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Toxicity of Linear Alkyl Benzene Sulfonate to Tarek (*Alburnus tarichi*) Larvae

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ABSTRACT
Linear alkyl benzene sulfonate is a group of anionic detergents widely used in domestic and industrial applications. It causes adverse effects by mixing with aquatic environments. Chemicals mixing with aquatic environments affect fauna and flora at different levels. This study was carried out to determine the toxicity of linear alkyl benzene sulfonate for the larvae of tarek (*Alburnus tarichi* Güldenstädt, 1814), a fish living in the Van Lake basin which has economic and ecological value. The chemical was administered at concentrations of 0.0, 0.6, 1.2, 1.8, 2.4, and 3.0 mg L^{-1} in the acute test and 0.0, 1.2, and 2.4 mg L^{-1} in the chronic test. Tests were carried out using 100 larvae in each group. Bioassays were carried out at mean temperature of 20.9 ± 0.4 °C. A median lethal concentration of 4.883 (4.099–6.482) mg L^{-1} at 96 hours was calculated for the larvae. Glutathione content, superoxide dismutase and glutathione S-transferase activity decreased significantly at 1.2 mg L^{-1} and increased significantly at 2.4 mg L^{-1} compared to controls ($P<0.05$). The malondialdehyde content increased significantly depending on the increase in chemical concentration ($P<0.05$). Linear alkyl benzene sulfonate is toxic to tarek larvae in terms of the measured biochemical parameters.

Keywords: Anionic surfactants, Antioxidant enzyme, Chronic test, Cyprinid fish, LC_{50}

1. Introduction

Linear alkyl benzene sulfonate (LAS) is an anionic surfactant. It has high cleaning capacity by reducing the surface tension of water. Therefore, the compound is widely used in household and industrial detergents. It enters the environment through wastewater discharge and may pose a potential risk to the receiving aquatic ecosystems (Zhang et al. 2015; Gouda et al. 2022; Filogh et al. 2023). Its microbial degradability is 6–22 days. Despite being biodegradable, conventional wastewater treatment plants are relatively inefficient at removing LAS. It can have a toxic effect on aquatic organisms (Manousaki et al. 2004; Zhang et al. 2015). Fish, an important group living in the aquatic environment, are very good bioindicators of aquatic contaminants. They react with great sensitivity to changes in water quality. Toxicity tests are performed to reveal and evaluate these reactions (Uedeme-Naa & Erondu 2014; Kankaya & Kaptaner 2016).

There are acute and chronic toxicity studies on many fish species related to LAS used in the experiment. Some of these studies examined the gill histology of *Oncorhynchus mykiss* fries by Hofer et al. (1995); the hatching of *Sparus aurata* fish eggs (Rosety et al. 2001); histopathology of LAS-absorbed sediment on different tissues of *Solea senegalensis* juveniles (Hampel et al. 2008); and histological effects on liver tissue of *Puntius ticto* fish by Varsha, Mishra & Govind (2013). In addition to these studies, Uedeme-Naa & Erondu (2014) evaluated the effects on plasma biochemical parameters of *Clarias gariepinus* juveniles, while Oyoroko & Ogamba (2017) studied the effects of detergents containing LAS on the behavior of *Heterobranchus bidorsalis*, *Clarias gariepinus* and *Heteroclarias*. Tarek (*Alburnus tarichi*, Güldenstädt 1814) is a member of the *Cyprinidae* family and is endemic to the Van Lake basin (Elp et al. 2014). It has economic value for the region with a catch of approximately 10,000 tons/year (TUIK 2022). It migrates to the rivers pouring into the lake for reproduction. Adult fish that have completed spawning return to the lake. Although the spawning calendar changes depending on the change in water temperature over the years, the spawning migration intensifies especially in May–June. Fertilization, hatching, hatching of larvae and the first feeding period after reproduction occur in the rivers. After a certain time, the fry leaves the rivers and enters the lake (Ünal et al. 1999; Kankaya & Ünal 2018). Fish are adversely affected because rivers are contaminated with different levels of pollutant load.
The aim of the study was to determine the acute and chronic toxic effects of LAS on tarek larvae.

2. Material and Methods

2.1. Collection of fish and larval production

Adult male and female tarek (*Alburnus tarichi*) were caught using a net from the Karasu river (43°13′38.46″ E, 38°35′20.41″ N) during the spawning migration period (May–June) (Figure 1). Fertilized eggs obtained by artificial insemination were hatched and larvae were obtained (Kankaya et al. 2015).

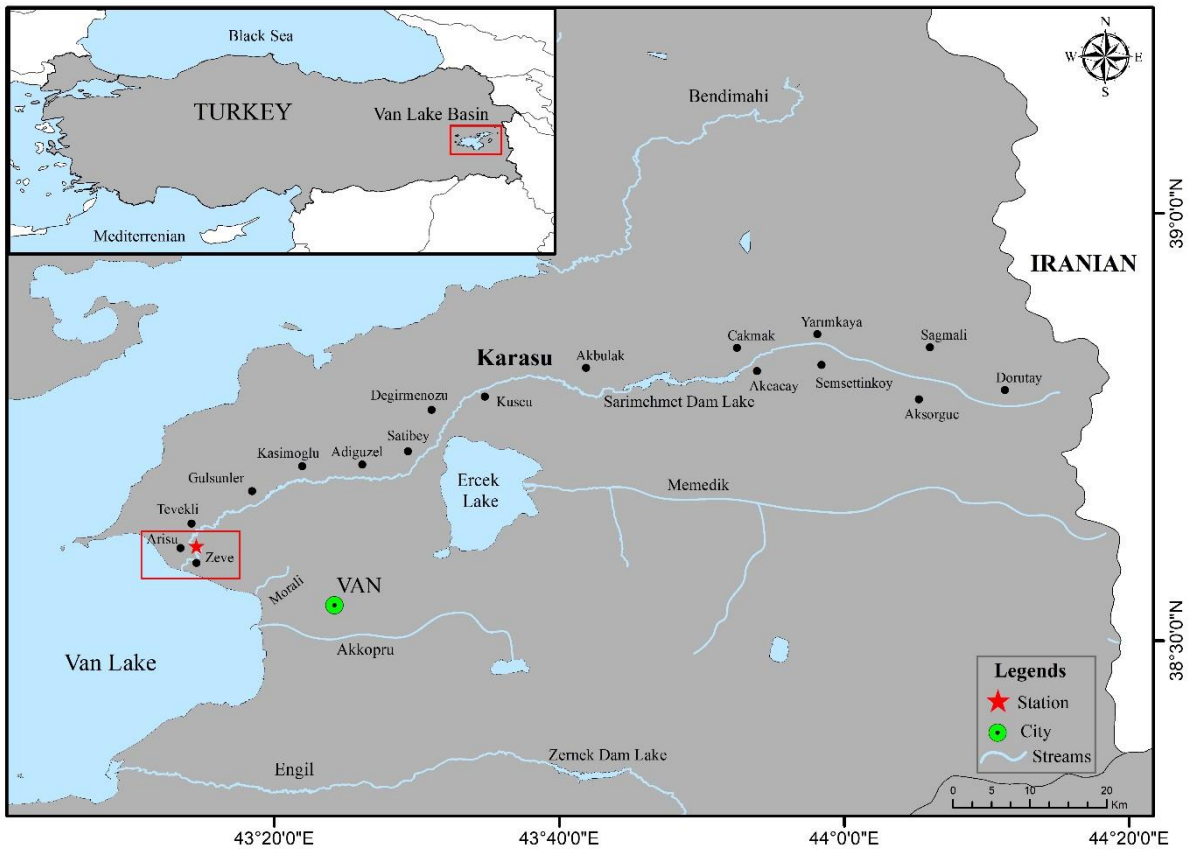

Figure 1- Fish sampling station on the Karasu river

2.2. Chemicals

LAS was purchased from Sigma-Aldrich (CAS: 25155-30-0). The superoxide dismutase (SOD) enzyme kit was obtained from Randox Laboratories Ltd. 5,5'-dithiobis 2-nitrobenzoic acid; glutathione (GSH); 1-chloro-2,4-dinitrobenzene and other chemicals were provided with analytical quality.

2.3. Experimental design

Concentrations of 0.0, 0.6, 1.2, 1.8, 2.4 and 3.0 mg L^{-1} LAS were applied in the acute toxicity test (Xu 1996; Rosety et al. 2001). In the chronic toxicity test, the amount of chemical was chosen as 1.2 and 2.4 mg L^{-1}, taking into account the median lethal concentration (LC_{50}). Applications were carried out in a glass beaker, using 100 larvae (12–24 hours) in each. Bioassays were carried out using the static test method (Wang et al. 2010). The acute test was continued for 96 hours and the chronic test for 7 days. The study was applied in natural photoperiods with 4 replications (USEPA 2002a; Çetinkaya 2010; OECD 2013; Kankaya & Ünal 2018).

2.4. Measurements and assessments

Water quality criteria (water temperature, pH, dissolved oxygen, oxygen saturation, electrical conductivity, salinity) were measured with a Hach HQ-40d multimeter regularly throughout exposure. Total hardness and total alkalinity were determined using the titrimetric method. In order to determine the actual LAS concentration of the experimental environment, water samples were analyzed using the methylene blue active substances method with Hach Lange DR 5000 UV/VIS spectrophotometer (APHA 1995; HACH 2005). During the chronic toxicity test, the morphological developmental stages of the larvae were followed (Unal et al. 2000; OECD 2013; Kankaya et al. 2015). Feed, content of which is given in Table 1, was given in the form

of powder for external feeding on the 6th day after hatching of the larvae. Morphological examination and imaging of larvae were performed using a Nikon SMZ 745 T stereo light microscope with XCAM digital camera attachment. At the end of the chronic toxicity test, 15–17 larvae in all application groups were placed in Eppendorf tube and 6 Eppendorf sample tubes were created for enzyme analysis. Samples were stored at -80 °C until biochemical analysis.

Table 1- Content of feed used for external feeding of larvae in the chronic test (Maripearls trademark)

Nutrient	***Value***
Protein (%)	38
Fat (%)	4
Raw fiber (%)	4.5
Raw ash (%)	10.5
Vit E	300 IU
Vit C	200 IU
Vit A	25000 IU
Vit D3	2000 IU
Enriched algae (%)	10

2.5. Tissue homogenization

Larval samples stored at -80 °C for analysis were weighed and homogenized with tissue lysis (Tissuelyser Qiagen) in an Eppendorf tube for 3 min in 50 mM KH_2PO_4 buffer (4 °C, 1:5 w/v).

The homogenate was centrifuged at 9500 rpm for 30 min at 4 °C with a refrigerated centrifuge (Inovia, INO-HR/T16M) (Marklund 1990). The obtained supernatant was used for GSH, glutathione S-transferase (GST), SOD, malondialdehyde (MDA) and catalase (CAT) measurements.

2.6. Biochemical Analyses

GSH content was determined according to Beutler (1984) by measuring at 412 nm and GST activity was measured at 340 nm using the method of Habig et al. (1974). In addition to these, SOD activity was determined at 505 nm using the procedure of the commercial kit manufacturer (Ransod, Randox Lab., UK), MDA content was measured at 532 nm (Jain et al. 1989) and CAT activity was measured at 240 nm by the spectrophotometric method according to Aebi (1984).

2.7. Statistical analysis

LC_{50} values and 95% confidence limits were calculated using a computer package program with the probit analysis method. The data obtained as a result of biochemical analyses were evaluated using one-way analysis of variance (ANOVA) and Duncan multiple comparison test. Values with $P<0.05$ in all analyses were considered statistically significant. Values are given as mean ± standard deviation (USEPA 2002b).

3. Results and Discussion

3.1. Water quality, actual LAS concentrations and LC_{50} value

The mean values for water temperature, pH, dissolved oxygen, oxygen saturation, electrical conductivity, salinity, total hardness and total alkalinity are given in Table 2. Theoretical and actual LAS concentration results are given in Table 3. The 96-hour LC_{50} value for larvae exposed to LAS was calculated as 4.883 (4.099–6.482) mg L^{-1}.

Table 2- Quality criteria and variation of water used in bioassay

Water Quality Criteria	***Mean ± SD***
Temperature (°C)	20.9 ± 0.4
pH	8.48 ± 0.08
Dissolved oxygen (mg L^{-1})	7.4 ± 0.2
Oxygen saturation (%)	102.2 ± 1.8
Electrical conductivity (µS cm^{-1})	719 ± 28
Salinity (‰)	0.34 ± 0.02
Total hardness ($CaCO_3$ mg L^{-1})	358 ± 23
Total alkalinity ($CaCO_3$ mg L^{-1})	557 ± 17

Table 3- Theoretical and actual LAS concentrations (n=2)

Theoretical LAS concentration (mg L^{-1})	*Actual LAS concentration (mg L^{-1})*
0.6	0.53±0.08
1.2	1.11±0.07
1.8	1.63±0.12
2.4	2.27±0.10
3.0	2.88±0.09

Products containing LAS are widely used in many areas of life and they are released into the environment. Therefore, in this study, the acute and chronic toxic effects of LAS on tarek were investigated. In similar studies on the subject Gholami et al. (2010) found the 96-hour LC_{50} value of LAS in *Rutilus frisii* fries was 11.62 mg L^{-1}; Spirita Sharmili et al. (2015) reported the 96-hour LC_{50} value was 27.31 mg L^{-1} in their study determining the toxicity of alkyl benzene sulfonate in zebrafish (*Danio rerio*). Shukla & Trivedi (2018) calculated the LAS 96-hour LC_{50} value as 34.40 mg L^{-1} in *Channa punctatus* fish weighing 25 g. Gouda et al. (2022) reported the LAS 96-hour LC_{50} value was 10 mg L^{-1} for Nile tilapia (*Oreochromis niloticus*) weighing 6.6–7.8 g. In the current study, the 96-hour LC_{50} value was calculated as 4.883 (4.099–6.482) mg L^{-1} in larvae exposed to LAS. According to the calculated LC_{50} values, it is clear that LAS is moderately toxic to tarek larvae.

3.2. Morphological observations and mortality rate in larvae with the chronic test

During the test, no significant difference was found between the groups in terms of total length, yolk sac consumption, pigmentation, swim bladder formation, appearance of fins, opening of the mouth and transition to external feeding in larvae. The determinations made regarding the morphological development of the larvae during the chronic test were as follows. First day: length 7.0–7.8 mm; eyes are large; pigmentation dorsal to the yolk sac, behind the eyes, along the head and back; blood circulation in the anterior of the yolk sac and along the notochord; and 2 pairs of otoliths are seen. Second day: length 7.4–8.5 mm; intestinal development began between the yolk sac and the notochord. Third day: length 7.5–8.5 mm; pigmentation is prominent in the head region, on the back up to the tail region, on the dorsal of the yolk sac from anterior to posterior; intestinal line is seen in a structure with yellow content up to the anus. Fourth day: length 8.0–8.9 mm; the first lobe of the swim bladder is visible; pigmentation shows as round spots, spread along the dorsal from the head to the tail, along the dorsal of the yolk sac, in the region between the anus and the caudal fin; pectoral fin prominent and mouth open. Fifth day: length 8.3–8.7 mm; yolk sac is rather thin; motility was detected in the larvae. Sixth day: length 8.7–9.1 mm; increased movements; powdered feed (Table 1) was given for external feeding. Seventh day: length 8.2–8.8 mm; red-colored food given on the sixth day can be noticed as red coloration in the digestive system up to the anus; larvae are free swimming (Figure 2). At the end of the bioassay, the mean total length of the larvae was determined as 8.3 ± 0.5, 8.0 ± 0.5, and 8.0 ± 0.5 mm at 0.0, 1.2 and 2.4 mg L^{-1} concentrations of LAS, respectively. The differences between larval sizes were found to be statistically insignificantly lower in the LAS-treated groups compared to the control group. The mean length of the yolk sac in the larvae was determined as 3.8 ± 0.2, 3.8 ± 0.2, and 3.7 ± 0.2 mm for 0.0, 1.2 and 2.4 mg L^{-1} concentrations of LAS, respectively. Yolk sac length decreased insignificantly in the 2.4 mg L^{-1} group ($P>0.05$). At the end of the test, the mortality rate in larvae was 1% in the control and 2% in the LAS groups.

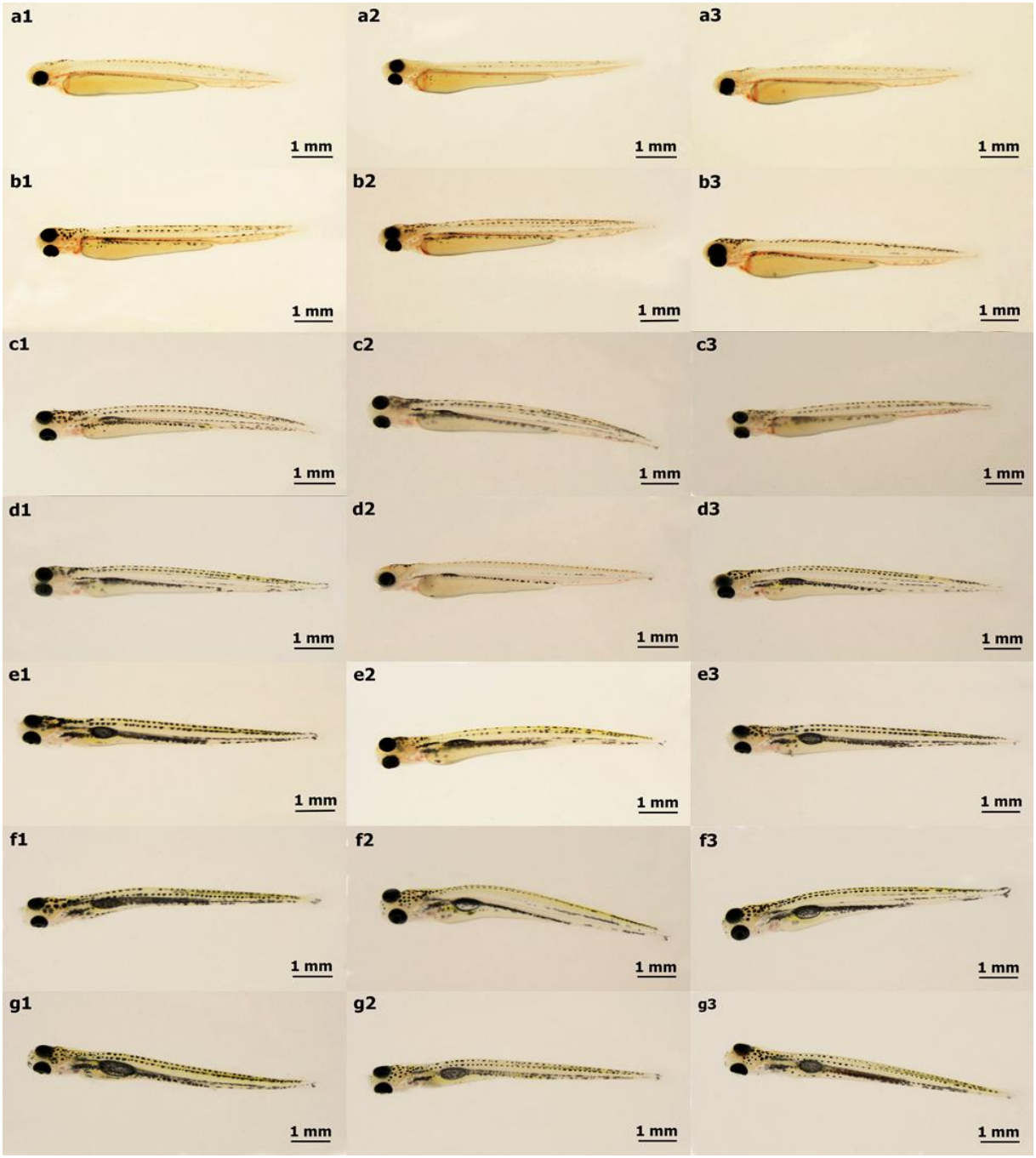

Figure 2- Microscopic images of larvae according to chemical application durations (a: 24 h, b: 48 h, c: 72 h, d: 96 h, e: 120 h, f: 144 h, g:168 h) and concentrations (0.0, 1.2 and 2.4 mg L^{-1}). The number '1' with each letter represents the control groups, '2' represents group administered 1.2 mg L^{-1} chemical and '3' represents the group administered 2.4 mg L^{-1} chemical)

Hampel et al. (2004) exposed *Sparus aurata* larvae sub lethally to LAS. They reported that the survival rate after 72 hours at 0.5 mg L^{-1} LAS concentration was 50% and severe edema could occur in the yolk sac. In the current study, the morphological development processes of the experimental group larvae during the test were similar both between the groups and to the results reported by Unal et al. (2000). No abnormality occurred in the larvae exposed to LAS for 7 days compared to the control (Sepil & Kankaya 2022). Larval length and yolk sac consumption were insignificantly different in all groups. Survival rate in LAS groups was 98%, similar to control. It was revealed that LAS did not have a significant effect in terms of yolk sac consumption, length and survival rate of tarek larvae.

3.3. Biochemical analyses

In the LAS chronic toxicity test, larvae exposed to 0.0, 1.2 and 2.4 mg L^{-1} concentrations had mean GSH content 7.59 ± 0.76, 3.79 ± 0.35, and 8.51 ± 0.97 µmol mg^{-1} protein, mean GST activity 1.23 ± 0.21, 0.44 ± 0.13, and 2.1 ± 0.64 nmol mg^{-1} protein, mean SOD activity 161.53 ± 21.24, 130.17 ± 19.28, and 203.12 ± 28.83 U g^{-1} protein, mean MDA content 7.22 ± 0.71, 25.62 ±

6.07, and 43.97 ± 3.56 nmol g^{-1} protein, and CAT activity was determined as 1.67 ± 0.19, 1.62 ± 0.30, and 3.69 ± 0.43 nmol g^{-1} protein, respectively (Figure 3). The GSH content, SOD and GST activity changed significantly between the groups. The MDA content increased significantly due to the increase in the concentration of the chemical, while CAT activity significantly increased at 2.4 mg L^{-1}.

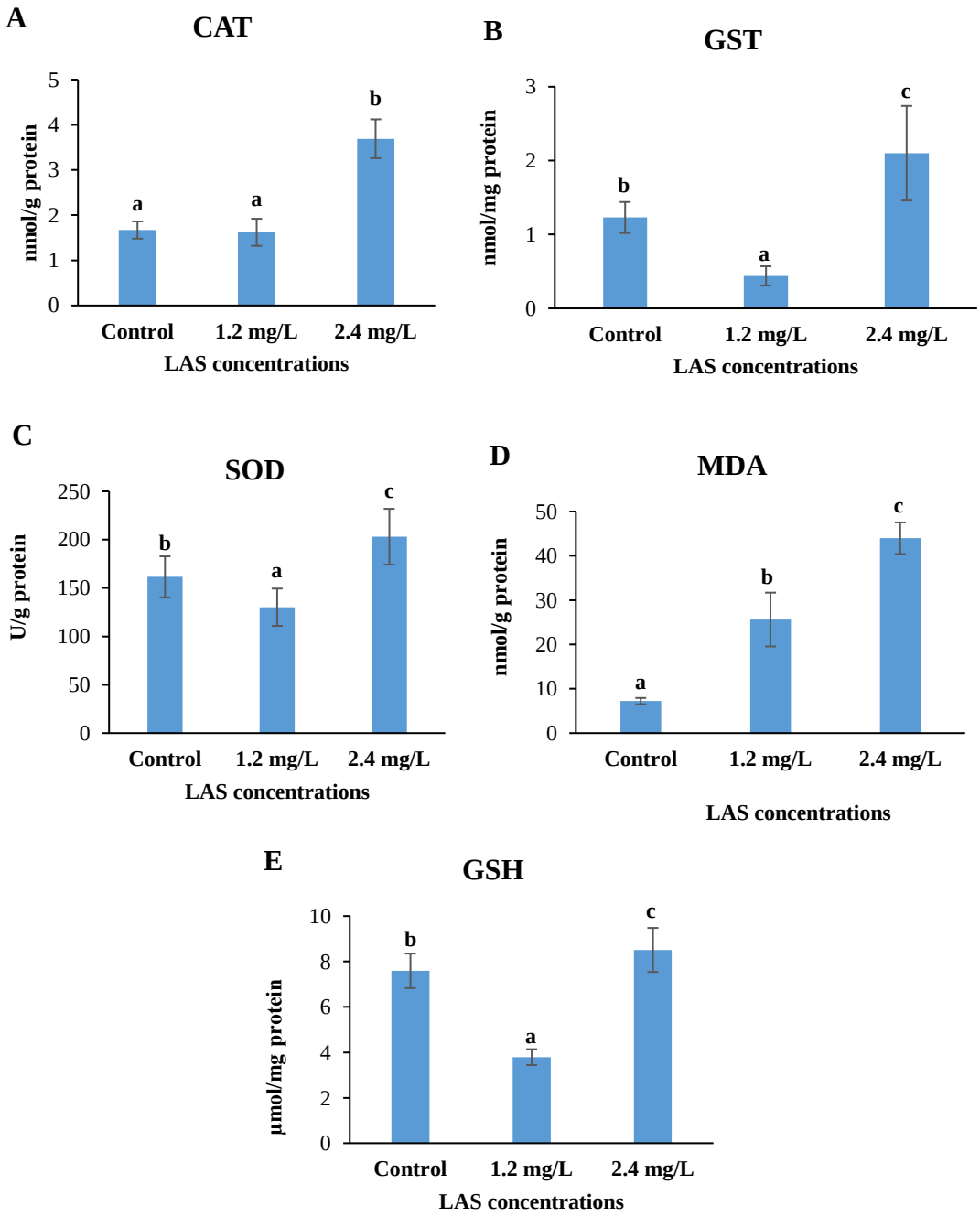

Figure 3- Changes in CAT (A), GST (B), SOD (C) activities, MDA (D) and GSH (E) contents according to the results of LAS chronic toxicity tests (0.0, 1.2 and 2.4 mg L^{-1} concentrations) in larvae

Hofer et al. (1995) reported in their study with *Oncorhynchus mykiss* that fries exposed to 0.2 mg L^{-1} concentration of LAS were significantly affected histologically and physiologically. In their study investigating LAS-induced oxidative stress and liver disorders in *Channa punctatus* fish, Shukla & Trivedi (2018) found that CAT and SOD activities determined in liver tissue increased significantly depending on the increase in concentration. Gouda et al. (2022), in their study determining the effects of LAS on the biochemical parameters of Nile tilapia (*Oreochromis niloticus*), reported that SOD and CAT activity and GSH content decreased depending on the increase in LAS concentration in serum samples. Ghosh et al. (2022) exposed *Oreochromis mossambicus* fish to sub lethal concentrations of alkyl benzene sulfonate and reported that CAT, GST, MDA, and SOD values increased significantly. In this study, no observed effect concentration (NOEC) value for CAT activity was 1.2 mg L^{-1} and the lowest observed effect concentration for GST, SOD activity and GSH, MDA content was 1.2 mg L^{-1}. The NOEC value was determined to be well above the value reported by Hofer et al. (1995) for *Oncorhynchus mykiss* fries. Since CAT has secondary

importance in protecting from the formation of oxygen radicals (Doğan & Çelik 2016), the activity value was similar to the controls at 1.2 mg L^{-1}. The increase at 2.4 mg L^{-1} exposure may be the result of an adaptation response against free radical formation.

Atıcı (2020) investigated the concentration level of LAS in the Karasu river. The mean LAS value was determined as 0.018 ± 0.001 mg L^{-1}. The LAS value was reported to be class I according to the surface water quality regulation. Atici (2021) studied the seasonal changes of LAS in the surface waters of Morali, Akköprü and Kurubaş rivers, which flow into Lake Van, carry domestic and industrial wastewater, and where tarek enters to spawn. In this study, the amount of LAS changed in the range of 0.032–0.184, 0.023–0.081, and 0.170–0.401 mg L^{-1} in Morali, Akköprü and Kurubaş rivers between April and July, respectively. Although the calendar of tarek spawning migration varies depending on the increase in water temperature of the rivers, considering that it generally occurs between April–July, it is possible that tarek will not have a problem in terms of LAS amount (Atıcı 2020) in Karasu river. But if they enter the Morali, Akköprü and Kurubaş rivers, tarek will encounter an amount much higher than the safe LAS concentration predicted for larvae. This situation may adversely affect the reproduction of tarek that will enter these rivers to spawn.

4. Conclusions

In conclusion, the existence of substances containing LAS in freshwater environments in ever-increasing amounts affects all organisms in the aquatic environment at different levels. Tarek larvae were exposed to sublethal concentrations of LAS and examined in terms of biochemical parameters and problems were revealed. It is necessary to treat wastewater discharged into rivers and to monitor in terms of LAS amount. In freshwater environments where the species reproduces, the LAS concentration should not exceed 0.049 mg L^{-1} for larvae.

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