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Bioavailability of biosolids-borne ciprofloxacin and azithromycin to terrestrial organisms: Microbial toxicity and earthworm responses

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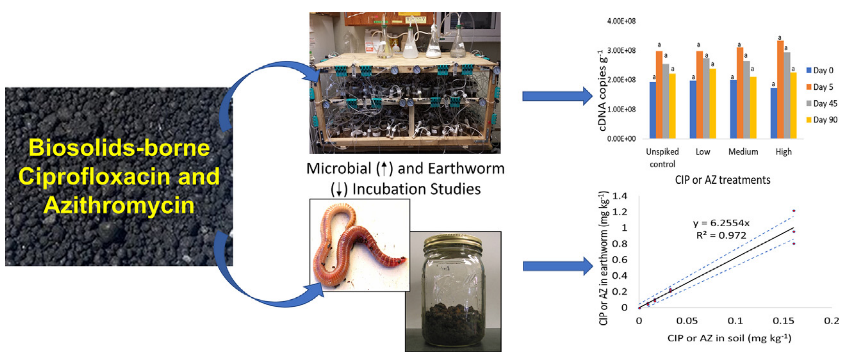
HIGHLIGHTS

- Biosolids-borne CIP and AZ were minimally toxic to microbes and earthworms.
- Earthworms accumulated biosolids-borne CIP and AZ.
- There is potential for CIP and AZ entry into ecological food web.

GRAPHICAL ABSTRACT



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ABSTRACT

Information on bioavailability of two antibiotic TOrCs, ciprofloxacin (CIP) and azithromycin (AZ), to terrestrial organisms is severely limited, especially in the biosolids context. Responses of two terrestrial organisms, earthworms and microbes, to a range of environmentally relevant concentrations of biosolids-borne CIP and AZ were assessed in laboratory incubation studies involving ^{3}H-labeled compounds. Earthworm assessments were based on the Earthworm Sub-chronic Toxicity Test (OCSPP 850.3100). Microbial impacts were assessed using respiration and reverse transcriptase-quantitative PCR (mRNA) analyses of nutrient (N and P) cycling genes as toxicity markers. Antibiotic extractability and stability during incubations were assessed using sequential extractions with $CaCl_2$, methanol:water, and accelerated solvent extraction and analyses using thin layer chromatography. Subsample combustion, in addition to sequential extraction, recovered nearly 100% of the added antibiotic. The two compounds persisted (estimated half-lives ≥ 3 y), but extractable fractions (especially of CIP) decreased over time. Neither biosolids-borne antibiotic significantly impacted overall respiration or N and P cycling. Microbial toxicity responses were minimal; complementary DNA (cDNA) concentrations of ammonia oxidizing bacterial genes were affected, but only initially. Similarly, earthworms showed no apparent response related to toxicity to environmentally relevant (and much greater) concentrations of biosolids-borne CIP and AZ. Earthworms, however, accumulated both compounds, and the bioaccumulation factor (BAF) values (dry weight basis) were ~4 (CIP) and ~7 (AZ) in depurated worms and ~20 (CIP and AZ) in un-depurated worms. The microbial and earthworm responses strongly to moderately correlated with "bioaccessible" fractions of the target TOrCs. The results suggest that biosolids-borne CIP and AZ toxicity to terrestrial microbes and earthworms is minimal, but there is a potential for target TOrC entry into ecological food web.

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1. Introduction

Human and environmental implications of trace organic chemicals (TOrCs) added to the terrestrial environment via land application of biosolids are uncertain. Data on two biosolids-borne antibiotic TOrCs, ciprofloxacin (CIP) and azithromycin (AZ), are especially limited. CIP and AZ are broad spectrum antibiotics widely used to treat various bacterial infections in humans (McClellan and Halden, 2010; Girardi et al., 2011; Parnham et al., 2014) and are, consequently detected in USA biosolids at concentrations up to 5 mg kg^{-1} (dw) for AZ and 41 mg kg^{-1} (dw) for CIP. Median concentrations (0.25 mg kg^{-1} (dw) for AZ and 5.4 mg kg^{-1} (dw) for CIP) are, however, much lower (USEPA, 2009). CIP and AZ are identified as high priority for research attention to close data gaps in fate and exposure for ultimate risk assessment in biosolids-soil systems (USEPA, 2009; McClellan and Halden, 2010).

Microbes and earthworms are an important part of biosolids-amended soil systems and can affect soil health by influencing, for example, nutrient cycling and availability, and enhancing contaminant bioaccessibility, biodegradation, and human and environmental exposure (Paul, 2014; Suter et al., 2000). In addition, earthworms are a significant fraction of the diet of many terrestrial vertebrates (Suter et al., 2000), and represent a potential pathway for entry of TOrCs (including CIP and AZ) into the ecological food web. Microbes are exposed to many TOrCs because of proximity to, and decomposition of, nutrient rich TOrC contaminated biosolids in amended soils. Similarly, earthworms are susceptible to biosolids-borne TOrC exposure because of thin and permeable cuticles, proximity to, and ingestion of contaminated soils (Suter et al., 2000). Microbes and earthworms can, therefore, be used in monitoring potential TOrC bioavailability and consequent assessment of environmental and human health risks (Jager et al., 2005; Snyder et al., 2011; ECETOC, 2013). Bioavailability herein is defined by the antibiotic concentration that induces a measurable response in a target receptor (e.g., microbes and earthworms). Assessing exposure of biosolids-borne CIP and AZ to terrestrial organisms is important because of limited data and reported immobility and persistence of the target compounds in the surface layer of amended soil (Ericson, 2007; Girardi et al., 2011; Gottschall et al., 2012). Several studies (e.g., Demoling et al., 2009; Girardi et al., 2011; Cui et al., 2014; Ding et al., 2014; Fang et al., 2014; Topp et al., 2016) suggest that TOrCs can adversely affect soil microbes. However, few studies address microbial responses specifically to CIP and AZ (e.g., Girardi et al., 2011; Cui et al., 2014; Topp et al., 2016), and only one (Youngquist et al., 2014) utilized biosolids-borne compound (CIP). Data directly pertaining to the influence of AZ on soil microorganisms are not present in the literature, but CIP reportedly can adversely affect soil respiration, N-cycling, and soil microbial community structure and function (Girardi et al., 2011; Cui et al., 2014).

Data on the bioavailability of biosolids-borne CIP and AZ to earthworms are essentially absent. Two studies assessed accumulation of inorganic metals (copper and cadmium) in earthworms in presence of CIP (Huang et al., 2009; Wen et al., 2011). Authors suggested that CIP accumulates in earthworm tissues and increases accumulation of metal ions like copper (Huang et al., 2009). Experimental details (freely bioavailable CIP at exceptionally high, g kg^{-1}, concentrations) limit the environmental relevance of the two studies. Scarcity of useful data on bioavailability of biosolids-borne CIP and AZ to terrestrial organisms warrants investigations, especially considering the reported soil microbe and earthworm responses to CIP.

The present work employed laboratory incubation studies to assess microbial and earthworm responses to a range of environmentally relevant concentrations of biosolids-borne CIP and AZ. Herein, environmentally relevant concentrations are defined in the context of biosolids-borne chemicals and are based on the targeted national sewage sludge survey (USEPA, 2009; see Supplementary Information Section I for details). Based on the minimal release of sorbed (bioaccessibility of) biosolids-borne CIP and AZ (Sidhu, 2018), we hypothesized minimal toxicity and bioaccumulation. To test the hypothesis, microbial and earthworm incubation studies were conducted using varying concentrations of biosolids-borne CIP and AZ. The studies focused on developing response curves, which are more transferrable and more representative of a range of soil conditions than point estimates (O'Connor, 1996). The microbial study evaluated toxicity from the target TOrCs using respiration and reverse transcriptase-quantitative PCR (RT-qPCR) analyses of genes involved in N and P cycles as response markers. The earthworm study was based on the Earthworm Subchronic Toxicity Test (OCSPP 850.3100) (USEPA, 2012), and evaluated toxicity and accumulation of biosolids-borne CIP and AZ. Changes in CIP and AZ extractability (potential bioaccessibility) and degradation (including mineralization) were also assessed and correlated with the microbial and earthworm responses.

2. Material and methods

2.1. Chemicals and reagents

^{3}H-CIP (CAS No. 85721-33-1; 97.4% radiochemical purity; specific radioactivity = 44.4 GBq $mmol^{-1}$) and ^{3}H-AZ (CAS No. 117772-70-0; 98.4% radiochemical purity; specific radioactivity = 29.6 GBq $mmol^{-1}$) were custom synthesized by Moravek Biochemicals (Brea, CA). Pharmaceutical secondary standards (>99% pure) of CIP and AZ, analytical grade calcium chloride ($CaCl_2$), and double deionized (DDI) water were purchased from Sigma Aldrich (St. Louis, MO).

2.1.1. Solid matrices

Anaerobically-digested, air-dried Class A (USEPA, 1995) biosolids was obtained from Metropolitan Water Reclamation District of Greater Chicago (MWRDGC), and contained relatively low CIP (1 mg kg^{-1} (dw)) and AZ (0.06 mg kg^{-1} (dw)) concentrations (analyzed by AXYS, BC, Canada). Soil used in the microbial incubation consisted of a sand (golf course topdressing "white sand") amended with cattle manure (4%, dw dw^{-1}) (hereafter referred to as "manured sand"). The manure was obtained from University of Florida Dairy Research Unit located in Hague, Florida and contained 0.01 mg AZ kg^{-1} (dw) and undetectable (i.e., less than the reporting limit of 0.035 mg kg^{-1} (dw)) CIP. The sand was sieved to <1 mm size. The manure was air dried and ground (<1 mm size) and mixed with the sand (4% dw dw^{-1}) in a concrete mixer for 30 min. Soils (ground and sieved to <1 mm size) used in the earthworm study included the sand and a silty clay loam (fine, mixed, superactive, mesic Typic Endoaquolls) with no known history of biosolids application from a site in Illinois (hereafter referred to as "control field soil"). A third soil used in the earthworm study was the silty clay loam from an adjacent field amended with an exceptionally high biosolids application (228 Mg ha^{-1}) in 2008 (hereafter referred to as "heavily amended field soil"). Biosolids-amended soils (sand, manured sand, and control field soils) were prepared by adding 0.25 g biosolids to 24.75 g soil (dry weight basis), corresponding to a typical agronomic rate of 1% by weight or ~20 Mg ha^{-1}. Select properties of the soils and the biosolids are listed in Table 1. The soil and biosolids properties (Table 1) were analyzed using the EPA 350.1 (NH_4-N; USEPA, 1993a), EPA 353.2 (NO_x-N; USEPA, 1993b); EPA 200.7 (P and K; USEPA, 1994), EPA 150.1 (pH; USEPA, 1983), EPA 351.2 (TKN; USEPA, 1993c), loss on Ignition (OM), and $BaCl_2$ compulsive exchange (CEC; Gillman and Sumpter, 1986) methods. Earthworms (*Eisenia fetida*) used in the laboratory studies were purchased from Carolina Biological (NC). Prior to use, the worms were grown in moist peat moss (growing medium) and fed worm food (Magic® Worm Food; Magic products Inc., WI) each day for at least 21 d.

2.2. Microbial incubation study

The microbial study involved manured sand amended with 1% (dw dw^{-1}) biosolids, and 100% biosolids. For the 100% biosolids