

Investigation and monitoring of tetracycline and degradation products in waters of trout farm

Alabalık çiftliği sularında tetrasiklin ve parçalanma ürünlerinin araştırılması ve izlenmesi

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Abstract

In this study, tetracycline and degradation products were determined in the basins of a trout farm and in the stream carrying the waters of trout farm. Tetracycline (TC), 4-epitetrasiklin (ETC), 4-epianhidrotetrasiklin (EATC) and anhidrotetrasiklin (ATC) concentrations were determined under the detection limit in upstream of trout farm. The highest and lowest tetracycline concentrations detected in trout farm are 7.64 ± 0.38 ppb and under detection limit, respectively. The highest and lowest 4-epitetrasiklin concentrations were 16.2 ± 0.8 and 1.85 ± 0.09 ppb, respectively while the highest and lowest 4-epianhidrotetrasiklin concentrations detected in trout farm were 25.8 ± 1.3 and 6.12 ± 0.30 ppb, respectively. The highest and lowest anhidrotetrasiklin concentrations detected in trout farm were 18.5 ± 0.9 and 6.24 ± 0.31 ppb, respectively. Mean tetracycline, 4-epitetrasiklin, 4-epianhidrotetrasiklin and anhidrotetrasiklin concentrations were 3.52 ± 0.17 ; 5.30 ± 0.26 ; 14.4 ± 0.7 and 9.64 ± 0.48 ppb in downstream of trout farm. When upstream and downstream were compared in terms of tetracycline and degradation products it could be said that Keban stream was affected as a result of activity of trout farm.

Keywords: Tetracycline, Degradation products, Trout farm, Stream, Turkey

Öz

Bu çalışmada, tetrasiklin ve parçalanma ürünleri bir alabalık çiftliğinin havuzlarında ve alabalık çiftliğinin sularını taşıyan dereye tespit edilmiştir. Tetrasiklin (TC), 4-epitetrasiklin (ETC), 4-epianhidrotetrasiklin (EATC) ve anhidrotetrasiklin (ATC) konsantrasyonları alabalık çiftliğinin yukarıdaki dereye dedeksiyon limitinin altında tespit edilmiştir. Alabalık çiftliğinde tespit edilen en yüksek ve en düşük tetrasiklin konsantrasyonları sırasıyla, 7.64 ± 0.38 ppb ve dedeksiyon limitinin altındadır. Alabalık çiftliğinde tespit edilen en yüksek ve en düşük 4-epitetrasiklin konsantrasyonları sırasıyla, 16.2 ± 0.8 ve 1.85 ± 0.09 ppb iken en yüksek ve en düşük 4-epianhidrotetrasiklin konsantrasyonları sırasıyla 25.8 ± 1.3 ve 6.12 ± 0.30 ppb olmuştur. Alabalık çiftliğinde tespit edilen en yüksek ve en düşük anhidrotetrasiklin konsantrasyonları sırasıyla, 18.5 ± 0.9 and 6.24 ± 0.31 ppb olmuştur. Alabalık çiftliğinin aşağısındaki dereye ortalama tetrasiklin, 4-epitetrasiklin, 4-epianhidrotetrasiklin ve anhidrotetrasiklin konsantrasyonları 3.52 ± 0.17 ; 5.30 ± 0.26 ; 14.4 ± 0.7 ve 9.64 ± 0.48 ppb olmuştur. Alabalık çiftliğinin yukarısında ve aşağısında bulunan dere tetrasiklin ve parçalanma ürünleri bakımından mukayese edildiğinde Keban Deresinin alabalık çiftliğinin faaliyetinden etkilendiği söylenebilir.

Anahtar kelimeler: Tetrasiklin, Parçalanma ürünleri, Alabalık çiftliği, Nehir, Türkiye

1 Introduction

Aquaculture is an alternative to extractive fishing [1],[2]. The demand for aquaculture products is increasing worldwide [3],[4]. Aquaculture is the fastest growing animal food-producing sector [5],[6].

The risk of bacterial infections among aquacultured fish is high [6] as a result of the non-hygienic and stressful conditions [7],[8] present in aquaculture facilities. Diseases in an aquaculture setting are important limiting factor to production and trade. In aquaculture situations, antibiotic are added directly into the water or as a part of the feed [9],[10]. Antibiotics may not be used in a responsible manner in aquaculture [6],[11].

Antibiotics kill the microorganisms or inhibit the growth of them [12]. A class of antibiotics, tetracyclines (TCs), are characterized by a broad spectrum of activity, a relatively high degree of safety, low production and sales costs [13]. A survey on the use of chemotherapeutics in European Union identified TCs as one of the most used antibiotics in fish farming [14]. Also, TCs are commonly used to mark fish. Because they have capacity to fluoresce [15]. TCs are used in microbial control

during the processes of creation and management of fish because of their low cost and easy accessibility [2],[16]. Since the Food and Drug Administration approved the use of oxytetracycline for humans and aquatic animals, the use of TC for the treatment of diseases in aquatic farms has increased [17]-[20].

One of the important source of drugs in the environment is fish farming. It has been estimated that around 70% of the drugs administrated is released into the environment [21],[22]. The heavy use of antibiotics in aquaculture has resulted in the increase of resistant strains [6],[23]. Widespread use of TCs has resulted in selection for resistant bacteria, and its imprudent use has caused a high prevalence of TC resistance [24]-[27].

Freshwater aquacultural fishing increased in recent years through the new opened dams in Turkey. Turkey ranks third in fish producing in Europe. Turkey produces annually 200.000 tons of trout and most of these are exported. Republic of Turkey Ministry of Food, Agriculture and Livestock gives subsidy support of 65 piaster-1 Turkish Lira per kg and 50% equipment. One of the cities those the investments escalate is Elazığ. Fish is one of the rare food product that Turkey exported to the European Nation. Turkey is the leader of Europe in fish

export. Fume, fresh and cooled trouts are especially exported to the countries of Germany, Holland, Austria, Poland, Romania [28]. Unfortunately, there is not any environmental legislation for the effluents of fish farms in Turkey.

To the best of our knowledge, there is not any study about the concentrations of TC and degradation products (DPs) in a trout farm in Turkey, although a large number of fish farms exist in the country. Because of this, determination of the occurrence and concentrations of antibiotic in fish farms, especially TC and DPs which is known to be one of the most common antibiotics used in fish farming would be worthwhile in order to understand the fate of them in a fish farm. The focus of this study was thus both to investigate the occurrence and concentrations of TC and DPs in a trout farm located in Turkey and the stream carrying the waters of the farm.

2 Materials and methods

2.1 Study area

The selected study area includes a trout farm and a stream (Keban Stream). It is one of the biggest trout farms in Turkey. Keban Stream is a stream that has continuous flow in all seasons. Keban Stream flows to Fırat River that located in downstream of Keban Dam Lake. Fırat River is an internationally important river. In the study, Keban Stream is evaluated as two different water bodies because of the aim of the determination of the effect of trout farm on receiving surface water. Upstream of trout farm was referred as water body 1 while downstream of trout farm was referred as water body 2. Water body 1 was selected as reference point in the study because there was not any trout farm. It is worth to noting that domestic wastewater discharge to the any point of the stream that could affect the concentration of the target pollutant. (Figure 1).

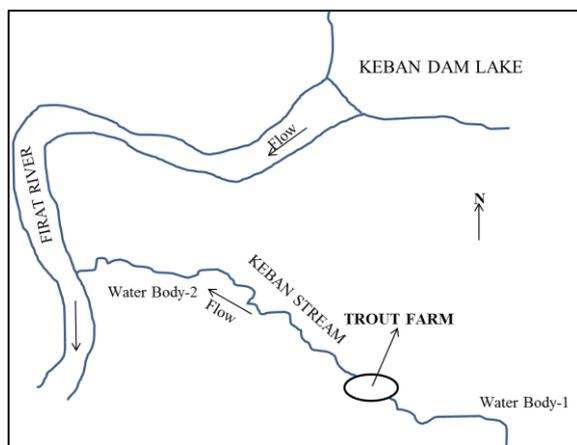


Figure 1: Study area.

2.2 Chemicals

TC, 4-epitetracycline (ETC), 4-epianhydrotetracycline (EATC) and anhydrotetracycline (ATC) were analyzed. Oasis HLB (500 mg, 6 cm³) and OasisMAX (60mg, 3cm³) which purchased from Waters Corporation, Milford, MA, USA were used as cartridges.

2.3 Sample Collection

All samples were taken during eight consecutive weeks in summer. Surface water samples were taken from 9 different points; one of them from Keban Stream (upstream of discharge

of trout farm waters), seven of them from different points of the trout farm and one of them from Keban Stream (downstream of discharge of trout farm waters). TC and DPs were under detection limit (UDL) in the surface water samples taken from Keban stream (upstream of discharge of trout farm waters). Sampling points of the trout farm are referred as follows;

Sampling point 1 (SP-1): basin of trout sale,

Sampling point 2 (SP-2): the other basin of trout sale,

Sampling point 3 (SP-3): basin of juveniles,

Sampling point 4 (SP-4): basin of trouts,

Sampling point 5 (SP-5): hatchery,

Sampling point 6 (SP-6): basin of fry trouts,

Sampling point 7 (SP-7): basin of broodstock.

Grab samples of surface water were taken from nine different points at each sampling point. Then the nine samples taken were mixed to obtain a homogenous sample from every sampling point. Sample volume was 500 mL. Samples were immediately transported to the laboratory and analyzed.

2.4 Sample extraction

Extraction of the samples was done according to the procedure of Jia et al. [29]. The samples were passed from SPE process for the determination of TC and DPs. Oasis HLB and Oasis MAX cartridges were used in SPE process. Oasis HLB cartridges were preconditioned with methylene chloride, methanol and ultrapure water containing 0.5 g/L Na₂EDTA. The samples were passed through the Oasis HLB cartridges. The HLB cartridges were rinsed with ultrapure water. They were dried under a flow of nitrogen and eluted with 6 mL of methanol. The eluates were collected in an amber vial and dried under a gentle flow of nitrogen. They were reconstituted to 0.3 mL with methanol. The extracts were diluted to 8 mL by ultrapure water (adjusted to pH 7.0 with 5% NH₃.H₂O). The solutions were then applied to the Oasis MAX cartridges (preconditioned with methanol, 5N NaOH, and ultrapure water). The cartridges were rinsed with 5%NH₃.H₂O, followed by methanol. Elution was performed with 3 mL of acetonitrile/water containing 1% formic acid (50/50, v/v) mixed reagents. The extracts were concentrated to 1.5 mL under a stream of nitrogen. Extracts were measured with ultra fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS).

2.5 UFLC-MS/MS

TC and DPs were analyzed using UFLC-MS/MS (Shimadzu Prominence UFLC coupled to 3200 Qtrap, Applied Biosystems). The brief description of the injection volume, mobile phase and gradient is given in Topal et al. [30].

LOD (limit of detection) and LOQ (limit of quantification) of TC and degradation products were calculated. LOD values calculated for TC, ETC, EATC and ATC were 0.307, 0.333, 0.479 and 0.733 ppb, respectively while LOQ values were 0.930, 1.009, 1.452 and 2.220 ppb, respectively [31].

2.6 Statistical analyses

Statistical studies were done using the IBM SPSS Statistics 21 programme (USA) (n=3).

3 Results and discussion

TC and DPs in water samples taken from different points of trout farm are given in Figure 2.

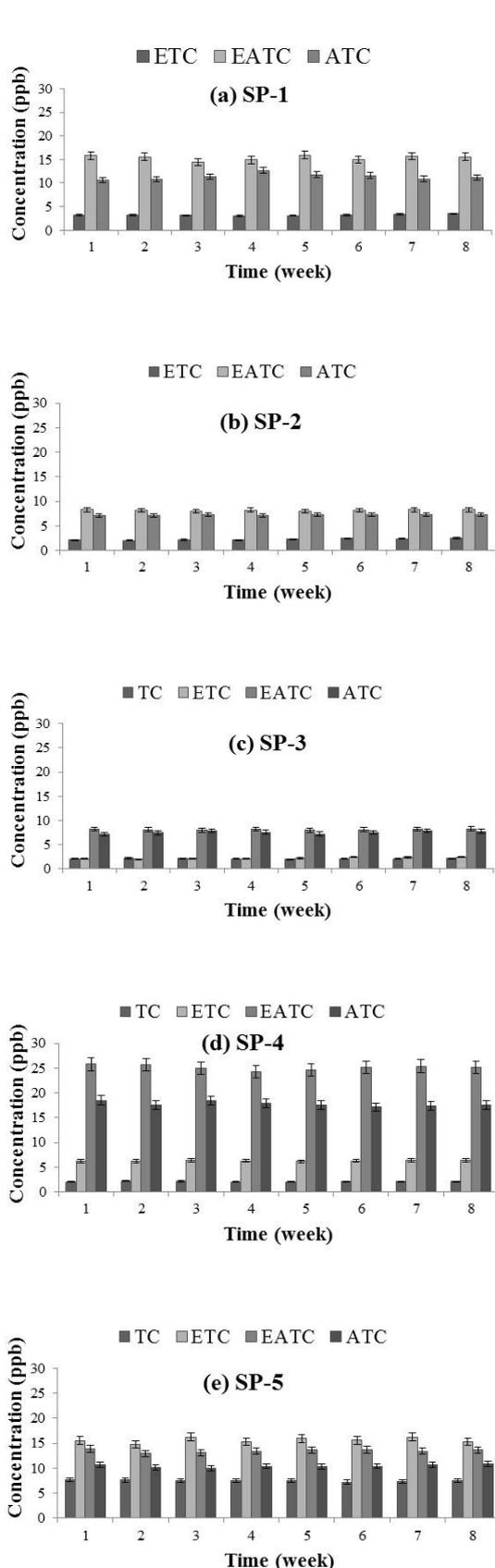


Figure 2. Concentrations of TC and DPs in water samples.

TC concentrations were between 2.0 ± 0.1 and 2.21 ± 0.11 ppb at sampling point 3 (Figure 2c) which the juveniles grown while TC concentrations were between 1.96 ± 0.1 and 2.14 ± 0.1 ppb at sampling point 4 (trout basin) (Figure 2d). TC concentrations were between 7.21 ± 0.36 and 7.64 ± 0.38 ppb at sampling point 5 (hatchery) (Figure 2e). TC concentration was under the detection limit in surface water of Keban Stream that was selected as reference point (upstream of trout farm wastewater discharge referred as water body 1). Mean TC concentration was 3.52 ± 0.17 ppb in surface water of Keban Stream (downstream of trout farm referred as water body 2). When water body 1 and water body 2 were compared in terms of TC it could be said that Keban Stream was affected as a result of activity of trout farm.

TCs are very unstable and decomposed rapidly under the influence of light and atmospheric oxygen, forming more than fourteen different DPs (e.g. the epi- and anhydro-compounds) [32],[33]. Therefore, DPs were also investigated in the present study.

As seen from Figure 2, the highest ETC concentration detected in trout farm was 16.2 ± 0.8 ppb in weeks 3 and 7 (SP-5) while the lowest ETC concentration was 1.85 ± 0.09 ppb in week 1 (SP-7). The situation could probably be caused by the transformation of TC to the degradation products and/or transformation of degradation products to each other. This situation is viable for the other degradation products. As reported by Halling-Sorensen et al. [34], TCs are known to possess limited stability in aqueous media. Also, as reported by Brain et al. [35], abiotic degradation products or reversible epimers may be formed through hydrolysis or photolysis, including epi-TCs and anhydro-TCs. ETC concentrations were under detection limit in surface water samples taken from Keban Stream at upstream of trout farm (reference point, water body 1). Mean ETC concentration was 5.30 ± 0.26 ppb in surface water samples taken from Keban Stream at downstream of trout farm (water body 2).

Concentration of EATC was maximum (25.8 ± 1.3 ppb) at SP-4 in week 1 while EATC concentration was minimum (6.12 ± 0.30 ppb) at SP-6 in week 7 (Figure 2). EATC concentration was under detection limit in the surface water samples taken from Keban Stream at upstream of trout farm (reference point, water body 1). Mean concentration of EATC was 14.4 ± 0.7 ppb in the surface water sample taken from Keban Stream referred as water body 2 (downstream of trout farm).

The highest ATC concentration detected was 18.5 ± 0.9 ppb in week 1 (SP-4). The lowest ATC concentration detected was 6.24 ± 0.31 ppb in weeks 5 and 7 (SP-6) (Figure 2). ATC concentration was detected under detection limit in surface water samples taken from Keban Stream at upstream of trout farm (water body 1, reference point) while mean ATC concentration was detected as 9.64 ± 0.48 ppb in surface water samples taken from Keban Stream at downstream of trout farm (water body 2).

According to Figure 2, the highest concentration in SP-1 and SP-2 was obtained for EATC. The decreasing order of TC and DPs was EATC>ATC>ETC. The highest EATC concentrations in SP-1 and SP-2 were 15.9 ± 0.8 and 8.27 ± 0.41 ppb, in week 5 and 8 respectively. The highest concentration in SP-3 and SP-4 was obtained for EATC, similar to SP-1 and SP-2. The highest EATC concentrations in SP-3 and SP-4 were 8.27 ± 0.41 and 25.8 ± 1.3 ppb, in week 8 and 1 respectively and the decreasing order of TC and DPs was EATC>ATC>ETC>TC. The highest concentration (16.2 ± 0.8 ppb) in SP5 was obtained for ETC in week 3 and 7. The decreasing order was ETC>EATC>ATC>TC. The highest concentration in SP-6 and SP-7 was obtained for ATC. ATC concentrations were 6.52 ± 0.32 and 6.88 ± 0.34 ppb, in week 1 and 2 respectively and the decreasing order was ATC>EATC>ETC.

Comparison of TC concentrations determined in sampling points is given in Figure 3.

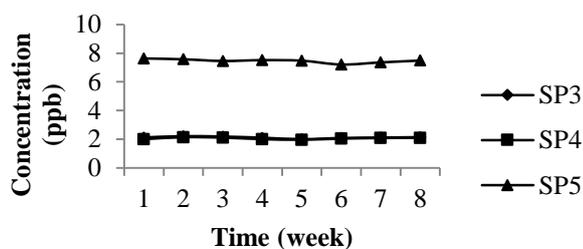


Figure 3. Comparison of TC concentrations determined in sampling points.

There was not any TC at SP-1 and SP-2 those the sale of the trouts are made. Also, it was determined that there was not any TC at sampling points of juveniles and broodstock basins (SP-6 and SP-7). The highest total concentrations (SP-3+SP-4+SP-5) of TC during sampling period was determined in week 2 as 11.93 ± 0.59 ppb. The order of concentrations of TC at sampling points was SP-5>SP-3>SP-4.

Comparison of ETC concentrations determined in sampling points is given in Figure 4.

The highest total concentrations (SP-1+SP-2+SP-3+SP-4+SP-5+SP-6+SP-7) of ETC during sampling period was determined in week 7 as 34.86 ± 1.74 ppb. Concentrations of ETC

determined at sampling points decreased in the order of SP-5>SP-4>SP-3>SP-1>SP-6>SP-2>SP-7.

Comparison of EATC concentrations determined in sampling points is given in Figure 5.

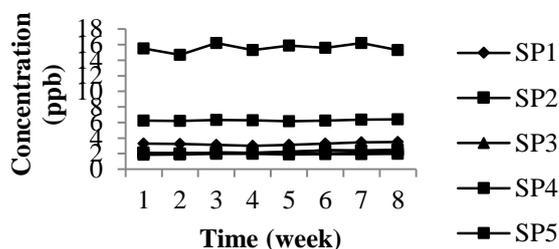


Figure 4: Comparison of ETC concentrations determined in sampling points.

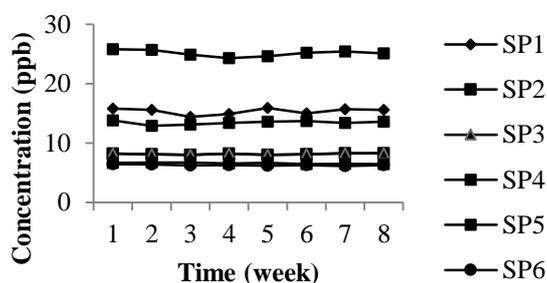


Figure 5: Comparison of EATC concentrations determined in sampling points.

The highest total concentrations (SP-1+SP-2+SP-3+SP-4+SP-5+SP-6+SP-7) of EATC during sampling period was determined in week 1 as 84.99 ± 4.25 ppb. The decreasing order of EATC concentrations in sampling points was SP-4>SP-3>SP-1>SP-5>SP-2>SP-7>SP-6.

Comparison of ATC concentrations determined in sampling points is given in Figure 6.

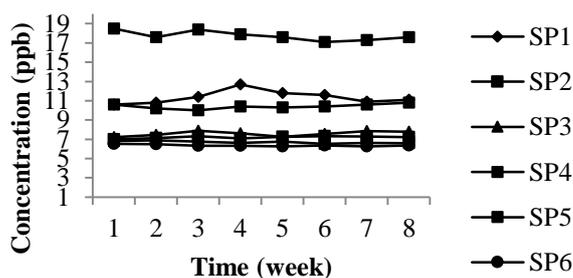


Figure 6: Comparison of ATC concentrations determined in sampling points.

The highest total concentrations (SP-1+SP-2+SP-3+SP-4+SP-5+SP-6+SP-7) of ATC during sampling period was determined in week 4 as 68.62 ± 3.43 ppb. ATC concentrations in sampling points followed the order of SP-4>SP-3>SP-1>SP-5>SP-2>SP-7>SP-6.

4 Conclusions

Elazığ has a big potential for the aquaculture production, especially trout production, due to its number of streams,

rivers, lakes and dams. With the spread of fishing in dams and rivers the production of trout is increasing day by day in the city. TC and DPs detected in the different sampling points of the trout farm generally followed the order of EATC>ATC>ETC>TC. In terms of TC and DPs, when upstream and downstream of the trout farm were compared, it could be said that Keban Stream was effected as a result of activity of trout farm.

5 References

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