

**DETERMINATION OF TETRACYCLINE GROUP
ANTIBIOTICS RESIDUES IN THE TIGRIS RIVER
AND AGRICULTURAL LANDS FED BY TIGRIS
RIVER AND REDUCTION OF UNCONSCIOUS USE
OF ANTIBIOTICS**

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Abstract

The recent increase in environmental pollutants is contaminating important natural resources and causing irreversible damage. Due to the limitations of traditional treatment techniques, studies on the problem of micro pollutant contamination of water resources have increased significantly. Antibiotics used in medicine and veterinary medicine cause significant pollution of water resources as residues of these contaminants remain unchanged in the aqueous environment. This has recently been recognised as a global environmental health threat. The ecosystem is vulnerable to residues of these contaminants from agriculture and aquaculture. In this study, the presence of tetracycline group antibiotics, the most commonly used in veterinary medicine, was determined in the Tigris river. This is the first study to date in which antibiotics from the tetracycline group have been identified in the Tigris river. Water samples were taken from 16 different points (80 samples) along the river. Sampling points were grouped as abattoirs, livestock markets, farmland, cafes and bridges near settlements. The samples were analysed for the presence of tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC). With a concentration range of 0.037 to 1.756 ng/L, oxytetracycline was found in 14 of the 16 sites. A concentration of 0.004–0.014 ng/L of tetracycline was found in five samples. Chlortetracycline was found in six samples, with concentrations ranging from 0.015 to 0.097 ng/L. According to the study, the tetracycline family

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of antibiotics was found in the water samples, and oxytetracycline was present in the highest concentrations in most of them.

Key words: Tigris river, tetracycline, oxytetracycline, chlortetracycline, antibiotic

Introduction. An antibiotic is a substance produced naturally by a microorganism that kills or inhibits the growth of another microorganism. In animal husbandry, antibiotics are used for both treatment and prophylactic purposes. It has been reported that 63151 ± 1560 tons of antibiotics per year are used in animal husbandry worldwide and this amount may increase by 67% until 2030 [1]. In veterinary medicine, the use of antibiotics has doubled compared with human medicine to promote growth in food animals. Although the mechanism of action of antibiotics used as growth-promoting agents is thought to be related to their interaction with the microbial population of the gut, it has not yet been clearly understood [2]. Studies have shown that up to 90% of antibiotics taken into the organism are excreted from the body without being metabolized. Therefore, a wide variety of antibiotics can be found in human and animal feces and consequently in domestic wastewater treatment systems [3]. Livestock and poultry manure constitutes a major source of antimicrobial contamination. Antibiotics were found in 80% of the manure from farm animals. The use of fertilizers in agricultural areas can lead to contamination of plants and soil, groundwater, and surface water by antibiotics. The inadequacy of conventional methods for antibiotic removal in the waste treatment of antibiotic production for the use in medical treatment, livestock production, and aquaculture also causes environmental antibiotic pollution. Antibiotics used in aquaculture farms cause contamination of sediments and surface waters. The presence of antibiotics in the environment can cause bacteria to acquire antibiotic resistance and toxic effects on aquatic and terrestrial organisms, thus resulting in disturbed ecological balance. Furthermore, antibiotics can accumulate in plant roots, affect their growth and development, and enter the human body through the food chain. Tetracyclines (TCs), discovered in the 1940s, are antibiotics with a broad spectrum of action against gram-positive and gram-negative bacteria, including chlamydiae, mycoplasmas, rickettsiae, and unicellular parasites. Tetracyclines were obtained from cultures of *Streptomyces* fungal species. The most common members of TCs include tetracycline (TC) [4], chlortetracycline (CTC) [5], and oxytetracycline (OTC), and all of them have very similar chemical structures [6]. TCS are relatively inexpensive antibiotics and are used to treat and prevent human and animal infections and added as growth promoters into animal feed below therapeutic doses [7]. Due to the use of tetracyclines and other antibiotics as growth promoters in animals, there is a growing concern that this application may lead to the development of resistant human pathogens. In this study, the concentrations of tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) were investigated in the Tigris river, which

is located in a region with intensive animal husbandry and agriculture. To the best of our knowledge, there is no study in the literature examining the Tigris river in terms of the presence of antibiotics, and our study is the first to address this environmental health issue.

Material and method. In this investigation, 80 samples were taken at 16 points, including slaughterhouses, animal markets, agricultural land, cafes, bridges, and animal farms close to the settlement centre, between the months of June 2022 and July 2022. Figure 1 shows the geographical location of the sampling points.



Fig. 1. Geographical position of study area and sampling points

Chemical agents. Oxytetracycline hydrochloride (CAS No. 2058-46-0) and chlortetracycline (CAS No. 64-72-2) were purchased from Applichem and Sigma-Aldrich. Tetracycline hydrochloride (CAS No. 64-75-5) and acetonitrile (CAS No. 75-05-8) were purchased from Merck as HPLC grade. Ammonium dihydrogenphosphate was supplied from Merck (CAS No. 7783-28-0).

Analysis of samples. Samples taken from influent and effluent were filtered immediately through a 0.22 μm membrane filter prior to OTC/TC/CTC analysis by HPLC (Shimadzu) with an AllureBiPh column (5 μm , 150 $\times$ 4.6 mm) after being taken periodically. The mobile phase was composed of a mixture of ammonium dihydrogenphosphate/acetonitrile (20/80, v/v). A mixture of 20 mM ammonium dihydrogenphosphate and HPLC-pure acetonitrile with pH adjusted to 2.45–2.55 was used as mobile phase. Flowrate and injection volume were maintained at 1.2 ml/min and 100 μL , respectively. OTC/TC/CTC were detected at 269 nm by a diode array detector [8]. The analysis conditions are given below.

Flow rate: 1.2 mL/min
 Max. Pressure limit: 20 mPa
 Detection: 269 nm
 mFA: 20 mM (NH₄)H₂PO₄ (pH: 2.45–2.55)
 mFB: HPLC-pure acetonitrile
 Column: RESTEK ALLURE Biph 150 × 4.6 mm
 Column temperature: 25 °C
 Analysis time: 13 min

The retention time was determined as 2.88, 3.90, and 7.95 min for oxytetracycline, tetracycline, and chlortetracycline, respectively.

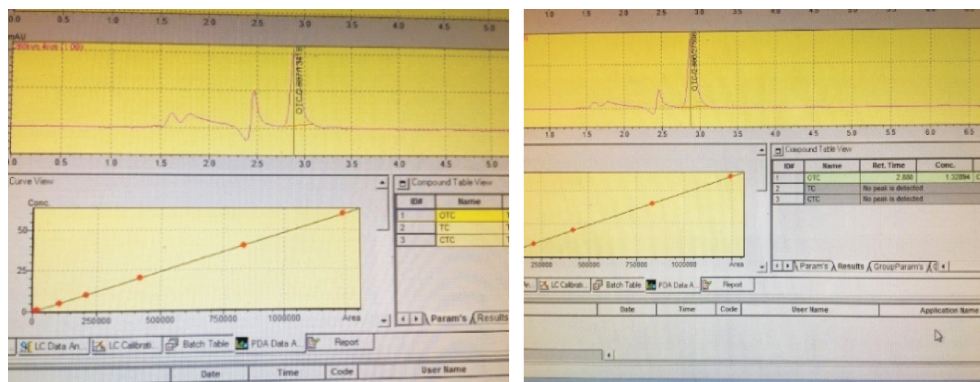


Fig. 2. High performance liquid chromatography (HPLC) peak images of analyzed samples

Results. OTC, TC, and CTC were detected in the water samples. OTC concentration was detected in fourteen samples with a minimum concentration of 0.037 ng/L and a maximum concentration of 1.756 ng/L. TC concentration was determined as minimum 0.04 ng/L and maximum 0.014 ng/L in five samples. CTC concentration was minimum 0.015 ng/L and maximum 0.097 ng/L in six samples (Fig. 3).

Discussion. To the best of our knowledge, this is the first study in which the tetracycline group of antibiotics was detected in the Tigris river. The levels of the tetracycline group of antibiotics detected in our study indicate the tetracycline contamination of the river. This can adversely affect the environmental and human health. Higher concentration of OTC was detected in fourteen samples, while TC and CTC were detected to a lesser extent in five and six samples, respectively. This may be due to antibiotics entering stream surface water via runoff [9]. TC and CTC may have also been detected in fewer samples and in lower quantities due to the location of the animal farms. Additionally, wastewater input may contribute to quantitatively higher OTC levels in a higher number of samples. This result is consistent with the results of previous studies on the presence of antibiotics in water sources. Higher and more abundant OTC concentrations are of great importance for the presence of antibiotics. It should be noted that excessive

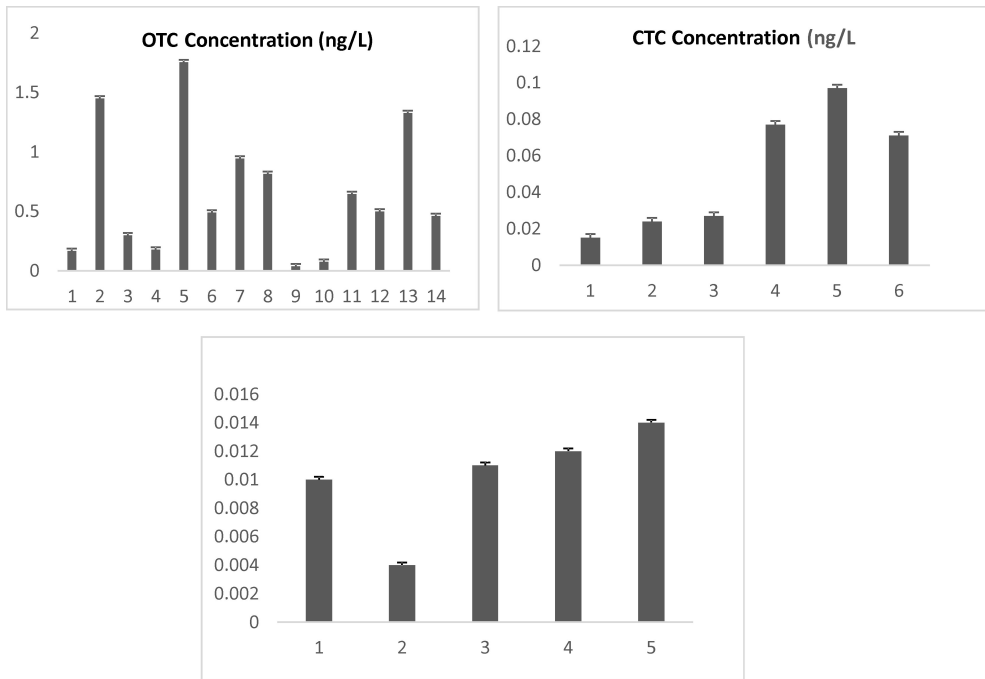


Fig. 3. Tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) concentration values

use of these drugs leads to their presence in water bodies due to the saturation of the soil capacity. This is called teraccumulation. When the soil is saturated with the TC group of antibiotics, rainwater carries the antibiotic to the groundwater. Detection of TC in water by binding to soil particles and remaining active poses a major problem. The presence of antibiotics in the aquatic environment also depends on the continuity of water discharges and the persistence of antibiotics in the environment. For example, penicillins are easily degradable, whereas ciprofloxacin and tetracyclines are much more stable [10]. Even low concentrations of these pollutants can have a negative impact on the survival, growth, and body weight of aquatic organisms [11]. Antibiotic concentrations in the water have been reported to be higher during winter. This suggests that TC, OTC, and CTC ratios would be higher in winter in our study. However, the environmental effect of antibiotics is based on the physical and chemical properties of the drug, amount, route of administration, treatment pattern and dosage, drug metabolism, degradation during the storage of manure, and the toxic effect on living organisms. Because of the biotransformation, the antimicrobial drugs transformed into polar, water-soluble, and compounds that are considered biologically active environmental pollutants. Accordingly, they can accumulate in the environment over time and negatively impact the ecosystem. In line with this conclusion, it has been reported in the literature that 80 different antibiotics can be found in the hospital discharges,

domestic wastewater, surface, and groundwater at $\mu\text{g/L}$ or ng/L levels. Indeed, different concentrations of tetracycline antibiotics were found in samples collected from different points of the Tigris river. Many studies have detected TC group antibiotics from river samples [12] supporting our results. In the Soeste river in Germany with nd-21 ng/L [13] Hong Kong with nd-1.1 ng/L [14], and Honghu Lake in China with $1070\text{--}2670 \text{ ng/L}$ [15], the presence of group antibiotics were reported. An extremely high TC concentration of up to $110\,000 \text{ ng/L}$ has been found in rivers in Brazil. Many studies have correlated the TC concentration in rivers with the season. Livestock are more susceptible to respiratory infections and diarrhea in the fall. TC is more commonly used for therapeutic and protective purposes, and thus higher TC concentrations were found in rivers in winter ($0.5\text{--}105 \text{ ng/L}$) than in summer ($0.1\text{--}29.3 \text{ ng/L}$) [16]. The widespread presence of TCs in rivers is due to their widespread use in areas such as pharmaceutical factories, hospitals, animal markets, animal care centres, slaughterhouses, and animal farms. Another reason is the excessive use of TCs as growth promoters by many livestock farms and the discharge of their residues into nearby rivers [17]. There are many reasons for the varied antibiotic concentrations in the aquatic environment. These include temperature, photolysis, pH, bacterial population, and residence time. The samples were not taken from a single point. These may be the most important reasons for concentration differences [18]. Additionally, the industrial structure of the region, the distribution of bacteria on the water body, the pharmaceutical and agricultural industry, climate, the medicine level, and the water discharge systems can be effective on the concentration of antibiotics in the rivers [19]. Current knowledge on the impact of TCs on the environmental and human health is still limited [20]. The occurrence of these antibiotic residues can over-accumulate in the food chain, inhibiting the growth and development of aquatic species, and potentially affecting human health. This may lead to joint disease, nephropathy, endocrine disruption, and central nervous system disorders. Thus, research is needed to clarify the negative effects of TC antibiotics.

Conclusion. This study aims to determine the presence of tetracycline group antibiotics in the Tigris river and to raise awareness. It was concluded that the Tigris river was contaminated by the tetracycline group of antibiotics. However, other types of antibiotics may also be present. Waste water from slaughterhouses, animal markets and animal farms can be considered a potential source of TC contamination of the river. In addition, the presence of antibiotics in the environment can lead to the mutation of antibiotic-resistant bacterial species and adversely affect those who use water from this source. Therefore, to prevent further damage to the environment, it is necessary to regularly monitor the presence of antibiotics used due to intensive livestock production in the region. In conclusion, we recommend further monitoring for the presence of antibiotic residues in water sources close to livestock farms.

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Methodology, F.Ö., N.B.A.; Formal analysis, F.Ö. and A.Ç.; Investigation, F.Ö., N.B.A. and A.Ç.; Resources writing—original draft preparation F.Ö.

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