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Article *in* Pakistan Journal of Biological Sciences · July 2012

DOI: 10.3923/pjbs.2012.658.665 · Source: PubMed

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Pakistan Journal of Biological Sciences 15 (14): 658-665, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.658.665

The Trace Element Analysis in Freshwater Fish Species, Water and Sediment in Iyidere Stream (Rize-Turkey)

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Abstract: Many environmental problems like dam construction, agricultural debris, flooding and industrial establishments threaten Iyidere stream (Rize, Turkey) on the southeastern coast of the Black Sea (Turkey). The trace element concentrations in water, fish and sediments in Iyidere stream (Rize, Turkey) were investigated in this study. The concentration of six different elements in ten freshwater fish species and sediment was determined using energy dispersive X-ray fluorescence method. A radioisotope excited X-ray fluorescence analysis using the method of multiple standard addition is applied for the elemental analysis of fish and sediments. Water samples for trace metals were analyzed using standard spectrophotometry methods. A qualitative analysis of spectral peaks showed that ten different freshwater fish samples (*Chondrostoma colchicum*, *Chalcalburnus chalcoides*, *Salmo trutta labrax*, *Alburnoides bipunctatus*, *Leuciscus cephalus*, *Barbus taurus escherichia*, *Capoeta tinca*, *Neogobius kessleri*, *Rutilus frisii*, *Lampetra lanceolata*) and sediment contained phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca) and titanium (Ti). Heavy metals as toxic elements for biota (Pb, Cd, Hg, Zn and Mn etc.) were not detected in fish, water and sediments. Thus, It can be declared that freshwater fish of Iyidere does not contains health risks for consumers in terms of metal pollution.

Key words: Energy dispersive X-ray fluorescence, freshwater fish, trace element analysis, water quality, heavy metals

INTRODUCTION

Environmental pollution in aquatic habitats is a very common problem everywhere in today's world. Many pollutants like acids and alkalis, anions, detergents, gases, heat, metals, nutrients, organic toxic wastes, pathogens, pesticides, polychlorinated biphenyls and radionuclides can be found in freshwater ecosystems. Especially metals are one of the most important inorganic pollutants for freshwater ecosystem (Kaur, 2010; Dogra, 2008; Jadhav and Purohit, 2008). Metals introduced in freshwater ecosystem as a result of geological processes like soil and rocks erosion and volcanic eruptions, from human activities involving the mining and processing of metals or using substances that contains metal pollutants (Haygarth and Jarvis, 2002; Abel, 1996).

Metal pollution causes serious environmental damage because metals remain in bottom sediments for many years and are non-biodegradable. Metal pollutants or heavy metals are digested with food and can be accumulated in tissues of aquatic organisms. Metal pollutants are assimilated by organisms and can be stored into tissues of species. Many fish species can be considered as monitors for environmental contamination with metals and other pollutants in aquatic ecosystems (Bervoets and Blust, 2003). Metal concentrations in many fish species, sediment and water have been studying for determination of metal pollution in freshwater and marine ecosystems (Amundsen *et al*., 1997; Catsiki and Strogyloudi, 1999; Widianarko *et al*., 2000; Karadede and Unlu, 2000; Turkmen *et al*., 2005).

In most of heavy metal analysis in water, fish and sediment, the method of assessment is atomic absorption spectrometry (Wong *et al*., 2001; Farkas *et al*., 2002; Castro *et al*., 1999; Giusti, 2001; De Souza Lima *et al*., 2002; Tarley *et al*., 2001; Diagomanolin *et al*., 2004). In this

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study, it has been used energy dispersive X-ray fluorescence method in fish and sediment samples. EDXRF spectrometry is well recognized as a tool for the qualitative and quantitative determination of major and minor elements in a wide range of sample types. EDXRF's versatility stems from its rapid, non-destructive, multi-element determinations from ppm to high weight percent of elements from Sodium (Na) through Uranium (U). This method (EDXRF) can be used the analysis of vegetables, aerosols, waters, sediments, soils, solid waste and other environmental samples. The EDXRF was used for the determination of trace elements of different samples such as tobacco, red mud, lichens, cole, emboli, etc. (Tirasoglu *et al*., 2005; Aslan *et al*., 2004; Cevik *et al*., 2003).

EDXRF offers significant advantages for multi-element applications in the analysis of a wide range of organic materials, despite the better detection limit levels achieved by some competing techniques. Aquatical, geological, metallurgical and ceramical materials can be analyzed successfully with this system. EDXRF systems are particularly appropriate for the analysis of geological, environmental, metallurgical and ceramic materials. The technique offers rapid, nondestructive analysis of materials presented as solids, powders, particulates collected on filter substrates and liquids. EDXRF is capable of measuring the elemental concentrations of almost all the elements in the periodic table (by convention from Na to U) to detection limits that, under optimum conditions, fall below the mg kg^{-1} level (Cevik *et al*., 2003).

In this study, fish, sediment and water samples from Iyidere stream were analysed to determine if the metal contamination is in Iyidere stream in Rize (TURKEY). *Salmo trutta labrax* as an endangered species prefers Firtina and Iyidere stream for reproduction on Turkish Black Sea coasts (Celikkale *et al*., 1999). Hence, Iyidere stream on the northeastern coasts of Turkey, one of the entrances of the *Salmo trutta labrax* to freshwater for reproduction and has a very rich biodiversity, was investigated in terms of metal pollution.

MATERIAL AND METHODS

Sampling sites: Iyidere stream is located between cities of Rize and Trabzon (Fig. 1). The stream is about 160 km long and 20 m wide. Substrate of the stream consisted of rock and stone. The average flow speeds are about is 2 and 4 msec in lower and upper basins, respectively. In Iyidere stream, the most frequently and abundantly fish species are *Salmo trutta labrax*, *Leuciscus cephalus*, *Neogobius kessleri*, *Alburnoides bipunctatus*, *Barbus taurus escherichia*, *Rutilus frisii*, Chalcalburnus chalcoides, *Capoeta tinca*, *Lampetra lanceolata*, *Cobitis splendens*, *Mugil cephalus* and *Liza aurata*. On the other hand, Turan *et al*. (2003) reported that *Eudontomyzon mariae*, *Neogobius fluviatilis*, *Neogobius eurycephalus*, *Cobitis taenia* and *Barbus plebejus escherichia* which had been recorded in previous studies in Iyidere stream were no longer present in the study area in Rize, Turkey.

Four stations in the southern part of Rize city (North Eastern Black Sea Coasts of Turkey) were chosen

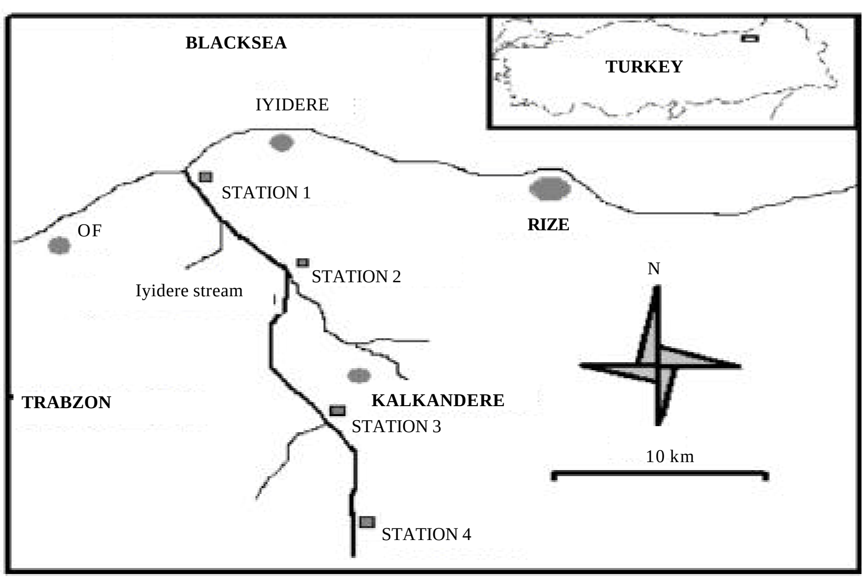

Fig. 1: A map showing the locations of Iyidere stream

as sampling sites for this biomonitor study. Stations of the study are Iyidere Kopru (Station 1), Kalkandere Yol Ayrimy (Station 2), Yokuslu (Station 3) and Guneyce villages (Station 4) (Fig. 1). Iyidere Kopru in the Iyidere province situated approximately 0.5 km south of Blacksea coast (latitude: 40°59' North, longitude: 40°19' East). Kalkandere at the 41 m altitude (latitude: 40°57' North, longitude: 40°23' East) and Yoku-lu (latitude: 40°53' North, longitude: 40°26' East, altitude: 141 m) are 10 and 20 km from the Black Sea coast, respectively. Guneyce village (latitude: 40°49' North, longitude: 40°28' East, altitude: 163 m) is mainly agricultural without industrial area, then this station was considered unaffected by anthropogenic emissions and suitable as a reference site.

Agricultural and industrial activity in the study area constitutes generally small scale industrial establishments, animal husbandry, tea plantation and tea processing factory. In the catchments area of Iyidere stream, there are 10 tea processing factory which has 929 tonnes/day production capacity (Anonymous, 2001).

Sample treatments: The fish species (*Chondrostoma colchicum*, *Chalcalburnus chalcoides*, *Salmo trutta labrax*, *Alburnoides bipunctatus*, *Leuciscus cephalus*, *Barbus taurus escherichia*, *Capoeta tinca*, *Neogobius kessleri*, *Rutilus frisii*, *Lampetra lanceolata*) were collected by electrofishing from Iyidere stream in Rize, Turkey. Ten fish samples for every species in all stations, four sediment and four water samples from each station were taken to the analysis.

The collected fish and stream bed sediment samples were transferred to the laboratory and washed with distilled water, dried in filter paper, homogenized, packed in polyethylene bags and stored below -20°C prior to analysis. Stream bed sediment samples were collected from undisturbed, continuously wetted and depositional zones of stream channel using a stainless steel dredge. Sediments were dried in an oven at 50°C for 48 h. About 200 mg of each sample was digested with HNO_3, HF and H_2O_2. Fish samples were dissected at the laboratory of the faculty. The whole body tissue of fish samples were taken to the analysis. The samples were heated to 55°C for 24 h and dried to constant weight. After homogenisation and digestion process of the fish and sediment samples, 50 mg powder of the each sample were used in the EDXRF measurements.

Quantitative elemental analysis of the fish and sediment samples was carried out with Energy Dispersive X-ray Fluorescence system. In order to describe the elemental composition in this arrangement, one radioactive source 1.85 GBq ^{55}Fe was used for direct excitation. The excitation energy is 5.96 keV for ^{55}Fe. ^{55}Fe radioisotope was used to obtain light and intermediate elements. Samples positioned according to the geometry of Fig. 2. The samples were analyzed in the form of pellets to obtain their characteristic X-ray spectra and the spectra were recorded with a PGT Si(Li) detector (FWHM = 160 eV at 5.9 keV, active area 13 mm^2, thickness 3 mm and Be window thickness = 30 μm) was used for element K_α and K_β lines measurement. The output from the preamplifier, with pulse pile-up rejection capability, was fed to a multi-channel analyzer interfaced with a personal computer provided with suitable software for data acquisition and peak analysis. The live time was selected to be 5000 sec for all elements. The samples were placed at 45° angle with respect to the direct beam and fluorescent X-rays emitted 90° to the detector.

Qualitative analysis of spectral peaks showed that the samples contained phosphorus, sulfur, chlorine, potassium, calcium and titanium. A representative example of a spectrum is given in Fig. 3 for elements excited by the ^{55}Fe radioactive source. Quantitative analysis for these elements was carried out using the method of multiple standard additions. In this method, certain amounts of the element to be analysed, called analyte, are added to samples (Cevik *et al*., 2003). In order to minimize the absorption effect, the SK_α/MnK_α intensity ratio was used instead of SK_α intensity obtained from ^{55}Fe.

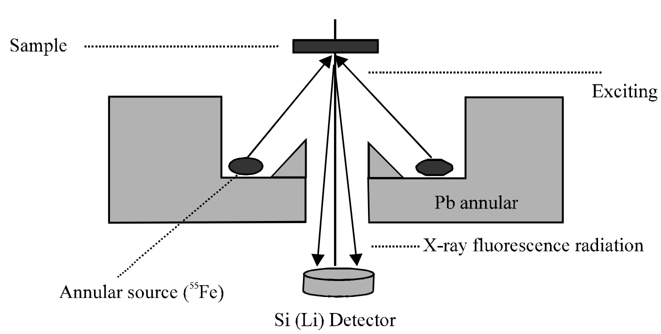

Fig. 2: Geometry of experimental setup (EDXRF system)

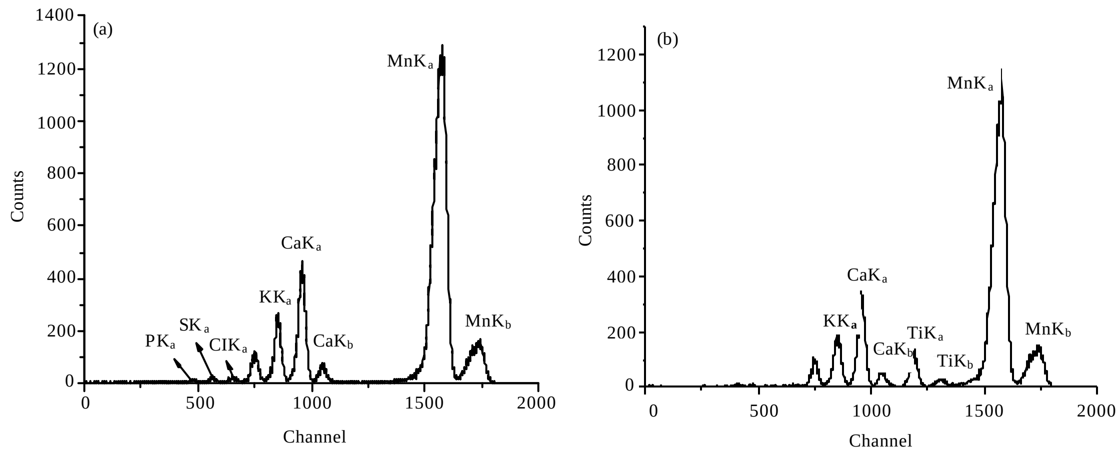

Fig. 3(a-b): Spectrum of (a) *Leuciscus cephalus* sample and (b) Sediment sample obtained with excitation by a ^{55}Fe radioactive source

Water samples were collected using polyethylene acid-washed containers according to Mackereth *et al.* (1989). The containers were rinsed with 5% nitric acid and distilled water and were washed with river water before sampling. Water samples were passed through Whatman glass micro fibre filters (GF/C). Samples were acidified with 1-2 mL of concentrated nitric acid (pH<2). The samples were transferred to the laboratory according to standard methods (APHA, AWWA, WEF, 1985). Water analysis for heavy metals in Iyidere was carried out with spectroquant NOVA30 photometer (Merck). In spectroquant photometric method, the component of a sample to be analysed is converted into a coloured compound in a specific reaction. It uses photometric test kits or cell tests. Some cell tests already contain all the necessary reagents in one and the same cell and the sample must merely be added using pipette. In other tests, it is necessary to separate the test into two or three different reagent mixtures. In the spectroquant NOVA 30 photometer, there are six narrow-band interference filters and photodiodes in array technology that take care of the measurements. This method contains 73 different cell tests to analysis water/wastewater and they have been approved by the American Environmental Protection Agency for the analysis of drinking water and waste water (ISO 8466-1 and DIN 38402 A 51).

Other physicochemical properties (pH, HCO_3, CO_2, BOD_5, Ca, Mg, total hardness, nitrite, amonium, phosphate, suspended solids and alkalinity etc.) of water samples were analysed according to standard methods (APHA, AWWA, WEF, 1985). Analysis of water samples for heavy metals and physicochemical properties was carried out along the year for four stations (Fig. 1).

Statistical analysis: In the determining of this study's results, the data were statistically analyzed using basic statistical parameters, analysis of variance (ANOVA) technique and means were compared using Duncan's multiple range test using SPSS statistical software package.

RESULTS AND DISCUSSION

The results of the trace element analysis of ten different freshwater fish species and sediment samples from Iyidere stream are presented in Table 1. Heavy metal concentrations in fish species and sediments were not detected but fish and sediment samples contained P, S, Cl, K, Ca and Ti elements. These elements are known as essential for life and present in all animal tissues. *L. cephalus* have the highest Ca and P concentrations. The K element concentrations in *C. colchicum* and sediment sample are higher than other fish species. The S element concentrations in *Neogobius kessleri* and *Rutilus frisii* are also higher than other fish species and sediment sample. Sediment sample has the Ca, K and Ti elements. The Ti concentration is detected only in sediment sample but in fish species are not. According to analysis of variance, there were significant differences ($p<0.05$) between element concentrations in different species. It could not have any comparison between this study's results and other studies due to no study about metals in water, biota and sediment for Iyidere stream. On the other hand, this study's results were checked for international standards in term of water, food and sediment materials.

Ca is a major element for freshwaters and is found in every terrestrial area in the world. It is necessary for organisms for creating their skeleton, tooth and shell.

Table 1: The concentration of trace elements in freshwater fish and sediment from Iyidere stream ($\mu g\ g^{-1}$) dry weight

	Trace elements ($\mu g\ g^{-1}$) dry wt.					
Fish species	P	S	Cl	K	Ca	Ti
Chondrostoma colchicum	12.40±0.24	1.60±0.06	3.40±0.10	49.60±2.97	6.70±0.18	nd
Chalcalburnus chalcoides	16.70±0.30	1.80±0.08	3.50±0.11	36.40±2.18	6.60±0.19	nd
Salmo trutta labrax	14.40±0.23	4.70±0.16	5.30+0.16	39.40±2.25	4.80±0.15	nd
Alburnoides bipunctatus	15.30±0.29	5.10±0.17	3.70±0.13	26.70±1.53	11.90±0.36	nd
Leuciscus cephalus	2.30±0.06	5.10±0.18	4.50±0.15	29.60±1.60	56.00±1.68	nd
Barbus taurus escherichia	13.10±0.22	3.20±0.12	3.60±0.12	22.50±1.36	10.20±0.30	nd
Capoeta tinca	8.90±0.15	2.40±0.10	2.40±0.08	15.40±1.10	4.70±0.14	nd
Neogobius kessleri	7.50±0.14	10.10±0.36	3.90±0.14	30.40±1.63	6.70±0.18	nd
Rutilus frisii+	18.70±0.31	7.70±0.22	3.40±0.10	31.60±1.68	15.30±0.44	nd
*Lampetra lanceolata**	0.60±0.01	2.30±0.09	1.10±0.04	28.00±1.55	nd	nd
Sediment sample	nd	nd	nd	28.30±1.59	18.80±0.56	6.20±0.13

Nd: Not detected, *The species contains maximum element concentration statistically, +The species contains minimum element concentration statistically

Table 2: Heavy metal concentration of water samples from Iyidere stream

	Concentrations ($mg\ L^{-1}$)					
					WQC for freshwater	
Elements (Detection limits; $mg\ L^{-1}$)	Upper	Mean	Lower	SD	TWPCR	USEPA
Pb (0.10-5.00)	UDL	UDL	UDL	-	0.050	0.065
Zn (0.20-5.00)	UDL	UDL	UDL	-	0.200	0.120
Fe (0.05-4.00)	0.05	0.045	0.00	0.026	1.000	1.000
Ni (0.10-6.00)	0.01	0.01	0.00	0.006	0.200	0.470
Cd (0.025-1.00)	UDL	UDL	UDL	-	0.005	0.002
Cu (0.05-8.00)	UDL	UDL	UDL	-	0.020	0.013
Mn (0.10-5.00)	UDL	UDL	UDL	-	0.100	0.100

TWPCR: Turkish water pollution control regulation water quality criteria for freshwater, USEPA: United States environment protection agency water quality criteria for freshwater, UDL: Under detection limits, WQC: Water quality criteria

Table 3: Physicochemical water quality of Iyidere stream

		Parameters								
Stations	Value	Water temp. (°C)	DO ($mg\ L^{-1}$)	O_2 saturation (%)	BOD ($mg\ L^{-1}$)	Conductivity ($\mu S\ cm^{-1}$)	Salinity (‰)	Flow rate ($m\ sec^{-1}$)	SS ($mg\ L^{-1}$)	pH
ST-1	Mean	8.30	8.58	55.89	2.64	70.11	0.06	0.07	26.56	7.61
ST-2		8.80	11.26	82.84	1.69	72.33	0.07	2.65	18.29	7.55
ST-3		6.29	11.95	80.97	2.41	45.00	0.00	2.52	16.61	7.48
ST-4		5.37	12.54	84.69	2.80	42.87	0.00	3.30	8.33	7.34
ST-1	Upper	11.80	11.00	91.30	4.00	82.50	0.10	0.50	43.00	8.58
ST-2		13.50	12.30	102.30	3.60	77.40	0.10	3.60	34.00	8.18
ST-3		9.40	12.70	98.50	4.20	51.40	0.00	5.20	31.70	8.24
ST-4		8.70	14.20	114.50	4.00	49.20	0.00	6.70	17.00	8.21
ST-1	Lower	6.00	4.90	17.50	0.80	48.50	0.00	0.00	2.00	6.61
ST-2		6.50	10.00	90.00	0.40	58.20	0.00	2.10	2.40	6.70
ST-3		4.20	10.30	87.00	0.65	36.60	0.00	1.35	2.70	6.50
ST-4		3.30	9.60	84.00	0.80	34.60	0.00	1.60	2.00	5.78
		Parameters								
	Value	Alkalinity ($mg\ L^{-1}$)	Ca^{++} ($mg\ L^{-1}$)	Mg^{++} ($mg\ L^{-1}$)	HCO_3 ($mg\ L^{-1}$)	CO_2 ($mg\ L^{-1}$)	TWH ($mg\ CaCO_3\ L^{-1}$)	NH_4 ($\mu g\ L^{-1}$)	NO_2 ($\mu g\ L^{-1}$)	PO_4 ($\mu g\ L^{-1}$)
ST-1	Mean	8.30	8.58	55.89	2.64	70.11	0.06	0.07	26.56	7.61
ST-2		8.80	11.26	82.84	1.69	72.33	0.07	2.65	18.29	7.55
ST-3		6.29	11.95	80.97	2.41	45.00	0.00	2.52	16.61	7.48
ST-4		5.37	12.54	84.69	2.80	42.87	0.00	3.30	8.33	7.34
ST-1	Upper	11.80	11.00	91.30	4.00	82.50	0.10	0.50	43.00	8.58
ST-2		13.50	12.30	102.30	3.60	77.40	0.10	3.60	34.00	8.18
ST-3		9.40	12.70	98.50	4.20	51.40	0.00	5.20	31.70	8.24
ST-4		8.70	14.20	114.50	4.00	49.20	0.00	6.70	17.00	8.21
ST-1	Lower	6.00	4.90	17.50	0.80	48.50	0.00	0.00	2.00	6.61
ST-2		6.50	10.00	90.00	0.40	58.20	0.00	2.10	2.40	6.70
ST-3		4.20	10.30	87.00	0.65	36.60	0.00	1.35	2.70	6.50
ST-4		3.30	9.60	84.00	0.80	34.60	0.00	1.60	2.00	5.78

DO: Dissolved oxygen, SS: Suspended solids, TWH: Total water hardness

Table 4: The comparison of present work results and Turkish and UN European Economy Commission and European Union (EU 74/440/EEC) water quality standards for fresh water

		Water quality standards TR			
		Water quality class		Drinking water	
Parameters	Present work results (Mean)	TR	UNECE	TR	EU
Water temperature (°C)	7.2	I	I	Accepted	12-25 Accepted
pH	7.5	I	I	≥6,5 ve ≤9,5 Accepted	6,5-8,5 Accepted
DO (mg L^{-1})	11.1	I	I	-	-
Oxygen saturation (%)	76.1	I	I	-	-
BOD_5 (mg L^{-1})	2.4	I	I	<3,5 Under limit	-
NO_2-N (mg L^{-1})	0.007	II	I	<0,5 Under limit	<0,1 Under limit
NH_4-N (mg L^{-1})	0.008	I	I	<0,50 Under limit	<0,05-0,5 Under limit
PO_4-P (mg L^{-1})	0.022	II	I	-	-

TR: Turkey, UNECE: United nations European economic commission, EU: European union, (Anonymous, 2004), UNECE (UNECE, 1994), EU (Soylak and Dogan, 2000), I: Very clean, II: Clean, III: Little polluted, IV: Polluted

Minor elements (>100 μg g^{-1}) as K, Cl, P and S) have limited value since they have many physiological roles for the animals. Ti elements have no environmental effects and has low toxicity on biota.

In our measurements maximum relative errors due to the counting system were of the order 2-6%. Errors originating from sample weighing, source intensity and system geometry were about 4%. The combined relative error in our results was according to the order of 5-8%. The concentration of trace P, S, Cl, K, Ca and Ti with this method provided a simple, accurate and reliable method for EDXRF determination. As has been shown, EDXRF spectrometry is a useful tool for qualitative and quantitative analysis of a wide variety of samples. Therefore, it presents some advantage over other spectrometric methods.

On the other hand, water quality and heavy metal concentrations of water samples from Iyidere stream are in Table 2. In the water analysis of Iyidere stream, it has been shown that the heavy metal concentration is not more than TWPCR (Turkish Water Pollution Control Regulations) and USEPA (United States Environmental Protection Agency) criterion value of fresh water. The heavy metal concentrations are below minimum detection limits and also suitable for TWPCR and USEPA criterion value of drinking water and freshwater sources.

On the other hand, the results of other physicochemical properties of Iyidere stream show that the average water temperature 7.20°C, BOD_5 2.40 mg L^{-1}, pH 7.50, electrical conductivity 57.60 μS cm^{-1}, dissolved oxygen 11.10 mg L^{-1} and flow speed 2.10 m sec^{-1} (Table 3). In this study, it compared the results of this work results with international water quality standards (Table 4). According to the results of heavy metal analysis of water samples from Iyidere stream, no heavy metal pollution is in Iyidere stream. If the water quality of Iyidere stream was determined by regulations compiled by TWPCR (Turkish Water Pollution Control Regulations) and UNECE (United Nations of Economy Commission of Europe) for preventing of freshwater aquatic life, Iyidere stream has a first quality water standard (very clean). And It was acceptable according to the drinking water standards of Turkey (17.02.2005/25730) and EU (75/440/EEC) was determined (Table 4). Hence, Iyidere stream water can be used in aquaculture, farming, recreational projects and as a drinking water source.

CONCLUSION

In the investigation of trace elements by the EDXRF method in the ten different fish species and sediment sample from Iyidere stream, six different elements were detected. Phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca) and titanium (Ti) elements are contained in the sample of fish and sediments. No heavy metal concentration in fish and sediment was determined by EDXRF method. The concentrations of trace elements in the fish and sediment from Iyidere stream were clearly below local and International Standards compiled USEPA. Hence, freshwater fish of Iyidere stream does not constitute health risks for human consumers. In water sample, heavy metal concentrations are below the detection limits. According to these results, the Iyidere stream has not got metal or inorganic pollution and it can be keep the natural conformation in terms of food web. Though tea plantation and factories in the Iyidere catchment area, any risk about metal pollution has not been occurring for a long time. But the catchment area of Iyidere stream has a very important potential for civilization, industrial establishment and agricultural developments. That's why, the environmental pollution will effect on aquatic ecosystem of Iyidere stream in near future with a great possibility. Measurements for controlling of environmental pollution on aquatic ecosystem should have done nowadays.

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