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Heavy Metals and Arsenic in Water, Sediment and the Muscle of Oncorhynchus *Mykiss* from the Tishgo River in the Central Andes of Peru

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ABSTRACT

The concentration of Cu, Pb, Zn and As in water, sediment and muscle of *Oncorhynchus mykiss* from the Tishgo river in the central Andes of Peru were analyzed. The water, sediment and fish samples were collected from 36 sites in three sampling sectors. The analytical determination was performed by flame atomic absorption spectrophotometry. The mean Pb concentrations in the water from the three sampling sectors showed significant differences (p < 0.05) which exceeded the values of the Peruvian standard and the WHO. The mean concentrations of Cu, Pb and As in sediment did not present significant differences (p > 0.05) in the three sectors evaluated. The redundancy analysis (RDA) revealed that the concentrations of As and Pb in water, and Cu, As and Zn in sediment significantly influence the concentration of these elements in the *O. mykiss* muscle. The mean concentrations of Cu, Pb, Zn and As in the *O. mykiss* muscle were lower than those established by international regulations. Therefore, the concentrations of heavy metals and As recorded in this study do not represent a threat to the consumption of *O. mykiss* from the Tishgo river.

Keywords: heavy metals, arsenic, water, sediment, Oncorhynchus mykiss, Tishgo river.

INTRODUCTION

Environmental contamination is one of the great problems facing the humanity today. Consequently, the planet is experiencing an accelerated deterioration of its quality. Anthropogenic processes, such as rapid industrialization, accelerated population growth and excessive consumerism, have led to the appearance of various pollutants in nature, exerting strong pressure on aquatic ecosystems (Haji Gholizadeh et al., 2016). The contamination of water bodies by heavy metals is a problem of great concern at the global level. Heavy metals represent an environmental risk due to their capacity for mobility and persistence in nature, bioaccumulation and biomagnification in the food chain (Monroy et al., 2014; Yi et al., 2017; Liu et al., 2018). The distribution of heavy metals in aquatic ecosystems is influenced by physicochemical characteristics, biogeochemical

processes, redox potential, pH, salinity, among others (Saiful Islam et al., 2015). It is also affected by diverse anthropogenic activities, like the discharge of urban and industrial residual waters, runoff of agrochemicals from agricultural areas, atmospheric deposition and pressures of natural order (Bonnail et al., 2016; Abdel Gawad, 2018).

The Tishgo river is a river of the Mantaro river watershed which is located in the central region of Peru. It is one of the rivers of this hydrographic basin the waters of which are used in the production of *Oncorhynchus mykiss* (rainbow trout) for export as well as for fishing. The health status of this river is being affected by acidic mine water runoff, the wastewater from population centers located in the areas adjacent to this river and by accidents, such as the one that occurred in 2010 where thirty tons of sodium hydroxide were dumped into the Tishgo river (SUTRAN, 2012). Because of the latter event, more attention has

The high content of heavy metals in water and sediment poses a serious threat to fish and consequently to human health. The consumption of contaminated fish can affect humans in many different ways, depending on the properties of the metal and the level of concentration in which it is found. The research on the effects of heavy metals report that cadmium (Cd), lead (Pb) and arsenic (As) are associated with serious health effects (Ahmed et al., 2015). Pb deteriorates the kidney function, affects IQ and interferes with metabolism (Moses & Etuk, 2015; Abarshi, Dantala, & Mada, 2017). As causes skin lesions, lung diseases and cancer. On the other hand, excessive intake of copper (Cu) and zinc (Zn) is linked to such diseases as nephritis and anuria, although these metals are essential for normal growth and development (Ali et al., 2020).

In this context and considering that it is fundamental to have a better understanding of the behavior of heavy metals and metalloids in aquatic ecosystems with fish potential due to their possible toxic effects on biological communities and human health. In addition, the fish consumption around the world has increased due to the information available on its nutritional and beneficial effects on the human health. The objective of the study was to analyze the concentration of Cu, Pb, Zn and As in water, sediment and the muscle of *Oncorhynchus mykiss* of the Tishgo river in the central region of Peru.

MATERIALS AND METHODS

Study area

The Tishgo river is located in the province of Yauli, in the Mantaro river watershed, a central region of Peru between 3725 and 3750 meters above sea level. The topography is complex, resulting in a variety of climatic conditions. The region's climate is predominantly semi-frigid with rainy seasons in summer and dry seasons in winter, with annual temperatures of 8°C, with a minimum of -4°C and a maximum of 10°C and an annual rainfall of more than 120 mm (CIIFEN, 2019). The study areas were made up of three sectors of the Tishgo river - Yauli - Junin. The sectors S1, S2 and S3 were established in the upper, middle and lower part of the river course, respectively, with twelve sampling sites for each sector (Figure 1).

Collection of water, sediment and fish samples

The water samples were collected in sectors S1, S2 and S3, between January and March



Figure 1. Location of the sampling sectors in the Tishgo river in the central Andes of Peru.

2018. In each sector, water, sediment and fish samples were collected from 36 sampling sites. Water samples were collected from three points at each sampling site in previously treated 1.5 liter plastic bottles and rinsed with double-distilled water (APHA, 2012). The sediment samples were collected using a modified Ekman dredge at the same water sample collection sites in each sector. The fish were collected using fishing nets and traps. The collected water, sediment and fish samples were transported by cold chain the same day of sampling to the laboratory for analytical determination.

Analysis of heavy metals and arsenic in water

The preparation of the sample consisted of adding 250 ml of water to a beaker, which was brought to a boil, until 100 ml was obtained. Immediately, 5 ml of nitric acid and 5 ml of ultrapure hydrochloric acid (supplied by Merck Germany) were added to achieve complete oxidation and reduce the interferences that could be caused by organic matter. The preparation was brought to a boil (until the water was consumed), allowed to cool and then 10 ml of distilled water was added. It was filtered and measured in a 100 ml basin, with 1% nitric acid (APHA/AWWA/WEF, 2012).

Analysis of heavy metals and arsenic in sediment

During the analysis, 1.0 g of sediment was weighed and deposited in 100 ml beakers. Ten milliliters of nitric acid were added to achieve the disintegration of organic matter and 10 ml of hydrochloric acid were left to act for one minute. The precipitation vessels containing the samples were brought to a boil until a sample of pasty consistency was obtained. It was left to cool and then 10 ml of hydrochloric acid was added to dissolve the sample residues adhering to the walls of the glass. Then, the sample was transferred to a 100 ml phial, homogenized and measured with distilled water at 100 ml. It was filtered and finally, the sample was quantified through the flame atomic absorption spectrophotometer (Varian AA240). Previously, standard solutions of Cu, Pb, Zn and As at 10, 20, 30 and 40% were available. Next, the readings of the standards were made at different wavelengths for each element.

Analysis of heavy metals and arsenic in Oncorhynchus mykiss muscle

Using a stainless steel knife, the dorsal muscle was removed from the fish body, packed into clean HDPE containers, labeled with a unique code and stored at -20 °C. The digestion of the rainbow trout muscle samples consisted of: placing 0.5 g of the sample in the digestion balloon, adding 5.0 ml of concentrated ultrapure 33% nitric acid and 5.0 ml of concentrated sulfuric acid to initiate the reaction. When the reaction decreases, the balloon with the sample was heated at 60 °C for 30 minutes. Then, the ball was remove from the heat and allowed to cool down. Afterwards, 10.0 ml of concentrated nitric acid was returned and return the balloon to the digester at a temperature of 120 °C. The temperature was increased to 150 °C for 6 hours and the balloon was removed. It was allowed to cool and 1.0 ml of H_2O_2 at 30% was added until the solution became clear without visible residues. The contents of the balloon were transferred to a 50.0 ml volumetric balloon and brought to volume with deionized water. The sample was quantified (Djedjibegovic et al., 2012).

Analysis of data

Since the assumptions of normality in the distribution of the evaluated data are not fulfilled, non-parametric statistics were used for the comparison of medians with a factor. The Kruskal-Wallis (KW) test was used to evaluate the differences between independently sampled groups (Kruskal & Wallis, 1952). Spearman's correlation was used to establish the correlation between the variables under study (Mukaka, 2012). The relationship study between two matrices of X and Y variables was carried out with the redundancy analysis (RDA) using the program Canoco 5, to test the relationship between heavy metals of the dependent variable (muscle) and heavy metals of the independent variable (water and sediment). This canonical analysis is the simultaneous analysis of two data matrices. Redundancy analysis combines two families of methods: regression and ordination. Regression is performed between the response variable table (metals in muscle), the explanatory variable table, and the ordination (PCA).

RESULTS AND DISCUSSION

Heavy metal and arsenic concentration in Tishgo river water

Table 1 shows the mean concentration, standard deviation, range and coefficient of variation of heavy metals and As determined in Tishgo River water. The decreasing order of mean heavy metal and As concentrations in water samples varied by the sampling sector. In S1, the decreasing order of mean concentrations was: Zn > Pb > Cu > As, in S2: Zn > Pb > As > Cu and in S3: Zn > Cu >Pb > As. The mean Cu concentration varied from 0.022 mg/L to 0.027 mg/L, Pb from 0.026 mg/L to 0.031 mg/L, Zn from 0.054 mg/L to 0.064 mg/L and As from 0.009 mg/L to 0.023 mg/L. The mean Cu concentrations recorded at all sampling sites in the respective sectors did not exceed the limit values of the World Health Organization standards (WHO) (2.0 mg/L) (WHO, 2011), United States Environmental Protection Agency (USEPA) (1.0 mg/L) (US EPA, 2006), or environmental quality standards for highland river water in Peru for drinking (2.0 mg/L), fish farming (0.2 mg/L) and conservation of the aquatic environment (0.1 mg/L) (MINEN, 2017). The mean Pb concentrations recorded in the three sectors under study exceeded the standard values of the WHO (0.01 mg/L) and MINEN for drinking water (0.01 mg/L), fish culture (0.0025 mg/L) and conservation of the aquatic environment (0.0025 mg/L). The Pb concentrations in S3 were higher than the Pb concentrations (0.0285 mg/L)

reported by Chanamé et al. (2017) in the waters of this river (Casaracra fish farm). This increase is due to run-off from the rivers running through mineralized areas, industrial effluent discharges from mining units and run-off from the agricultural areas. The measured Zn concentrations at all sampling sites in the three sectors under study did not exceed the national and international standards. The mean concentrations of As recorded in sectors S2 and S3 exceeded the standard values of the WHO (0.01 mg/L) and MINEN of drinking water (0.01 mg/L). However, in none of the sampling sites of the studied sectors did the waters register the values higher than the standards of water quality for fish culture (0.1 mg/L) and conservation of the aquatic environment (0.15 mg/L).

The decreasing order of the coefficient of variation of the average concentrations of heavy metals and As at the sampling sites of S1 and S2 was: Cu > Zn > Pb > As and at S3: Zn > Cu > Pb > As. The results obtained also reveal that the S1 sampling sites presented the lowest concentrations of the elements studied.

The mean concentrations of heavy metals and As in water of the three sampling sectors according to the Kruskal Wallis' analysis showed significant differences (p < 0.05) (. The sectors located in S1 and S2 showed lower concentrations of heavy metals and As in relation to S3. The mean concentrations of Cu, Pb and Zn showed a similar trend to the sites established along the river, since statistically the distributions do not tend to be differentiated (p < 0.05) (Figure 2). The test of correlation between variables showed a significant

Element	Descriptive statistic	Sampling sector / river course			WHO	USEPA	MINEN		
		S1	S2	S3	Drinking water	Drinking Water	Drinking water	Water for fish culture	Conservation of the aquatic environment
Cu	Rank	0.008-0.058	0.008-0.063	0.011-0.052					
	Mean±SD	0.022±0.002	0.026±0.002	0.027±0.01	2.0	1.0	2.0	0.2	0.1
	CV	75.65	63.53	43.34					
	Rank	0.008-0.043	0.023-0.045	0.013-0.043					
Pb	Mean±SD	0.026±0.01	0.031±0.008	0.027±0.009	0.01	0.0	0.01	0.0025	0.0025
	CV	39.76	26.42	32.46					
	Rank	0.014-0.117	0.028-0.109	0.008–0.106					
Zn	Mean±SD	0.064±0.03	0.064±0.025	0.054±0.031	3.0	5.0	3.0	1.00	0.12
	CV	44.57	39.63	57.66					
	Rank	0.005-0.015	0.012-0.021	0.024–0.038					
As	Mean±SD	0.009±0.003	0.017±0.003	0.031±0.005	0.01	0.0	0.01	0.1	0.15
	CV	29.72	17.79	15.87					

Table 1. Descriptive statistics of heavy metals and arsenic in water from the Tishgo River in the Central Andes of Peru, expressed in mg/L.



Figure 2. Distribution and comparison of heavy metal and arsenic concentrations in Tishgo river water, according to the sampling sector.

negative correlation (p < 0.05) between Cu – Zn (rho = -0.48) and a significant positive correlation between Cu – As (rho = 0.41).

Lead, copper, zinc and arsenic concentration in the Tishgo river sediment

Table 2 shows the descriptive statistics of heavy metals and As in the Tishgo river sediment. In S1 and S2, the decreasing order of the mean concentrations of the elements studied was: Zn > As > Pb > Cu and S3: Zn > Pb> As > Cu. The mean Cu concentration varied from 15.19 mg/Kg to 21.51 mg/Kg, Pb from 30.30 mg/Kg to 49.67 mg/Kg, Zn from 168.82 mg/Kg to 236.20 mg/Kg and As from 33.01 mg/Kg to 41.32 mg/Kg. The mean Cu concentrations recorded in all sampling sites of S1, S2 and S3 did not exceed the mean values of the IAEA-SL-1 reference material (30.00 mg/Kg) of the International Atomic Energy Agency – IAEA (International Atomic Energy Agency, 1999) and the Interim Sediment Quality Guidelines of the Canadian Council of Ministers of the Environment (ISQG) (18.70 mg/Kg) (Canadian Council of Ministers of the Environment, 2001). The mean Pb concentrations in S1 and S2 did not exceed the value of the reference material, but they did exceed ISQG. The mean concentrations of As in the three sectors studied exceeded the values of the reference material and ISQG. The mean Zn concentrations at the S1 and S2

Element	Descriptive	Sam	pling sector / river	Reference	ISQG Canadian interim sediment quality guideline	
Element	statistic	S1	S2 S3			
	Rank	12.76–22.08	12.2–16.93	18.17–25.14		
Cu	Mean±SD	17.96±2.85	15.19±1.41	21.51±2.33	30.00	18.70
	CV	15.87	9.28	10.81		
	Rank	20.15-43.96	25.46-33.53	38.34–65.2		
Pb	Mean±SD	34.95±7.2	30.30±2.93	49.67±9.97	37.70	30.20
	CV	20.6	9.65	20.08		
	Rank	142.7–216.56	143.45–188.25	181–290.1		
Zn	Mean±SD	177.93±24.3	168.82±14.54	236.2±39.1	223.00	124.00
	CV	13.65	8.61	16.55		
	Rank	27.94-36.37	32.24-43.35	32.28-45.21		
As	Mean±SD	33.01±2.74	41.32±3.42	40.13±3.66	27.60	7.24
	CV	8.3	8.26	9.12		

Table 2. Descriptive statistics of heavy metals and arsenic in sediment from the Tishgo river in the Central Andes of Peru, expressed in mg/Kg.

IAEA: International Atomic Energy Agency.

sampling sites did not exceed the values of the reference material (223.0 mg/Kg), but they did at the S3 sampling sites. As well as the values of the sediment quality guidelines in all sampling sites that included the studied sectors.

The variation in the concentration of heavy metals and As recorded in the three sampling sectors would be mainly influenced by anthropogenic activities such as the discharge of mining waste transported by tributary rivers and domestic and industrial wastewater from human settlements concentrated on the river bank. The high content of toxic metals found in the lower part of the Tishgo river is of great concern for the diversity of water use.

The mean concentrations of Zn in the sediment varied significantly along the Tishgo river. At S3, the concentrations are significantly high p<0.05) compared to S2 and S1. The mean concentrations of Cu, Pb and As did not show significant differences in the three sectors evaluated (Figure 3).

Heavy metal and arsenic concentration in the muscle Oncorhynchus mykiss from Tishgo river

Table 3 shows the descriptive statistics of heavy metals and As in the muscle of *Oncorhynchus mykiss* from the Tishgo river. In S1, the decreasing order of the average concentrations of the studied elements was: Zn > Pb > Cu > As, in S2: Zn > Cu > Pb > As and S3: Zn > Cu > Pb >As. The mean concentration \pm SD of Cu in the muscle tissue in this study varied from 0.165 \pm $0.05 \ \mu g/g$ to $0.240 \pm 0.11 \ \mu g/g$, from Pb from $0.171 \pm 0.04 \ \mu g/g$ to $0.219 \pm 0.05 \ \mu g/g$, from Zn from 2.521 \pm 0.07 µg/g to 2.578 \pm 0.09 µg/g and from As from 0.009 \pm 0.003 $\mu g/g$ to 0.09 \pm 0.03 µg/g. The mean Cu, Pb, and Zn concentrations measured in this study were lower than the concentrations reported by Chanamé et al. (2017) in the *O. mykiss* muscle from fish farms in the study area (Cu 0. 33 µg/g, Pb 0.82 µg/g and Zn 4.77 µg/g, respectively) and those reported by Monroy et al. (2014) who registered high concentrations of Cu (108.84 μ g/g) and Zn $(102.22 \ \mu g/g)$ in this species in Titicaca Lake. The mean Pb concentrations did not exceed the regulatory limits established by the FAO and European Union Food Codex (0.3 μ g/g) for fish meat (WHO/FAO, 2007) and by NOM-027-SSAI-1993 (Official Mexican Norm-OMN-027-SSA1, 1993) (1.0 μ g/g) The mean As concentrations registered in this study were lower than the concentrations reported by Varol et al., (2018) in the O. mykiss grown in net cages and the concentrations of Bonsignore et al., (2018) in the muscle tissues of other salmonids and international food standards (Makedonski et al., 2015; Gallego Ríos et al., 2018).

The mean concentrations of heavy metals and As in the *O. mykiss* muscle for the three sectors evaluated showed significant differences (p < 0.05). In the S1 and S2 sectors, the As concentrations were significantly lower. Similar behavior was presented by the Pb, since its concentrations were significantly higher than the those of the S1 and S2 sectors. In turn, the Cu



Figure 3. Distribution and comparison of heavy metal and arsenic concentration in the Tishgo river sediment, according to the sampling sector.

Element	Descriptive statistics	Sampli	ng sector / river	course	Codex	FAO⁵	CEC℃	EPAd
		S1	S2	S3	Commission ^a			
	Rank	0.083–0.241	0.122–0.485	0.106–0.491				
Cu	Mean±SD	0.165±0.05	0.236±0.11	0.24±0.11		30.0		
	CV	31.12	47.67	43.88				
	Rank	0.12-0.221	0.137–0.259	0.111-0.264				
Pb	Mean±SD	0.171±0.04	0.195±0.05	0.219±0.05	0.3		0.3	
	CV	20.85	25.62	20.52				
	Rank	2.443-2.644	2.475–2.714	2.465–2.751				
Zn	Mean±SD	2.521±0.07	2.578±0.09	2.531±0.08		30.0		
	CV	2.84	3.35	3.13				
	Rank	0.004-0.013	0.011-0.042	0.025–0.122				
As	Mean±SD	0.009±0.003	0.024±0.009	0.09±0.03				1.3
	CV	34.9	37.47	37.48				

Table 3. Descriptive statistics of heavy metals and arsenic in muscle of *Oncorhynchus mykiss* from the Tishgo river in the Central Andes of Peru (in $\mu g/g$).

^aWHO/FAO (2007), ^bFAO (1983), ^cCommission of the European Communities-CEC (2006), ^dBurger & Gochfeld (2005)

and Zn concentrations in the *O. mykiss* muscle in the sectors evaluated showed similar behavior (Figure 4).

Redundancy analysis according to the concentration of heavy metals and arsenic in water and muscle of *Oncorhynchus mykiss*

The redundancy analysis (RDA) significantly explained the distribution of the observations and the effect of the independent vectors (concentration of heavy metals and arsenic in water) that are reflected in the sum of the eigenvalues of the first four axes of 0.44 and an explained variation of 44.3% (Figure 5). The first axis obtained the significant own value of 0.4 of the total explaining 40.02% of the variation. Considering the relation between the concentration of elements in water and *O. mykiss* muscle, it is observed that As is determinant and transcendental in the differences between the sectors, being the sector S3 the one that has a greater concentration in water, with significant correlations to the first axis (p < 0.05) with percentages of explanation in the similarity of distribution in the matrices of 39.1%. Therefore, it is inferred that the concentration of As and Pb in water significantly influences the concentration in the muscle of *O. mykiss*. The perceptual map of the matrix interaction according to RDA shows the correlation of the Pb and Zn



Figure 4. Distribution and comparison of heavy metal and arsenic concentration in *Oncorhynchus mykiss* from Tishgo river, according to sampling sector.



Figure 5. Redundancy analysis of correlations between the concentration of elements in *Oncorhynchus mykiss* muscle and water variables (explanatory variables represent 44.3%).

correspondences between muscle and water. Cu has a similar distribution, but the correspondence is not significant, indicating that water is not the only source of Cu. Atmospheric deposition and infiltration of heavy metals from urban, mining and agricultural activities or even acid rain that breaks down soils and releases them constitute major contributors to heavy metals in water bodies (Goher et al., 2019). Pb is the element with the greatest contribution in the distribution and relation of vectors, indicating that Pb in water tends to be the element that is significantly concentrated in the muscle.

Redundancy analysis according to the concentration of heavy metals and arsenic in the *Oncorhynchus mykiss* muscle and sediment

The RDA significantly explains the distribution of the observation and the effect of the independent vectors (concentration of heavy metals and arsenic in the sediment) with a cumulative value of 0.50 and an adjusted variation of 98%, indicating that the matrices of the dependent and independent variables adjust their distributions based on the first and second axis specifically. Considering the relation of heavy metal and arsenic concentration in the sediment and muscle of *O. mykiss*, three significant correlations of Cu, As and Zn are observed. Therefore, the concentration of these elements in sediment significantly influences the concentration in the muscle of *O. mykiss*. The perceptual map of the matrix interaction according to the RDA shows the correlation of Cu, As and Zn correspondences between the muscle and sediment. Cu is the main element that determines the distribution and relation of vectors and indicates the tendency to concentrate significantly in muscle (Figure 6).

The Cu and Zn are essential metals in several vital processes in plants, animals and aquatic microorganisms. However, at high concentrations, these trace metals become toxic. Cu forms insoluble salts and soluble complexes that are deposited and concentrated in the sediment (Ibrahim et al., 2019). The Zn concentrations in all O. mykiss muscle samples did not exceed the FAO standard value (30 μ g/g). Pb is a toxic metal; its widespread use has caused extensive environmental pollution and health problems in many parts of the world. Several studies have reported that Pb has a significant impact on biological processes. In children, it affects the cognitive development, causes attention deficit disorder, delayed psychomotor development and decreased intelligence quotient (Dórea, 2019). As in its inorganic form is considered toxic, so



Figure 6. Redundancy analysis of correlations between trout muscle element concentration and sediment variables (explanatory variables represent 60.1%).

in different countries there are limits established for greater control. This metalloid enters the aquatic environment through natural erosion of the rocks, mining and smelting processes, affecting important areas such as the waters destined for agriculture and fishing. Tolerable levels in the case of domestic animals are 50 mg/kg, being higher than those established for the water for human consumption and those approved by European legislation.

CONCLUSIONS

In this study, the concentrations of heavy metals and arsenic in water, sediment and muscle of *O. mykiss* from the Tishgo river in central Peru were determined. Most of the mean heavy metal and arsenic concentrations analyzed in the study area exceeded the values of national and international standards for water, sediment, and muscle of *O. mykiss*. The concentration of Pb in the water samples was higher than the environmental quality standards for water from rivers in the Peruvian highlands in their various uses and the WHO. In the sediment, the mean Pb concentrations in the three sampling sectors

exceeded the provisional CCME sediment quality guidelines and only S3 exceeded the values of the IAEA-SL-1 reference material. The mean Zn concentrations behaved similarly to Pb at S3. The mean As concentrations in the three sampling sectors exceeded the provisional sediment quality guidelines of the CCME and only S3 exceeded the values of the IAEA-SL-1 reference material. In the O. mykiss muscle, the decreasing order of the mean concentrations of the elements studied at S1 was: Zn > Pb > Cu >As, at S2: Zn > Cu > Pb > As and S3: Zn > Cu> Pb > As. The mean concentrations of heavy metals and As in O. mykiss muscle were lower than those established by the Food Codex, FAO, CEC and EPA. Therefore, we can conclude that these metals do not represent a threat to the consumption of O. mykiss.

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