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# Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (*Oncorhynchus mykiss*) using ICP-OES technique

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## ABSTRACT

The objective of this research was to determine the differences between farmed and wild rainbow trout in terms of heavy metal and trace element accumulation in edible tissues. The samples were analyzed for As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr and Zn by inductively coupled plasma-optical emission spectrometry (ICP-OES); and for Hg by cold vapor atomic absorption spectrometry (CVAAS). The results were expressed as µg/g of dry weight. With the exception of Ba and Sr, liver had significantly higher heavy metal and trace element concentrations compared to the muscle in farmed or wild fish. Higher levels of Ba, Cr, Fe, Mn and Zn, as well as lower levels of Cu and Sr were found in tissues of wild rainbow trout compared to its farmed relative. Levels of Cd in 41.6% of farmed fish samples and 45.8% of wild fish samples exceeded the European Commission regulation. Regarding the Pb, concentrations in 50% of farmed fish samples and 62.5% of wild ones were above the European Commission limit. However, levels of Hg and As in all of the examined samples were lower than the legislated limits. The differences in heavy metal and trace element accumulation observed between farmed and wild fish were probably related to the differences in their environmental conditions and dietary element concentrations.

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## 1. Introduction

Owing to consumers demand for high-quality healthy food, seafood products can be an important part of a balanced human diet. They are rich in protein, and contain high portions of valuable ω-3 polyunsaturated fatty acids, liposoluble vitamins and essential minerals [1,2]. The Nutrition Committee of the American Heart Association advocates consumption of fish at least twice a week in order to prevent cardiovascular diseases [3]. Nevertheless, seafood products can be a potential source of inorganic contaminants such as heavy metals some of which are highly toxic. Hence, the need for the determination of metals in seafood products is evident and has gained much attention in recent decades [4,5].

Anthropogenic activities are the main source of metal pollution in the aquatic ecosystems. The pollutants are bioaccumulated by aquatic inhabitants and subsequently transferred to humans through the food chain [6–8]. Fish have been considered as a subject of investigation on heavy metals accumulation and monitoring programs in aquatic

environments since they occupy higher trophic levels and are an important component of the human diet [9,10]. It has been demonstrated that the accumulation of heavy metals in fish tissues is influenced by a number of factors such as environmental conditions (temperature, salinity, pH etc.), biological variations (species, sex, size and age), nourishment sources and seasonal changes [11].

Rainbow trout (*Oncorhynchus mykiss*) is a member of the Pacific trouts and belongs to the Salmonidae family. They survive in cold, clear and well-oxygenated lakes, rivers and streams with the ideal temperature, ranging between 13 °C and 15.5 °C. However, the temperature tolerance of rainbow trout is from 0 °C to over 25 °C. Rainbow trout ranks among the top five most sought game fish in North America. Concurrently, it is also widely used as a farmed fish in many countries around the world due to its rapid growth and high nutritional value [12,13]. Besides, rainbow trout is the main freshwater fish species farmed in Iran. Its farming started in 1959 in Iran, and production increased from 599 tons in 1978 to 62,630 tons in 2009 [14].

The fish quality differences of wild and farmed origin are always a subject of discussion. Several investigators have reported differences in chemical composition between farmed and wild fish; and the variability in environmental conditions and diet is regarded as the main reason for the observed distinctions [15–18]. Referring to existing scientific literature, few studies have addressed the comparative metal

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accumulation in farmed and wild fish. In addition, no data have been published to determine differences between farmed and wild rainbow trout in terms of metal contents. Therefore, the objective of this study was to compare and contrast the concentrations of heavy metals and trace elements in edible tissues of farmed and wild rainbow trout for the first time.

## 2. Experimental

### 2.1. Sample collection and preparation

Wild rainbow trout were captured from 12 fishing sites of Zayandeh-Rood River in Chaharmahal-va-Baghtiari province, Iran. Owing to its unique aquatic ecosystem, the river is the natural habitat of endangered wild rainbow trout, hence the government has banned catching rainbow trout from the river except during June to September. In this study, 3–5 fish were caught from each of the fishing sites of the river during June to September 2010. Farmed rainbow trout were obtained at the same period from 12 trout farms (5 fish from each farm) located in Chaharmahal-va-Baghtiari province, the first rank farmed rainbow trout production province in Iran [14]. The cultured system in all trout farms was race-way system (a kind of super intensive cultured system); and the water source was spring water. Farmed fish were hand fed with commercial pellet two times a day.

Both wild and farmed fish were slaughtered by immersing in ice-cold water and transported to the laboratory within 3–5 h after harvesting. The mean weight and length (mean  $\pm$  SEM) of fish were  $251.41 \pm 4.83$  g and  $25.33 \pm 2.7$  cm for wild and  $265.75 \pm 7.19$  g and  $28.42 \pm 3.7$  cm for farmed fish, respectively. It has been demonstrated that the size may be an important factor in metal accumulation of aquatic animals [19]. There were no significant differences ( $P > 0.05$ ) in weight and length between farmed and wild rainbow trout.

The fish were beheaded, eviscerated, deboned, skinned and filleted. The fillets and livers of fish samples from each of the trout farms or fishing sites were separately pooled and used as a unit. The samples were packed in polyethylene pouches and stored at  $-20^\circ\text{C}$  prior to analysis.

### 2.2. Analytical procedures

The samples were dried in an oven at  $60\text{--}65^\circ\text{C}$  until a constant weight was obtained, allowed to cool, and then ground in a household food mill. Digestion of the samples was performed by using high-pressure decomposition vessels, known as digestion bomb, according to the method of our previous studies [7,20] with some minor modifications. A sample (0.65 g) was mixed with 8 ml of 68% nitric acid (super purity quality; Romil Ltd., Cambridge, UK) and 4 ml of 30% hydrogen peroxide (suprapure quality; Merck, Darmstadt, Germany) in a Teflon container. The system was heated up to  $140^\circ\text{C}$  for 130 min. After cooling to ambient temperature, the solution was filtered through a  $0.45\ \mu\text{m}$  nitrocellulose membrane filter, followed by transfer to an acid-washed volumetric flask (25 ml) and made up to volume with double deionized water. Blank digest was also carried out in the same way.

Analysis of the elements (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr and Zn) was carried out by inductively coupled plasma-optical emission spectrophotometer (ICP-OES Horiba Jobin-Yvon Ultima 2 CE). Details of the instrumental operating conditions are depicted in Table 1. Total mercury analysis was performed by PerkinElmer 4100 atomic absorption spectrophotometer (Norwalk, CT, USA) equipped with MHS 15 CVAAS system according to the method of our previous study [20]. In order to validate our methods, TORT-2 certified reference material of lobster hepatopancreas (NRCC, Canada) was analyzed for corresponding elements. The concentrations found were within 92–105% of the certified values for all measured elements.

**Table 1**  
ICP-OES instrumental operating conditions.

Parameter	
RF generator power (W)	1200
Frequency of RF generator (MHz)	40.68
Plasma gas flow rate (l/min)	12
Auxiliary gas flow rate (l/min)	0.2
Nebulization gas flow rate (l/min)	0.85
Sample uptake rate (ml/min)	1
Type of detector	Solid state
Type of spray chamber	Cyclonic
Injector tube diameter (mm)	0.3
Measurement replicates	3
Element ( $\lambda$ /nm)	As 193.695; Ba 233.527 Cd 228.802; Co 228.616 Cr 267.716; Cu 324.754 Fe 259.939; Mn 257.610 Mo 202.301; Ni 231.604 Pb 220.353; Se 196.026 Sr 407.771; Zn 213.856

### 2.3. Statistical analysis

The data were statistically analyzed using *t*-test of GraphPad Prism software version 3 ([www.graphpad.com](http://www.graphpad.com)) to evaluate differences in element concentrations between muscle and liver in farmed or wild fish; and between the same tissues in farmed and wild fish. The levels were considered significantly different at  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Element concentrations in commercial feed mixtures

The mean concentrations of the elements in commercial feed mixtures used in trout farms are depicted in Table 2. Our study showed that the contents of heavy metals and trace elements in commercial feeds were different. These alterations may be ascribed to differences in raw materials used in feed preparation and addition of different amounts of mineral premixes to feed mixtures. Fe, Zn, Mn and Cu were predominant in studied commercial feed mixtures.

There is few and limited knowledge available on the acceptable and non-hazardous amount of trace elements in fish feed. One case has been reported that the dietary requirements of Fe, Cu, Mn and Zn in rainbow trout were 30, 3, 13 and  $30\ \mu\text{g/g}$ , respectively [21,22]. These elements are essential for growth, reproduction and energy metabolism in all living organisms [23,24]. Fish feed is the major source of the mentioned elements that all of which have low concentrations in seawater [24]. Our results indicated that the contents of Fe,

**Table 2**  
Concentration of the elements in commercial feed used in trout farms.

Element	Mean $\pm$ SEM ( $\mu\text{g/g}$ )	Range ( $\mu\text{g/g}$ )
As	$1.154 \pm 0.355$	0.000–3.562
Ba	$3.487 \pm 0.563$	0.618–5.743
Cd	$0.704 \pm 0.155$	0.000–1.213
Co	$0.998 \pm 0.235$	0.000–2.017
Cr	$0.984 \pm 0.237$	0.000–2.968
Cu	$7.369 \pm 0.941$	4.146–13.510
Fe	$48.631 \pm 4.684$	34.177–77.549
Hg	$1.088 \pm 0.252$	0.088–2.753
Mn	$18.730 \pm 2.017$	12.871–30.047
Mo	$0.381 \pm 0.117$	0.000–1.014
Ni	$0.951 \pm 0.268$	0.000–3.904
Pb	$2.409 \pm 0.408$	0.992–5.317
Se	$3.391 \pm 0.646$	1.587–8.329
Sr	$4.742 \pm 0.681$	0.801–7.964
Zn	$37.058 \pm 3.522$	28.856–66.313

Cu, Mn and Zn in commercial feed mixtures provided the requirements of rainbow trout (Table 2).

### 3.2. Element concentrations in rainbow trout

The concentrations of heavy metals and trace elements in edible tissues of farmed and wild rainbow trout are presented in Table 3. The results showed that with the exception of Ba and Sr, liver had significantly higher ( $P < 0.05$ ) heavy metal and trace element concentrations compared to the muscle in both farmed and wild fish. The similar pattern has been extensively documented in several studies for various fish species [25–28]. The difference in accumulation potential between muscle and liver can be due to the greater tendency of the elements to react with the metallothioneins (proteins that are present in liver but not in the muscle), thus accumulate at high concentrations in liver [28,29].

The elements such as Fe, Cu, Mn and Zn are essential elements because of their important role in biological systems. Our study showed that tissues of wild rainbow trout contained significantly higher concentrations ( $P < 0.05$ ) of Fe, Mn and Zn, while tissues of farmed rainbow trout had significantly higher contents ( $P < 0.05$ ) of Cu (Table 3). Higher Fe content in muscle of wild fish compared to farmed fish might be due to the dominance of dark muscles in the body of wild fish. It has been demonstrated that the dark muscles contain higher Fe concentrations than light muscles [30]. In previous studies, no significant differences were found in levels of Fe, Cu, Mn and Zn between farmed and wild common carp [31], sea bass [32] and gilthead seabream [33]. However, higher levels of Cu were observed in liver of the wild white seabream [34] and muscle of wild salmon [35], Fe in muscle of the wild sea bass [36], and Mn in muscle of the wild yellow perch [37] compared to their farmed counterparts. Our data are in agreement with published results for Fe, Cu, Mn and Zn

contents in muscle of rainbow trout caught from the Atatürk Dam Lake in southeastern Turkey [38]. However, Mendil et al. [39] reported remarkably higher levels of Fe and Zn in muscle of rainbow trout from Yeşilirmak River in Tokat, Turkey.

Selenium is an essential element acting as antioxidant, anti-carcinogenic, regulator of thyroid hormone metabolism and an antagonistic agent to the toxicological effects of mercury [40]. The concentrations of Se in tissues of farmed rainbow trout compared to its wild relative were not significantly different ( $P > 0.05$ ), which is consistent with the study of Kelly et al. [41] on farmed and wild salmon. Also, our obtained results were below the proposed toxicity threshold for aquatic biota i.e. 10 µg/g of dry weight [42].

Chromium is considered as a heavy metal and pollutant due to its use in a wide range of industrial applications. On the other hand, Cr is a trace element playing an essential role in insulin function and glucose metabolism [43]. No statistically significant difference ( $P > 0.05$ ) was observed in muscular Cr contents between farmed and wild rainbow trout, while it was significantly higher ( $P < 0.05$ ) in liver of wild fish than in farmed fish (Table 3). In previous studies, no difference was found in muscular Cr contents between farmed and wild fish species [23,36,37,41].

Cobalt, an essential nutrient, is an integral part of vitamin B<sub>12</sub>. The element has an important role in blood pressure regulation and is necessary for proper thyroid function [44]. Molybdenum is another important essential trace element involved in metabolism through metalloenzymes [23]. Nickel is not an essential element but in a few trace amounts it may be beneficial to activate some enzyme systems. The absorption of Ni is very low and its chronic intake is associated with an increased risk of lung cancer [45]. Our study showed that the levels of Co, Mo and Ni in tissues of farmed rainbow trout compared to its wild relative were not significantly different ( $P > 0.05$ ). The results are in agreement with some previous investigations comparing

**Table 3**

Concentration of heavy metals and trace elements in edible tissues of farmed and wild rainbow trout (minimum and maximum in parenthesis). The results are expressed as µg/g of dry weight.

Element	Farmed		Wild	
	Muscle	Liver	Muscle	Liver
As	0.934 ± 0.293 <sup>a</sup> (0.000–2.389)	3.546 ± 0.413 <sup>b</sup> (0.735–5.228)	1.119 ± 0.366 <sup>a</sup> (0.000–3.416)	2.988 ± 0.447 <sup>b</sup> (0.688–5.199)
Ba	1.800 ± 0.339 <sup>A</sup> (0.211–3.395)	2.423 ± 0.464 (0.207–4.508)	3.121 ± 0.530 <sup>B</sup> (0.214–5.337)	2.828 ± 0.445 (0.209–5.017)
Cd	0.097 ± 0.058 <sup>a</sup> (0.000–0.655)	1.872 ± 0.558 <sup>b</sup> (0.000–6.079)	0.130 ± 0.068 <sup>a</sup> (0.000–0.769)	2.202 ± 0.666 <sup>b</sup> (0.000–7.231)
Co	0.186 ± 0.053 <sup>a</sup> (0.000–0.496)	0.960 ± 0.199 <sup>b</sup> (0.000–2.157)	0.216 ± 0.062 <sup>a</sup> (0.000–0.553)	0.998 ± 0.207 <sup>b</sup> (0.000–2.340)
Cr	0.565 ± 0.151 <sup>a</sup> (0.000–1.587)	1.318 ± 0.317 <sup>bA</sup> (0.000–3.154)	0.630 ± 0.178 <sup>a</sup> (0.000–2.053)	3.200 ± 0.503 <sup>bB</sup> (0.487–5.192)
Cu	21.813 ± 2.791 <sup>ab</sup> (7.483–39.902)	58.494 ± 9.807 <sup>bb</sup> (16.233–115.745)	8.398 ± 1.681 <sup>aA</sup> (0.717–17.211)	33.687 ± 5.872 <sup>bA</sup> (9.965–70.105)
Fe	15.469 ± 3.126 <sup>aA</sup> (3.228–39.865)	67.528 ± 7.975 <sup>bA</sup> (21.388–111.347)	32.459 ± 3.686 <sup>ab</sup> (16.884–54.128)	128.782 ± 20.772 <sup>bb</sup> (38.026–312.455)
Hg	0.314 ± 0.195 <sup>a</sup> (0.000–2.025)	1.261 ± 0.237 <sup>b</sup> (0.097–2.226)	0.292 ± 0.181 <sup>a</sup> (0.000–1.952)	1.408 ± 0.217 <sup>b</sup> (0.088–2.148)
Mn	6.262 ± 0.888 <sup>aA</sup> (2.125–12.987)	18.871 ± 2.286 <sup>bA</sup> (5.995–35.147)	13.932 ± 1.456 <sup>ab</sup> (5.984–26.145)	31.794 ± 4.573 <sup>bb</sup> (7.211–62.148)
Mo	0.114 ± 0.042 <sup>a</sup> (0.000–0.374)	0.425 ± 0.085 <sup>b</sup> (0.000–0.738)	0.121 ± 0.046 <sup>a</sup> (0.000–0.447)	0.417 ± 0.079 <sup>b</sup> (0.000–0.794)
Ni	0.379 ± 0.108 <sup>a</sup> (0.000–0.998)	1.802 ± 0.238 <sup>b</sup> (0.381–2.967)	0.406 ± 0.120 <sup>a</sup> (0.000–0.948)	1.731 ± 0.212 <sup>b</sup> (0.409–2.713)
Pb	1.108 ± 0.400 <sup>a</sup> (0.000–4.453)	2.885 ± 0.565 <sup>b</sup> (0.713–6.118)	1.201 ± 0.373 <sup>a</sup> (0.000–3.188)	2.907 ± 0.536 <sup>b</sup> (0.744–7.136)
Se	2.046 ± 0.509 <sup>a</sup> (0.000–4.863)	4.928 ± 0.632 <sup>b</sup> (0.951–8.163)	2.324 ± 0.593 <sup>a</sup> (0.000–6.014)	5.167 ± 0.709 <sup>b</sup> (1.129–9.887)
Sr	3.615 ± 0.608 <sup>bb</sup> (1.067–6.983)	0.490 ± 0.126 <sup>a</sup> (0.212–1.798)	1.495 ± 0.405 <sup>bA</sup> (0.433–4.551)	0.537 ± 0.146 <sup>a</sup> (0.215–1.607)
Zn	20.973 ± 4.424 <sup>aA</sup> (6.741–59.092)	81.068 ± 8.920 <sup>bA</sup> (29.146–118.079)	46.742 ± 6.390 <sup>ab</sup> (19.501–81.327)	125.250 ± 16.037 <sup>bb</sup> (58.419–213.458)

<sup>a,b</sup>Means ± SEM for each element with different lowercase superscript letters are significantly different between muscle and liver in farmed or wild fish ( $P < 0.05$ ).

<sup>A,B</sup>Means ± SEM for each element with different uppercase superscript letters are significantly different between the same tissues in farmed and wild fish ( $P < 0.05$ ).

farmed and wild sea bass [36], yellow perch [37], haddock [23] and common carp [31]. In another study, Co and Ni in the flesh of wild sea bream were lower than those of cultured sea bream [24].

Strontium is a non-essential element because of its unknown metabolic function in organisms. The element can be used as a marker to distinguish between fish and meat due to the fact that its concentration is considerably higher in fish than in meat [46]. The content of Sr was significantly higher ( $P < 0.05$ ) in muscle of farmed rainbow trout, whereas Ba concentration was significantly higher ( $P < 0.05$ ) in muscle of wild rainbow trout. However the levels of both elements were not significantly different ( $P > 0.05$ ) in liver of farmed and wild rainbow trout (Table 3).

The elements such as As, Cd, Pb and Hg, categorized as toxic elements, do not play any metabolic function but can be harmful for humans, even at low concentrations, when ingested over a long time period [47]. The concentrations of the mentioned elements in tissues of farmed rainbow trout were not significantly different ( $P > 0.05$ ) compared to wild rainbow trout (Table 3). According to the European Commission (EC) [48], the permissible limits for Cd, Pb and Hg in fish for human consumption are 0.05, 0.3 and 0.5  $\mu\text{g/g}$  of wet weight, respectively. The US Environmental Protection Agency has considered an upper limit of 1.3  $\mu\text{g/g}$  of wet weight for As [49]. In order to compare the data obtained in this study with prescribed limits, all concentrations were recalculated to wet weight. Levels of Cd in 16.6% of muscle and 66.6% of liver samples from farmed fish; and 16.6% of muscle and 75% of liver samples from wild fish exceeded the EC limit. Regarding the Pb, concentrations in 33.3% of muscle and 58.3% of liver from farmed fish; and 41.6% of muscle and 83.3% of liver samples from wild fish were above the EC regulation. However, concentrations of Hg and As in all of the examined samples were lower than the legislated limits.

#### 4. Conclusion

This study provides information on the heavy metal and trace element accumulation in farmed and wild rainbow trout. Some elements (Ba, Cr, Cu, Fe, Mn, Sr and Zn) were found to be significantly different ( $P < 0.05$ ) between the tissues of farmed and wild fish. Owing to the fact that the wild rainbow trout is an endangered species, we recommend consumption of farmed rainbow trout (specially flesh part) in the human diet. Moreover, farmed fish have the advantage of being cultivated under controlled conditions; hence the probable hazards for the consumers could be more easily managed. Regular monitoring of heavy metal concentrations should be conducted in the future since the levels of Cd and Pb exceeded the legislated limits in some of the examined samples.

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