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Phytoremediation of Tetracycline and Degradation Products from Aqueous Solutions

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ABSTRACT: The present study aims at phytoremediation of *Lemna gibba* L. in aqueous solutions with different concentrations of TC and Degradation Products (DPs). It also tries to determine whether there are differences in TC, ETC, EATC, and ATC levels, accumulated by *Lemna gibba* L. Exposure concentrations of 50, 100, and 300 ppb have been selected for TC and DPs, showing that the highest TC50, TC100, and TC300 concentrations in the plant have been 23.5±1.1, 80.1±3.9, and 274±13 ppb, respectively, while the highest ETC50, ETC100, and ETC300 have proven to be 39.5±1.9, 47.8±2.4, and 168±8.4 ppb, respectively. The highest EATC50, EATC100, and EATC300 concentrations in the plant have been 45.3±2.3; 65±3.0 and 173±9.0 ppb, respectively, whereas the highest ATC50, ATC100, and ATC300 concentrations in *Lemna gibba* L. have been 34.7±1.7, 39.6±0.2, and 114±5.6 ppb, respectively. TC, ETC, EATC, and ATC concentrations in *Lemna gibba* L. have increased with the increase of initial TC, ETC, EATC, and ATC concentration.

Keywords: Antibiotics, duckweed, *Lemna gibba* L., metabolites

INTRODUCTION

Antibiotics have aroused much concerns, due to their excessive application in various areas (human beings, agriculture, and planting) (Luo et al., 2010; Yuan et al., 2011; He et al., 2014), reaching the aquatic environment after use. In particular, the presence of antibiotics in aquatic environment has created two major concerns: The immediate one is potential toxicity of these compounds for both aquatic organisms and humans through drinking water. In addition, there is growing concern that release of antibiotics to the environment contributes to the emergence of strains of pathogenic bacteria that are resistant to high doses of these drugs (Chee-Sanford et al., 2001; ASM, 2002; Yang & Carlson, 2003).

Tetracycline (TC) antibiotics are widely used to treat infectious diseases in both humans and animals (Liu et al., 2013). TC antibiotics fall into a broad-spectrum bacteriostatic compounds as they bind reversibly to the 30S subunit of ribosome and prevent the binding of aminoacyl transfer to DNA, inhibiting protein

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synthesis and cell growth of sensitive bacteria (Halling-Sørensen, 2000; Brain et al., 2005).

Significant fractions of pharmaceuticals are excreted in either un-metabolized form or as metabolites (active or inactive), reaching to wastewater treatment plants (WWTPs) (Amrita et al., 2010; Aleksnadra et al., 2011; Prieto-Rodriguez et al., 2012). Conventional WWTPs are designed to remove conventional parameters, such as BOD, TSS, and ammonia (Matamoros et al., 2012), but they are not currently designed to cope with pharmaceuticals. Advanced reclamation systems, e.g. ozonation, ultrasound, activated carbon, membrane bioreactors, photo-fenton, and reverse osmosis, have been evaluated with promising results (Deng, 2009; Rosal et al., 2010; Shariati et al., 2010; Maroga-Mboula et al., 2012; Avila et al., 2013; Garcia-Rodríguez et al., 2013). However, due to the costs of an advanced treatment process, they are not widely used (Avila et al., 2013).

Phytoremediation falls under a group of technologies, based on the use of natural occurrence or genetically-modified plants, to reduce, remove, break, or immobilize pollutants, working as an alternative to wastewater treatment (Lasat, 2002; Teles Gomes et al., 2014). The use of a macrophyte is an attractive phytoremediation approach as it is a low-cost, easily managed and environmentally friendly in situ technology, especially for large water volumes (Gao et al., 2012; Wang et al., 2012; Wang et al., 2013; Lu et al., 2014).

Biologically-based treatment systems (e.g. ponds and constructed wetlands) have proven successful in reducing classical parameters, e.g. suspended solids, BOD, nitrogen, and phosphorus in water (Vymazal, 2007; Vymazal, 2008), currently targeted as possible cheap and effective systems for elimination of emerging pollutants (Matamoros & Bayona, 2008; Hijosa-Valsero et al., 2010; Matamoros et al., 2012).

The present study aims at phytoremediation of *Lemna gibba* L. in aqueous solutions with different concentrations of TC and degradation products (DPs). It also attempts to determine whether there are any differences in TC, 4-epitetracycline (ETC), anhydrotetracycline (ATC), and 4-epianhydrotetracycline (EATC) levels, accumulated by *Lemna gibba* L.

MATERIAL AND METHODS

The study was conducted in the reactors (600 mL), planted with *Lemna gibba* L. plants, with 5 g of *Lemna gibba* L. added to each of the reactors. Then, 400 mL of solutions with 50, 100, and 300 ppb of TC, ETC, EATC, and ATC were placed inside the reactors (Figure 1). In the reactors the *Lemna gibba* L. were harvested in various hydraulic retention times (HRTs). Afterwards, the concentrations of TC and DPs (ETC, EATC, and ATC) were determined.

Fig. 1. The reactors

The study used TC-98%, ETC-97%, ATC-97%, and EATC-97% purities. TC was purchased from Sigma-Aldrich (USA), while the DPs (ETC, ATC, EATC) were purchased from Acros Organics (NJ, USA). The methanol from Carlo Ebra, methylene chloride from Fisher Chemical, and acetonitrile and formic acid from J.T. Baker (USA) were all of HPLC grade, whereas hydrochloric acid (HCl) (J.T. Baker), sodium hydroxide (NaOH) (Acros Organics), ammonia solution ($NH_3.H_2O$) (Carlo Ebra), and ethylenediamine

tetraacetic acid disodium (Na_2EDTA) were of analytical reagent grade, having been purchased from Sigma-Aldrich (USA). The study also employed solid-phase extraction (SPE) cartridges, Oasis HLB (500 mg, 6 cm^3), and Oasis MAX (60 mg, 3 cm^3) cartridges, which had been purchased from Waters Corporation (Milford, MA, USA). The ultrapure water, used throughout the study, was supplied by Zeneer power water purification system.

The aquatic plant *Lemna gibba* L., identified according to the procedure in Flora, Turkey (Davis, 1988), was collected from local fresh water bodies in Elazığ region (Turkey). *Lemna gibba* L. was settled to the jerrycan with a volume of 2 L, next to be brought to the laboratory of Fırat University Environmental Engineering Department. In the laboratory, the collected plants were washed with distilled water to remove probable pollutants on the plant surface prior to being placed in the reactors.

The plant samples, harvested from the reactors, got extracted via the method, used by Lillenberg et al. (2010), in which 250 mg of dried *Lemna gibba* L. was extracted with 10 mL of 1:1 (v/v) mixture of acetonitrile and 1% acetic acid, then to be homogenized with laboratory homogenizer DIAX 900 (Heidolph Instruments, Germany) at 25,000 rpm, sonicated (5'), vortexed (1'), and centrifuged at 8,000 rpm. The supernatant was then separated and dried with nitrogen stream. Approximately 15 mL of 1 % acetic acid was added to the 1 mL of evaporation residue.

Analyses were performed by means of Solid-Phase Extraction (SPE) method, reported by Jia et al. (2009). The samples were filtered with a glass microfiber filter (0,7 µm, Whatman, Maidstone, England) and after filtration, 16 mL of the sample was added to 0.5 g/L Na_2EDTA, and acidified to pH = 3.0 with HCl. Oasis HLB cartridges were preconditioned with 6 mL of methylene chloride, 6 mL of methanol, and 6 mL of ultrapure water, containing 0.5 g/L of Na_2EDTA (adjusted to pH = 3.0 with HCl). The samples were passed through these HLB cartridges. The flow rate was approximately 3 mL/min. The HLB cartridges were rinsed with 10 mL of ultrapure water, dried under a flow of nitrogen, and then 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% of $NH_3 \cdot H_2O$). The solutions were then applied to Oasis MAX cartridges (preconditioned with 1mL of methanol, 1 mL of 5N NaOH, and 1 mL of ultrapure water). All cartridges were rinsed with 1 mL of 5%$NH_3 \cdot H_2O$, followed by 1 mL of methanol. Elution was performed with 3 mL of acetonitrile/water, containing 1% formic acid (50/50, v/v) and mixed reagents. The extracts were concentrated to 1.5 mL under a stream of nitrogen and measured with LC–MS/MS soon after preparation.

Concentrations of TC and DPs (ETC, ATC, EATC) in duckweed (*Lemna gibba* L.) samples were analyzed, using LC-MS/MS (Shimadzu Prominence UFLC coupled to 3200 QTRAP, Applied Biosystems). TC and DPs got separated with a Waters ACQUITY UPLC BEH C18 column (1.7µm; 2.1mm×100mm). The injection volume was 10µL (full loop). The mobile phases included Acetonitrile (A) and ultrapure water, containing 0.1% of formic acid (v/v) (B). The gradient was as follows: The initial 10% A was increased linearly to 20% in 5 min, a further 20% A was increased to 90% in 4 min and stayed so for 0.5 min, followed by a final increase to 100% A at which it stayed for 1 min. Eventually, the gradient returned to the initial conditions of 10% A and remained for 2 min to allow for equilibration. The flow rate was 0.2 mL/min. The column was maintained at 30°C with the sample room temperature being 20°C. Mass spectrometry was performed, using an AB Applied Biosystems (triple-quadrupole)

detector, equipped with electrospray ionization.

The concentration range of the calibration standarts was 0.1, 0.5, 1, 2, 3, 4, 5, 10, 30, 50, 100, 300, and 500 µg/L and mean coefficients of determination (R^2) were 0.9761, 0.9850, 0.9996, and 0.9998 for ATC, EATC, ETC, and TC, respectively.

Experimental results were analyzed, using the IBM SPSS Statistics 21 programme (USA), and the illustraterd values were the means of three replicates (n=3) with the error bars representing the Standard Deviation (SD).

RESULTS AND DISCUSSION

Figure 2 shows TC concentrations in *Lemna gibba* L. for the initial TC concentration of 50 ppb. The lowest TC concentration in the plant was 1.76±0.08 ppb, while the highest was 23.5±1.1 ppb. The concentration of 23.5±1.1 ppb of the initial TC concentration (50 ppb) was accumulated by *Lemna gibba* L, with the remaining concentration (≈25ppb) of the initial TC concentration probably being photodegradated. Garcia Rodriguez et al. (2013) proved that photodegradation was a removal method for TC. In the current study, concentrations of TC in *Lemna gibba* L. ascended with the increase of the HRT; however, TC concentration did not change a lot from day 6 to day 10 (having increased from 20.4±1.0 ppb to 23.5±1.1 ppb).

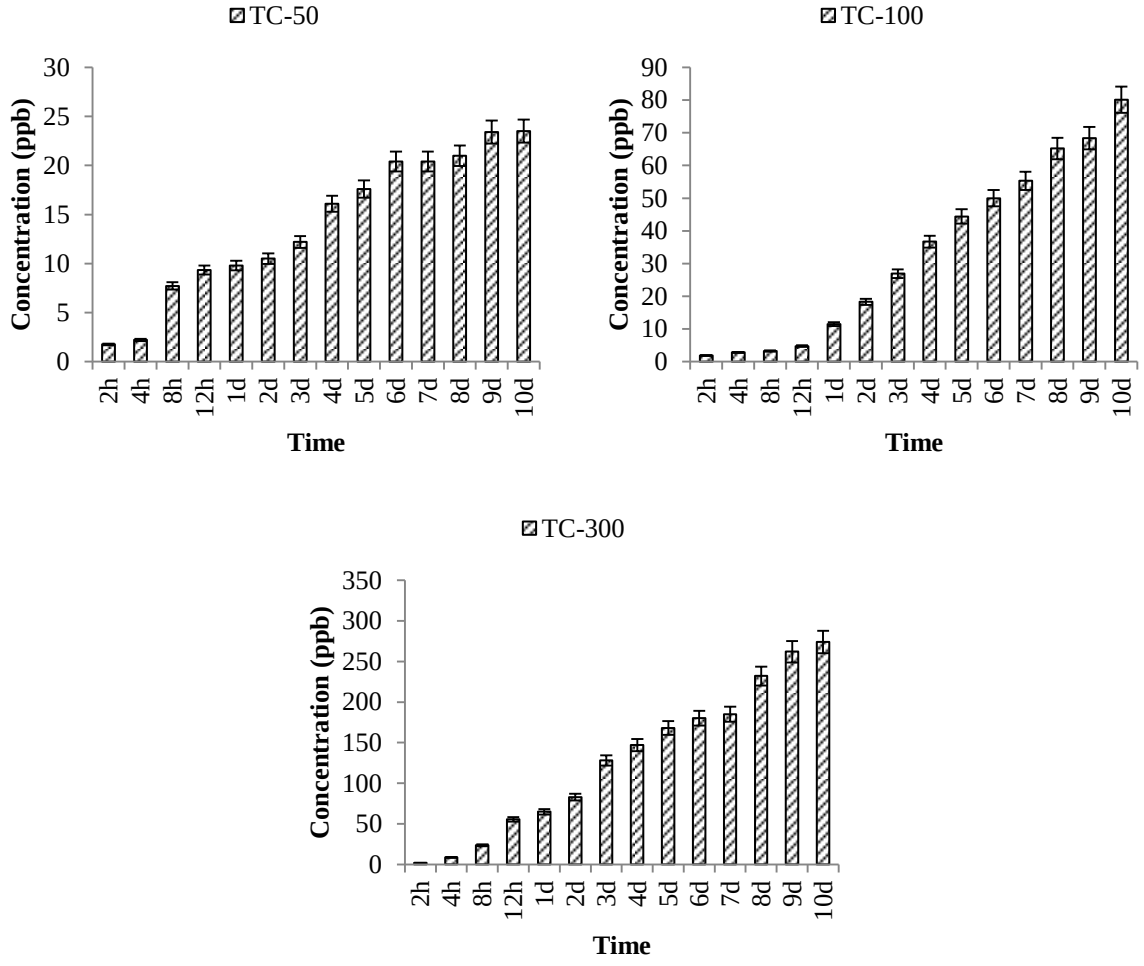

Fig. 2. TC concentrations versus time

Figure 2 demonstrates TC concentrations in *Lemna gibba* L. for initial TC concentration of 100 ppb. It was revealed that the lowest TC concentration was 1.87±0.1 ppb and the highest one, 80.1±3.9 ppb. TC concentration in *Lemna gibba* L. ascended with the increase of HRT, similar to the initial TC concentration of 50 ppb. Figure 2 also presents TC concentrations in *Lemna gibba* L. for the initial TC concentration of 300 ppb, in which the lowest and the highest TC concentration was 1.89±0.1 ppb and 274±13 ppb, respectively. Similar to the initial TC concentrations of 50 and 100 ppb, TC concentration in *Lemna gibba* L. rose with the increase of HRT.

Figure 3 demonstrates ETC concentrations in *Lemna gibba* L. for the initial ETC concentration of 50 ppb, showing the lowest and highest ETC concentrations in the plant as 6.26±0.31 ppb and 39.5±1.9 ppb, respectively. ETC concentration in *Lemna gibba* L. increased with the HRT.

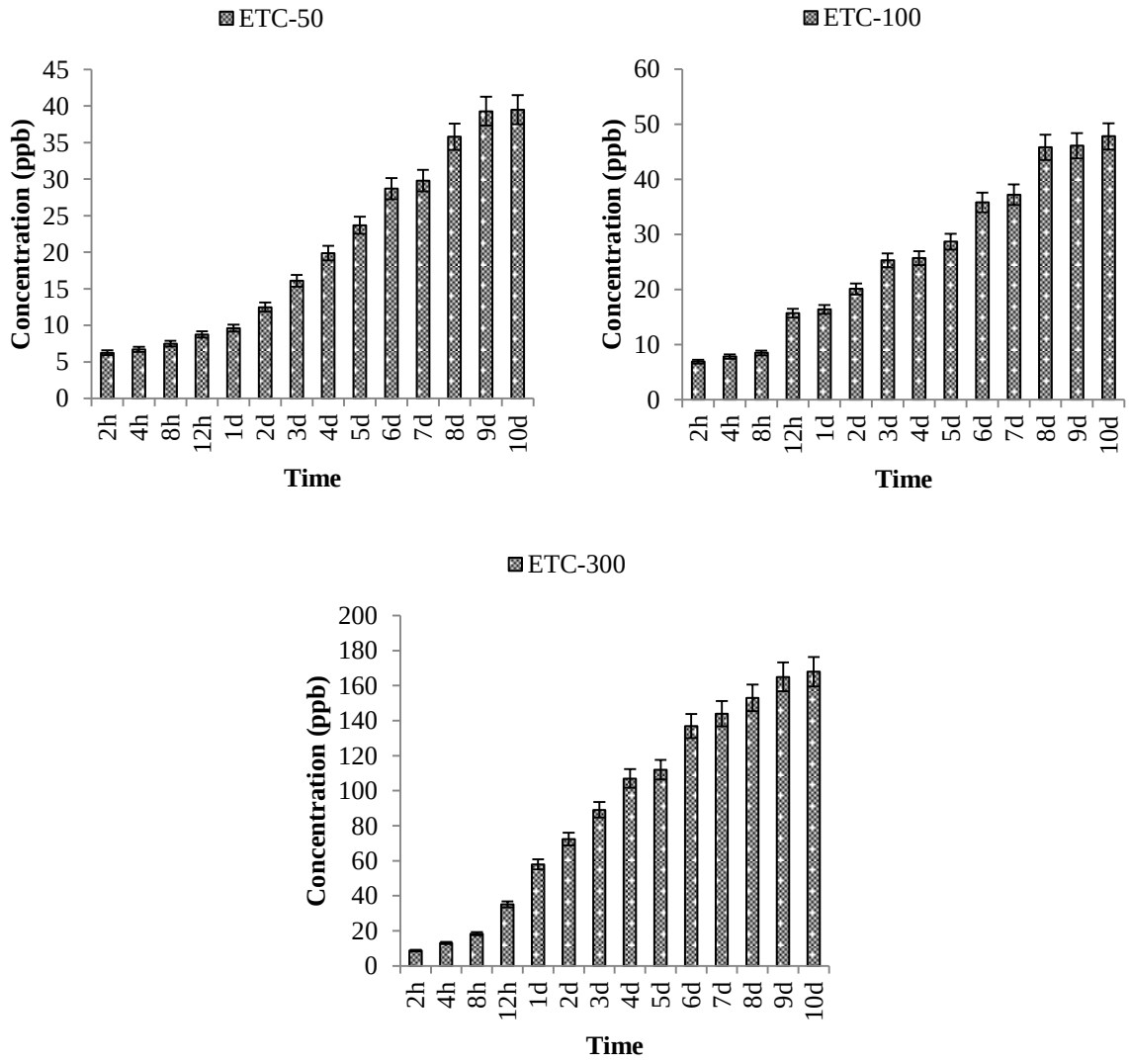

Fig. 3. ETC concentrations versus time

Furthermore, Figure 3 illustrates ETC concentrations in *Lemna gibba* L. for the initial ETC concentration of 100 ppb, for which it was determined that the lowest and the highest ETC concentrations were 6.93±0.34 ppb and 47.8±2.4 ppb, respectively. ETC concentration in the plant grew with the increase of HRT. The figure also presents ETC concentrations in *Lemna gibba* L. for the initial ETC concentration of 300 ppb, for which the determined lowest and highest ETC concentrations in *Lemna gibba* L. plant were 8.75±0.43 ppb and 168±8.4 ppb, respectively. Here also the increase of HRT meant increased ETC concentrations in the plant.

Figure 4 gives EATC concentrations in *Lemna gibba* L. for initial concentration of 50 ppb, showing the lowest EATC concentration as 6.04±0.3 ppb and the highest one as 45.3±2.3 ppb. EATC accumulated more in *Lemna gibba* L. with the increase of HRT.

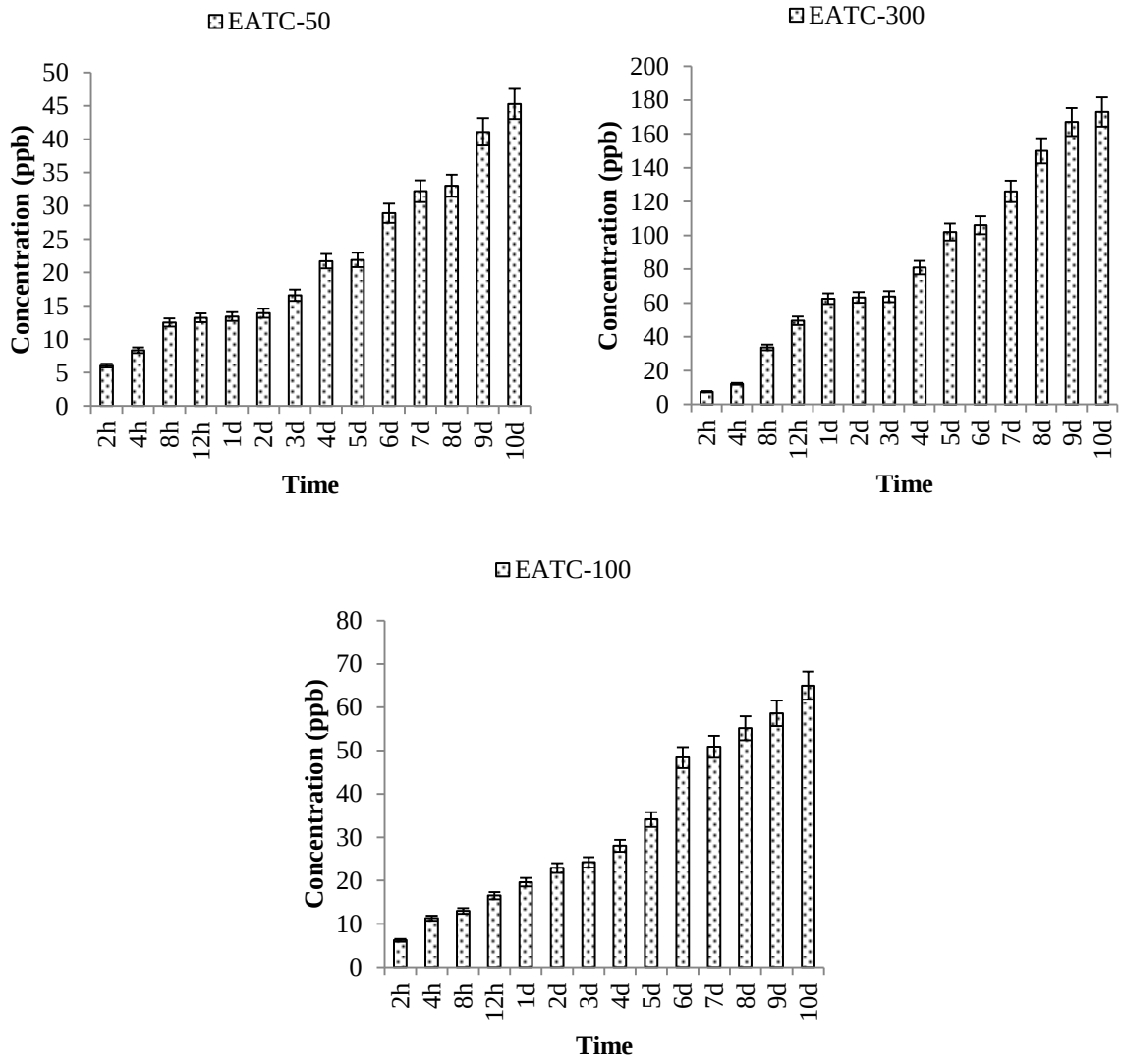

Fig. 4. EATC concentrations versus time

The figure presents EATC concentrations in *Lemna gibba* L. for initial concentration of 100 ppb, too. There the lowest EATC concentration in *Lemna gibba* L. was 6.2±0.3 ppb, while the highest one was 65±3.0 ppb. The accumulation of EATC in *Lemna gibba* L. increased with the increase of HRT. Additionally, the figure illustrates EATC concentrations in *Lemna gibba* L. for initial concentration of 300 ppb, showing the lowest EATC concentration as 7.47±0.3 ppb and the highest as 173±9.0 ppb.

Figure 5, offers ATC concentrations in *Lemna gibba* L. for initial ATC concentration of 50 ppb, with the lowest and highest ATC concentrations being 7.3±0.3 ppb and 34.7±1.7 ppb, respectively. ATC concentrations in *Lemna gibba* L. ascended with the increase of HRT.

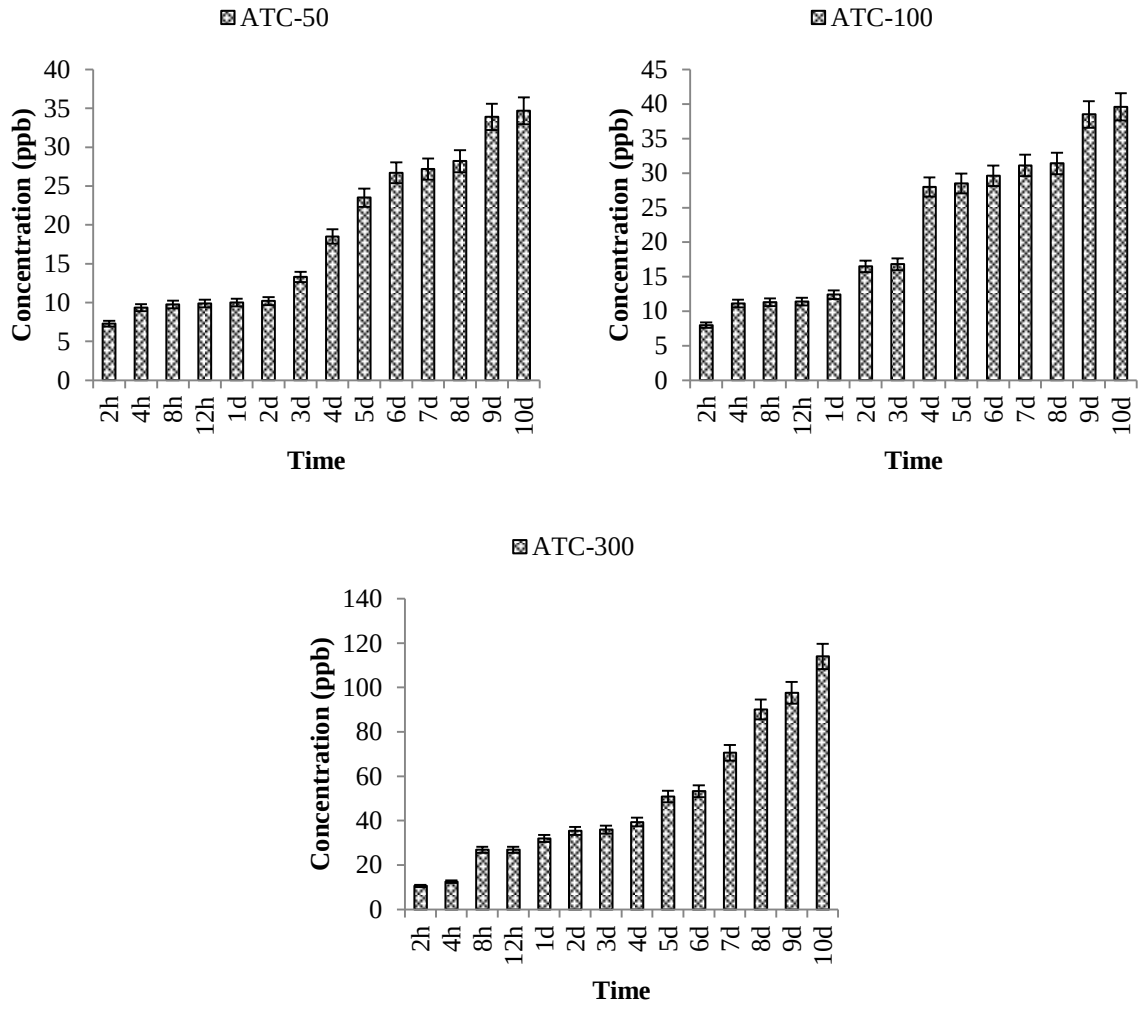

Fig. 5. ATC concentrations versus time

Additionally, Figure 5 shows ATC concentrations in *Lemna gibba* L. for initial concentration of 100 ppb, in which the lowest and highest ATC concentrations in *Lemna gibba* L. were 7.97±0.4 ppb and 39.6±0.2 ppb, respectively. ATC concentration increased with the increase of HRT. Finally, this figure demonstrates ATC concentrations in *Lemna gibba* L. for initial concentration of 300 ppb and it can be seen that the lowest ATC concentration in the plant was 10.6±0.5 ppb, while the

highest one was 114±5,6 ppb. Also, ATC concentration in *Lemna gibba* L. plant increased with the increase of HRT.

In a 2-hour period, TC50, ETC50, EATC50, and ATC50 concentrations in *Lemna gibba* L. plant were 1.76±0.08, 6.26±0.31, 6.04±0.3, and 7.3±0.3 ppb, respectively, arranged in the following order: ATC50>ETC50>EATC50>TC50. In a 12-hour period, however, TC50, ETC50, EATC50, and ATC50 concentrations in the plant were 9.34±0.4, 8.75±0.26, 13.2±0.6, and 9.87±0.5 ppb, respectively, wherein TC50 and the degradation products in the plant fluctuated with HRT.

It was determined that in a 2-hour period concentrations of TC100, ETC100, EATC100, and ATC100 in *Lemna gibba* L. were 1.87±0.1, 6.93±0.34, 6.2±0.3, and 7.97±0.4 ppb, respectively, following the descending order of ATC100>ETC100>EATC100>TC100. What is more, in a 12-hour period, concentrations of TC100, ETC100, EATC100, and ATC100 were 4.75±0.4, 15.7±0.8, 16.5±0.8, and 11.4±0.6 ppb, respectively, set in the following descending order: EATC100>ETC100>ATC100>TC100. Also, in a period of 10 days, TC100, ETC100, EATC100, and ATC100 concentrations were determined as 80.1±3.9, 47.8±2.4, 65±3, and 39.6±0.2 ppb, respectively. They followed the decsending order of TC100>EATC100>ETC100>ATC100.

As for the concentrations of TC300, ETC300, EATC300, and ATC300 in *Lemna gibba* L. and in a 2-hour period, it was observed that they amounted to 1.89±0.1, 8.75±0.43, 7.47±0.3, and 10.6±0.5 ppb, respectively, with their determined order being as follows: ATC300>ETC300>EATC300>TC300.

When the concentrations of TC50, TC100, and TC 300 in *Lemna gibba* L. were compared, they were 23.5±1.1 ppb, 80.1±3.9 ppb, and 274±13 ppb, respectively in a period of 10 days, which showed that TC concentrations in *Lemna gibba* L. rose significantly as the initial TC concentration was increased; therefore, the highest TC concentration in the plant belonged to the initial concentration of TC300.

When the concentrations of ETC50, ETC100, and ETC300 were compared in the plant, they were 39.5±1.9 ppb, 47.8±2.4 ppb, and 168±8.4 ppb, respectively in a period of 10 days, showing that ETC concentrations in the plant increased with the increase of initial ETC concentration. It was observed that the highest ETC concentration in *Lemna gibba* L. belonged to the initial concentration of ETC300.

As for initial concentrations of EATC50, EATC100, and EATC300, they were 45.3±2.3 ppb, 65±3.0 ppb, and 173±9.0 ppb, respectively in a period of 10 days. It was determined that EATC concentrations in *Lemna gibba* L. increased with the increase of initial EATC concentration.

Finally, when the concentrations of ATC50, ATC100, and ATC300 were compared in the plant, they were 34.7±1.7 ppb; 39.6±0.2 ppb and 114±5.6 ppb, respectively in a period of 10 days, showing a similar trend to the abovementioned findings.

CONCLUSION

The highest initial TC and DPs concentrations (300 ppb) resulted in the highest concentrations in plants after a period of 10 days. It was seen that *Lemna gibba* L. efficiently bioaccumulated TC and DPs at concentrations of 50, 100, and 300 ppb, respectively; therefore, It could be said that the plant is suitable for usage as biomonitor of TC and DPs contamination in water. Using *Lemna gibba* L. to eliminate these micropollutants is a possible, effective, and low-cost system.

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