

Loosely Bound Oxytetracycline in Riverine Sediments from Two Tributaries of the Chesapeake Bay

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The fate of antibiotics that bind to riverine sediment is not well understood. A solution used in geochemical extraction schemes to determine loosely bound species in sediments, 1 M MgCl₂ (pH 8), was chosen to determine loosely bound, and potentially bioavailable, tetracycline antibiotics (TCs), including oxytetracycline (5-OH tetracycline) (OTC) in sediment samples from two rivers on the eastern shore of the Chesapeake Bay. Bottom sediments were collected at sites upstream from, at, and downstream from municipal sewage-treatment plants (STPs) situated on two natural waterways, Yellow Bank Stream, MD, and the Pocomoke River, MD. Concentrations of easily desorbed OTC ranged from 0.6 to approximately 1.2 μg g⁻¹ dry wt sediment in Yellow Bank Stream and from 0.7 to approximately 3.3 μg g⁻¹ dry wt sediment in the Pocomoke River. Concentrations of easily desorbable OTC were generally smaller in sediment upstream than in sediment downstream from the STP in the Pocomoke River. STPs and poultry manure are both potential sources of OTC to these streams. OTC that is loosely bound to sediment is subject to desorption. Other researchers have found desorbed TCs to be biologically active compounds.

Introduction

The use of antibiotics in animal husbandry in the United States has increased from 16 million pounds per year during the 1980s to between 17.8 (Animal Health Institute) and 25 (Union of Concerned Scientists) million pounds per year in 2001 (1). Of the 3 million pounds of tetracycline antibiotics (TCs) used in animal agriculture each year, more than 1 million pounds are used in the poultry industry. Much of the antibiotics administered to poultry become part of poultry litter. Another source of pharmaceuticals to the environment is sewage treatment plants (STPs) (2, 3). A portion of the pharmaceuticals that enter STPs is not decomposed in the sewage treatment process and enter streams via STP effluent.

Reports in the literature show that pharmaceuticals used in human medicine and animal husbandry are present in soil, sediment, surface water, and groundwater throughout the western world (3–5). Efforts have been made to determine the fate of these compounds in the environment, but there is a limited amount of information about environmental processes involving pharmaceuticals. Once thought to readily degrade in the environment, recent studies indicate that TCs in sediments and soils are persistent. Researchers have found that several antimicrobial agents, including oxytetracycline (OTC), persist in marine sediments (6–13). Hamscher et al.

(14) reported that tetracycline from animal manure applied to crop fields was persistent at original concentrations for more than a year. Included among the reported environmental effects of TCs are changes in antibiotic resistance of indigenous bacteria (15) and changes in the kinetics of nitrite oxidation by soil and sediment bacteria (3, 16). Some of the pharmaceuticals that reach streamwaters remain in the water column, but others, such as the TCs, tend to bind with geochemical phases in sediment. Kolpin et al. (2) identified TCs in less than 2.5% of the water-column samples from 139 streams that they sampled throughout the United States.

Oxytetracycline is commonly used in aquaculture (4, 7, 10, 17). Studies of marine sediments collected from sites in close proximity to fish farms have reported the loss of OTC from these sediments and attributed this loss to diffusion from the sediment, out-washing, and/or decomposition determined by measuring the decrease in the concentrations of OTC in sediment over time (6–13, 18). Desorption could be a mechanism for loss of OTC from marine sediment. Concentrations of OTC in marine sediment in these studies ranged from 0.2 to 285 μg g⁻¹.

The data presented in this paper are based on analyses of riverine rather than marine sediment. These data indicate that desorption is a mechanism for removal of OTC from riverine sediment. Laboratory studies in which buffers were used to desorb TCs from soils and clays showed that the desorbed TCs retained their integrity and remained effective antibiotics (19, 20). The affect of desorbed TCs on microbial populations in riverine sediment merits study.

In this study MgCl₂ solutions were chosen to extract sediments because a goal of this study was to determine the fraction of TCs in sediment that could be readily released and might be, therefore, bioavailable. MgCl₂ solutions are used to remove exchangeable or loosely bound analytes, including metals and phosphate, from sediment and soil samples in geochemical extraction schemes (21–26). At a pH of 8 the dissolution of carbonates and metal oxyhydroxides in the samples is minimized and the TCs will be present as anions available for complexation with Mg²⁺. Liquid chromatography with electrochemical detection was used because the extraction solutions containing 1 M MgCl₂ and extracted OTC can be injected directly into the instrument.

This study is part of an ongoing U.S. Geological Survey (USGS) investigation to determine if geochemical processes in bottom sediments of streams reflect differences in land-use practices in watersheds of the Chesapeake Bay Basin. The main purpose of this paper is to present data for the concentrations of loosely bound tetracycline antibiotics obtained by extraction with 1 M MgCl₂ (pH 8) solutions of riverine sediments from locations in the Chesapeake Bay watershed.

Experimental Section

Sample Collection. Sample sites near STPs were selected in two streams located in the Chesapeake Bay Watershed (Figure 1). The density of commercial poultry farms differs, with more than 10 times more chicken houses being located in the Pocomoke River study area than are located in the Yellow Bank Stream study area. In 1997, more than 256 million broilers (approximately 4% of all the broilers produced in the United States) were produced in three Maryland counties (Somerset, Wicomico, and Worcester) through which the Pocomoke River flows (26). The broilers are raised in “poultry houses”, buildings large enough to accommodate hundreds of chickens. For economic reasons, when the poultry houses

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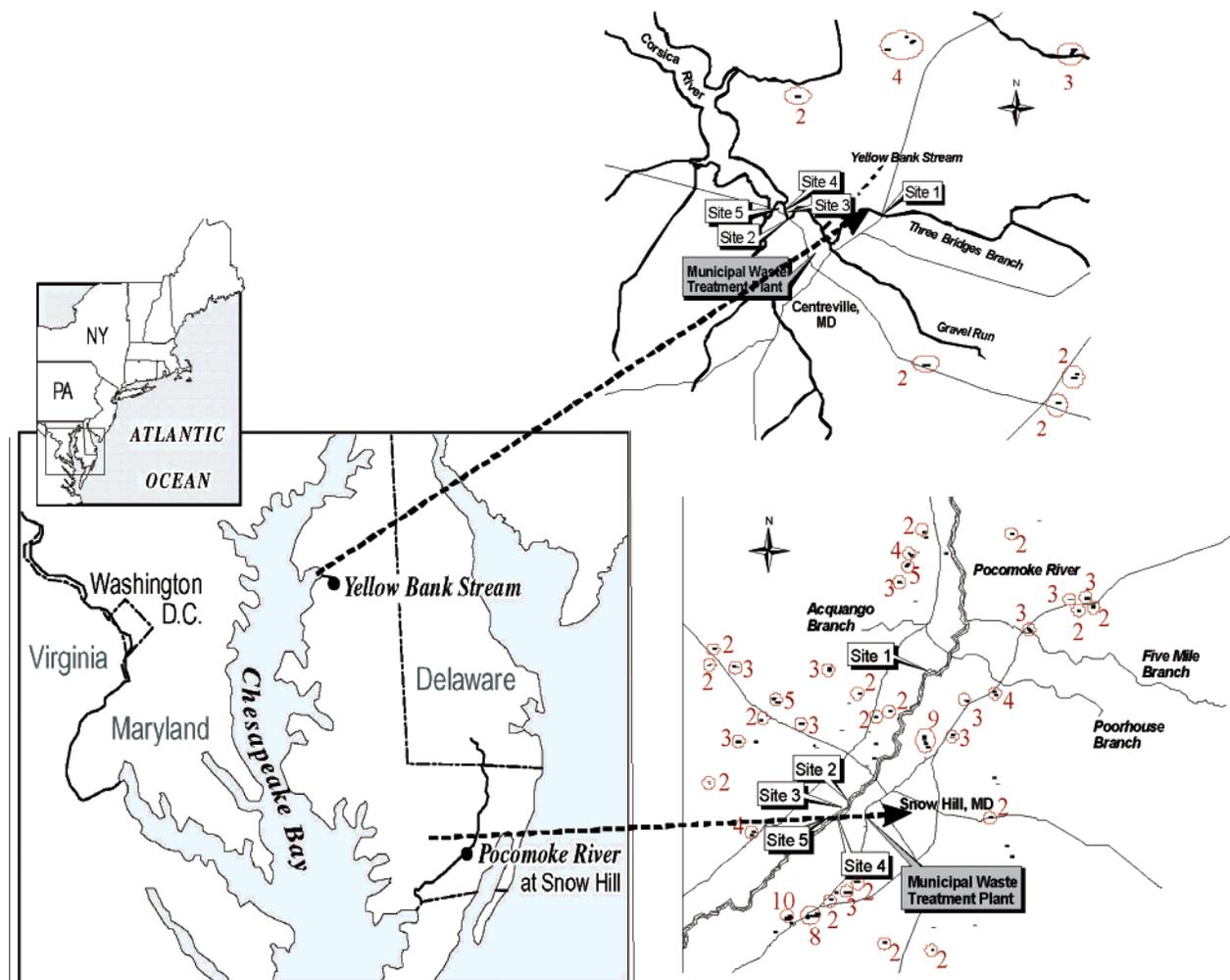


FIGURE 1. Map of the Chesapeake Bay showing study Areas. The maps of Yellow Bank Stream, MD, and the Pocomoke River, MD, have individual poultry houses marked with a dot. The circles indicate that more than one poultry house exists, and the number of the poultry houses is indicated by the number next to the circle.

are cleaned, the litter is disposed of by applying it to local fields.

Yellow Bank Stream drains into the Corsica River, which flows into the Chester River, a tributary to the Chesapeake Bay. The Pocomoke River is the southern most tributary to the Chesapeake Bay on the eastern shore of Maryland. The distribution of poultry houses in the areas of Centreville, MD, and Snow Hill, MD, are shown in Figure 1.

The sites within each watershed were selected so that the first location in the sampling gradient was (a) above the head of tides and (b) upstream from a municipal STP, the location of which is marked on a USGS 7.5 min quadrangle map. Sediment samples were collected from Yellow Bank Stream, MD, on October 23, 2001. Sediment samples were collected from the Pocomoke River, MD, on September 18, 2001. Site 1 was selected to provide bottom sediment that was unaffected by a STP. The second site was downstream from, and close to, the outfall of a municipal STP. Three additional sites were sampled in a gradient downstream from each STP outfall. Sites in the vicinity of the town of Centreville, MD, on Yellow Bank Stream are designated YB-1–5. These sites are located approximately 0.06 km apart. Sites in the vicinity of the town of Snow Hill, MD, on the Pocomoke River are designated PR-1–5. These sites are located approximately 0.5 km apart.

Water-column and sediment samples were collected at each site. Samples were filtered through a 0.2 μm polycarbonate membrane and refrigerated at approximately 4 $^{\circ}\text{C}$.

Bottom sediment was collected using a petit ponar grab sampler (Wildlife Supply Company, Buffalo, NY).

The top 1 cm of sediment was freeze-dried. Freeze-drying stops microbial activity. The dry samples were sieved and separated into <75 μm (slightly larger than the 63 μm cutoff for silt), 75–125 μm (fine sand), 125–250 μm (sand), 250 μm to 1 mm (coarse sand), and >1 mm (very coarse sand) fractions. Weights of several size fractions were less than required for the extraction procedure. Therefore, the size fractions were combined and ground to a particle size <125 μm . The combined, ground sediment was analyzed for total phosphorus (P), total iron (Fe), organic carbon (C) and nitrogen (N), and easily desorbable OTC.

Chemicals. Chemicals were ACS reagent grade with the following exceptions. Methanol was high purity solvent from Burdick and Jackson. TCs were purchased from Sigma Chemicals, Inc. and used without further purification. The reported purities of the TCs were 95% for tetracycline (TC) and oxytetracycline (OTC) and 80 and 98% for chlortetracycline (CTC) and doxycycline (DOX), respectively.

Care must be taken to use high purity base and acid when adjusting the pH of the MgCl_2 (pH 8) extracting solution. Extraneous peaks can occur in chromatograms if even small concentrations of electroactive contaminants are present. Isothermally distilled ammonium hydroxide and hydrochloric acid are suggested for use in adjusting the pH of the MgCl_2 extracting solution.

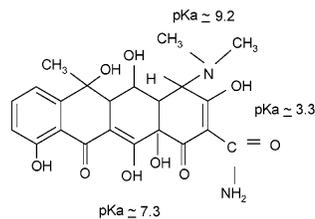


FIGURE 2. Schematic of OTC. pK_a's of functional groups are shown.

TC Desorption Method. OTC has several pH-dependent functional groups, as depicted in Figure 2. At pH 8, OTC exists primarily as a net negatively charged ion (+ - -), which can complex with Mg²⁺ ions (28, 29). The mechanism for the desorption of TCs from sediment is thought to be similar to the mechanism for phosphate desorption. Phosphate desorption is attributed to formation of Mg²⁺ complexes and mass action displacement by Cl⁻ (26). OTC's solubility in water is approximately 1 mg mL⁻¹ (30). In the presence of complexing agent, the solubility of OTC is increased because complexation increases OTC stability in water. Complexation results in OTC being "salted in" (31).

Sample Preparation. The analyte of interest in this study was loosely bound or potentially desorbable OTC. To extract easily desorbed OTC, sediment samples weighing approximately 0.1 g were extracted with 10 mL of 1 M MgCl₂ (pH 8) using sonification for 2 h (26). The pH of the extracting solution was measured with a pH meter; pH values varied between 7.8 and 8. After centrifugation and filtration through a 0.2- μ m filter, extracts of samples were injected into a liquid chromatograph with an electrochemical detector (LCEC). A supplement to analysis of 1 M MgCl₂ (pH 8) extracts of sediment samples without modification was analysis of 1M MgCl₂ (pH 8) extracts using the method of standard additions. Extractions of sediment were run in triplicate and the OTC spike was added at the start of the extraction. For each sample three aliquots were weighed out. Sample A was not spiked with OTC, sample B was spiked with 25 μ g of OTC, and sample C was spiked with 50 μ g of OTC. The native concentration of loosely bound OTC was determined from a linear regression analysis of the concentration data.

Recovery of OTC from Sediment. A kinetic study of the release of OTC from sediment using 1 M MgCl₂ (pH 8) was done using triplicate aliquots of sediment from each of four study sites, including YB-1, YB-2, PR-2, and PR-4. The extractions were carried out for 3 h, and an aliquot of the extracting solution was removed at 15, 30, 60, 120, and 180 min for OTC analysis.

Experiments were run to test the recovery of OTC from spiked sediment samples. Sediment samples from sites in both streams in close proximity to the STP and a site downstream from the STP were studied. The site above the STP in the Yellow Bank Stream watershed was also studied; there was insufficient material from the site above the STP in the Pocomoke River watershed at Snow Hill, MD, for experimentation. To spike sediment with OTC, approximately 10 μ g g⁻¹ of OTC in water was added to an aliquot of 2–4 g of wet sediment in 10 mL of native water with a pH of approximately 7; the sample was sonified for 30 min and shaken several times during the treatment period. The slurry was centrifuged, and the solids were immediately frozen, freeze-dried, and ground. Grinding to a particle size <125 μ m homogenized the samples. One set of OTC spiked sediment samples was extracted using 1 M MgCl₂ (pH 8) using the procedure described above. A set of sediment samples was extracted a second time with 1 M MgCl₂ (pH 8) to determine the added recovery of a second extraction. In addition, a set of samples was extracted to test the effectiveness of deionized water, artificial seawater (32), 10 ppm K₂HPO₄, artificial seawater containing 10 ppm K₂HPO₄, and

artificial seawater containing 1 ppm K₂HPO₄ for the removal of OTC from sediment. All extraction solutions except deionized water were adjusted to a pH of approximately 8. The extraction conditions, including the 2 h extraction period, were the same as those used with 1 M MgCl₂ (pH 8). Background concentrations of easily desorbed OTC were subtracted during the calculation of recoverable OTC.

Analysis for TCs. The method of Kazemifard and Moore (33) for the identification and quantification of TCs was modified for use with these samples. A different column and eluent were used in this study. A Bioanalytical Systems (BAS; West Lafayette, IN) Model BAS 200B liquid chromatograph was used with a glassy carbon working electrode (BAS electrode) (LCEC) set at an oxidation potential of 1050 mV with respect to a Ag/AgCl reference electrode. The eluent was a 55:45 mixture of 0.18 M trifluoroacetic acid (adjusted to a pH of 2 with ammonium hydroxide (NH₄OH) and high purity grade methanol (Burdick and Jackson). Before use, 0.1 g L⁻¹ ethylenediaminetetraacetic acid (EDTA) was added and the eluent filtered. The separation of TC, OTC, chlortetracycline (CTC), and doxycycline (DOXY) was tested using buffer-to-methanol ratios ranging from 60:40 to 40:60.

Chromatography was on a 250 mm \times 4.6 mm Luna C₁₈ column (Phenomenex) with a 5 μ m particle size packing using a Luna C₁₈ precolumn. Standards and samples were chromatographed at a flow rate of 0.5 cm³ min⁻¹. Calibration standards of OTC with concentrations ranging from 0.1 to 1 mg L⁻¹ in 1 M MgCl₂ (pH 8) were prepared daily from a stock solutions of 100 ppm OTC in methanol. These standards were used to produce a calibration curve. Stock solutions of OTC were prepared monthly and stored at 4 $^{\circ}$ C. Solutions of OTC with a pH from 3 to 9 are stable for 30 days at a temperature of 5 $^{\circ}$ C (30). Calibration curves were determined and limit of detection analysis were calculated. The response of the amperometric detector was linear but not constant, that is, the response of the detector, as measured by peak area of standards, degraded linearly with time. To compensate for changes in detector response, a standard solution of OTC was run after every two samples. Also, approximately 4 mL of each sample or standard was spiked with 10 μ L of 0.1 g of phenol L⁻¹ to provide a reference peak. This was done to ensure that if, for any reason, the retention times changed, the chromatographic run contained an internal reference. In addition to analysis using an external calibration curve, a set of samples was analyzed by creating a calibration curve in the sample matrix to minimize matrix affects (method of standard addition). Aliquots of sample extracts containing approximately 0.2 μ g of OTC were spiked with 0.2 and 0.4 μ g of OTC standard.

To confirm the presence of OTC in sediment extracts, the effluent from the electrochemical cell was directed through a column containing MgO to provide Mg²⁺ to react with TCs and to make the eluent alkaline before continuing through a fluorescence detector. The fluorescence detector, a BAS Model FL45A, was set at an excitation wavelength of 390 nm and an emission wavelength of 512 nm. The samples were analyzed by creating a calibration curve in the sample matrix to minimize matrix affects (method of standard addition). Aliquots of sample extracts containing approximately 0.2 μ g of OTC were spiked with 0.2 and 0.4 μ g of OTC standard.

Columns containing different packing materials provide different separation characteristics that can be used to help in the identification of an analyte. Extracts of bottom sediment were chromatographed on (1) the Luna C₁₈ column with precolumn described above and (2) a 100 mm \times 2.1 mm PRP-1 (styrene divinylbenzene reverse phase column) with a 3- μ m particle size packing and PRP-1 precolumn (PRP-1, Hamilton Co.). The Luna C₁₈ and the PRP-1 contain reverse phase column packing materials with different compositions. The C₁₈ is a nonpolar octadecyl bonded phase silica packing

material. The PRP-1 is a non-silica polymeric styrene divinylbenzene packing material. The eluent described above was used with both columns. The chromatographic conditions using the PRP-1 column included a flow rate of 0.2 cm³ min⁻¹ and the use of a gel ice pack to chill the precolumn to improve separation of the salts in the sample from the analytes.

Experiments were run to determine the linearity of response of the detector and the sensitivity of the method to small concentrations of OTC. The linearity of the response of the electrochemical detector was examined by measuring the response of the detector to a series of solutions of OTC ranging in concentration from 0.1 to 100 μg L⁻¹. The range of the solutions covered 2 orders of magnitude in concentration. The sensitivity of the method was evaluated by measuring the change in precision, i.e., relative standard deviation, with change in OTC concentration injected into the instrument.

Other Analyses. Concentrations of Cl⁻, NO₃⁻, and SO₄²⁻ in water-column samples that had been collected at the same time as the sediment samples were determined using a Dionex DX120 ion chromatograph. Na⁺, Ca²⁺, and Mg²⁺ concentrations in water-column samples acidified with HNO₃ were determined using a Perkin-Elmer Optima 4300 optical emission spectrometer and the method suggested by the manufacturer.

In preparation for determination of total P and metals, sediment samples were digested with concentrated HNO₃ and HF acids using the microwave sample preparation system, Model MDS-2100, manufactured by CEM Corp. The molybdenum blue method of Murphy and Riley as described in Rand et al. (34) was used to determine orthophosphate concentrations in microwave digests of sediment samples. Total P concentration determined for NIST 1646a Estuarine Sediment was within 4% of the certified concentration value. Details of the analyses for total P and metals in sediment samples are given in Simon et al. (35). A Perkin-Elmer Optima 4300 optical emission spectrometer and the method suggested by the manufacturer were used to determine Fe concentrations in microwave digest solutions. Total C and N concentrations were determined in approximately 20 mg of each sample by flash oxidation and separation of the gaseous products using a Carlo Erba EA 1108 elemental analyzer.

Results and Discussion

Desorption of TCs. A kinetics study of the release of OTC from sediment using 1 M MgCl₂ (pH 8) shows that a 2 h extraction period is optimal for maximum removal of OTC from samples (S6 in Supporting Information). Sediment samples had been spiked with OTC as described under "Recovery of OTC from Sediment". A 2 h extraction time used with the samples in this study is in agreement with findings of Martin (28) and Berthon et al. (29) relating to complexation between OTC and Ca²⁺. They reported that the Mg²⁺ complex of OTC is stronger than the Ca²⁺ complex of OTC. Brion and others (36) calculated that at a pH of 7.4, 82% of OTC originally present in solution is present as M₂L²⁺ (M = Ca²⁺) after a 2-h reaction period. The complexation reaction between OTC and Mg²⁺ to form M₂L²⁺ would, therefore, be expected to be more than 82% complete after 2 h, the length of time for the MgCl₂ extraction used in this study.

Extraction with 1 M MgCl₂ (pH 8) can provide total extraction of OTC only if all of the OTC in the sample is loosely bound. Variation in the concentrations of easily desorbed OTC determined in spiked sediment samples using 1 M MgCl₂ (pH 8) is dependent on the homogeneity and sorption characteristics of the sediments. Average concen-

TABLE 1. Results of OTC Recovery Experiment for Sediment Samples from Yellow Bank Stream (YB) at Centreville, MD, and from the Pocomoke River (PR) near Snow Hill, MD^a

sample	added OTC, μg g ⁻¹	OTC, μg g ⁻¹	±SD, n = 4	% recovery
YB-1	10	6	2	60
	5	5	1	82
YB-2	10	8	4	79
	8	5	1	74
YB-3	9	10	5	113
PR-2	10	10	6	102
	6	5	2	82
PR-4	10	9	12	93
	5	4	0.2	74

^a The extracting solution is 1 M MgCl₂ (pH 8).

TABLE 2. Results of OTC Recovery Experiments for Sediment from Yellow Bank Stream (YB-1) at Centreville, MD^a

ppm phosphate	concn OTC spike ^b	recovered OTC ^c	±SD, n = 3	% recovery
1	10	0.9	0.7	9
10	10	4.6	3.3	43

^a The extracting solutions are artificial seawater plus 1 ppm KH₂PO₄ or 10 ppm KH₂PO₄. ^b Units are μg/g dry wt sediment. ^c Units are μg/g dry wt spiked sediment.

trations of easily desorbed OTC recovered by extraction from replicate sediment samples spiked with OTC are reported in Table 1. Concentrations of easily desorbed OTC originally present in the samples were subtracted in the calculations. The efficiency of the extractions ranged from 60 to 113%. Differences in the calculated concentration of easily desorbed OTC in sediment containing small concentrations will reflect the variation inherent in the method at the lower end of method's linear range. Inhomogeneity of and large concentrations of organic carbon in sediment material will contribute to variation in the data.

A second extraction with 1 M MgCl₂ (pH 8) removed an average of 0–7% more OTC from sediment samples. This implies that if all of the OTC added as a spike is present as loosely bound OTC, more than 90% is removed by one extraction with 1 M MgCl₂ (pH 8) (S7 in Supporting Information). The data presented in this paper do not include total concentrations of OTC sediment from the study sites. It is unclear how much of the total concentration of OTC in these samples remains after extraction with 1 M MgCl₂ (pH 8) and whether the residual OTC is bioavailable.

Sample YB-1 was chosen for further study because the recovery OTC from YB-1 sediment spiked with OTC was the lowest of all the samples extracted with 1 M MgCl₂ (pH 8) (Table 1). Phosphate was added to two extraction solutions because Soulides (20) determined that phosphate buffers efficiently remove TC from sediment and because phosphate concentrations in sediment interstitial water can be in excess of 1 ppm. Water, 1 ppm K₂HPO₄ in water, and artificial seawater (32) did not remove detectable OTC from OTC spiked sediment from site YB-1. Artificial seawater containing 1 or 10 ppm K₂HPO₄ adjusted to a pH of 8 removed approximately 1–4 μg g⁻¹ OTC from spiked sediment (Table 2). The phosphate amended artificial seawater was less efficient than 1 M MgCl₂ (pH 8) in recovering OTC. It is possible that the association of OTC with sediment from YB-1 is unlike the association of OTC with sediment from the other study sites. Further work is indicated to determine if natural waters, in particular sediment interstitial waters

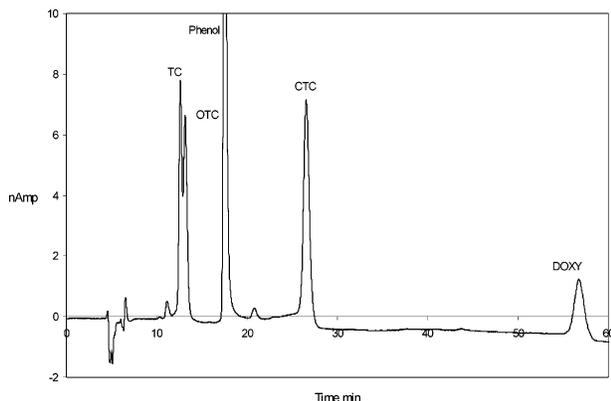


FIGURE 3. Chromatogram of standard containing 1 ppm of TC, OTC, CTC, and DOXY. Chromatography conditions: 250 mm Luna C₁₈ column with Luna C₁₈ precolumn; eluent 55:45, 0.18 M trifluoroacetic acid adjusted to pH 2 plus methanol; flow rate 0.5 cm³ min⁻¹; amperometric glassy carbon electrode, 1050 mV; sensitivity 10 nA.

containing elevated concentrations of phosphate, could release OTC from some contaminated bottom sediments.

Analysis for TCs. Electrochemical detection can be used in the analysis of TCs (33, 37–39). An amperometric detector is “blind” to any constituent in a sample that does not undergo an electrochemical reaction at the voltage setting of the electrode. Less sample cleanup is required when the detector is selective (40) and samples needed only filtration before injection into the LCEC. In amperometric detection of TCs, oxidation occurs at phenolic and dimethylamino functional groups in the ring structure. Hydrovoltamograms indicate that the maximum voltage for oxidation of OTC in this system exceeds 1300 mV. The higher the potential of the electrode in an amperometric detector, the more rapidly the electrode is fouled and the more rapid detector sensitivity is lost. A detector potential of 1050 mV was chosen because this potential permits the use of the detector for up to 8 h when sediment extracts are being analyzed.

Liquid chromatography with fluorescence detection has been used to analyze samples for TCs (40–43). To confirm the identification of OTC in the sediment extracts, the effluent from the LCEC electrochemical cell was directed through a postcolumn to adjust the pH of the eluent to 8 and to add Mg²⁺ before the effluent passed through a fluorescence detector. The postcolumn broadened the peak produced by the fluorescence detector. The retention times for responses of both the electrochemical detector and the fluorescence detector were the same for OTC standards and 1 M MgCl₂ (pH 8) extracts of sediments (S8 in Supporting Information).

Trifluoroacetic acid solution (TFA) adjusted to a pH of 2 was selected for the eluent because trifluoroacetic acid does not promote epimerization of TCs as do phosphate, oxalate, and citrate ions (44) and because at pH 2 the formation of the C4 epimer of TCs or anhydrotetracyclines is minimized (45). Peak definition is improved by adding EDTA to the eluent. The separation of TC, OTC, CTC, and DOXY was tested using TFA buffer-to-methanol ratios ranging from 60:40 to 40:60. A ratio of 55:45 buffer:methanol solution gave the best separation of all four compounds for an elution time within 1 h (Figure 3). The retention times for TC and OTC differ by 0.5 min; the resolution (R_s) of the two peaks is calculated to be 0.7.

The retention times for OTC standards matched peaks in the extracts of bottom sediments on both the Luna C₁₈ and PRP-1 columns. This reinforces the identification of the unknown peaks as being OTC. The same mobile phase was used with both columns so that the only difference between the analyses was the change in column packing. Chromatograms obtained using a Luna C₁₈ column of (a) 1 M MgCl₂

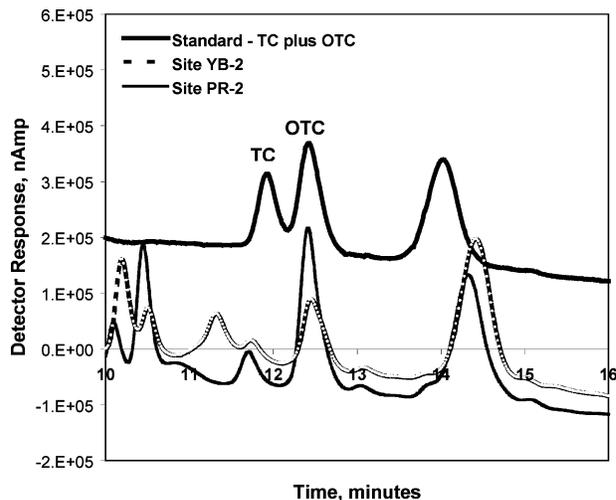


FIGURE 4. Chromatograms of 1 M MgCl₂ (pH 8) extractions of sediment from the Pocomoke River at PR-5 (light gray line) and sediment from Yellow Bank Stream at YB-2 (dotted line). The solid black line is a chromatogram of 1 ppm TC and OTC in 1 M MgCl₂ (pH 8). Chromatography conditions: 250 mm Luna C₁₈ column with Luna precolumn; amperometric glassy carbon electrode, 1050 mV; sensitivity 0.2 nA; flow rate 0.5 cm³ min⁻¹.

(pH 8) solution extract of bottom sediment from the Pocomoke River, (b) 1 M MgCl₂ (pH 8) solution extract of bottom sediment from Yellow Bank Stream, and (c) a standard containing 0.02 ppm TC and OTC made up in 1 M MgCl₂ (pH 8) are presented in Figure 4. The retention time of the peak for the OTC standard matched the retention time for peaks in chromatograms of the samples.

A calibration curve for OTC using this method is linear between 2 and 100 μg L⁻¹ (S9 in Supporting Information). If linearity is evaluated between 5 and 1000 μg L⁻¹, the response of the detector per unit concentration is constant for replicate samples (*n* = 6) until the concentration of OTC in the injected sample is 5 μg L⁻¹ or less (S10 in Supporting Information). Upon plotting the %RSD versus the OTC concentrations in standard solutions with concentrations ranging from 1000 to 5 μg L⁻¹ OTC, the %RSD remains within ±5% of a constant value until the concentration of OTC in the injected sample is 5 μg L⁻¹ or less (S11 in Supporting Information). This concentration is at least 1 order of magnitude larger than would be expected in environmental water samples (2) but smaller than the level for detection of OTC that is expected to be of potential biological importance (8–200 μg g⁻¹) (46). A concentration of 0.5 μg g⁻¹ dry wt can be detected in a sediment sample, but confidence in concentration data and quantification of OTC in sediment samples improves as calculated concentrations increase to values greater than 1 μg OTC g⁻¹ dry wt.

The samples in this study were analyzed using three different analytical schemes (Table 3). Data were reproducible for samples analyzed using the electrochemical detector and the method of standard additions. Sample aliquots of the MgCl₂ (pH 8) solution used to extract OTC from a sediment sample were spiked with a solution of the OTC standard to provide an internal calibration and to minimize any matrix affects. The standard deviations of concentrations of OTC in sediment extracts determined using fluorescence detection with the method of additions were acceptable, but the fluorescence method used with these samples is less sensitive than electrochemical detection; standard deviations for replicate samples analyzed using electrochemical detection with calibration curves were not satisfactory. The use of the method of standard additions is recommended to minimize matrix affects.

TABLE 3. Oxytetracycline (OTC) Concentrations in Sediment Samples from Yellow Bank Stream (YB) at Centreville, MD, Collected October 23, 2001, and from the Pocomoke River (PR), near Snow Hill, MD, Collected September 18, 2001

sample	OTC concentration (<i>n</i> = 3), $\mu\text{g g}^{-1}$					
	electrochem detection method of standard additions	SD	fluorescence detection method of standard additions	SD	electrochem detection calibrn curve	SD
Yellow Bank Stream						
YB-1	1.16	0.39	0.67	0.67	0.3 ^a	0.4
YB-2	1.9	0.06	2.2	0.59	0.1 ^a	0.1
YB-3	0.65	0.23	0.87	0.41	0.7	0.5
YB-4	0.93	0.32	0.50	0.50	0.9	0.5
YB-5	0.94	0.03	0.93	0.43	2.2	2.2
Pocomoke River						
SH-1	1.1	0.03	1.4	0.04	0.6	0.7
SH-2	3.28	0.31	1.99	0.38	7	7.4
SH-3	2.71	0.55	2.17	0.12	3.9	2.2
SH-4	0.7	0.39	0.8	0.47	1.3	0.1
SH-5	1.34	0.55	1.4	0.39	4.6	3

^a Approximate concentration of OTC determined using a 0.2-g sample.

TABLE 4. Analytical Data for Sediment Samples from Yellow Bank Stream (YB) at Centreville, MD, Collected October 23, 2001, and from the Pocomoke River (PR), near Snow Hill, MD, Collected September 18, 2001

sample	total P, $\mu\text{mol g}^{-1}$	%C	%N	total Fe, mmol g^{-1}	fraction of particles <250 μm
Yellow Bank Stream					
YB-1	45.2	5.1	0.5	20.5	0.46
YB-2	30.5	3.2	0.3	20.9	0.87
YB-3	133.3	3.9	0.4	28.8	0.75
YB-4	20.6	4.0	0.4	25.4	0.79
YB-5	135.8	1.7	0.2	31.3	0.71
Pocomoke River					
PR-1	31.0	35.7	1.9	31.1	0.82
PR-2	13.2	3.6	0.2	17.2	0.15
PR-3	35.5	11.9	0.7	50.7	0.99
PR-4	31.0	2.5	0.2	9.8	0.17
PR-5	50.6	11.4	0.8	24.4	1.00

Easily Desorbed OTC in Bottom Sediment. Concentrations of easily desorbed OTC in sediments from Yellow Bank Stream, near Centreville, MD, range from approximately 0.6 to 1.2 $\mu\text{g g}^{-1}$ dry wt. (concentrations determined using electrochemical detection and method of standard additions). Concentrations of easily desorbed OTC in sediments from the Pocomoke River near Snow Hill, MD, range from 0.7 to 3.3 $\mu\text{g g}^{-1}$ dry wt (concentrations determined using electrochemical detection and method of standard additions) (Table 3). There appears to be no covariance between the concentrations of easily desorbed OTC and total P and total Fe or between easily desorbed OTC and percents C and N (Table 4). Covariance between easily desorbed OTC and these chemical parameters might be expected on the basis of the fact that OTC and P can have similar environmental sources, such as STPs and agricultural fields, and both OTC and P bind with Fe and organic C. Small particle size provides large surface areas, favoring increased sorption. However, there is no covariance of easily desorbed OTC and the physical parameter of particle size (Table 4) in these sediment samples.

Water-column data indicate the difference in salinity between the two study areas (Table 5). Water-column water from site 1 (YB-1) in the Yellow Bank Stream had concentrations of dissolved ions similar to the concentrations of dissolved ions in the Pocomoke River. The water column downstream from site 1 (YB-1) of Yellow Bank Stream contained concentrations of Ca^{2+} approximately 10 times

TABLE 5. Data for Water-Column Samples from Yellow Bank Stream at Centreville, MD, Collected October 23, 2001, and from the Pocomoke River near Snow Hill, MD, Collected on September 18, 2001

sample	concentration, ppm				
	Cl^{-}	SO_4^{2-}	Na^{+}	Ca^{2+}	Mg^{2+}
Yellow Bank Stream					
YB-1	21	16	12	41	6
YB-2	5430	736	2610	127	342
YB-3	5430	743	2660	129	361
YB-4	5330	722	2530	126	351
YB-5	5210	713	2600	124	349
Pocomoke River					
PR-1	16	na	14	11	3
PR-2	14	9	16	11	3
PR-3	13	9	13	10	3
PR-4	13	9	18	16	3
PR-5	13	9	16	15	3

greater and concentrations of Mg^{2+} approximately 100 times greater than the concentrations of Ca^{2+} and Mg^{2+} in the water column of the Pocomoke River. It is unclear whether the concentrations of OTC in sediment collected from Yellow Bank Stream are less than the concentrations of OTC in Pocomoke River sediment because the concentrations of Ca^{2+} and Mg^{2+} , complexors of OTC, were larger in the water column of Yellow Bank Stream than they were in the water column of the Pocomoke River.

In another geochemical study of Pocomoke River sediments, Gupta and Karupiah (48) found that concentrations of Cd, Cu, and Zn were larger in sediment and that concentrations of As, Cd, Cu, and Zn in sediment porewater were larger, at a site 1.6 km downstream from the STP at Snow Hill, MD, than in the sediment from a site adjacent to the STP. Gupta and Karupiah attributed the larger metal concentrations in the sediment to a poultry farm close to the downstream site. The antihelminthic medication fed to poultry is an organoarsenical compound. They found arsenic was present in the porewater at this site but not in the porewater from a site adjacent to the STP. Site PR-5 is located approximately 1.5 km downstream from the STP at Snow Hill, MD. The concentration of easily desorbed OTC in sediment from PR-5 was not larger than the concentrations of easily desorbed OTC in the sediment from the site at the Snow Hill STP. It is unclear if the poultry farm is a source of OTC.

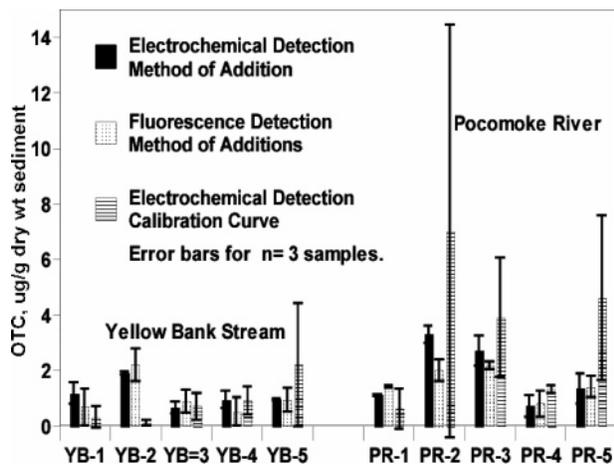


FIGURE 5. Concentrations of easily desorbed OTC determined by extraction with 1 M MgCl₂ (pH 8). Sediment samples were collected from the Yellow Bank Stream, MD, on October 23, 2001, and from the Pocomoke River, MD, on September 18, 2001. Solid bars are concentrations of easily desorbed OTC in sediment determined with electrochemical detection and method of additions; bars with dots are concentrations of easily desorbed OTC in sediment determined with fluorescence detection and method of additions; striped bars are concentrations of easily desorbed OTC in sediment determined using electrochemical detection and calibration curves. Error bars are \pm one standard deviation.

There could be a common source for both easily desorbable OTC and total P in samples analyzed for this study. The study sites receive agricultural runoff and effluent from STPs (Figure 1). Differences are small among data for easily desorbable OTC concentrations (electrochemical detection and method of additions) in sediments from sites in Yellow Bank Stream, and variation among concentration data for each site hinders statistical comparison. The data for concentrations of easily desorbable OTC (electrochemical detection and method of additions) in sediments from the Pocomoke River indicate that easily desorbable OTC is present in smaller concentrations in sediments upstream and downstream from the STP than in sediment collected from sites in close proximity to the STP (Figure 5). The data for easily desorbable OTC in sediments from the Pocomoke River points to, but does not prove, that the STP is a source of OTC.

Weston et al. (47) spiked both marine and freshwater sediments with OTC. Of the six sediment samples amended with OTC and tested for bacterial sensitivity, two sediment samples, one a freshwater sediment and the other a marine sediment, showed diminished bacterial numbers and bacterial growth at concentrations of 15–19 $\mu\text{g OTC g}^{-1}$ dry wt of sediment. The concentration of OTC, 3.3 $\mu\text{g of OTC g}^{-1}$ (standard deviation = $\pm 0.3 \mu\text{g of OTC g}^{-1}$) determined for the Pocomoke River sediment sample collected in close proximity to the STP is the largest concentration of OTC determined in this study, and this concentration value falls short of the concentration range tested for antibiotic sensitivity by Weston et al. (47). Experiments using material from these study sites could address the question of bacterial sensitivity to OTC for microorganism in these sediments. Soulides (20) and Misra (49) reported that desorbable TCs have the same chemical structure as the TC compounds that were sorbed. The concentrations of desorbable antibiotics were determined in the studies of Pinck et al. (19) and Soulides (20), by testing samples for antibiotic activity. The desorbable TCs retained their biological activity. Thus, it is possible that sorbed OTC in riverine sediments, when released to associated water, has an effect on the resident microbial community.

More important than identification of the source of OTC to these sediments are the implications associated with the presence of easily desorbable OTC, or other easily desorbable TCs, in sediments. TCs can be desorbable from sediment without loss of chemical or biological integrity (19, 20, and 49). Experiments reported in this study with phosphate-amended artificial seawater indicate that solutions having ionic strengths similar to seawater and containing elevated phosphate concentrations can lead to release of OTC and, by implication, possibly other TCs, from sediment.

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Supporting Information Available

Six figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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