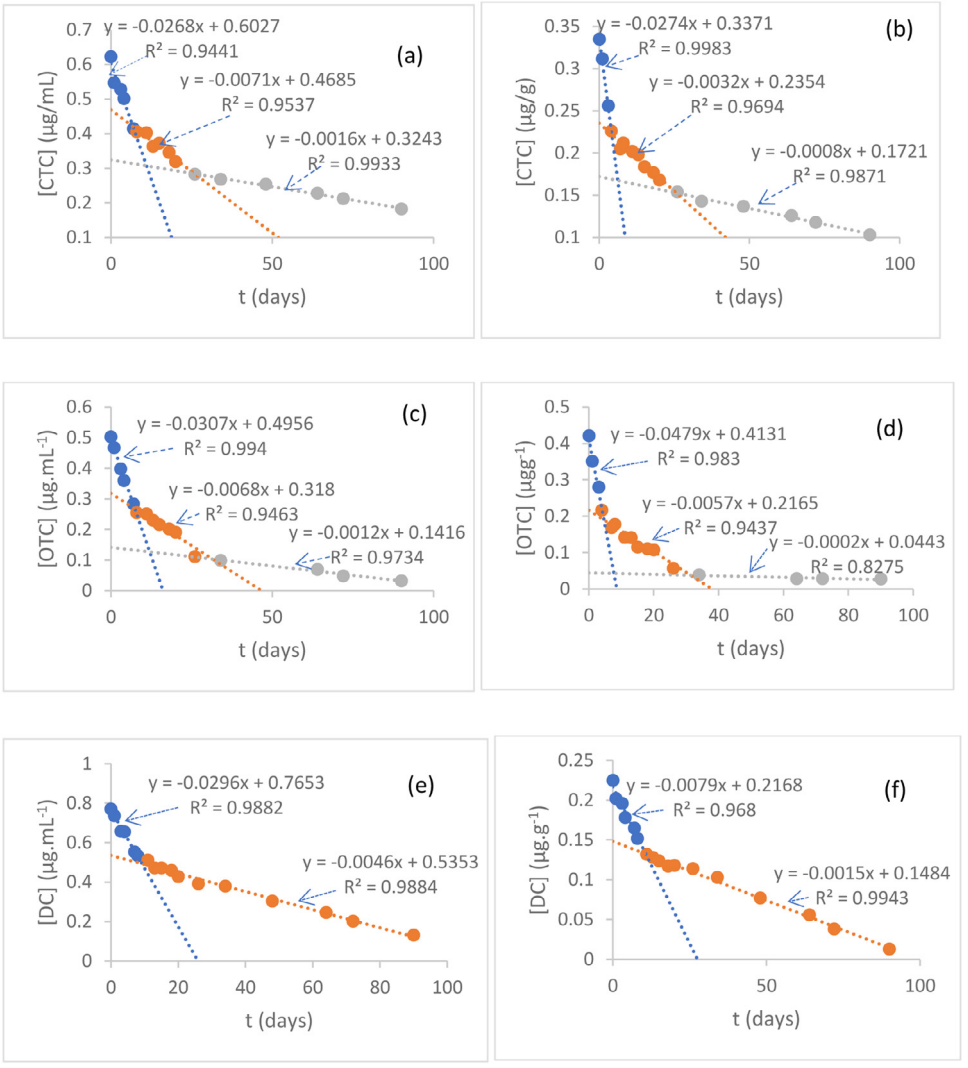

Fig. 1. Multi-phase linear residual concentration curves for CTC (water phase (a) and sediment phase (b)), OTC (water phase (c) and sediment phase (d), and DC (water phase (e) and sediment phase (f)) in the microcosm experiment.

Table 1
Day zero $B_{ACC(max)}$, $B_{ACC(min)}$, K_p, and zero order dissipation rate constants[a] $k_{o(wp)}$, $k_{c1(wp)}$, $k_{c2(wp)}$, $k_{o(sp)}$, $k_{c1(sp)}$ and $k_{c2(sp)}$ values for oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DC).

Property	CTC	OTC	DC
$B_{ACC(min)}$	0.1451	0.4629	0.5358
$B_{ACC(max)}$	1.1653	1.0849	1.5486
$K_{p\ (0)}$	1.5408	1.1976	3.4311
$k_{o(wp)}$ (µg.mL^{-1}day^{-1})	0.0268	0.0307	0.0296
$k_{c1(wp)}$ (µg.mL^{-1}day^{-1})	0.0071	0.0068	
$k_{c2(wp)}$ (µg.mL^{-1}day^{-1})	0.0016	0.0012	0.0046
$k_{o(sp)}$ (µg.g^{-1}day^{-1})	0.0274	0.0479	0.0079
$k_{c1(sp)}$ (µg.g^{-1}day^{-1})	0.0032	0.0057	
$k_{c2(sp)}$ (µg.g^{-1}day^{-1})	0.0008	0.0002	0.0015

[a] Given by the slopes of the linear portions of the residual concentration curves in Fig. 1.

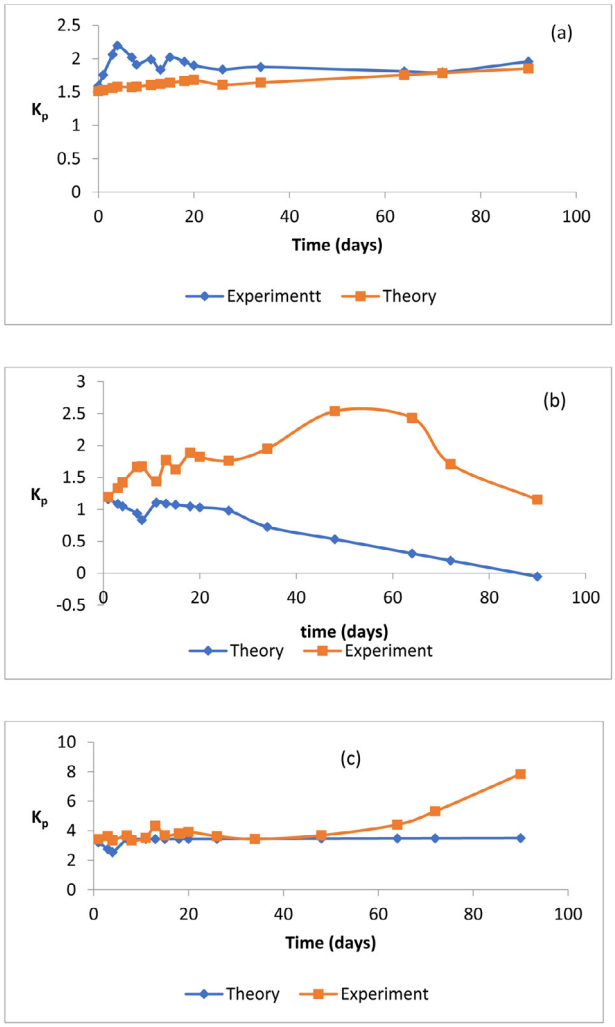

Fig. 2. Experimental bioaccessibility coefficient ($B_{AAC(max)}$) of doxycycline (DC), oxytetracycline (OTC) and chlortetracycline (CTC) in the aquatic microcosm experiment as a function of time.

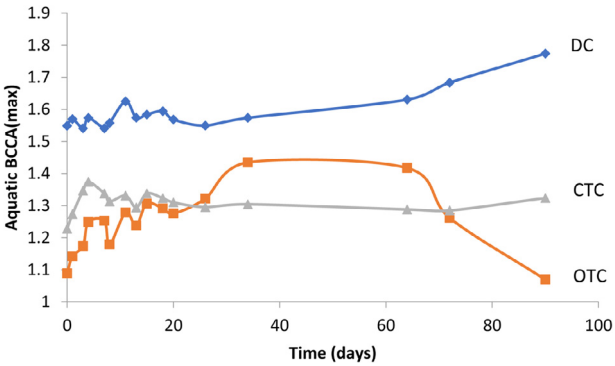

Fig. 3. Water-column/sediment partition coefficient (K_p) of (a) chlortetracycline (CTC), (b) oxytetracycline (OTC) and (c) doxycycline (DC) in Wayerera river microcosm: Experimental versus Theoretical.

0.5029 μgmL^{-1} (water phase) and 0.4205 μgg^{-1} (sediment phase), it can be shown that of the total 0.08 g OTC charged into the experiment, 0.0008 g (1.0%) was in the sediment, while 0.0402 g (50.25%) remained in the water phase. The remainder of 0.039 g (48.75%) is assumed to be adsorbed onto the walls of the container. In the case of DC with day zero analysis results of 0.0.7720 μgmL^{-1} (water phase) and 0.2250 μgg^{-1} (sediment phase), it can be shown that of the total 0.08 g charged into the experiment, 0.0005 g (0.625%) was in the sediment, while 0.0618 g (77.25%) remained in the water phase and 0.0177 g (22.1%) was adsorbed onto the walls of the containers. Similarly for CTC, the day zero analysis results of 0.6920 μgmL^{-1} (water phase) and 0.4350 μgg^{-1} (sediment phase) translate to 0.0498 (62.25%) and 0.0007 (0.875%) in the water phase and sediment phase respectively, while 0.0237 (36.875%) is assumed to be adsorbed on to the walls of the container.

Dissipation kinetics

Fig. 1(a) and (c) show the persistence curve for the degradation of CTC and OTC respectively in the water phase of the microcosm experiment. Both curves are made up of three portions, which can be resolved into highly linear curves, $R^2 = 0.9441$ to 0.994. The three linear portions of the triphasic linear degradation curves were interpreted as representing the dissipation of three speciation forms of CTC all dissolved in the water phase of the microcosm experiment: (a) free molecules of the antibiotic, (b) molecules of the antibiotic adsorbed to non-settling colloidal particles type 1, and (c) molecules of the antibiotic adsorbed to non-settling colloidal particles type 2.

Fig. 1(b) and (d) show the persistence curves for the degradation of CTC and OTC in the sediment phase of the microcosm experiment. As with the water phase, dissipation in the sediment follows a triphasic linear rate law. The three linear portions of the triphasic linear degradation curves are interpreted as representing the dissipation of (i) free molecules of the antibiotic dissolved in the sediment pore water, and (ii) molecules of the antibiotic adsorbed to settling colloidal particles types 1 and 2.

Fig. 1(e) and (f) shows that, unlike OTC and CTC, dissipation of DC in both water and sediment phases follows a biphasic linear rate law. The biphasic linear degradation rates in the water phase are interpreted as representing the dissipation of (i) dissolved free antibiotic molecules, and (ii) antibiotic adsorbed to non-settling colloidal particles. The biphasic linear degradation rates in the sediment phase are interpreted as representing the dissipation of (i) free antibiotic dissolved in the sediment pore water, and (ii) antibiotic molecules adsorbed to settling colloidal particles. The slopes of the linear portions of the biphasic and triphasic degradation curves give the respective pseudo-zero order rate constants, k_o, for the degradation of the different speciation forms, and these are summarized in Table 1

Calculation of the speciation coefficients α and x_i

Calculation of the speciation coefficients α and x_i requires knowledge of the initial concentration ($[A]_{0,}\mu gg^{-1}$) of each speciation form. The values of $[A]_o$ for the first and second linear portions in the multi-phase dissipation curve in Fig. 1, are given by the difference between the intercepts in the regression equations of the linear portion under consideration and the succeeding linear portion as shown in Table 2. The value of $[A]_o$ for the final linear portion is given by the intercept for that linear portion, The values of the speciation coefficients α, x_1 and x_2 are given by $[A]_o/\Sigma[A]_o$. The values of $A_{o(w)}$, $A_{o(s)}$, and speciation coefficients α, x_1 and x_2 obtained are shown in Table 2.

Determination of ϕ, the fraction of the adsorbed speciation form that is desorbed upon contact with the intestinal fluid

Bioaccessibility is accessed using in vivo, ex vivo, in situ and in vitro methods [17,18], although in general in vitro methods are more commonly used. Several such in vitro methods, designed to simulate gastric and small intestine digestion, have been developed [17,22]. In general, the sample is digested after the sequential addition of simulated saliva at pH 6.5 ± 0.2, gastric juice (pH 1.07±0.07), and lastly duodenal juice (pH 7.8 ± 0.2) and bile (pH 8.0 ± 0.2). The mixture is heated to 37±2 °C, mixed by tumbling, and then centrifuged. The released bioactive substance in the supernatant fluid (or chyme) is then determined using a suitable analytical method. The analysis is done before and after the in vitro digestion [22]. For the determination of ϕ, the fraction of the adsorbed speciation form that is desorbed upon contact with the intestinal fluid, the analytical method must be capable of differentiating between free antibiotic molecules and colloidal-particle-adsorbed antibiotic molecules in the chyme. A possible way to achieve this discrimination of free antibiotic molecules and colloidal-particle-adsorbed antibiotic molecules in the chyme is the use of semi-permeable cellulose dialysis, in place of the centrifugation separation step in the method described above [17,32,33]. The approach has been used previously to study the bioavailability of polyphenols, for which molecular size, degree of polymerization or glycosylation, and conjugation with other phenolics affect their absorption and metabolism [17]. Applied to the study of the bioaccessibility of different CTC speciation forms, a further difficulty envisaged is the ability of the semi-permeable cellulose dialysis to differentiate between different colloidal-particle adsorbed speciation forms, namely antibiotic adsorbed to colloidal particles type 1 and antibiotic adsorbed to colloidal particles type 2. Thus a lot of work still remains to be done in regard to the determination of ϕ, the fraction of the adsorbed speciation form that is desorbed upon contact with the intestinal fluid. In the present work a value of unity is assumed for ϕ.

Table 2
Initial concentration ($[A]_{0,}\mu gg^{-1}$), and Speciation Coefficient (α, x_1 and x_2) for the degradation of tetracycline antibiotics in the water phase and sediment phase of the microcosm experiment.

Spec form[a]	$[A]_0$ calculation	$[A]_0$ (μgg^{-1})	Rate constant CTC	Spec Coefficient
$A_{fs(w)}$	0.6027 – 0.4685	0.1542	0.0268	0.2476 (= α)
$A_{c1(w)}$	0.4685 - 0.3243	0.1442	0.0071	0.2316 (= x_1)
$A_{c2(w)}$	0.3243 - 0.0	0.3243	0.0016	0.5208 (= x_2)
$\Sigma[A]_{o(w)}$		0.6227		
$A_{fs(s)}$	0.3371 – 0.2354	0.1017	0.0274	
$A_{c1(s)}$	0.2354 – 0.1721	0.0623	0.0032	
$A_{c2(s)}$	0.1721 – 0.0	0.1721	0.0008	
$\Sigma[A]_{o(s)}$		0.3371		
			OTC	
$A_{fs(w)}$	0.4956 – 0.318	0.1776	0.0307	0.3584 (= α)
$A_{c1(w)}$	0.318 - 0.1416	0.1764	0.0068	0.3559 (= x_1)
$A_{c2(w)}$	0.1416 - 0.0	0.1416	0.0012	0.2857 (= x_2)
$\Sigma[A]_{o(w)}$		o.4956		
			DC	
$A_{fs(w)}$	0.7653 - 0.5363	0.229	0.0296	0.2992 (= α)
$A_{c1(w)}$				
$A_{c2(w)}$	0.5363 - 0.0	0.5363	0.0046	0.7008 (= x_2)
$\Sigma[A]_{o(w)}$		0.7653		

[a] A = antibiotic molecules; Subscripts fs = free in solution, c1 = adsorbed to colloidal particle type 1; c2 = adsorbed to colloidal particle type 2, w = water phase and s = sediment phase respectively; $\Sigma[A]_o$ = total day zero antibiotic concentration.

Bioaccessibility of tetracyclines on day zero

Table 1 shows that, depending on the values of χ_i and ϕ_I, $BACC_{(wp)}$ for DC lies in the range 0.5 - 1.5, while $B_{ACC(wp)}$ for CTC falls in the range 0.1 - 1.2, and that for OTC falls in the range 0.5 – 1.1. The minimum value of $B_{ACC(wp)}$ depends on the value of $\alpha_{(w)}$ which in turn depends on the nature of organic pollutant and adsorbent particle, and is expected to be highly variable depending on the dominant colloidal particles in a given aquatic ecosystem. The maximum value of $B_{ACC(wp)}$, on the other hand, depends on the partition or distribution coefficient, K_p, of the pollutant between the water column and the sediment. The distribution coefficient largely depends on the properties of the water phase and the sediment, which can easily be established, see Table S1 (Supplementary Material). While the dependence of $B_{ACC(wp)}$ on specific properties of sediments has still to be studied, it is reasonable to assume that classification of organic pollutants on the basis of their maximum $B_{ACC(wp)}$ values is the only practical way for classifying aquatic organic pollutants in the absence of values of α, χ_i and ϕ_i.

From Table 1 the aquatic $B_{ACC(wp)}$ of OTC is the lowest at 1.09. The bioaccessibility of DC, on the other hand, is the highest at 1.55. The aquatic bioaccessibility of CTC is intermediate at 1.23. On the basis of the classification proposed above, the results suggest that OTC and CTC exhibit moderately high aquatic bioaccessibility (1.0 – 1.5), while DC exhibits extremely high aquatic bioaccessibility (1.5 – 2.0). These results show that the aquatic bioaccessibility coefficient of organic substances in a given aquatic ecosystem differs for different substances.

Meaningful comparisons of these results to results reported in the literature, obtained using existing methods comprising in vitro, ex vitro, in situ, and in vivo methods all based on Eq. (1), are not possible as the existing methods do not take into account partitioning of the pollutant between the water column and the sediment, as well as any speciation undergone by the substance.

Effect of antibiotic structure on day-zero aquatic bioaccessibility

Tetracyclines differ from one another chemically at positions 5, 6 and 7 by variation of substituents at these positions, see Supplementary Material Fig. S1. In addition, TCs differ in their three pK_a values, corresponding to the phenolic diketone, dimethylamine and tricarbonylamide groups (Fig. S1) for each TC member [34,35]. In an effort to establish the parameters on which the aquatic bioaccessibility of TCs depend on, a Pearson's correlation analysis was carried out involving the following parameters obtained in this study for the CTC, OTC and DC, see Table 1: the day zero $B_{CCA(max)}$, $B_{CCA(min)}$, water-column/sediment partition coefficient $K_{p(o)}$, the zero order rate constants $k_{o(wp)}$, $k_{c1(wp)}$, $k_{c2(wp)}$, $k_{o(sp)}$, $k_{c1(sp)}$, $k_{c2(sp)}$, and pKa1, pKa2 and pKa3 values from the literature [34,35], see Table S3 (Supplementary Material). It is apparent from Table S3 that neither $B_{CCA(max)}$, $B_{CCA(min)}$, nor the zero order rate constants $k_{o(wp)}$, $k_{c1(wp)}$, $k_{o(sp)}$, $k_{c1(sp)}$, $k_{c2(sp)}$ show significant correlations with the pKa values. Thus the phenolic diketone, dimethylamine and tricarbonylamide substituent groups have little or no effect on the bio-accessibility coefficient. This is in agreement with the finding by Figuera et al. [36] that substituents effects amongst members of TC compounds had only minor effects on sorption. This suggests that the A and the B, C and D rings are the complexing sites, and is collaborated by the fact that at neutral pH and low concentration, TC adsorption by

Table 3
$k_{c2(wp)}$ and $t_{[kc2(wp)]}$: Predicted versus Experimental.

	CTC	OTC	DC
$k_{c2(wp)}$ Predicted	0.0016	0.0011	0.0044
$k_{c2(wp)}$ Experimental	0.0016	0.0012	0.0046
$t_{[kc2(wp)]}$ predicted	621	912	225
$t_{[kc2(wp)]}$ experimental	625	833	217

soils and clay has been attributed mainly to surface complexation [37] (Li et al., 2010). Such a complex should be common to all TCs, and the strength of adsorption bond should be approximately the same for all TCs, except for minor variations arising from the differences in the substituents at positions 5. 6 and 7. For example, through the resonance effect, the presence of the Cl ligand at position 7 for CTC should reduce the strength of the adsorption bond if the bond involves the LUMO orbital of the antibiotic molecule, or increase the strength of the adsorption bond if the bond involves the HOMO orbital of the molecule. $B_{ACC(min)}$ shows a significant positive correlation coefficient with $k_{c2(wp)}$ (Pearson's correlation coefficient 0.998 ($p = 0.035$)), which is related to the strength of the adsorption bond of the most persistent adsorption speciation form of the antibiotic in the water phase. The fact that $k_{c2(wp)}$ is common to all three TCs (see Table 1) suggests $k_{c2(wp)}$ corresponds to the adsorption bond involving the A, B, C and D rings as discussed above, while the fact that $B_{ACC(min)}$ correlates positively with $k_{c2(wp)}$ suggests that $B_{ACC(min)}$ depends on the strength of this adsorption bond. From Table 1 the rate constant for the k_{C2} process is higher for CTC than for OTC suggesting a weaker adsorption bond and hence a higher bio-accessibility coefficient for CTC, and that the adsorption bond involves the LUMO of the antibiotic molecule.

$B_{ACC(min)}$ further shows a significant positive correlation coefficient with $K_{p(o)}$ (Pearson's correlation coefficient 1.0000 ($p = 0.013$)). Thus $B_{ACC(min)}$ is expected to increase with an increase in $K_{p(o)}$. $K_{p(o)}$ can therefore be used to predict $B_{AAC(min)}$. For tetracyclines the regression equation is (see, Fig. S2):

$$B_{AAC(min)} = 0.206K_{p(o)} + 0.8427 \tag{8}$$

The dependence of $B_{ACC(min)}$ on $K_{p(o)}$ follows from Eq. (6) above. $k_{c2(wp)}$ is related to the strength of the adsorption bond of the most persistent adsorption speciation form of the antibiotic in the water phase, hence the fact that $B_{ACC(min)}$ correlates positively with $k_{c2(wp)}$ suggests that $B_{ACC(min)}$ depends on the strength of this adsorption bond, while the fact that $k_{c2(wp)}$ is common to all three TCs suggests that the strength of this adsorption bond should be approximately the same for all three TCs, except for minor variations arising from the differences in the substituents at positions 5. 6 and 7.

Other significant correlations are $k_{c2(wp)}$ versus $K_{p(o)}$ (Pearson's correlation coefficient 0.999, $p = 0.022$), and $k_{c2(sp)}$ versus $k_{o(sp)}$ (Pearson's correlation coefficient 0.998, $p = 0.037$). Thus, $K_{p\,(o)}$ can also be used to predict $k_{c2(wp)}$, and hence the rate of degradation and the life-time of antibiotic adsorbed to colloidal particles type 2 in the water phase. For tetracyclines the regression equation is (see, Fig. S2):

$$k_{c2(wp)} = 0.0015\mathrm{Kp}_{(o)} - 0.0007 \tag{9}$$

From Fig 1, the $k_{c2(wp)}$ speciation form is the most persistent speciation form in the water phase. Because the $k_{c2(wp)}$ speciation form is available for ingestion by higher fauna, knowledge of its rate of degradation, and hence its life-time, is important.

According to the multi-phase pseudo-zero order rate law, persistence is characterized in terms of its life-time, t_o, in the environment, given by Eq. (10) [38]:

$$t_o = [C]_o/k_o \tag{10}$$

where $[C]_o$ denotes initial concentration of the substance, and k_o is the zero-order rate constant. When persistence is defined in terms of the number of days required to degrade 1 μg of pollutant per gram of medium and the rate of degradation is expressed in $\mu g g^{-1}$ day^{-1}, persistence is given by the reciprocal of the zero-order rate constant (or the linear rate of degradation), i.e.,

$$t_{(k_o)} = \frac{1}{k_o}\left(\frac{\mu g g^{-1}}{\mu g g^{-1} day^{-1}}\right) \tag{11}$$

Thus the life-time (in days) of antibiotic adsorbed to colloidal particles type 2 in the water phase is given by

$$t_{[k_{c2(wp)}]} = \frac{1}{k_{c2(wp)}} = \frac{1}{0.0015K_{p(o)} - 0.0007} \tag{12}$$

Table 3 shows the values of $k_{c2(wp)}$ and the life-times, $t_{[kc2(wp)]}$ (in days), predicted for CTC, OTC and DC on the basis of Eqs. (9) and 12 respectively, and $K_{p(o)}$ values in Table 1. Good agreement is obtained in all cases.

The dependence of $k_{c2(wp)}$ on $K_{p(o)}$ is understandable if cognisance is taken of the fact that $k_{c2(wp)}$ is directly proportional to the initial concentration, $[A]_{c2(wp)}$, of the antibiotic adsorbed to colloidal particles type 2 in solution in the water phase according to Eq. (13) [24]:

$$k_{c2(wp)} = k''_{C2} = k'_{C2}\left[A(C_2)_y\right]_{o(w)} \tag{13}$$

where k'_{c2} is the first order rate constant for the degradation of the antibiotic adsorbed to colloidal particles type 2 in solution in the water phase. As Table 2 shows, the concentration of the antibiotic adsorbed to colloidal particles type 2 ($[A]_{c2(wp)}$ (= $[A(C_2)_y]_{o(w)}$ in Eq. (13)), is a major contributor to the total concentration of antibiotic in the water phase.

Contribution from adsorbed speciation forms in the sediment

Multi-phase dissipation rates are possible provided (a) chemical and photochemical degradation affects the free dissolved speciation form only [39,40], (b) rates of degradation of speciation forms differ significantly [24], and (c) rates of desorption of adsorbed speciation forms are less than the rates of microbial binding of the antibiotics, i.e., desorption is rate limiting for the microbial degradation of adsorbed forms of the antibiotic [38]. If the rate of desorption of an adsorbed speciation form is equal to or faster than the rate at which degrading microorganisms can bind freely dissolved molecules of antibiotic, then degrading microorganisms cannot differentiate that adsorbed speciation form from the freely dissolved speciation form, and to all intents and purposes that adsorbed speciation is non-existent. It follows therefore that since the rate of degradation of adsorbed speciation forms is limited by the rate of desorption, binding of adsorbed antibiotic by degrading microorganisms occurs immediately upon desorption. Applied to adsorbed speciation forms in the sediment phase, this means that adsorbed antibiotic molecules in the sediment phase are bound by degrading microorganisms immediately upon desorption, and will not be available for sediment-to-water-column equilibrium exchange. In other words, sediment/water column equilibrium exchange is possible only while the free dissolved speciation form lasts. Thus provided the antibiotic is susceptible to microbial degradation, there is no sediment/water column equilibrium exchange involving adsorbed speciation forms, as microbial binding occurs immediately upon desorption. Resuspension of adsorbed antibiotic can however occur [23]. It is therefore safe to assume that in the absence of such resuspension, adsorbed speciation forms in the sediment are not available for diffusive sediment/water column equilibrium exchange, and therefore are not bioaccessible to fish and other higher fauna.

Bioaccessibility as a function of time

It has been shown above (Eq. (8)) that bioaccessibility correlates linearly with the water-column/sediment distribution ratio, K_p. Hence changes in bioaccessibility as a function of time should be reflected in changes in K_p as function of time. Changes in K_p as function of time can arise due to (a) diffusive water-column/sediment equilibrium transfer, (b) changes in the concentration of adsorbed speciation forms in the water phase and sediment phase as a result of differences in degradation rate constants of adsorbed speciation forms as shown in Table 2, and (c) resuspension of settling adsorbed speciation forms of the antibiotic in the sediment.

Effect of diffusive water-column/sediment equilibrium transfer on k_p

Fig. 3 shows that, as with B_{acc}, the experimental K_p does not remain constant as a function of time, and that its behaviour as a function of time differs significantly for all three antibiotics. This behaviour of K_p as a function of time may be attributed to diffusive water-column/sediment equilibrium transfer of free molecules of antibiotic in solution in the water phase and the sediment pore water [41,42], driven by differences in the rates of degradation of antibiotic speciation forms in the water phase and sediment phase.

If we take into account speciation of the antibiotic in the water and sediment phases as shown in Table 2, Eq. (5) can be expressed as

$$K_p = \frac{[A]o(w)}{[A]o(s)} = \frac{[A]_{fs(w)} + [A]_{c1(w)} + [A]_{c2(w)}}{[A]_{fs(s)} + [A]_{c1(s)} + [A]_{c2(s)}} \tag{14}$$

Diffusive water-column/sediment equilibrium transfer will involve free molecules of antibiotic in solution in the water phase and in solution in the sediment pore water, i.e., $[A]_{o(w)}$ and $[A]_{o(s)}$. The value of $[A]_{o(w)}$ in turn is affected by adsorption/desorption equilibria between $[A]_{o(w)}$ and $[A]_{c1(w)}$, and between $[A]_{o(w)}$ and $[A]_{c2(w)}$. Similarly the value of $[A]_{o(s)}$ is affected by adsorption/desorption equilibria between $[A]_{o(s)}$ and $[A]_{c1(s)}$, and between $[A]_{o(s)}$ and $[A]_{c2(s)}$. However, since the rate of degradation of adsorbed forms is limited by the rate of desorption [24], adsorption/desorption equilibria between $[A]_{o(s)}$ and $[A]_{c1(s)}$, and between $[A]_{o(s)}$ and $[A]_{c2(s)}$ are disrupted as adsorbed antibiotic molecules undergo microbial degradation as soon as desorption takes place. Therefore diffusive sediment-water column equilibrium transfer is only expected to involve the original free dissolved speciation forms only. However as diffusive sediment/water-column equilibrium transfer occurs to maintain the sediment-water column equilibrium partition coefficient, K_p, the direction of transfer is towards the phase with the greater degradation rate, but the partition coefficient, is expected to remain constant, in agreement with the findings by Geschwend and Wu [23]. Thus diffusive sediment/water-column equilibrium transfer has no effect on K_p and hence $B_{ACC(wp)}$. The value of K_p will depend on the degradation rates of the adsorbed speciation form, as changes in the values of $[A]_{c1(w)}$, $[A]_{c2(w)}$, $[A]_{c1(s)}$, and $[A]_{c2(s)}$ will affect the value of K_p in Eq. (14).

Effect of differences in the degradation rate constants of adsorbed antibiotic speciation forms in the water phase and sediment phase on K_p.

It can be shown that in the absence of sediment/water-column equilibrium transfer, the rate of change of K_p as a function of time is given by

$$\frac{d(K_p)}{dt} = \frac{d(C_w/C_s)}{dt} = \frac{1}{C_s}\frac{dC_w}{dt} - \frac{C_w}{C_s^2}\frac{dC_s}{dt} \tag{15}$$

Eq. (15) shows that $d(K_p)/dt$ is a complex function of the concentration of the antibiotic in the water column, C_w, and the sediment phase, C_s, as well as the rates of its degradation in either phase. In the case of multi-phase linear dissipation kinetics, C_w, and C_s represent the initial water column concentration and sediment phase concentration for the respective water phase and sediment phase linear portions of the dissipation curve in Fig 1, and $dC_w/dt$and dC_s/dt are the respective rates of dissipation. Thus $d(K_p)/dt$ can be evaluated if C_w, C_s, dC_w/dt and dC_s/dt are known. In the case of multi-phase zero-order dissipation kinetics, $dC_w/dt = k_{o(w)}$ and $dC_s/dt = k_{o(s)}$, where $k_{o(w)}$ and $k_{o(s)}$ are the zero-order dissipation rate constants for the water phase and sediment phase respectively in Fig. 1. The values of C_w and C_s are computed from the intercept exhibited by each regression equation in Fig. 1 as shown in Table 2. Hence substituting for dC_w/dt and dC_s/dt in Eq. (15) and integrating, Eq. (15) becomes

$$K_p = K_{p_{(o)}} + \sum_x \left\{ \frac{1}{C_s}\left(k_{o(w)} - \frac{C_w}{C_s}k_{o(s)}\right)t_{\lim t=0-t_x} \right\} \tag{16}$$

where Σ sums over the number of linear portions in the multi-phase linear rate law, and t_x = the point in time where one linear portion in multi-phase linear rate curve ends. .

Eq. (16) does not take into account sediment-water column equilibrium transfer, hence the experimental value of K_p as a function of time will be greater or lower than the theoretical value depending on the direction of sediment-water column equilibrium transfer. In the absence of sediment-water column equilibrium transfer, Eq. (16) shows that K_p as a function of time will increase when $(C_w/C_s)k_{o(s)} < k_{o(w)}$, decrease when $(C_w/C_s)k_{o(s)} > k_{o(w)}$, and remain constant when $(C_w/C_s)k_{o(s)} = k_{o(w)}$.

Fig. 3 shows the theoretical K_p curves computed using Eq. (16). The theoretical K_p curve for CTC in Fig. 3 shows a steady increase. The theoretical K_p curve for OTC on the other hand shows a steady decrease from a K_p value of about 1.2 at day zero to a value of zero at day 90, while that of DC remains virtually constant at a value just under 4.

Effect of re-suspension of adsorbed antibiotic on k_p

It is apparent from Fig. 3 that in all cases the experimental K_p curve deviates considerably from the theoretical curve. The observed deviation of the experimental K_p curve from the theoretical can only be attributed to re-suspension of the antibiotic adsorbed to settling colloidal particles. *Re*-suspension of the substances adsorbed to settling colloidal particles has been alluded to previously [23]. Such re-suspension of absorbed antibiotic cannot be avoided during the sampling of sediment samples, hence care should be exercised when collecting water samples for analysis. The large deviations of the experimental from the theoretical curve observed for OTC and DC highlight the problem associated with taking water samples from shallow water bodies. In addition, because higher fauna such as birds, wild and domestic animals, often wade into the shallow waters to drink, aquatic bio-accessibilities of pollutants experienced by such higher fauna will be higher than predicted theoretically. In the natural aquatic environment, resuspension of adsorbed organic pollutant species can also occur through other processes such as bioturbation, bioirrgation by benthic organisms, or through flooding [41].

Conclusions

From the foregoing discussion the following conclusions can be made:

(a) The proposed new method has the ability (i) to predict the aquatic bio-accessibility of any substance on the basis of its water column/sediment partition coefficient, K_p, on the basis of Eq. (4), and (ii) on the basis of Eq. (16) to predict the time dependence of the K_p of any substance resulting from differences in the rates of degradation of the substance in the water column and sediment phase.

(b) The new method further provides a basis for classifying organic aquatic pollutants on the basis of their maximum possible $B_{ACC(wp)}$ values predictable on the basis of K_p.

(c) In the case of tetracycline antibiotics, aquatic bia-accessibility correlates linearly with K_p and $k_{c2(wp)}$, the rate constant for the degradation of the most persistent adsorption speciation form in the water phase, hence the new method has the ability to predict the aquatic persistence of tetracycline antibiotics on the basis of K_p.

(d) A further significant contribution is the introduction of the new terms bio-accessibility coefficient, speciation coefficients α, and χ, which like K_p, are thermodynamic parameters characteristic of the substance under consideration for a given aquatic ecosystem.

(e) Sediment-to-water column equilibrium exchange, expected for the free dissolved speciation forms, has no effect on the theoretical value of K_p. However, the experimentally determined K_p may deviated considerably from the predicted values as a result of resuspension of antibiotic adsorbed to settling colloidal particles. Hence care should be exercised when collecting water phase samples for analysis.

(f) Limitations of the proposed new approach include the fact that the speciation coefficients α, χ and ϕ cannot be predicted theoretically. The speciation coefficients α and χ can only be determined experimentally using microcosm experiments, while ϕ can only be determined using gastrointestinal (GI) simulation studies. In addition, bio-accessibility as defined in this paper cannot be applied to soil systems, and cannot be used to describe aquatic bioaccessibility to microorganisms. Future research should therefore focus on extending the theoretical approach proposed in this paper to soil systems, and to include bio-accessibility and bioavailability of organic pollutants to microorganisms.

Declaration of Competing Interest

None.

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Supplementary materials

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