Environ Monit Assess (2021) 193: 755
https://doi.org/10.1007/s10661-021-09549-2

Physicochemical water quality of Karabel, Çaltı, and Tohma brooks and blood biochemical parameters of *Barbus plebejus* fish: assessment of heavy metal concentrations for potential health risks

Tuğba Demir · Ekrem Mutlu · Seyit Aydın · Nejdet Gültepe

Received: 25 May 2021 / Accepted: 19 October 2021 / Published online: 29 October 2021

Abstract The present study aims to comparatively examine the physical quality parameters of water samples taken from Karabel, Çaltı, and Tohma brooks in Sivas province and the blood biochemical parameters of blood samples of *Barbus plebejus* fish obtained from these waters. In periods when chemical pollution in water increased and decreased, it was determined that GLU and UA among blood biochemical parameters were significantly affected. Moreover, the potential risk levels of lead (Pb), copper (Cu), cadmium (Cd), and ferrous (Fe) for human health were compared to the international standards. Metal (Fe, Pb, Cu, and Cd) concentrations in the water were determined using atomic absorption spectrophotometer. The highest $HQ_{ingestion}$ values of Cd were found in Brook Çaltı, which were 0.0018 for adults and 0.1980 for children. THQ upper limit set by the United States Environment Protection Agency (USEPA) is < 1. It was determined that $HQ_{ingestion}$, HQ_{dermal}, and THQ values of all the heavy metals were much lower than this limit. It was concluded that water quality parameters of samples taken from Karabel, Çaltı, and Tohma brooks on monthly basis for 12 months were not higher than the limits and the water qualities of brooks were determined to be "good".

Keywords Water quality · Fish quality · Heavy metal · Human health · Risk assessment

T. Demir (✉)
Faculty of Veterinary, Department of Food Hygiene and Technology, Sivas Cumhuriyet University, 58140 Sivas, Turkey
e-mail: tugba@cumhuriyet.edu.tr

E. Mutlu
Faculty of Fisheries, Department of Aquaculture, Kastamonu University, Kastamonu, Turkey

S. Aydın
Vocational School Veterinary Department, Laboratory and Veterinary Health Program, Ankara Yıldırım Beyazıt University, Ankara, Turkey

N. Gültepe
Fisheries Faculty, Department of Fisheries Fundamental Sciences, Atatürk University, 25240 Erzurum, Turkey

Introduction

Being an important factor for the survival of organisms, water is one of our most important resources. Deterioration of water quality or balance for various reasons also causes water pollution. Used mainly in agricultural, industrial, and domestic activities, the quality of water is of significant importance depending on the area of use (Mutlu et al., 2018). Throughout the history, human beings have always conducted various activities, polluted and changed the nature, and caused the deterioration of nature's balance (Aydın et al., 2021). Due to the environmental pollution with gradually increasing importance nowadays, the number of studies on air metal pollution and its effects constantly increases (Mutlu et al., 2013). It was reported that the levels of heavy metals in water sources significantly increased due to the rapid growth of population and the industrialization (Milošković et al., 2014). As known, although fishes constitute an important protein source, they also constitute an important ring in the biological circle of water ecosystems. Fishes in an aquatic environment polluted by heavy metals are exposed to severe levels of heavy metal pollution (Yanbo et al., 2006). It is known that high concentrations of heavy metals have potentially toxic effects on the living organisms in aquatic ecosystems.

An increase in heavy metal concentrations in an aquatic environment also causes significant changes in water's physical and chemical properties. Aquatic organisms that are exposed to heavy metals together with these changes in water tend to accumulate these metals in their bodies and fishes are generally affected more than other organisms (Milošković et al., 2014). These changes also result in various metabolic and physiologic changes, as well as accumulation of heavy metals in tissues and organs of aquatic organisms. These changes may sometimes result in the death of an organism (Wang et al., 2021). Pollution of rivers, lakes, and seas limits the lives of species living there and causes the annihilation of them or puts them under the risk of annihilation (Adams et al., 2020). Clinical blood tests have been widely used in examining the fishes exposed to toxic materials. Besides the hematologic and biochemical parameters, fish blood provides information also about the ecosystem's health and the effects of environmental and anthropogenic stress factors (Bopp et al., 2008). Another point to emphasize here is that, if a freshwater source is used by humans for table water or irrigation water needs, then the high levels of heavy metals become very harmful. Although they can be found at very low levels in water resources, heavy metals may become toxic when accumulated at the toxic levels in the course of time (Finn, 2007).

Brook Karabel, examined here, is formed by the merge of various creeks streaming towards the north. It originates from Zara district of Sivas province. Brook Çaltı is located in Divriği district of Sivas province and originates from Karagöl Mountains in Kangal district. Brook Tohma is located in Gürün district of Sivas province and it originates from foot of Tahtalu Mountain and merges into Fırat River. In literature, there is no study examining the quality parameters of water samples taken from Karabel, Çaltı, and Tohma brooks and the blood parameters of *Barbus plebejus* fishes collected from the study area. From this aspect, the novelty of the present study comes to the forefront. The present study aims to comparatively examine the physicochemical quality characteristics of water samples annually collected from Karabel, Çaltı, and Tohma brooks and the biochemical characteristics of fishes, and potential risks of Pb, Cu, Cd, and Fe heavy metals in the water for human health, and was performed in accordance with international standards.

Materials and methods

Study area

For this research, approval certificate was obtained from Kastamonu University Animal Experiments Local Ethics Committee (Decision No: 19.12.2014/2014.09).

The study area consists of three stations. The first station is Brook Karabel (39°32′39″N-37°44′05″E), the second station is Brook Çaltı (39°21′07″N-38°15′03″E) and the third station is Brook Tohma (38°39′35″N-37°28′28″E).

Water samples

A total of 48 water samples were collected monthly during the year 2015–2016, including 4 samples from each station. The total volume of 1000 mL water samples was collected in polyethylene bottles (twice rinsed with deionized water) and stored in an ice box and transported to the laboratory for further analysis. 100 mL of filtered water samples was acidified with concentrated HNO_3 (20 mL) at 100 °C. The acidified water was cooled down to room temperature, diluted, and filtered through Whatmann-42 filter paper.

Fish samples

During the study, *Barbus plebejus* species, widely caught in the region, were collected monthly. Three groups have been formed and each group has 17 samples. All samples wrapped in polyethylene bags, then an ice stored transportation was made to the laboratory for the biometrics, dissection, and collection of fish tissue for heavy metal analysis. In the laboratory, washing was performed with tap water for surface cleaning (Maurya et al., 2019).

Fish blood parameters

After applying light anesthesia to the fish, the blood samples were dried with a towel and the tail sections were cut with a sharp scalpel in a single blow and collected from the tail vein (1–1.5 mL) into vacuum-gel biochemical blood tubes. Then, the samples were centrifuged at 5000 rpm for 10 min. The obtained serums were stored at − 20 °C until the analysis period. Serums were analyzed using Beckman UniCel DXC 800 and DXI 800 Synchron (Beckman Coulter Inc., CA, USA) auto-analyzers. Trinder method was used for glucose determination and esterase method was used for cholesterol. Triglyceride was measured by the glycerol phosphate oxidase method. The biuret colorimetric method in serum was used for total protein. Albumin bichromatic analysis method was used. Globulin was obtained by subtracting albumin from the total protein. Creatine was determined with the Jaffe method; uric acid was determined by the uricase method and the Urea urease method. IFCC pyridoxal phosphate-free ALT activation was calculated by IFCC pyridoxal phosphate-free AST activation. The other biochemical parameters: HDL (High-Density Lipoprotein), LDL (Low-Density Lipoprotein), VLDL (very Low-Density Lipoprotein), LDH (Lactate Dehydrogenase), TBIL (Total Bilirubin) and DBIL (Direct Bilirubin) were analyzed using laboratory type biochemical analyzer.

Experimental analysis

Temperature, pH, dissolved oxygen, and electrical conductivity parameters were measured inplace via land-type measurement devices. Dissolved oxygen and temperature were measured via YSI brand S2 model oxygen-meter, pH measurement was conducted with Orion brand 420A model pH-meter, and saltiness (ppt) and the electrical conductance (µs/cm) were measured by using YSI brand 30/50 FT model conductance-meter. Among other parameters determining water quality; total alkalinity, total hardness, ammonium nitrogen, nitrite, nitrate, phosphate, sulfite, sulfate chloride, sodium, potassium, suspended solid matter (SSM), chemical oxygen demand (COD), biological oxygen demand (BOD), calcium, magnesium, ferrous, lead, copper, zinc, nickel, mercury and cadmium analyses of water samples analyses (APHA, 1995) were conducted in the laboratory of Kastamonu University, Department of Aquaculture, Faculty of Fisheries.

Measurements were carried out on atomic absorption spectrometer (AAS, GBC, Avanta) equipped with a graphite furnace autosampler. AAS was used to measure Zn, Pb, Cu, Cd, and Cr in the samples (water and fish) collected. About 0.5 g of oven dried sample as mentioned above was digested with concentrated nitric acid in glass tube at 80 ˚C for 48–72 h then heated up to 120 ˚C for 4–8 h to have clear solution as previously described. Samples then were cooled and diluted with distilled water up to 25 mL, filtered using small glass funnels prewashed with sulfuric acid (El-Nahhal et al., 2013). The purity of standard and acetylene gases was 99.999 to 99.99%, respectively. Atomic signals for Zn, Pb, Cu, Cd, and Cr were measured in peak area mood. The concentration of heavy metals in water sample was calculated using the following formula.

$$Heavymetalconcentration\left(\mu\frac{g}{ml}\right) = \frac{AASreadingxV}{Volumeofthesample(ml)_{11}}$$

$$where, V = volumeofdilutionsolution_{11}$$

The concentration of heavy metals in fish tissue was calculated using the following formula;

$$Heavymetalconcentration\left(\mu\frac{g}{ml}\right) = \frac{AASreadingxV}{Weightofthesample(gm)}$$

$$where, V = volumeofdilutedsolution$$

Estimated daily intake of metals

The estimated daily intake of heavy metals was calculated using the following equation.

$$EDI = \frac{CxV}{BW_{11}}$$

where, C is the mean heavy metals concentration in fish muscle (µg/g) of dry weight basis. For conversion from dry weight to wet weight, 4.8 conversion factor is taken (Rahman et al., 2012). FIR (Food Ingestion

Rate) is the daily consumption of freshwater fish (gram per day (g day^{-1}) per capita. The average FIR was 0.019 g $person^{-1}$ day^{-1} (FAO, 2016). BW is the average body weight, 70 kg for adults (USEPA, 2011).

Target hazard quotient (THQ)

The THQ is the estimate of non-carcinogenic risk level due to heavy metals exposure (Islam et al., 2015). It was calculated using the following equation (USEPA, 2011).

$$THQ = \frac{EfrxEDxFIRxCx0.001}{RfDxBWxATn}$$

where Efr (Exposure frequency) is 365 day^{-1}, and ED (Exposure Duration) is 70 years (as set for this study). RfD (Reference Dose) assesses the health risk of consuming fish, and ATn is the time of average exposure for non-carcinogenic (365 day *x* no. of exposure year (USEPA, 2011).

Risk assessment for human health

The detailed methodology for the risk assessment related to human health has been described elsewhere (Li & Zhang, 2010; Saleem et al., 2014; Wu et al., 2009). Trace metals exposure to human beings could happen through inhalation, dermal adsorption and direct ingestion; however, ingestion and dermal routes are usually considered for the water intake (Singh et al., 2018; USEPA, 2004; Wu et al., 2009). The mathematical expression used for risk assessment has been obtained from USEPA Risk Assessment Guidance for Superfund (RAGS) methodology (USEPA, 2004):

$$ADD_{ing} = \frac{C_{water}xIRxEFxED}{BWxAT}$$

$$ADD_{derm} = \frac{C_{water}xSAxK_{p}xETxEFxEDxCF}{BWxAT}$$

where, ADD_{ing} shows average daily dose by ingestion (μg kg^{-1} day^{-1}); ADD_{derm} indicates average daily dose through dermal absorption (μg kg^{-1} day^{-1}); C_{water} reveals concentration of the metals in surface water (μg L^{-1}); IR depicts ingestion rate (2.2 L d^{-1} for adults and 1.8 L d^{-1} for children); EF stands for exposure frequency (350 d y^{-1}); ED shows exposure duration (70 y for adults and 6 y for children); BW indicates average body weight (70 kg for adults and 15 kg for children); AT shows averaging time (25,550 d for adults and 2190 d for children); SA reveals exposed skin area (18,000 cm^2 for adults and 6600 cm^2 for children); ET denotes exposure time (0.58 h d^{-1} for adults and 1 h d^{-1} for children); CF stands for unit conversion factor (0.001 L cm^{-3}); and K_p indicates dermal permeability coefficient (0.001 cm h^{-1} for Cd, Cu, Fe and 0.004 cm h^{-1} for Pb (USEPA, 2004; Wu et al., 2009; Li & Zhang, 2010).

The potential non-carcinogenic risk was estimated by comparing the exposures through each pathway with the reference dose (RfD) to find out the hazard quotients (HQ) using the following relationship:

$$HQ_{ing/derm} = \frac{ADD_{ing/derm}}{RfD_{ing/derm}}$$

where, $HQ_{ing/derm}$ refers to hazard quotient through ingestion and dermal routes. If the HQ value exceeds unity, there might be concern for non-carcinogenic risk.

Statistical analysis

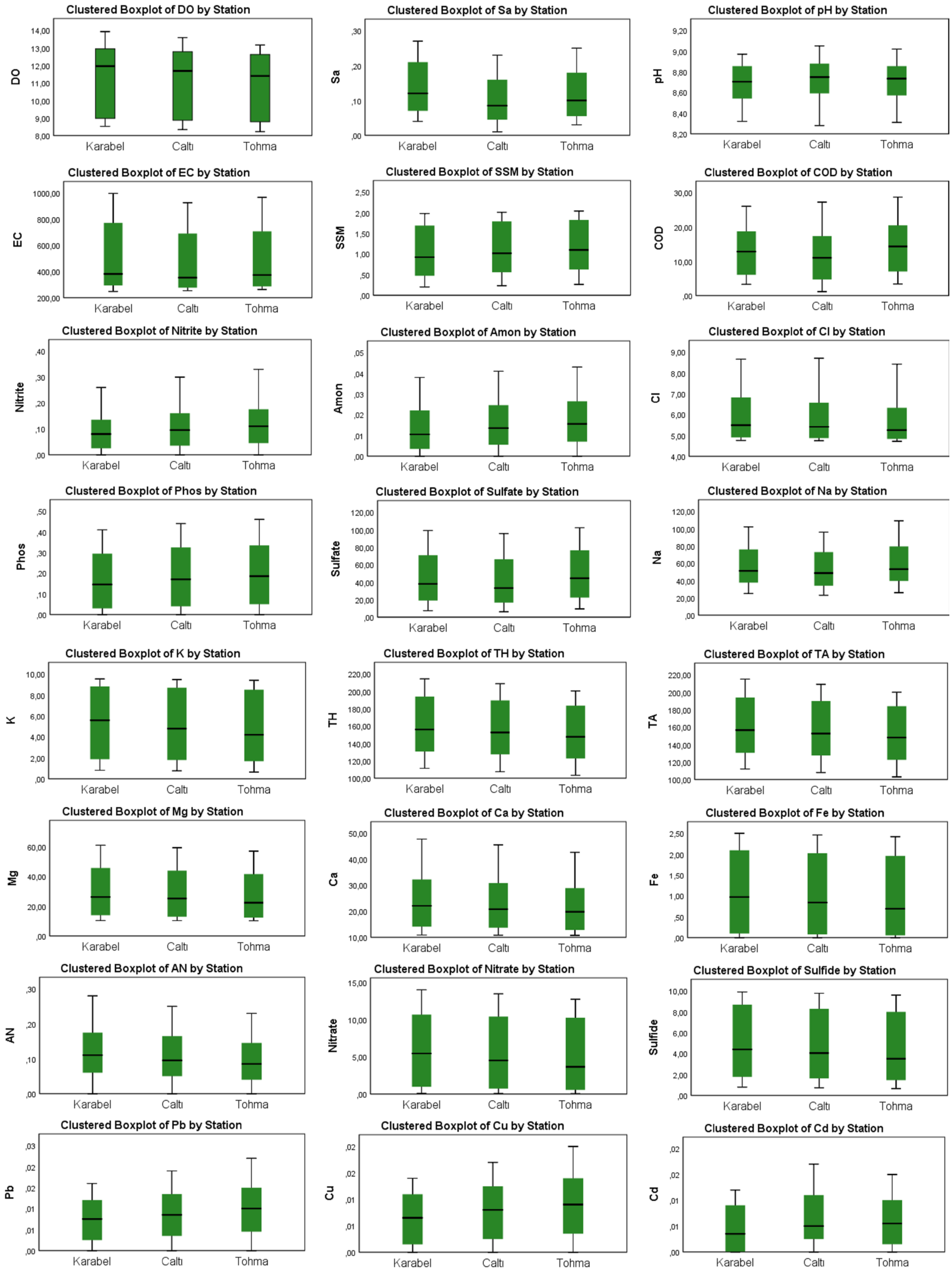
The data were statistically analyzed using the statistical package SPSS (version 22.0). The mean ± standard deviations of the metal concentration in fish species were calculated. Regarding the correlation coefficient level, if $p < 0.05$, it was evaluated as there was a statistically significant difference between the groups.

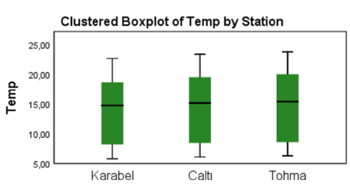
Results and discussion

Water quality parameters

Water quality parameters of Brook Karabel, Brook Çaltı, and Brook Tohma were analyzed throughout the year. Figure 1 is presented that Clustered Boxplot diagrams of water quality parameters measured in Karabel, Çaltı, and Tohma brooks throughout the

Fig. 1 Clustered boxplot diagrams of water quality parameters measured in Karabel, Çaltı, and Tohma brooks throughout the year ▸





DO; Dissolved oxygen (mg/L), **Sa;** Saltiness (%0.), **pH**, **EC**; Electrical conductivity, **SSM**; Suspended solid matter (mg/L), **COD**; Chemical oxygen demand (mg/L), **Nitrite**; Nitrite(NO_2) (mg/L), **Amon**; Ammonia, **Cl**; Chloride (mg/L), **Phos**; Phosphate (PO_4)(mg/L), **Sulfate**;Sulfate (**SO_4**)(mg/L), **Na**; Sodium (mg/L), **K**; Potassium (mg/L), **TH**; Total hardness (mg/L), **TA**; Total alkalinity (mg/L), **Mg**; Magnesium (mg/L), **Ca**; Calcium (mg/L), **Fe**; Iron (mg/L), **AN;** Ammonium nitrogen (mg/L), **Nitrate;** Nitrate (NO_3)(mg/L), **Sulf;** Sulfide (SO_3)(mg/L), **Pb**; Lead, **Cu**; Copper, **Cd;** Cadmium, **T°C**; Temperature.

year. Dissolved oxygen concentration is an important parameter for developing a balanced aquatic fauna. Dissolved oxygen is as necessary for aquatic life as it is for biochemical oxidations (Atay & Pulatsu, 2000; Mutlu et al., 2016). Dissolved oxygen levels of Brook were determined to be at the highest level of 13.920 ± 2.160 mg/L in January, whereas the annual mean value was found to be 11.198 mg/L. The level of dissolved oxygen decreased in June and then started to increase again in November. This finding can be related to temperature because temperature increases in months, when dissolved oxygen level decreased, vice versa. For aquatic life, freshwater sources must have a minimum dissolved oxygen level of 5 mg/L (Mutlu et al., 2013). The values found in the present study indicate Class I quality according to WPCR.

Salinity refers to the amount of salts dissolved in a liter of water salinity is measured by g/L or %. Salinity is closely related with temperature and electrical conductivity (Mutlu et al., 2018). Salinity values of Brook Karabel ranged between 0.040 and 0.270 ± 0.070. The values increasing the average were measured in September. Examining from the seasonal aspect, it can be stated that salinity increased in autumn and decreased in winter seasons. The salinity level of the brook can be defined as brackish.

Electrical conductivity and suspended solid matter values were ranged between 248.00 µs/cm and 998.00 µs/cm and between 0.20 mg/L and 1.98 mg/L, respectively. The highest electrical conductivity value was found in September, whereas the highest level of the suspended solid matter was detected in August. Examining the changes in pH, a trend that is parallel with electrical conductivity was found. The highest pH level was observed in September. Electrical conductivity (EC) indicates the amount of solid matter dissolved salts in water and it varies depending on the serological structure and the level of precipitation. In a previous study carried out on Lake Kurugöl (Sivas), electrical conductivity and salinity values showed a parallel decrease in winter and an increase in summer (Mutlu et al., 2013). When compared to the results achieved in the present study, the results are consistent with the literature. From the aspect of electrical conductivity, the water samples were found to be very good according to WPCR.

Chemical oxygen demand and nitrite values reached their highest levels in September and the lowest values in February. The mean values achieved were 12.962 ± 7.735 mg/L and 0.092 ± 0.082 mg/L, respectively.

Examining the dissolved oxygen level of Brook Çatlı, which is another station, the highest value was found to be 13.580 ± 2.160 in January and the annual mean value was found to be 10.974 mg/L. Dissolved oxygen levels decreased in June and then increased again in November. This finding can be related to temperature and temperature and pH since temperature increased in months when the level of dissolved oxygen showed an increase, and vice versa.

The annual mean salinity value of Brook Çaltı (%) was found to be 0.104 ± 0.071 (0.010–0.230). The value increasing the average was the data recorded in September. Considering from the seasonal aspect, it was determined that salinity increased in autumn and decreased in winter.

Chemical oxygen demand is an important parameter used in determining the pollution levels of waters and wastewaters. Chemical oxygen demand (COD) level higher than 25 mg/L indicates pollution, whereas levels higher than 50 mg/L indicate severe pollution and also a potential toxic effect for aquatic organisms (Li et al., 2018). The highest level of chemical oxygen demand was found in September (27.24 mg/L) and the lowest level in July (1.16 mg/L). Affected by seasonal transitions, chemical oxygen demand is affected by pH changes at most. The highest level of pH was found in September (9.05).

The sources of nitrogen mixing into surface water generally have generally domestic, natural, industrial, and agricultural origins (Mutlu et al., 2013). The levels of ammonium nitrogen (NH_4^+), nitrite, and nitrate, which are nitrogen derivatives, in Brook Çaltı were found to be very low. The highest level of nitrite was found in April (0.6 ± 0.16), that of ammonia in August (0.041 ± 0.013), and those of phosphate and sulfate in October and September, respectively. The water quality parameters of Brook Çaltı showed remarkable changes in autumn. Nitrogenous compounds have significant effects on water pollution, and they have important effects on oxygen and eutrophication (Du et al., 2017). From the aspect of the aforementioned parameters, the quality of Brook Çaltı was found to be good.

Comparing with oxygen values of Brook Tohma, the highest values were observed in February and the lowest ones in February. The mean level of dissolved oxygen was found to be 10.781 ± 1.97 mg/L.

The brook was found to be Class I in terms of water quality. The highest salinity (%) was found in July (1.19%) and showed a stable course in other months. Electrical conductivity, chemical oxygen demand, and nitrite values reached their highest values in September and the results were 968 ± 61.78, 28.7 ± 8.44, and 0.33 ± 0.1, respectively. Electrical conductivity is directly proportional to temperature and salinity. Considering the monthly measurements, it had an effect on the standard deviation. It was determined that electrical conductivity, chemical oxygen demand, and nitrite levels increased and decreased with pH values and increasing pH resulted in an increase in these parameters (Fig. 1).

Total alkalinity and total hardness values were found to be close to and parallel with each other throughout the year. In waters in lime soils, total alkalinity and total hardness values are generally close to or same as each other (Yetiş et al., 2019). Among the water quality parameters examined for Brook Tohma, the highest levels of total hardness, total alkalinity, Mg, Ca, Fe, and ammonium nitrogen were observed in June and the lowest values in February. The mean total hardness value of Brook Tohma was found to be 150.454 mg/L $CaCO_3$ and it indicates hard water according to WPCR. The highest temperature in Brook Tohma was found to be 23.8 °C (September) and the lowest value to be 6.3 °C (February).

Comparing all of the analyzed water samples, it can be seen that the higher levels were observed in Brook Karabel for dissolved oxygen, in Brook Tohma for salinity, electrical conductivity, and suspended solid matter, and in Brook Çaltı for pH. The highest temperature was found in Brook Tohma, followed by Çaltı and Karabel, respectively.

Being one of the natural anions of water, sulfate is necessary for the increase of biological productivity in natural waters (Wang et al., 2021). Comparing the mean sulfate levels, it was determined that the highest value was found in Brook Tohma, followed by Karabel and Çaltı (102.340, 99.160, and 95.730, respectively) and all the values indicate Class I water quality.

The sulfite measured in the present study was Na_2SO_4 sodium sulfite and the mean sulfite values were found to be 9.770 mg/L, 9.890 mg/L, and 9.600 mg/L in Çaltı, Karabel, and Tohma brooks. These values were less than 1/10 of the maximum sulfite level and it indicates that the water quality is very good in all study areas.

Blood parameters of Barbus plebejus fishes collected from Karabel, Çaltı, and Tohma stations

Together with water samples, *B. plebejus* fish samples were collected from Karabel, Çaltı, and Tohma brooks on monthly basis and their blood parameters were investigated. Fishes are used as bioindicators in determining the conditions and changes in the aquatic environment. For this reason, depending on the changes in the ecosystem, fishes respond to these changes at various levels and the level and forms of responses should be known (El-Nahhal, 2018; El-Nahhal et al., 2016; Sula et al., 2020). The hematological and biochemical parameters of fish blood are indicators of aquatic vertebrae and they indicate the effects of the environment- and human-related stress factors, as well as the health of the ecosystem (Ural et al., 2013).

Besides the minimum and maximum values, the mean values and standard deviations of biochemical parameters of fish samples taken from all the stations are presented in Table 1. Each biochemical parameter was measured in three replicates in 17 fish (Table 1). As can be seen, the monthly differences in GLU parameter were found to be statistically significant and the highest value was found in July (517.26 ± 8.69). Biochemical parameters increasing in June, when seasonal temperatures started increasing, were found to be as follows: CHOL (Cholesterol) (601.94 ± 8.88), TG (Triglyceride) (411.88 ± 8.11), ALB (Albumin) (7.29 ± 0.18), GLO (Globulin) (7.24 ± 0.18), HDL (521.08 ± 11.34), VLDL (129.90 ± 2.75), LDH (1251.95 ± 2.77), and ALT (Alanine Aminotransferase) = GPT (609.23 ± 3.83). In September, when temperatures started seasonally decreasing, CRE, UA, UREA, BUN, ALP, TBIL, DBIL, Ca, Fe, and Mg values were found to be at the highest levels. Among the biochemical parameters, LDL reached its lowest value in this month (92.71 ± 2.44).

Besides them, Cl (122.91 ± 16.98) values were found to be high in October, Na and K values (150.85 ± 0.06, 8.42 ± 0.01) in August, and TP and AST = GOT values (8.51 ± 0.07; 107.41 ± 2.90) in April. In conclusion, the highest level of LDL was found in February (411.84 ± 4.97).

In fishes, stress arising from any reason may affect the level of glucose. Stress factor causes higher energy to need during metabolism's reaction and it

Table 1 Blood parameters of *B. plebejus* fishes collected from stations

Blood biochemistry	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
GLU	116.0 ± 4.63	137.68 ± 2.69	280.94 ± 7.48	434.00 ± 5.87	457.18 ± 5.83	482.12 ± 5.81	517.24 ± 8.69*	186.76 ± 4.19	335.76 ± 8.84	409.82 ± 43.19	355.53 ± 6.14	168.47 ± 5.12
CHOL	250.8 ± 8.65	221.32 ± 4.84	403.18 ± 11.55	475.18 ± 5.43	526.06 ± 8.73	601.94 ± 8.88*	566.18 ± 8.16	480.47 ± 21.82	329.47 ± 11.80	373.24 ± 7.26	434.35 ± 8.93	339.59 ± 3.76
TG	130.4 ± 4.81	127.47 ± 4.32	159.71 ± 6.19	343.76 ± 5.54	374.06 ± 6.76	411.88 ± 8.11*	315.65 ± 5.37	267.28 ± 68.64	275.71 ± 46.23	224.12 ± 4.17	204.94 ± 6.04	178.71 ± 3.92
TP	6.62 ± 0.14	6.06 ± 0.13	7.21 ± 0.16	8.51 ± 0.07*	7.82 ± 0.15	3.61 ± 0.08	3.85 ± 0.21	4.19 ± 0.09	4.48 ± 0.08	4.79 ± 0.09	5.24 ± 0.13	5.62 ± 0.13
ALB	1.91 ± 0.08	1.60 ± 0.08	5.29 ± 0.16	5.85 ± 0.14	6.49 ± 0.17	7.29 ± 0.18*	2.20 ± 0.08	3.21 ± 0.11	3.98 ± 0.26	4.44 ± 0.11	3.61 ± 0.14	2.52 ± 0.10
GLO	3.43 ± 0.01	3.40 ± 0.01	5.69 ± 0.07	6.19 ± 0.08	6.69 ± 0.05	7.24 ± 0.06*	3.44 ± 0.01	3.54 ± 0.04	4.24 ± 0.33	5.22 ± 0.03	3.66 ± 0.02	3.48 ± 0.01
CRE	0.56 ± 0.02	0.60 ± 0.01	0.64 ± 0.01	0.72 ± 0.01	0.85 ± 0.01	1.02 ± 0.02	1.24 ± 0.03	1.50 ± 0.07	1.65 ± 0.02*	0.93 ± 0.02	0.77 ± 0.01	0.67 ± 0.01
UA	1.42 ± 0.01	1.39 ± 0.01	1.46 ± 0.01	1.58 ± 0.02	1.87 ± 0.03	1.95 ± 0.02	2.58 ± 0.33	2.71 ± 0.28	3.86 ± 0.72*	2.16 ± 0.20	1.67 ± 0.02	1.50 ± 0.01
UREA	0.64 ± 0.01	0.61 ± 0.01	0.67 ± 0.01	0.80 ± 0.01	1.01 ± 0.03	1.31 ± 0.03	1.91 ± 0.02	2.23 ± 0.16	2.37 ± 0.23*	1.45 ± 0.02	0.87 ± 0.02	0.73 ± 0.01
BUN	0.68 ± 0.01	0.65 ± 0.01	0.70 ± 0.01	0.79 ± 0.01	0.98 ± 0.02	1.13 ± 0.04	1.59 ± 0.04	2.07 ± 0.23	2.18 ± 0.08*	1.32 ± 0.04	0.88 ± 0.02	0.73 ± 0.01
HDL	224.09 ± 4.82	205.24 ± 7.20	311.78 ± 6.33	353.31 ± 5.83	456.87 ± 10.24	521.29 ± 11.34*	487.31 ± 12.73	409.19 ± 10.17	244.67 ± 9.20	289.13 ± 9.13	330.44 ± 6.36	262.23 ± 5.03
LDL	393.18 ± 7.67	411.84 ± 4.97*	228.76 ± 5.46	197.06 ± 2.54	162.65 ± 3.55	92.71 ± 2.44	148.41 ± 2.96	180.06 ± 4.49	336.00 ± 7.53	259.88 ± 4.78	211.59 ± 5.20	297.88 ± 6.50
VLDL	60.33 ± 0.70	56.57 ± 1.38	68.09 ± 0.77	76.11 ± 1.07	90.69 ± 1.73	129.86 ± 2.75*	108.02 ± 3.03	82.81 ± 0.93	62.09 ± 0.93	65.80 ± 0.82	83.75 ± 53.31	64.06 ± 0.82
LDH	1051.06 ± 66.02	1010.63 ± 2.59	1058.90 ± 244.22	1150.71 ± 2.08	1193.65 ± 4.46	1251.94 ± 2.77*	1219.65 ± 3.59	1197.12 ± 121.93	1056.00 ± 5.47	1090.65 ± 5.75	1138.41 ± 3.68	1075.94 ± 3.67
ALT = GPT	334.84 ± 2.39	314.94 ± 2.68	400.76 ± 3.04	458.04 ± 2.04	536.69 ± 1.82	609.18 ± 3.83*	578.42 ± 4.31	489.30 ± 4.09	331.42 ± 3.22	377.49 ± 2.77	430.56 ± 3.11	350.08 ± 5.25
AST = GOT	89.88 ± 1.36	86.00 ± 1.27	92.12 ± 1.17	107.41 ± 2.90*	96.41 ± 1.58	65.06 ± 0.83	67.59 ± 1.12	68.41 ± 1.28	72.53 ± 1.28	74.00 ± 1.17	77.06 ± 0.90	82.00 ± 1.54
ALP	394.19 ± 100.51	420.05 ± 2.20	424.35 ± 0.86	443.12 ± 3.57	441.98 ± 112.70	500.24 ± 3.38	519.82 ± 2.43	540.53 ± 2.29	556.82 ± 3.68*	484.82 ± 2.48	454.59 ± 2.35	433.12 ± 1.93
TBİL	1.29 ± 0.05	1.36 ± 0.19	1.88 ± 0.04	2.10 ± 0.09	2.40 ± 0.10	2.58 ± 0.10	3.29 ± 0.09	3.61 ± 0.09	3.82 ± 0.08*	3.12 ± 0.09	1.71 ± 0.08	1.32 ± 0.08
DBİL	0.79 ± 0.02	1.06 ± 0.19	1.48 ± 0.04	1.55 ± 0.05	1.65 ± 0.05	1.85 ± 0.05	2.54 ± 0.06	2.80 ± 0.09	3.09 ± 0.09*	2.40 ± 0.11	1.29 ± 0.09	0.95 ± 0.05
Cl	116.14 ± 0.05	115.90 ± 0.03	116.93 ± 0.73	116.64 ± 0.06	117.48 ± 0.47	119.53 ± 0.07	120.75 ± 1.95	122.12 ± 0.07	114.88 ± 26.18	122.91 ± 16.98*	118.19 ± 0.07	119.26 ± 12.14
Ca	8.89 ± 0.02	8.92 ± 0.01	8.94 ± 0.01	9.00 ± 0.01	9.09 ± 0.01	10.11 ± 0.01	10.67 ± 0.02	10.85 ± 0.03	10.91 ± 0.05*	10.02 ± 0.02	9.03 ± 0.01	8.97 ± 0.01
Na	141.89 ± 0.33	141.79 ± 0.03	142.78 ± 0.09	142.50 ± 0.04	143.46 ± 0.04	146.27 ± 0.05	147.59 ± 0.05	150.85 ± 0.06*	149.05 ± 0.24	145.71 ± 1.41	144.22 ± 0.05	142.27 ± 0.03
K	6.37 ± 0.01	6.35 ± 0.01	6.46 ± 0.01	6.42 ± 0.01	6.51 ± 0.01	6.95 ± 0.01	7.66 ± 0.01	8.42 ± 0.01*	8.10 ± 0.09	6.79 ± 0.01	6.62 ± 0.01	6.39 ± 0.01
Fe	18.54 ± 0.01	18.48 ± 0.01	18.74 ± 0.01	18.96 ± 0.01	19.17 ± 0.06	19.37 ± 0.02	19.50 ± 0.03	19.71 ± 0.25	19.87 ± 0.01*	18.85 ± 0.02	18.70 ± 0.01	18.60 ± 0.01
P	14.10 ± 0.02*	14.01 ± 0.02	13.89 ± 0.01	13.70 ± 0.01	13.56 ± 0.02	13.39 ± 0.01	11.92 ± 3.98	13.22 ± 0.01	13.16 ± 0.01	13.49 ± 0.01	13.63 ± 0.01	13.77 ± 0.01
Mg	0.83 ± 0.01	0.88 ± 0.01	0.91 ± 0.01	0.99 ± 0.01	1.11 ± 0.01	1.29 ± 0.01	1.35 ± 0.01	1.38 ± 0.01	1.44 ± 0.01*	1.18 ± 0.02	1.04 ± 0.01	0.95 ± 0.01

results in the catalysis of glucose in body stores (Sula et al., 2020). In all three stations, seasonal increases in temperature and pH values were found to be in parallel with blood GLU levels. Thus, in parallel with the literature, it was determined that varying stress factors have an effect on GLU values.

In previous studies, it was determined that the blood cholesterol level of fishes may vary depending on factors such as nutrition, feed, stress, water pollution, and temperature (Lemaire et al., 1991). In the present study, the changes in blood cholesterol levels can be related to temperature. As seen in Table 1, the increases in CHOL and TG parameters in the months of seasonal temperature increase were higher than the increases in winter. On the other hand, considering the pollution factors in study areas, it was determined that chemical pollutants increased in summer months and dissolved oxygen level decreased in this period (June, Table 1) and it might affect the TG values.

It was reported in previous studies that nutritional status and stress might affect the total protein values (Lemaire et al., 1991; Ural et al., 2013). In the present study, TP, ALB, and GLO values reached their maximum in April and June. In the same period, when comparing Na, total hardness, and total alkalinity, it was determined that Na was significantly affected and other parameters (CHOL, TG, LDL, and HDL) were slightly affected. In the literature review, it was stated that an increase in water temperature may a play role in increase of TP, ALB, and GLO values and especially the GLO values may show monthly and seasonal variation (Finn, 2007). These results are consistent with the results reported in the literature and affected by the temperature increase and monthly changes.

In previous studies, it was reported that blood UA and UREA concentrations changed depending on the factors such as sudden changes in environmental conditions, stock intensity, water temperatures, season, and nourishment (Ural et al., 2013). In the present study, CRE, UA, and UREA values and water temperature reached their maximum levels in September.

Assessment of metal concentrations in water and fishes

Cl, Na, K, Mg, Ca, and Fe concentrations of Karabel, Çaltı, and Tohma brooks were measured on monthly basis. Moreover, the fish blood parameters were measured on monthly basis and compared to water levels. First, it was determined that the highest blood Cl levels were found in October when the water Cl level reached its minimum. On the other hand, biochemical parameters of blood were found to be low in months when chlorine ion increased (Fig. 2). In previous studies, it was reported that an increase in chlorine ions

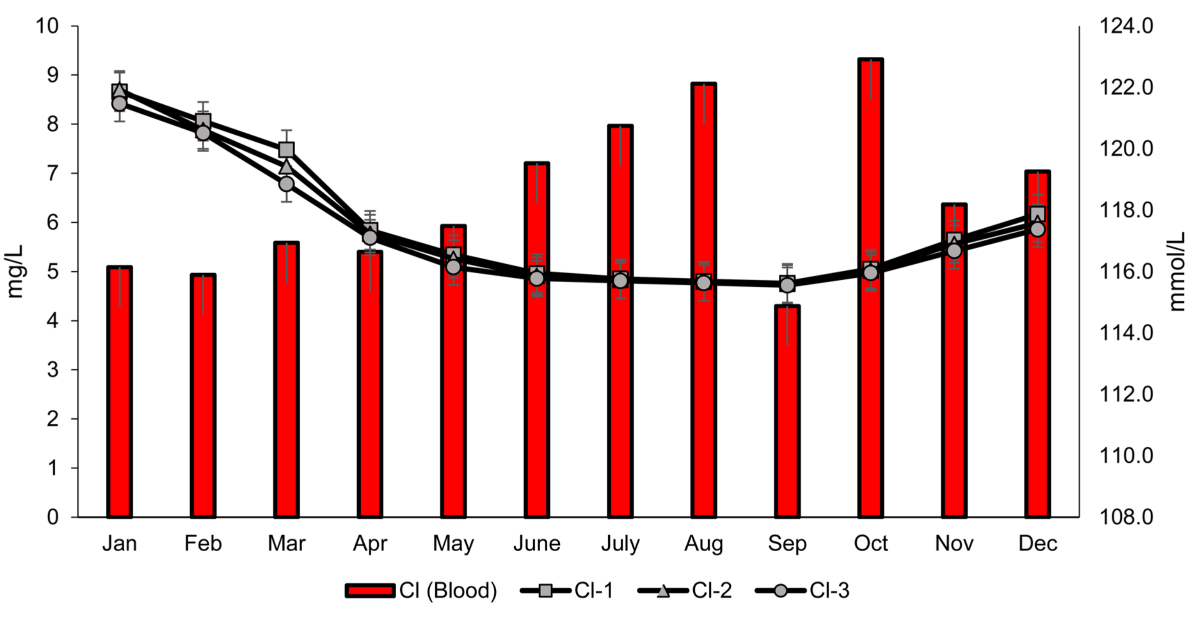


Fig. 2 The relationship between chloride (Cl) levels measured in blood biochemistry and water quality parameters

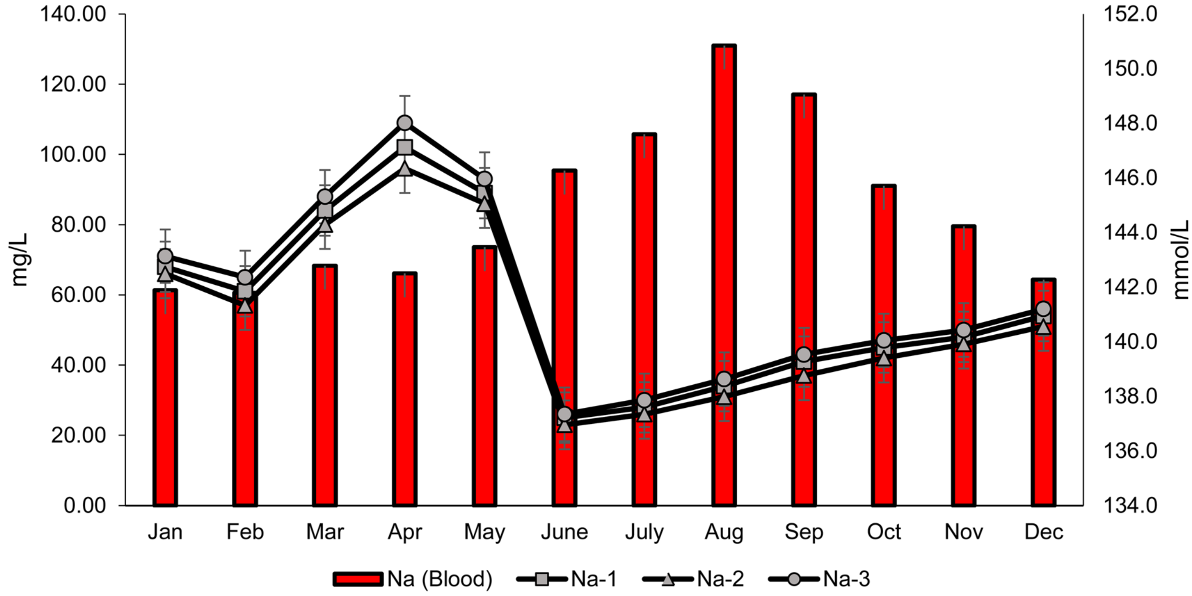


Fig. 3 The relationship between sodium (Na) levels measured in blood biochemistry and water quality parameters

in the medium may protect fish from nitrite poisoning by preventing the intake of nitrite (Yanbo et al., 2006). In the present study, the water quality parameters of all three stations showed that (Fig. 1) nitrite values reached the lowest level in September (lowest level of chlorine ions) and nitrite values decreased to zero in months with high levels of chlorine ions. These findings are consistent with the literature. Examining the sodium (Na) levels, it can be seen that sodium in water decreased and was used for bioavailability when blood sodium levels increased (Fig. 3). Moreover, it was also reported that an increase in sodium decreased heavy metal toxicity (Adams et al., 2020). Examining Fig. 4 comparing potassium (K) levels among blood

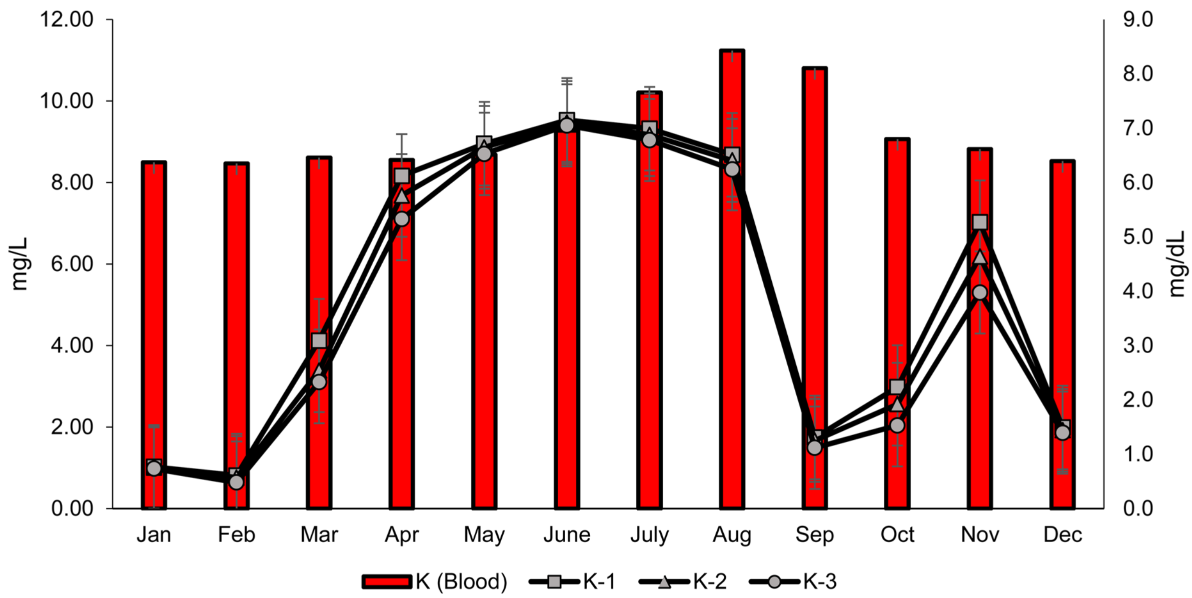


Fig. 4 The relationship between potassium (K) levels measured in blood biochemistry and water quality parameters

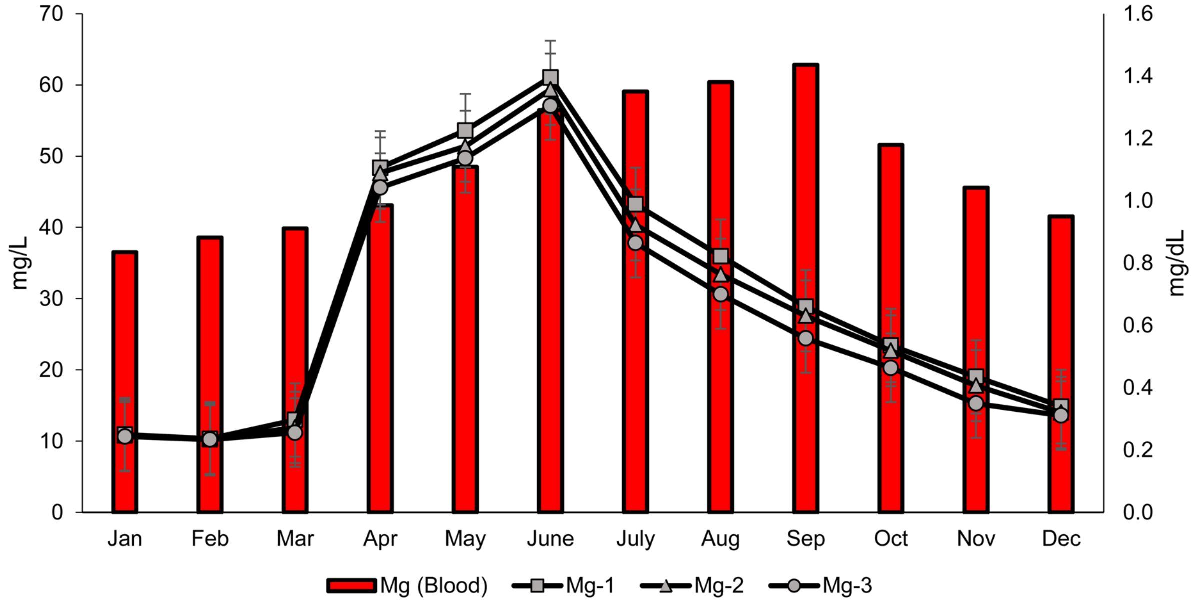


Fig. 5 The relationship between magnesium (Mg) levels measured in blood biochemistry and water quality parameters

biochemistry and water quality parameters, it can be stated that blood potassium levels were not significantly affected by the changes in water.

Calcium (Ca^{++}) and magnesium (Mg^{++}) ions were related to the hardness of the water. Since water hardness affects the physiological functions of fishes, it was reported that fish would get osmotic stress when optimum values are exceeded (Romano et al., 2020). Given Ca and Mg values in the present study, it was determined that the results in blood and water were in parallel. In other words, in the months when water hardness might have increased (Apr, May, June), it was determined that both of these ions increased but not exceeded beyond the optimum levels (Fig. 5).

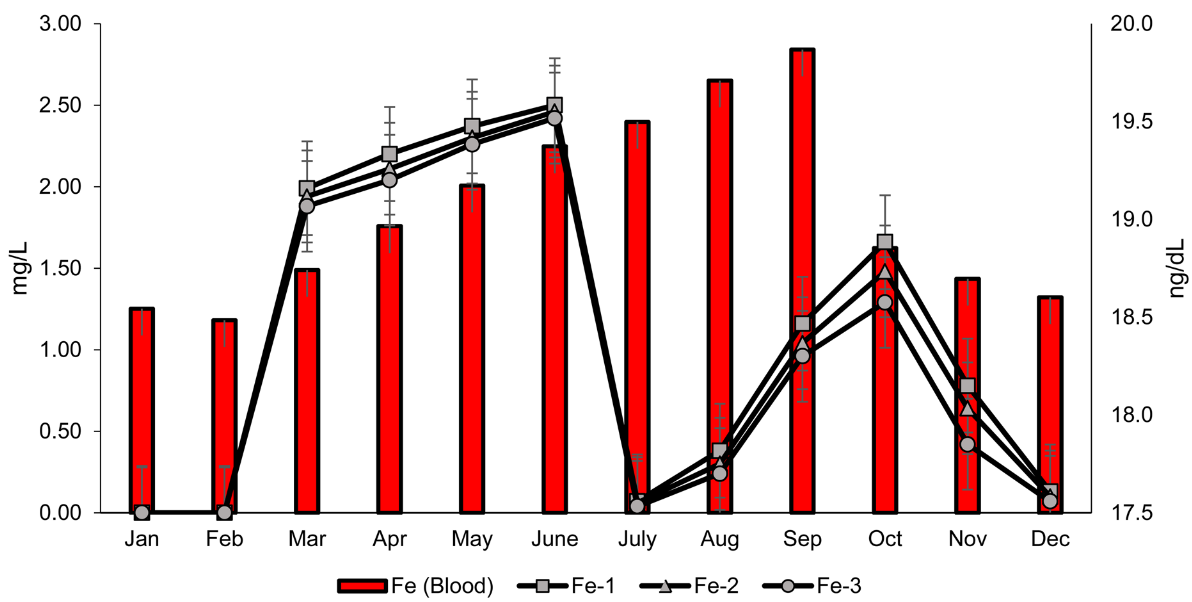


Fig. 6 The relationship between iron (Fe) levels measured in blood biochemistry and water quality parameters

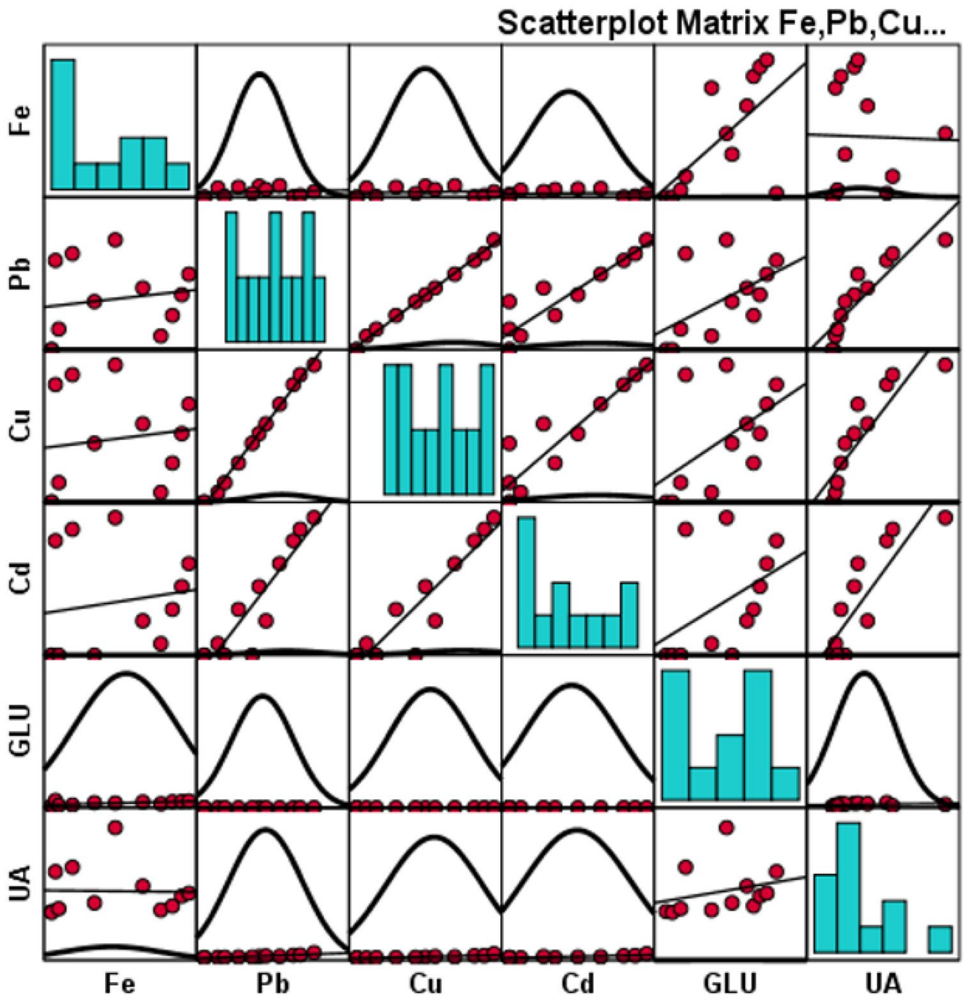


Fig. 7 Distribution of the relationships between GLU and UA levels in fish blood and metal concentrations

The highest ferrous level in the blood was found in September. In January and February months, when the level of ferrous in natural waters is almost zero, fishes are not affected by these ferrous concentrations and they keep the ferrous ion, which they use for bioavailability, in their bodies because ferrous is an element that is necessary for metabolism.

Fish are harmed generally by low oxygen content and low pH in winter and, in this season, iron is in its soluble form "ferro" (Ranjitha et al., 2020). In the present study, the levels of ferrous ion in blood parameters of fish were low especially in winter months and they are not harmful to living organisms (Fig. 6).

Scatterplot Matrix was used that Cd, Cu, Pb and Fe heavy metals for all stations. GLU and UA values, which are mostly affected by blood biochemical values, were compared in the same matrix. Since each parameter was measured over twelve months, the matrix was used to compare the increase and decrease. Figure 7 shows that distribution of the relationships between GLU and UA levels in fish blood and metal concentrations.

Examining heavy metals for health risk

Natural waters containing high metal concentrations are harmful to both aquatic organisms and human health. In the present study, the detectable heavy metals' carcinogenic/non-carcinogenic health risks were determined for adults and children. As seen in Table 2, annual mean levels of Fe, Pb, Cu, and Cd

Table 2 Risk levels and cancer risk assessments of toxic metals found in Karabel, Çaltı, and Tohma brooks

Heavy metals	Study area	HQ (Ingestion)		HQ (Dermal)		THQ	
		Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**
Fe	Karabel	6.30E-08	7.06E-08	1.70E-06	2.18E-06	2.82E-04	3.55E-04
	Caltı	5.92E-08	6.63E-08	1.60E-06	2.05E-06	2.65E-04	3.34E-04
	Tohma	5.53E-08	6.20E-08	1.49E-06	1.92E-06	2.48E-04	3.12E-04
Pb	Karabel	1.75E-03	1.96E-03	3.15E-03	4.04E-03	3.78E-03	4.44E-03
	Caltı	2.01E-03	2.25E-03	3.62E-03	4.64E-03	4.33E-03	5.10E-03
	Tohma	2.31E-03	2.59E-03	4.15E-03	5.33E-03	4.98E-03	5.86E-03
Cu	Karabel	2.61E-04	2.93E-04	7.05E-03	9.05E-03	6.69E-02	8.41E-02
	Caltı	3.09E-04	3.46E-04	8.34E-03	1.07E-02	7.90E-02	9.94E-02
	Tohma	3.60E-04	4.03E-04	9.71E-03	1.25E-02	9.21E-02	1.16E-01
Cd	Karabel	1.31E-03	1.47E-03	1.41E-01	1.82E-01	4.19E-03	5.27E-03
	Caltı	1.83E-03	2.05E-03	1.98E-01	2.54E-01	5.87E-03	7.38E-03
	Tohma	1.69E-03	1.89E-03	1.83E-01	2.34E-01	5.41E-03	6.80E-03

heavy metals collected from three stations were used in health risk calculations. The reference doses (RfD) declared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and determined by the United States Environment Protection Agency were used in acceptable daily intake (ADI) values. The calculations were separately assessed for adults and children as specified in international standards.

For human health analysis, $HQ_{ingestion}$, HQ_{dermal}, and THQ values were calculated for each metal. Examining the results, it was determined that all the values were much lower than the limits. For each metal taken in through digestion and skin, both carcinogenic and non-carcinogenic risks were calculated and assessed for adults and children.

Among heavy metals, the highest $HQ_{ingestion}$ and HQ_{dermal} values of Fe were found in Brook Karabel, followed by Caltı and Tohma brooks (adult and children), whereas highest Pb and Cu values were found in Brook Tohma, followed by Çaltı and Karabel brooks. Cd values of Brook Çaltı were found to be higher than those of Tohma and Karabel brooks for adults and children (Table 2). $HQ_{ingestion}$ values of cadmium were found to be 0.0013, 0.0018, and 0.0016 for adults and 0.1414, 0.1980, and 0.1825 for children (Karabel, Çaltı, and Tohma brooks). The limit of THQ set by USEPA is <1 (USEPA). Examining the results, it was found that THQ values found were much lower than this limit and there was no severe health risk.

Conclusion

In the present study, physicochemical water quality parameters of water samples collected from Karabel, Çaltı, and Tohma brooks on monthly basis for 12 months were analyzed. It was determined that quality parameters of water samples were not higher than the limits and the samples were found to have good quality. Moreover, biochemical blood parameters of fishes monthly sampled from the same stations were examined and the results were analyzed in terms of the effects of water quality on the fish metabolism. It was found that chemical pollutants in the water had significant interaction with fish metabolism. Moreover, the annual heavy metal concentrations of water were determined and analyzed for the long-term potential health risk by using the methods and measurement techniques set by international standards (USEPA, FAO, and WHO). It was found that none of the heavy metals exceeded the acceptable limits but Cd values were closer to the limits. Researches on this subject should be deepened and required measures should be taken.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P. H., & Spry, D. (2020). Bioavailability assessment of metals in freshwater environments: A historical review. *Environmental Toxicology and Chemistry, 39*(1), 48–59.

APHA. (1995). *American Public Health Association*. America Public Health Association.

Atay, D., & Pulatsu, S. (2000). Su kirlenmesi ve kontrolü. Ankara Üniversitesi Ziraat Fakültesi, Publication No: 1513, Ankara.

Aydın, H., Ustaoğlu, F., Tepe, Y., & Soylu, E. N. (2021). Assessment of water quality of streams in northeast Turkey by water quality index and multiple statistical methods. *Environmental Forensics, 22*(1–2), 270–287.

Bopp, S. K., Abicht, H. K., & Knauer, K. (2008). Copper-induced oxidative stress in rainbow trout gill cells. *Aquatic Toxicology, 86*(2), 197–204.

Du, Y., Ma, T., Deng, Y., Shen, S., & Lu, Z. (2017). Sources and fate of high levels of ammonium in surface water and shallow groundwater of the Jianghan Plain, Central China. *Environmental Science: Processes & Impacts, 19*(2), 161–172.

El-Nahhal, Y., Tubail, K., Safi, M., & Safi, J. M. (2013). Effect of treated waste water irrigation on plant growth and soil properties in Gaza Strip Palestine. *American Journal of Plant Sciences, 4*(09), 1736–1743.

El-Nahhal, Y., Hamdona, N., & Alshanti, A. (2016). Toxicological data of some antibiotics and pesticides to fish, mosquitoes, cyanobacterial mats and to plants. *Data in Brief, 6*, 871–880.

El-Nahhal, Y. (2018). Toxicity of some aquatic pollutants to fish. *Environmental Monitoring and Assessment, 190*(8), 1–15.

FAO. (2016). Food and Agriculture Organization of United Nations, http://www.fao.org/news/story/en/item/421871/icode

Finn, R. N. (2007). The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. *Aquatic Toxicology, 81*(4), 337–354.

Islam, S. M. S., Alam, D. S., Wahiduzzaman, M., Niessen, L. W., Froeschl, G., Ferrari, U., & Lechner, A. (2015). Clinical characteristics and complications of patients with type 2 diabetes attending an urban hospital in Bangladesh. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 9*(1), 7–13.

Lemaire, P., Drai, P., Mathieu, A., Lemaire, S., Carriere, S., Giudicelli, J., & Lafaurie, M. (1991). Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (cholesterol, triglycerides) of sea-bass (*Dicentrarchus labrax*). *Aquaculture, 93*(1), 63–75.

Li, J., Luo, G., He, L., Xu, J., & Lyu, J. (2018). Analytical approaches for determining chemical oxygen demand in water bodies: A review. *Critical Reviews in Aanalytical Chemistry, 48*(1), 47–65.

Li, S., & Zhang, Q. (2010). Risk assessment and seasonal variations of dissolved trace elements and heavy metals in the Upper Han River. *China. Journal of Hazardous Materials, 181*(1–3), 1051–1058.

Maurya, P. K., Malik, D. S., Yadav, K. K., Kumar, A., Kumar, S., & Kamyab, H. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology Reports, 6*, 472–481.

Milošković, A., Dojčinović, B., Simić, S., Pavlović, M., & Simić, V. (2014). Heavy metal and trace element bioaccumulation in target tissues of three edible predatory fish species from Bovan Reservoir (Serbia). *Fresenius Environmental Bulletin, 23*, 1884–1891.

Mutlu, E., Demir, T., Kutlu, B., & Yanık, T. (2013). Determination of water quality parameters in Sivas-Kurugöl Lake. *Turkish Journal of Agriculture-Food Science and Technology, 1*(1), 37–43.

Mutlu, E., Kutlu, B., & Demir, T. (2016). Assessment of Çinarli Stream (Hafik-Sivas)'s water quality via physicochemical methods. *Turkish Journal of Agriculture-Food Science and Technology, 4*(4), 267–278.

Mutlu, E., Kutlu, B., Demir, T., & Yanik, T. (2018). Assessment of metal concentrations and physicochemical parameters in the waters of Lake Tecer. *Kastamonu University Journal of Forest Faculty, 18*(1), 1–10.

Rahman, M. S., Molla, A. H., Saha, N., & Rahman, A. (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka. *Bangladesh. Food Chemistry, 134*(4), 1847–1854.

Ranjitha, T., & Sharath Chandra, S. P. (2020). Biological and ecological impact of iron and iron nanoparticles across diverse array of fish models: A review. *Ecology, Environment and Conservation, 26*(1), 180–184.

Romano, N., Egnew, N., Quintero, H., Kelly, A., & Sinha, A. K. (2020). The effects of water hardness on the growth, metabolic indicators and stress resistance of largemouth bass Micropterus salmoides. *Aquaculture*, *527*, 735469.

Saleem, M., Iqbal, J., & Shah, M. H. (2014). Dissolved concentrations, sources, and risk evaluation of selected metals in surface water from mangla lake, Pakistan. *The Scientific World Journal*, *2014*.

Singh, U. K., Ramanathan, A. L., & Subramanian, V. (2018). Groundwater chemistry and human health risk assessment in the mining region of East Singhbhum, Jharkhand, India. *Chemosphere, 204*, 501–513.

Sula, E., Aliko, V., Barceló, D., & Faggio, C. (2020). Combined effects of moderate hypoxia, pesticides and PCBs upon crucian carp fish, Carassius carassius, from a freshwater lake-in situ ecophysiological approach. *Aquatic Toxicology*, *228*, 105644.

Ural, M. Ş, Parlak, A. E., & Alayunt, N. Ö. (2013). The comparison of some blood parameters of rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792) in different rearing habitats. *Journal of Science, 25*, 19–26.

USEPA, (2004). Risk Assessment Guidance for Superfund. In: Human Health Evaluation Manual (Part E), Supplemental Guidance for Dermal Risk Assessment, vol. 1. Office of Superfund Remediation and Technology Innovation, United States Environmental Protection Agency, Washington DC. Final EPA/540/R/99/005, OSWER 9285.7–02EP PB99–963312 July 2004.

USEPA, (2011). Regional Screening Level (RSL) Summary Table—November 2011. Available at http://www.epa.gov/region9/superfund/prg/

Wang, J., Zhao, X., Wu, A., Tang, Z., Niu, L., Wu, F., & Fu, Z. (2021). Aggregation and stability of sulfate-modified polystyrene nanoplastics in synthetic and natural waters. *Environmental Pollution*, *268*, 114240.

Wu, B., Zhao, D. Y., Jia, H. Y., Zhang, Y., Zhang, X. X., & Cheng, S. P. (2009). Preliminary risk assessment of trace metal pollution in surface water from Yangtze River in Nanjing Section, China. *Bulletin of Environmental Contamination and Toxicology, 82*(4), 405–409.

Yanbo, W., Wenju, Z., Weifen, L., & Zirong, X. (2006). Acute toxicity of nitrite on tilapia (*Oreochromis niloticus*) at different external chloride concentrations. *Fish Physiology and Biochemistry, 32*(1), 49–54.

Yetiş, R., Atasoy, A. D., Yetiş, A. D., & Yeşilnacar, M. İ. (2019). Hydrogeochemical characteristics and quality assessment of groundwater in Balikligol Basin, Sanliurfa. *Turkey. Environmental Earth Sciences, 78*(11), 1–17.