# Assesment of trace elements in rainbow trout (Oncorhynchus mykiss) cultured in the marine aquaculture cages on the Black Sea Coast

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# ASSESMENT OF TRACE ELEMENTS IN RAINBOW TROUT (*Oncorhynchus mykiss*) CULTURED IN THE MARINE AQUACULTURE CAGES ON THE BLACK SEA COAST

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## SUMMARY

The concentrations of trace elements were determined in various tissues (internal organs, liver, abdominal and dorsal muscle) of individuals of cultured fishes obtained from two marine cage farms located on the coasts of Rize, Turkey. The concentrations of seven trace elements (sulphur (S), chloride (Cl), potassium (K), calcium (Ca), iron (Fe), barium (Ba), and tin (Sn) were determined in ten specimens (5 from each farm) using energy dispersive X-ray fluorescence method. A radioisotope excited X-ray fluorescence analysis using the method of multiple standard additions is applied for the elemental analysis of fish. The results obtained from these analyses showed similar patterns of distribution among farms and organs. The averages of trace elements did not statistically differ among farms, but did differ among the organs. Regardless of organs, K (1.64 %) was the highest followed by Cl (1.64 %), Fe (0.57 %), S (0.48 %), and Ca (0.20 %). The other trace elements (Sn and Ba) regarded as heavy metals with Fe had the lowest concentrations in fish. The results also showed that the liver accumulated the highest concentrations of all elements combined (0.48 %) followed by dorsal and abdominal muscle, and interior organs. Concentrations of K (2.35 %), Cl (2.27 %), S (1.22 %), and Fe (0.85 %) in liver were much higher than the concentrations of the other elements. Accumulations of Sn and Ba as heavy metals, on the other hand, were the lowest in liver with averages of 0.03 $\mu gg^{-1}$ and 0.00 $\mu gg^{-1}$, respectively. Despite the variability in concentrations of the heavy elements, concentrations of those elements in the tissue samples are not higher than the toxic level.

**KEYWORDS:** Freshwater fish, trace elements, aquaculture, rainbow trout, heavy metals.

## INTRODUCTION

Environmental pollution in aquatic habitats is one of the biggest problems. The problem is getting more and more important due to the fact that fish dairy products are consumed much since they contain almost all kinds of proteins, biological important lipids, and lip soluble vitamins. On the other hand, fish can also be a source of contamination, because of the amounts of trace elements they may contain. Thus, fishes can be considered as monitors for environmental contamination with metals and other pollutants in aquatic ecosystems [1]. Concentrations of trace elements have been widely studied in many fish species, sediments and waters all around the world [2-8].

The increase in consumption of fish as food has unfortunately diminished some fish stocks, which has stimulated a new area of industry, aquaculture, in order to meet the demand. Among the fish species, the trout is the most widely cultured fish species in both freshwater and saltwater throughout the world. Despite the importance of this fish species for human consumption, the degree of accumulation of heavy metals in the tissues of this species reared in cages is known little. Knowing the level of heavy elements concentrations in tissue of fishes is very important in areas where industry has been developed. There are more than one hundred tea processing factories (Salarha stream) and a copper mine (Çayeli stream) in the area where the farms providing fish specimens for this current study [9]. The waste of these factories is deposited to the sea through the Salarha Stream. Thus, the determination of the magnitude of heavy elements concentration will allow us to assess the potential danger of accumulation to the human organism. The objective of this study is to determine the concentration of some trace elements including three heavy elements (Fe, Sn, and Ba) in the tissues of cultured rainbow trout.

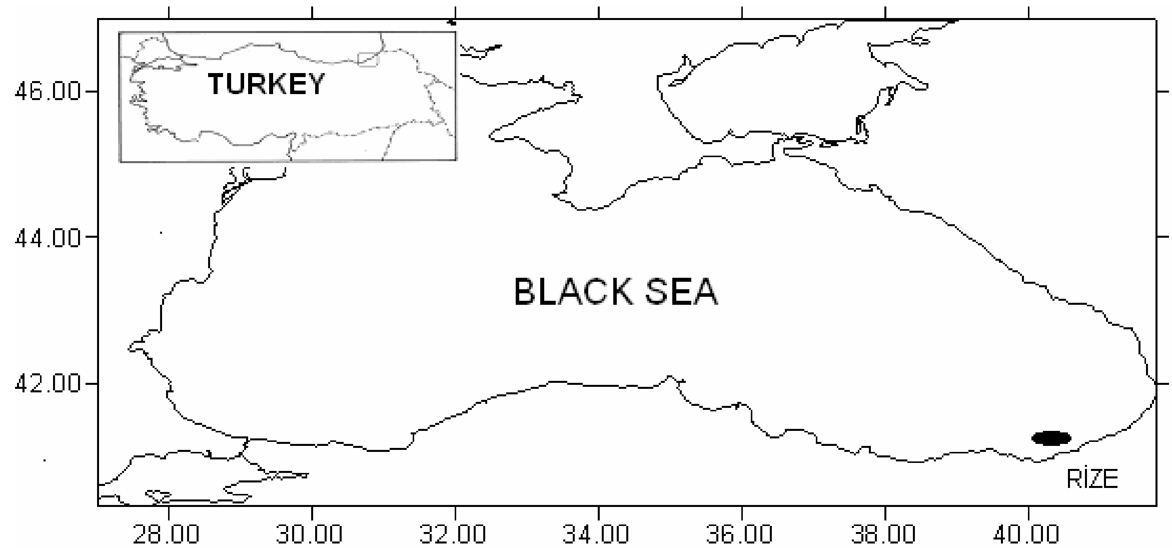


**FIGURE 1 - Location of the coast of Rize, Turkey.**

## MATERIAL AND METHODS

### Fish collection and experimental procedure

The specimens of rainbow trout (*Oncorhynchus mykiss*) for analysis were obtained from two marine aquaculture farms (namely Dortfarm and Ozfarm) located on the coasts of Rize, a city of Turkey located on the coasts of Black Sea (Fig.1). These farms have also freshwater fish rearing facilities located near to the Salarha stream. In order to prevent fish loss due to high temperatures during summer, these farms transfer their fish to those freshwater facilities.

The obtained samples of specimens were brought to a laboratory in the Faculty of Fisheries at the University of Rize. Then the samples were washed with distilled water, dried in filter paper, homogenized, packed in polyethylene bags and stored below -20 °C prior to analysis. Quantitative elemental analysis of the fish samples (50 mg dried weight) was done with an Energy Dispersive X-ray Fluorescence system. In order to describe the elemental composition one radioactive source (1.85 GBq $^{55}$Fe) was used for direct excitation. The excitation energy for $^{55}$Fe is 5.96 keV. $^{55}$Fe radioisotope was used to obtain light and intermediate elements. The samples were 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=160eV at 5.9 keV, active area 13 mm$^2$, thickness 3 mm and Be window thickness=30 μm) used for element $K_\alpha$ and $K_\beta$ 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 s for all elements. The samples were placed at 45° angles with respect to the direct beam and fluorescent X-rays emitted 90° to the detector.

Quantitative analysis for these elements was carried out using the method of multiple standard additions [10]. In this method, certain amounts of the element to be analyzed, called analyte, are added to samples. This method was explained in an earlier paper [10]. In order to minimize the absorption effect, the $SK_\alpha/MnK_\alpha$ intensity ratio was used instead of $SK_\alpha$ intensity obtained from $^{55}$Fe.

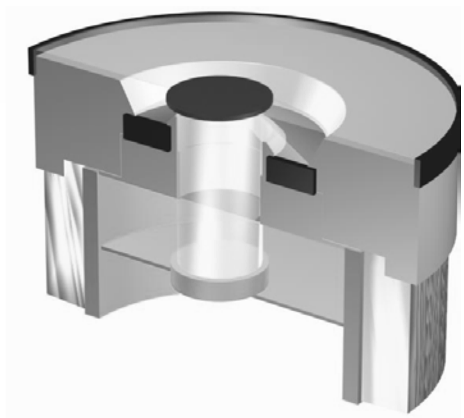

**FIGURE 2 - The geometry of the experimental setup.**

### Statistical analysis

A two-way ANOVA was used to test for significant differences in concentrations of each element among farms and organs. Prior to analysis, the data were subjected to normality (Kolmogorov-Smirnov test) [11] and homogeneity of variances tests (Levene's test) [12]. All the data except Ca met the assumptions of normality and homogeneity of variances. Concentrations of Ca were normalized with log transformations. When a significant difference ($P<0.05$) for main effects (farms and organs) was detected, Student Newman-Keul (SNK) multiple comparison test [13] was used to test for significant mean differences.

## RESULTS AND DISCUSSION

Qualitative analysis of spectral peaks showed that the samples contained sulphur, chlorine, potassium, calcium, iron, tin and barium (Fig. 3 and 4). A representative example of a spectrum is given in Fig. 3 for elements excited by the $^{55}$Fe radioactive source and Fig. 4 for elements excited by the $^{241}$Am radioactive source.

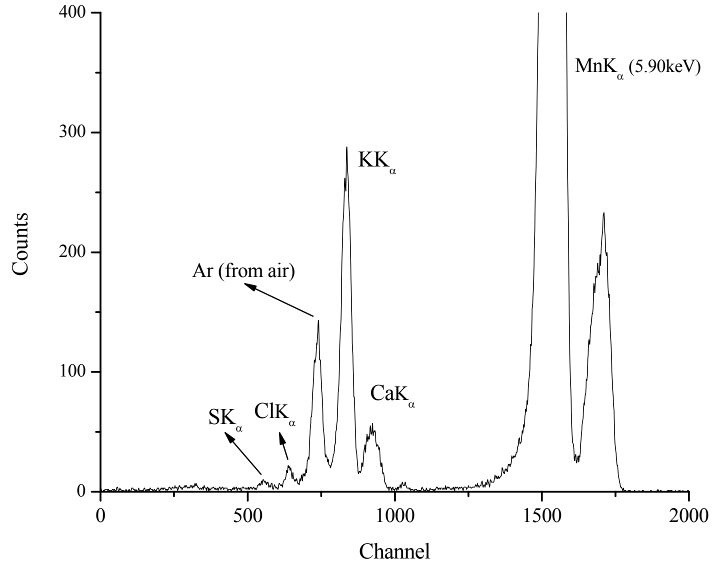


**FIGURE 3 - The XRF spectrum of one sample recorded with the $^{55}$Fe source and Si (Li) detector.**

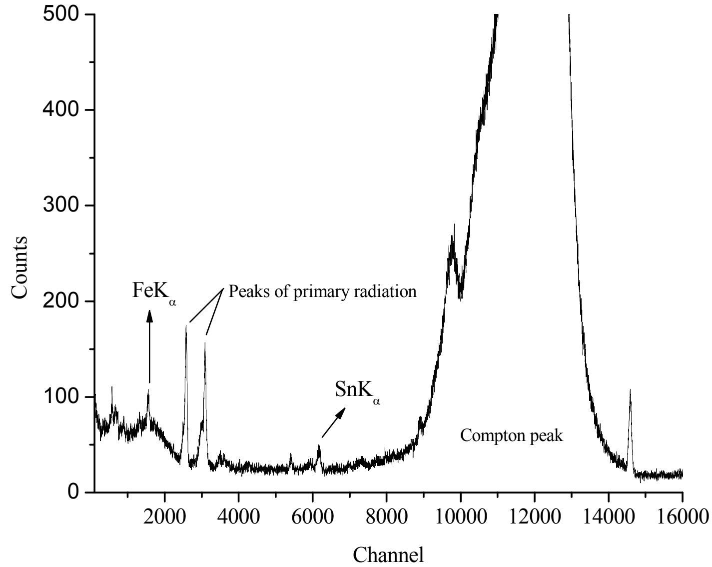


**FIGURE 4 - The XRF spectrum of one sample recorded with the $^{241}$Am source and Si (Li) detector.**

Percent concentrations of seven elements which are known to be essential to life and to be present in all animal tissue, e.g., K, Ca, Fe, S, Cl, Ba, and Sn were determined to be lower than the toxic level. The mean concentrations of elements pooled across farms and organs were determined as K (1.64%), Cl (1.14%), Fe (0.57%), and S (0.48%), Ca (0.20%), Sn (0.01%), and Ba (<0.01%). The differences in mean concentrations of pooled data were highly significant (P<0.001) (Fig. 5 and 6).

The concentrations of K pooled across farms and tissue were marginally significant than the other elements (P<0.001). Similarly, the concentrations of Cl were significantly lower than the concentrations of K and higher than the rest of the elements measured. The concentrations of Fe and S did not differ significantly (P<0.001). The remaining elements (Ca, Sn, and Ba) did not significantly higher than each other (P<0.001). The obtained results from this study agree with the findings of Carvalho et al. (2005) [14]. They found high concentrations of K in edible parts of various fish species captured from the natural environment. However, the concentrations of K measured in edible parts (abdominal and dorsal muscle) were marginally higher than their results. The higher potassium concentrations measured in this study may be attributed to the high potassium levels of the fish food. A closer examination of K in various tissues of fishes revealed that edible parts (dorsal and abdominal muscle) had higher K concentrations (Table 1).

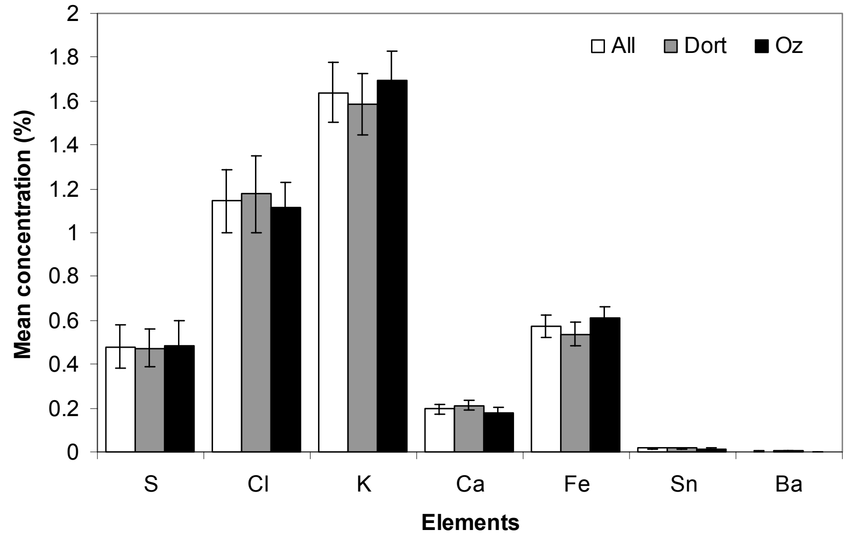


**FIGURE 5 - Mean percent concentration of elements pooled across tissue (bars ± S.E).**
(All = Dortfarm+Ozfarm; Dortfarm = Dortmevsim marine fish farm; Ozfarm = Özkafes marine fish farm)

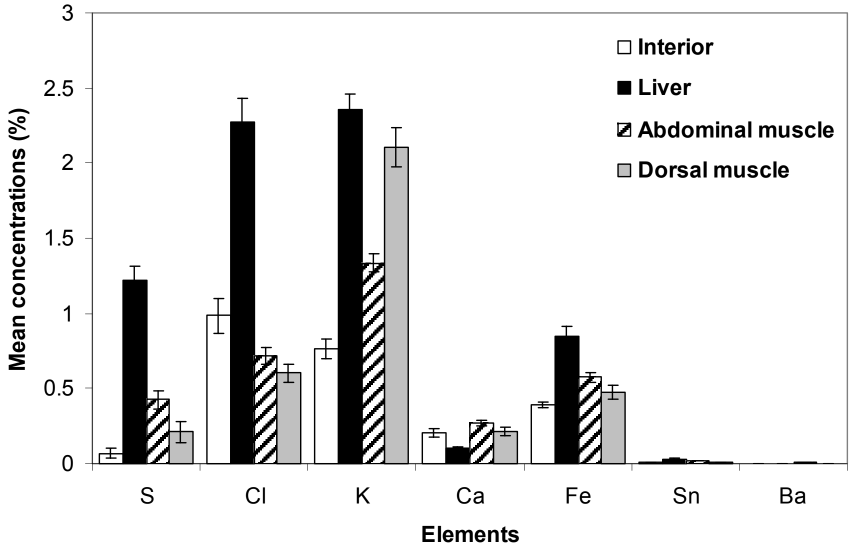


**FIGURE 6 - Mean percent concentration of elements pooled across farms (bars ± S.E).**

**TABLE 1 - Mean trace element concentrations in Rainbow trout cultured the marine fish farms on the Rize coasts.**

| Element (µg/g dw) | Dort Mevsim Marine Fish Farm (Dortfarm) | | | | Özkafes Marine Fish Farm (Ozfarm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Internal organs | Liver | Abdominal muscle | Dorsal muscle | Internal organs | Liver | Abdominal muscle | Dorsal muscle |
| S | 1.33±1.30 | 9.07±3.05 | 4.38±1.87 | 4.21±2.59 | ND | 15.36±1.05 | 4.13±1.72 | ND |
| Cl | 8.08±1.64 | 26.07±5.87 | 7.00±1.68 | 5.75±1.42 | 11.59±4.27 | 19.24±1.51 | 7.39±1.53 | 6.27±1.90 |
| K | 7.09±0.90 | 22.49±4.70 | 11.32±1.75 | 21.48±2.36 | 8.19±2.70 | 23.34±2.94 | 15.55±1.83 | 20.67±5.02 |
| Ca | 3.56±0.67 | 0.83±0.33 | 2.64±0.27 | 1.48±0.62 | 0.52±0.31 | 1.17±0.15 | 2.70±0.68 | 2.78±0.80 |
| Fe | 4.00±0.57 | 3.86±1.89 | 4.98±0.81 | 3.56±1.40 | 3.86±0.55 | 8.13±2.00 | 6.50±1.13 | 5.99±1.14 |
| Sn | 0.12±0.03 | 0.12±0.03 | 0.18±0.03 | 0.06±0.02 | 0.12±0.04 | 0.27±0.05 | 0.15±0.04 | 0.09±0.03 |
| Ba | ND | ND | 0.042±0.034 | ND | ND | ND | 0.019±0.017 | ND |

N.D.: Not detected, d.w.: dry weight

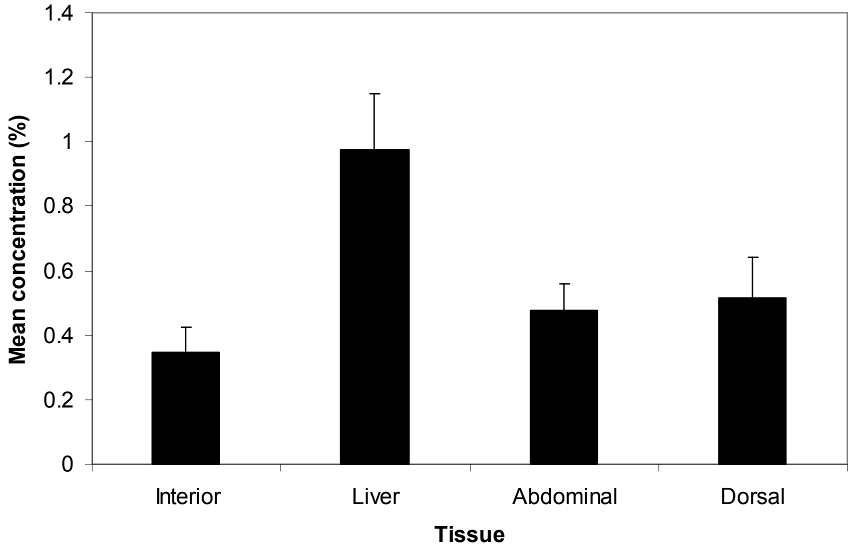


**FIGURE 7 - Mean percent accumulation of all elements pooled across farms and elements (bars ± S.E).**

Although the concentrations elements pooled across elements and organs were slightly higher in the *Oz*farm (0.59%) than in the *Dort*farm (0.57%), the differences were not statistically significant (Fig. 5 and 6). Moreover, the concentrations of each element in each tissue were not statistically significant among the farms ($P>0.05$). This was an expected result because both farms are very close to each other and feed the fish with similar food containing the approximately same amount of elements (Fig. 5).

The results of concentrations of trace elements determined in pooled liver, abdominal and dorsal muscles as well as in internal organs are displayed in Figure 6. Although some exceptions exist, generally the levels of trace elements in liver, irrespective of source of sample, were higher than in the rest of the tissue (i.e. abdominal and dorsal muscle, and interior organs). The concentrations of K, whose concentration was measured to be the highest among the elements, were significantly higher in liver and dorsal muscle than those in other tissue ($P<0.0001$). The high level of K, together with some other elements, in liver could be attributed to the functions of liver. Liver is the principal organ responsible for the detoxification, transformation, and storage of toxic materials and it is an active site of pathological effects induced by contaminants [15].

Concentrations of heavy metals measured in tissues of rainbow trout were determined to be low (0.016% and 0.002% for Sn and Ba, respectively). The concentrations of these metals did not change significantly among the farms ($P=0.655$ and $P=0.131$ for Sn and Ba, respectively) (Fig. 5 and 6). The concentrations of Sn, however, significantly varied among the organs. The concentrations of Sn in liver (0.03%) were, as expected, much higher than those in other tissues.

The higher concentration in liver is again due to its physiological duty. Liver is the principal organ responsible for the detoxification, transformation, and storage of toxic materials and it is an active site of pathological effects induced by contaminants. The concentrations measured in various tissues were below the toxic level. In spite of the lower levels of these metals in fish tissue, their toxicity, persistence, and bioaccumulation nature make these metals important components of pollution threatening life.

Iron, which is one of the essential elements for fish nutrition, did not vary among the farms, but varied among the tissues. The highest and lowest concentrations of iron in tissue ranged from 0.085% (liver) to 0.039% (internal organs) (Fig. 6 and 7). The concentration in liver was significantly higher than those in other tissues (P=0.045). The measured iron concentration in all tissues combined ranged from 4 to 6 µg $g^{-1}$. This was well below the values obtained from other fish species. For example, determining the concentrations of various elements in edible parts of several marine fishes, Carvalho et al. [14] found that iron ranged from 6 to 106 µg $g^{-1}$. They related this discrepancy to the fact that the Fe content is dependent on species, individuals, and sampling period. They also found out that fish feeding on the bottom leaded to higher concentration of Fe than in pelagic fish, which suggested that high concentrations could be a function of individual fish diet. Almost the same concentrations of iron measured in each farms suggested that the major Fe pathway was probably via feeding (Table 1).

Chloride was the second most abundant ion in tissues from which concentrations were determined. Chloride concentrations varied significantly among tissue (P>0.001), but did not vary among farms (P=0.763). The liver, again, had the highest concentration (2.42%) followed by internal (0.98%), abdominal muscle (0.72%), and dorsal muscle (0.60%) (Fig. 5-7). The highest chloride ion concentration in liver reflects its concentration in blood. Chloride ions constitute more than half of the ionic composition of the blood. Therefore, the high concentration of chloride in liver could be related to chloride ion in the blood. In addition, chloride ion could have been uptaken from the sea water through osmoregulation progress.

Ca is one of the major elements widely found in various habitats including freshwater saltwater as well as terrestrial. Calcium serves mainly building materials for both plants and animals in their skeleton, teeth, the egg's shell. Calcium is also one of the main elements circulating throughout the body with blood. The calcium concentration like other elements did not significantly vary among farms (P=0.375), but significantly changed among tissues (P=0.027) (Fig. 5-7). Unlike other elements, the calcium concentration in liver was lower than those in the other tissues. The highest concentrations were measured in abdominal and dorsal muscle. The high concentrations in muscle could be attributed to the fact that calcium is the primary element in contraction and relaxation of muscles.

## CONCLUSIONS

Measured elements concentrations in four different tissues of cage-cultured rainbow trout were under the toxic level set by both local (Turkish Water Pollution Protection & Control Regulations [16], Turkish Fisheries Regulations [17]) and international (United States Food & Drug Agency [18], European Union Directives [19]) regulations. Therefore, it seems that cultivation of fish in these waters where many tea processing factories operate is found to be safe at the moment. However, building new factories and any other facilities may increase the level of these elements in fish tissues.

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