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A persistent antibiotic partitioning and co-relation with metals in wastewater treatment plant—Chlortetracycline

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ABSTRACT

The addition of high concentration of chlortetracycline (CTC) antibiotic to livestock feed and its resistance toward degradation results in accumulation of CTC in the environment. The strong chelation activity of CTC with metal ions has impact on numerous factors, such as solubility, stability, degradation, and antibacterial activity of CTC in wastewater treatment plant (WWTP). Further, no studies have been carried out on the chelation behavior of CTC with metal ions and its effect on removal efficiency from WWTP. This study attempts to provide the information about CTC distribution in wastewater (WW) and wastewater sludge (WWS) and the role of CTC–metals complexation on this behavior. The analytical method developed in this study combines an existing pre-treatment technique of solid-phase extraction (SPE) with laser diode thermal desorption (LDTD) coupled with tandem mass spectrometry (MS/MS). LDTD–MS/MS analysis has been recently developed to enhance the high throughput capacity in MS by reducing LC–MS/MS runs of 5–30 min to 10–30 s in LDTD–MS/MS run. This study considered the most common, polluting and efficient metal ions (Al(III), Ca(II), Co(II), Cu(II), Fe(III), Mg(II), Ni(II) and Zn(II)) which have a tendency to complex with CTC in WW. Chlortetracycline–metal complex and its mobility in WWTP were well-correlated which showed that CTC concentration in WWS was higher than the WW. These results showed that the water soluble CTC became insoluble and/or relatively less soluble after chelation with metal ions. Furthermore, the results signify the importance of chelation property of CTC and serve as a tool in determining the wastewater treatment plant matrix to be treated and also the type of treatment method to be used.

Introduction

Tetracyclines are old and broad-spectrum antibiotics; among tetracyclines, chlortetracycline (CTC) was the first member of the tetracycline family discovered in the late 1940s. The mechanism of action of these antibiotics to inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the bacterial ribosome is well established [1]. The promising antibacterial properties of CTC and the absence of major adverse side effects has led to their common use in the treatment of human and animal infections. Apart from the treatment of infections, CTC is also used for prophylactic purposes; added at sub-therapeutic levels to animal feeds as growth promoters and to improve feed efficiency [2]. Currently, across the world, more than half of the antibiotics are used in the livestock, which contributes to the development of resistance. This has led to a ban of some antibiotics in humans, among them CTC is one [3]. This antibiotic application at higher concentrations for therapeutic and prophylactic use and lower concentrations for growth promotion usually led to CTC, its degradation products and its epimers being discharged into the environment. Wastewater treatment plant (WWTP) effluent, biosolids and animal manure application to agricultural fields as fertilizer are regular sources of antibiotics into the environment [4,5]. Continuous usage of antibiotics for both infectious and non-infectious diseases resulted in global issues, such as antibacterial resistance in both human and veterinary medicine.

Abbreviations: CTC, chlortetracycline; QUC, Quebec Urban Community; Na_2H_2EDTA, disodium ethylenediamine tetraacetate; IDL, instrument detection limit; LDTD–MS/MS, laser diode thermal desorption-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; SPE, solid phase extraction; WW, wastewater; WWS, wastewater sludge; WWTP, wastewater treatment plant.

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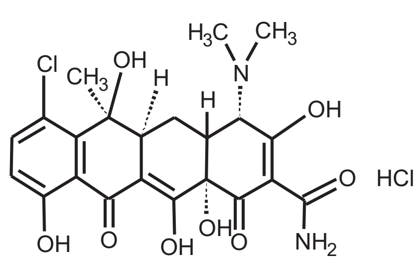
Heavy metals are essential in trace amounts for humans, animals and plants but large amounts of any of them may cause acute or chronic toxicity. Due to industrialization and urbanization, large amounts of heavy metal ions are released into the environment and become abundant [6]. Because of their high solubility in the aquatic environments, heavy metal ions can be absorbed and accumulated easily by living organisms and are toxic even at low concentrations. Metal ions in WW do not degrade naturally like organic pollutants. Toxicity of metal ions is due to direct generation of free radical species and depletion of antioxidants. The toxicity widely varies depending on the allotrope or oxidation state of the metal [7]. Metal ions have inhibitory effects on the biological treatment process at the WWTPs and also limit the application of biosolids as fertilizer [8]. Even though there are many treatment methods for removal of these toxic metal ions, but still, several metals are lacking robust pathways for effective removal from the WW. Apart from this, metal ions can get easily chelated with organic pollutants and remain in the WW at elevated pH and also change the chemical and clinical properties of chelated compounds [9].

Chlortetracycline is the most regularly used pharmaceutical in intensive animal farming. Moreover, most of the studies so far have confirmed the presence of CTC in WW and WWS [10–12]. Physico-chemical properties of CTC are shown in Table 1. However, partitioning of CTC in the WWTP and the effect of presence of metal ions on partition has not been explored. Chlortetracyclines are strong chelating agents and their antimicrobial and pharmacokinetic properties are influenced by this chelation property. The presence of high concentrations of metal ions in WW is understood to account for the significant differences in distribution of CTC between WW and WWS. Further, the presence of metal ions in WWTP not only affect the partitioning of CTC but also have effect on other numerous factors such as stability, degradation, antibacterial activity. Chelation activity causes changes in the antibacterial properties leading to inferior antibacterial activity. This chelated CTC–metal complex undergoes pH-dependent speciation and therefore, might occur as charged species in the environment. Several studies have indicated positively charged metal–CTC complex traverse the outer membrane of gram-negative enteric bacteria leading to accumulation [13]. Uptake of metal–CTC complex across the cytoplasmic membrane is energy dependent and influenced by the pH of the surrounding environment [14].

Table 1
Physico-chemical properties of chlortetracycline hydrochloride.

Structure



Molecular formula	$C_{22}H_{23}ClN_2O_8{\cdot}HCl$
Molecular weight	515.34 Da
Density	1.7 ± 0.1 g/cm^3 at 25 °C
Water solubility	8.6 mg/mL at 25 °C
Melting point	210–215 °C
Boiling point	821.1 ± 65.0 °C at 760 mmHg
Vapor pressure	0.0 ± 3.1 mmHg at 25 °C
log $K_{o/w}$	-0.53 ± 0.82
pK_a [a]	3.33 ± 0.30
	7.55 ± 0.02
	9.33 ± 0.30
Storage	−20 °C
Appearance	The yellow crystalline solid
Use	An antimicrobial and antibacterial agent

[a] pK_a values were measured by potentiometric method [27]

Therefore, the present study was conducted with following objectives; (i) to develop a new, rapid and sensitive method for quantification of CTC in WW and WWS by using LDTD–MS/MS method; (ii) to evaluate the fate and partitioning of CTC in WWTP; (iii) to estimate different metal concentrations in the WW and WWS and; (iv) to find a correlation between CTC and metal ion concentration in WW and its critical link to distribution of CTC in WWTP.

Materials and methods

Chemicals and standards

Isochlortetracycline (Iso-CTC, purity > 97%) was purchased from Toronto Research Chemicals (TRC) (Toronto, Canada). Clomiphene citrate (purity > 95%) salt was purchased from Sigma Aldrich (USA). Nitric acid (Trace metal™ grade, 67–70%), reagent grade perchloric acid ($HClO_4$, 67–71%), hydrofluoric acid (HF, 48–51%), methanol (HPLC grade, purity > 99.8%), acetic acid (HPLC Grade, purity > 99.7%), and ammonium hydroxide (NH_4OH, 28–30% w/w) were purchased from Fisher Scientific (Ontario, Canada). Disodium ethylenediamine tetraacetate (Na_2H_2EDTA, 99%) was purchased from E-bay (Tokyo, Japan). Standards for metal analysis were purchased from SCP Science, plasmaCAL, Quebec. Standard reference materials (SRM): peach leaves (SRM 1547) and Tart-3 (Lobster Hepato pancreas) were purchased from National Research Council of Canada, Ottawa, Canada. Sep-pack® C_{18} Plus Short Cartridge (360 mg Sorbent and 37–55 μm Particle Size per Cartridge) for solid phase extraction (SPE) were purchased from Waters (Milford, MA, USA). HPLC grade water was prepared in the laboratory using milli-Q/Millli-Ro Milli pore system (Milford, MA, USA)

Wastewater sampling and analysis

Samples were collected from Quebec Urban Community (QUC) WWTP (Quebec city, Quebec, Canada) in October 2013. Samples were collected in pre-cleaned high-density polyethylene (HDPE) containers from six points of wastewater treatment plant, such as influent, grit, primary sludge, secondary sludge, mixed sludge (60% of primary sludge and 40% secondary sludge) and effluent on the same day. Samples were transported to laboratory and stored under dark conditions at 4 ± 1 °C, until used. Wastewater control parameters, such as pH, total solids (TS), suspended solids (SS), volatile solids (VS), total chemical oxygen demand (TCOD), ammonia–nitrogen, phosphate, total organic carbon (TOC) and alkalinity were analyzed in accordance with the Standard Methods (APHA, 2005) [15].

Sample preparation for metal analysis

General

Overall estimation of metal content in all liquid samples [influent, effluent and liquid fraction of sludge (primary sludge, secondary sludge and mixed sludge)] and solid samples (primary sludge, secondary sludge, mixed sludge and solid fraction of primary sludge, secondary sludge, and mixed sludge) was carried out by acid digestion using autoclave to get clear samples for ICP-AES metal analysis.

Liquid sample preparation

The liquid fraction was separated from sludge by centrifugation at $7650 \times g$ for 25 min and the supernatant was hence transferred