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Bioaccumulation of heavy metals in *Oncorhynchus mykiss* for export at production centers in the Peruvian Central Highlands

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ABSTRACT

The bioaccumulation of the heavy metals Cu, Zn, Fe and Pb was determined in the livers, kidneys and muscles of *Oncorhynchus mykiss* trout at seven production centers in the province of Yauli, Junín-Peru. The determination and quantification of total heavy metals in water samples collected monthly from the production sites and in 28 trout that averaged 250 g and 27 cm was performed by atomic absorption spectrophotometry, according to the methodology recommended by FAO. Levels of Zn, Fe and Pb were found to exceed the environmental quality standards established by the Peruvian Ministry of the Environment for the rivers of the coast and highlands, as well as the quality standards of the European Union for the cultivation of trout, while levels of Cu conformed with those standards. Concentration of Cu, Zn, Fe and Pb in the livers, kidneys and muscles exceeded the maximum permissible limits established by the European Union for fish meat and by the Mexican official standard, NOM-027-SSA1-1993, for fresh, refrigerated and frozen fishery products, in the case of Pb. The correlation between the concentrations of copper, zinc, iron and lead in the water and the concentrations of these metals in the livers, kidneys and muscles is low and not significant, except for copper, which had a significant correlation.

Keywords: atomic absorption spectrophotometry, bioaccumulation, heavy metals, trout.

Bioacumulação de metais pesados em *Oncorhynchus mykiss* para exportação dos centros de produção dos Andes Centrais do Peru

RESUMO

A bioacumulação de metais pesados de Cu, Zn, Fe e Pb no fígado, rim e músculo da truta *Oncorhynchus mykiss* foi determinada em sete centros de produção na província de Yauli, Junín-Peru. A determinação e quantificação do total de metais pesados em amostras de água coletadas mensalmente nos locais de produção e em 28 trutas de 250 g e 27 cm em média foram realizadas por espectrofotometria de absorção atômica, de acordo com a metodologia recomendada pela FAO. Com exceção do Cu, alguns níveis de Zn, Fe e Pb foram encontrados na água em padrões de qualidade ambiental superiores aos estabelecidos pelo Ministério do Meio Ambiente do Peru para os rios do litoral e planalto, e aos padrões de qualidade da União

Europeia para o cultivo da truta. A concentração de Cu, Zn, Fe e Pb no fígado, nos rins e nos músculos excedeu os limites máximos permitidos pela União Europeia para a carne de peixe; e pela NOM-027-SSA1-1993 mexicana para os produtos frescos da pesca, refrigerados e congelados, no caso de Pb. A correlação entre a concentração de cobre, zinco, ferro e chumbo em água e a concentração destes metais no fígado, rim e no músculo, é baixa e não significativa, exceto para cobre que teve correlação significativa.

Palavras-chave: bioacumulação, espectrofotometria de absorção atômica, metais pesados, truta.

1. INTRODUCTION

Fish have been recognized as an integral component of a well-balanced diet, and provide an important source of energy, high quality proteins, vitamins and a wide range of other nutrients (Pieniak et al., 2010), such as Omega-3 polyunsaturated fatty acids, whose health benefits have been widely recognized (Swanson et al., 2012; Olmedo et al., 2013). In contrast to these benefits, the frequent presence of chemical pollutants in farmed fish is of great concern (Demirak et al., 2006; Martorell et al., 2011).

The balance of aquatic ecosystems has been altered by increased discharges of organic and inorganic pollutants (Dudgeon et al., 2006; Rizzo et al., 2010), and of these, the heavy metals are among the most worrisome.

Heavy metal pollution resulting from mining activity and increasing industrialization (Lozano et al., 2010) represents a special environmental risk due to its long-term persistence in nature and possible bioaccumulation and biomagnification in the trophic chain (Agah et al. 2009; Kehrig et al., 2009, Lajeunesse et al., 2011, Zenker et al., 2014).

Metals such as copper (Cu), zinc (Zn), cobalt (Co) and iron (Fe) are considered a hazard to the aquatic biota unless they reach concentrations higher than those required for growth and reproduction (Canli and Atli, 2003). However, cadmium (Cd), mercury (Hg) and lead (Pb) present in wastewater discharged in rivers by mineral concentrators in the highlands (Padilla, 2005; Hurtado et al., 2006) has significant adverse effects for the aquatic biota and for human beings (Jiménez et al., 2000; Gammons et al., 2006; Kojadinovic et al., 2007).

The discharge of heavy metals in the Mantaro River watershed, Junin Region, Peru, as a result of mining and metallurgical activities, especially in La Oroya-Yauli, is seriously degrading water quality and species diversity due to its toxicity, persistence and cumulative behavior (García Cambero, 2002; Bandowe et al., 2014). Despite this, the production of rainbow trout in this watershed for export to United States and European markets has increased.

Due to the limited knowledge available on the concentration of heavy metals in the water and rainbow trout of highland ecosystems, more information is needed regarding pollution levels in the waters and rainbow trout specimens of Yauli province production centers. Therefore, the objectives of the study were: (a) to determine the level of accumulation of copper, zinc, iron and lead in livers, kidneys and muscles of trout of commercial size and weight (28 cm and 250 g on average) and (b) to determine the concentrations of copper, zinc, iron and lead in the water of the production centers.

2. MATERIAL AND METHODS

2.1. Study area

The study area included the production centers of rainbow trout for export located in the micro watershed of the Huari and Tishgo Rivers, tributaries of the Mantaro River, in the Huari, Casaracra and Paccha Districts of Yauli Province, Junín Region. The "El Paraiso" (CP1) and

"El Pedregal" (CP2) production centers are located in the district of Huari at 3670 masl (409735E, 8713106N) and at 3654 masl (410467E, 8712942N), respectively. The "Sol Radiante" (CP3) center at 3856 masl (398175E, 8735716N), "Manantial Agua de Vida" (CP4) at 3853 masl (398038E, 8735583N), "Contratistas Véliz" (CP5) at 3845 masl (397549 E, 8735250N) and Eloim (CP6) at 3842 masl (397159E, 8734904N) are located in the district of Casaracra and the "Casaracra" (CP7) production center at 3801 masl (396041E, 8733599N) in the district of Paccha (396041E, 8733599N). (Figure 1).

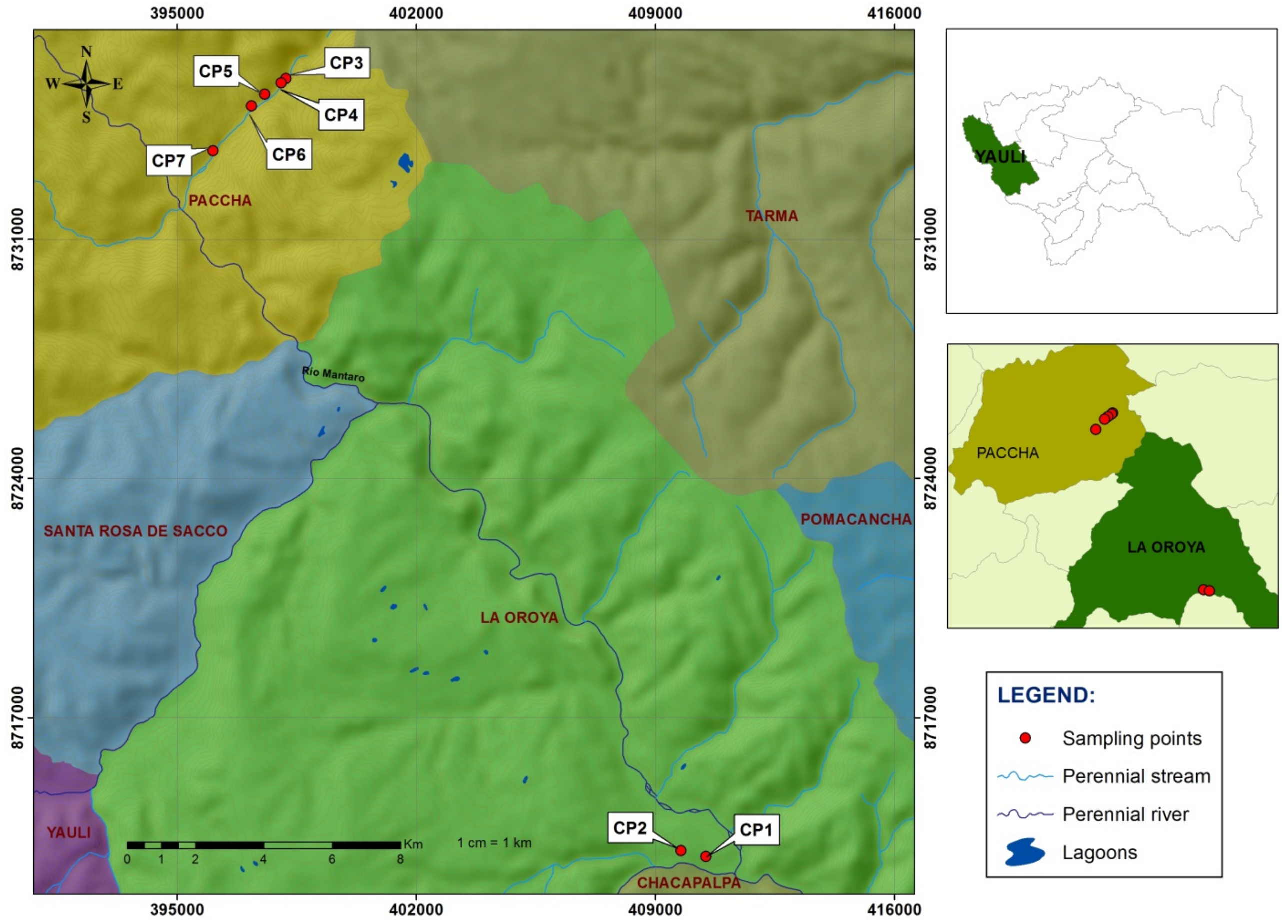

Figure 1. Location of production centers of rainbow trout for export (CP) in the Huari and Tishgo Rivers, Mantaro River Watershed.

2.2. Methods

2.2.1. Determination of heavy metals in livers, kidneys, and muscles

A total of 28 trout that averaged 250 g and 27 cm were collected from seven production centers in the province of Yauli. These were transported in dry ice the same day of their capture to the Laboratory of Instrumental Analysis of the Faculty of Chemical Engineering of the Universidad Nacional del Centro del Peru for chemical analysis. The next day the livers, kidneys and dorsal muscles were separated from each fish. Ten g of each tissue were weighed into a porcelain capsule and calcined on a heating plate. Subsequently, the capsules were placed in the muffle for the destruction of organic matter at 500° C for three hours. For the preparation of the sample, the ash was dissolved with a solution of 6% nitric acid and 3% hydrochloric acid at a ratio of 1: 1, filtered and diluted in a 100-ml dilution flask with 1% nitric acid (Dybern et al., 1983). The samples were analyzed for three days.

2.2.2. Determination of heavy metals in water used for the production of trout

The water samples were collected in disposable 1.5 L plastic bottles, previously treated with 10% nitric acid solution for 24 hours and rinsed with bi-distilled water. 1.5 mL of concentrated nitric acid was then added to one liter of water from each of the samples to preserve them (APHA, 2012). In addition, temperature, dissolved oxygen and pH were determined in situ by Hanna Instruments portable equipment (HI 991301 Microprocessor pH / temperature and HI 9146 Microprocessor dissolved oxygen) and portable computers. The equipment had previously been calibrated in the respective sampling sector.

The next day the sample was prepared by placing 250 mL of water in a beaker which was brought to boiling, until 100 mL remained. Immediately, 5 mL of nitric acid and 5 mL of concentrated hydrochloric acid were added to destroy organic matter and the sample was boiled again until the water achieved a pasty consistency. The sample was allowed to cool and then 10 mL of distilled water was added, filtered and diluted in a 100-mL dilution flask with 1% nitric acid (APHA, 2012). The samples were analyzed for one day.

The quantitative determination of heavy metals (copper, zinc, iron and lead) was performed by the flame atomic absorption spectrophotometry method, according to the methodology recommended by Dybern et al. (1983), using a Shimadzu-brand AA-6800 Atomic Absorption Spectrophotometer. Standard solutions of copper, zinc, iron and lead were previously prepared and read in increasing order of concentration to determine the calibration curve, and then the samples readings were done.

2.2.3. Analysis of data

Data were expressed as the mean ± standard deviation and, in order to compare the concentration level of metals present in the water and trout tissues between production centers, were subjected to analysis of variance (ANOVA) with the SPSS 21 program. To determine significant differences between averages, the Tukey's multiple comparisons test was applied with a 5% error. Also, a correlation analysis was performed between the concentration of metals in water and in the trout tissues, using the linear correlation coefficient (Clifford y Taylor, 2008).

2.2.4. Elaboration of the calibration curve

With the standard 1000 ppm of Pb, Cu, Fe, Zn, As; an average standard of 100 ppm concentration was prepared. Then, working standards of 0.001 were prepared; 0.01, 0.1, 1.0 and 2.0 ppm, with nitric acid at 1%. The absorbance readings of the standards were then performed at different wavelengths for each element in the atomic absorption spectrophotometer. Finally, the calibration curve was plotted: concentration vs absorbance and the concentration of the samples was read using the respective calibration curve in the atomic absorption spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Concentration of heavy metals in trout tissues

When analyzing the average concentrations of copper, zinc, iron and lead in the livers, kidneys and muscles of trout obtained from the production centers of the province of Yauli-Junín, high levels of these metals were registered, which exceeded the maximum permissible limits established by the current international legislation, mainly for muscle. These results are supported by Cuéllar Carrasco (2000), who report that the presence of metals in water facilitates their accumulation in the livers and muscles of fish. The kidneys were also exposed to water metals because blood flows from the gills to the carotid artery, which supplies blood to the kidney. Likewise, the results are corroborated by Jezierska and Witeska (2006), who point out that metals accumulate in the bodies of fish in different quantities, due to the different affinities of the metals with the tissues of the fish, at different rates of uptake, deposition and excretion. However, they considered that the accumulation of metals in fish depends on pollution, and may differ for different species of fish that live in the same body of water.

3.2. Heavy metals in liver

The mean concentration of heavy metals in trout livers (Table 1) showed considerable oscillations between production centers. For copper, the lowest concentration was observed in CP4 and the highest in CP5, with values of 2.55 ± 0.23 and 17.55 ± 14.33 µg/g, respectively, with significant statistical differences ($p \geq 0.05$) between production centers. In the case of zinc, the lowest concentration was found in CP7 and the highest in CP1, with values of 12.77 ± 1.53 and 38.12 ± 22.30 ug/g, respectively, with significant statistical differences ($p \geq 0.05$) between production centers. For iron, the lowest concentration was observed in CP7 and the highest in CP1, with values of 33.58 ± 5.85 and 111.78 ± 22.06 µg/g, respectively, with statistically significant differences ($p \geq 0.05$) between production centers. In the case of lead, the lowest concentration was found in CP6 and the highest in CP1, with values of 1.41 ± 0.34 and 6.11 ± 3.92 µg/g, respectively, with no significant statistical differences ($p \geq 0.05$) between production centers.

The liver was the organ that accumulated the highest concentration of heavy metals, concentrated mostly iron, followed by zinc, copper and lead. Copper was basically accumulated in the liver, although levels of it were also detected in the kidneys and a little in the muscles. In a study by Robinson and Avenant-Oldewage (1997) in *Tilapia mossambica*, it was observed that the liver accumulated the largest amount of copper with respect to other organs, indicating that this high concentration of copper is attributable to the union of this metal with the metallothioneins to form complexes as detoxification mechanisms.

There are studies on the bioaccumulation of copper, zinc, iron and lead in the livers of freshwater fish (García Cambero, 2002), that report a copper concentration of 18.47 to 22.61 µg/g for the "barbo" (*Barbus barbus*), and a concentration ranging from 20.88 to 27.79 µg/g for zinc. These results are similar to those found in this work. In relation to lead, the content varied from 0.07 to 0.15 µg/g. These results are very low in relation to those obtained in this study.

Table 1. Mean concentration of heavy metals in trout livers.

Metals (µg/g wet weight)	Production Center						
	CP1	CP2	CP3	CP4	CP5	CP6	CP7
Copper	12.10 ± 4.63**a**	5.22 ± 1.48**b**	9.43 ± 6.16**a**	2.55 ±0.23**b**	17.55 ± 14.33**a**	10.48 ±7.00**a**	14.57 ± 9.18**a**
Zinc	38.12 ± 22.30**a**	29.31 ±10.23**a**	29.09 ± 11.75**a**	16.64 ± 5.44**b**	29.26 ±5.86**a**	18.90 ±2.49**b**	12.77 ± 1.53**b**
Iron	111.78 ± 22.06**a**	73.98 ±23.28**b**	40.45 ± 5.35**b**	37.91 ± 13.08**b**	76.32 ±34.39**a**	40.73 ±2.21**b**	33.58 ± 5.85**b**
Lead	6.11 ± 3.92**a**	3.54 ± 0.85**a**	5.17 ± 3.83**a**	1.78 ± 0.43**a**	4.04 ±2.18**a**	1.41 ±0.34**a**	2.18 ± 1.14**a**

Note: Unequal letters in horizontal form, show that there are significant statistical differences between mean concentrations ($p \geq 0.05$).

3.3. Heavy metals in kidneys

The mean concentration of heavy metals in trout kidneys (Table 2) showed fluctuations between production centers. For copper, the lowest concentration was recorded at CP6 and the highest concentration was recorded at CP2, with values of 1.54 ± 0.80 and 4.74 ± 2.45 μg/g, respectively, with no statistically significant differences ($p \geq 0.05$) between production centers. In the case of zinc, the lowest concentration was registered at CP6 and the highest was registered at CP1, with values of 15.30 ± 4.47 and 32.53 ± 18.10, respectively, and there were no significant statistical differences ($p \geq 0.05$) between production centers. For iron, the lowest concentration was recorded at CP4, and the highest concentration was registered at CP1, with values of 37.33 ± 9.20 and 108.02 ± 51.55 μg/g, respectively, with significant statistical differences ($p \geq 0.05$) between production centers. In the case of lead, the lowest concentration was recorded at CP5 and the highest was registered in CP2, with values of 2.35 ± 0.41 and 5.98 ± 4.50 μg/g, respectively, with no significant statistical differences ($p \geq 0.05$) between production centers.

Regarding the accumulation of heavy metals in the kidneys, this organ mostly concentrated iron, followed by zinc, lead and copper. There are reports of research on the bioaccumulation of copper, zinc and iron in the kidneys of freshwater fish, such as the one made by García Cambero (2002) for the "barbo" (*Barbus barbus*), which found a concentration of copper in the kidneys that varied from 1.52 to 2.05 μg/g, and a concentration of 15.17 to 17.28 μg/g for zinc. These results are lower than those found in this work. As to the content of lead in kidney, a concentration of 0.05 to 0.07 μg/g was found; these concentrations are very low in comparison with those found in this work.

Table 2. Mean concentration of heavy metals in trout kidneys.

Metals (μg/g Wet weigh)	Production Center						
	CP1	CP2	CP3	CP4	CP5	CP6	CP7
Copper	4.23 ± 2.31**a**	4.74 ±2.45**a**	2.64 ± 0.99**a**	2.34 ± 1.56**a**	1.98 ±0.86**a**	1.54 ±0.80**a**	1.64 ± 0.81**a**
Zinc	32.53 ± 18.10**a**	28.17 ±2.81**a**	28.38 ± 10.33**a**	24.13 ± 4.80**a**	24.02 ±3.07**a**	15.30 ±4.47**a**	19.78 ± 7.15**a**
Iron	108.02 ± 51.55**a**	65.93 ±33.68**b**	55.39 ± 10.06**b**	37.33 ± 9.20**b**	66.50 ±28.10**b**	53.37 ±11.41**b**	57.63 ± 18.92**b**
Lead	3.92 ± 2.35**a**	5.98 ±4.50**a**	2.75 ± 0.59**a**	2.65 ± 1.36**a**	2.35 ±0.41**a**	2.97 ±1.18**a**	3.17 ± 0.84**a**

Note: Unequal letters in horizontal form, show that there are significant statistical differences between mean concentrations ($p \geq 0.05$).

3.4. Heavy metals in muscle

The mean concentration of heavy metals in trout muscles (Table 3), showed notable variations among production centers. For copper, the lowest concentration was found at CP6 and the highest at CP1, with values of 0.14 ± 0.06 and 1.81 ± 0.86 μg/g, respectively, with significant statistical differences ($p \geq 0.05$) between production centers. In the case of zinc, the lowest concentration was observed at CP7 and the highest at CP3, with values of 4.77 ± 1.80 and 5.82 ± 4.80, respectively, and there were no significant statistical differences ($p \geq 0, 05$) between production centers. For iron, the lowest concentration was found at CP1 and the highest at CP3, with values of 1.80 ± 1.59 and 7.10 ± 3.99 μg/g, respectively, with no statistically significant differences ($p \geq 0.05$) between production centers. In the case of lead, the lowest concentration was observed at CP1 and the highest at CP3, with values of 0.54 ± 0.12 and

4.74 ± 3.82 μg/g, respectively, with significant statistical differences ($p \geq 0.05$) between production centers.

Concerning the accumulation of heavy metals in muscle, this tissue concentrated mostly zinc, followed by iron, lead and copper. The results of copper concentrations in muscles found in this research are lower than those reported by Sauval (2000), who found a concentration of <0.5 μg/g in wet weight for "rainbow trout" (*Oncorhynchus myk*iss) and other freshwater fish such as "pejerrey bonaerense" (*Odontesthes bonariensis*), common carp (*Cyprinus carpio*), perch bocona (*Percichthys colhuapiensis*), periquita (*Percichthys altispinnis*) and "catfish otuno" (*Diplomystes viedmensis*). Argota Coelho et al. (2005) who, working with biological matrices, found a concentration of 3 μg/g for the total mass of fish.

Regarding zinc, the results obtained in this work are higher than those found by Sauval (2000) in the muscle of "rainbow trout", which showed a concentration of 3.3 μg/g, and, in other freshwater fish such as "pejerrey", 4.7 μg/g, in "common carp", 4.4 μg/g, in "perch", 3.1 μg/g, and in "periquita", 5.1 μg / g in wet weight. Argota Coelho et al. (2005), working with biological matrices, found a concentration of 42 μg/g for the total mass of fish.

As for iron, the results found in this research are higher (except for CP1 and CP6) than those found by Sauval (2000), which report a content of 3.1 μg/g for "rainbow trout" muscle. Other freshwater fish showed the following concentrations: "pejerrey bonaerense", 1.0 μg/g; "common carp", 5.4 μg/g; "perca bocona", 4.8 μg/g; "parakeet", 3.5 μg/g; and "catfish Otum ", 5.4 μg/g by wet weight. Argota Coelho et al. (2005), working with biological matrices, found a concentration of 31.3 μg/g for the total mass of fish.

For lead, the concentrations obtained are very high compared with those found by Sauval (2000), who reported a concentration <0.15 μg/g, in wet weight, for "rainbow trout" muscles and for other freshwater fish Such as "pejerrey bonaerense", "common carp", "perca bocona", "periquita" and "catfish otuno".

In this study, very high levels of lead were found in muscles, far exceeding the permissible maximum limit (PML) established by the European Union, which is 0.3 μg/g for fish meat and by the Mexican official standard, NOM-027-SSAI-1993, which is 1.0 μg/g for fresh, chilled and frozen fishery products.

The concentrations of copper, zinc, iron and lead registered in livers, kidneys and muscles of trout was high, and in the specific case of lead in muscles, the content exceeded the maximum limits established by current international legislation. This is possibly due to the mining and metallurgical companies, which discharge their effluents into the waters that are used in the intensive production of trout without any treatment. Consequently, the concentration of heavy metals in the water increases, and the metals become concentrated in the tissues of fish through the process of bioaccumulation.

Table 3. Mean concentration of heavy metals in trout muscles.

Metals (μg/g Wet weigh)	Production Center						
	CP1	CP2	CP3	CP4	CP5	CP6	CP7
Copper	1.81 ± 0.86**a**	1.07 ± 1.37**a**	0.39 ± 0.28**a**	0.42 ± 0.39**a**	0.32 ± 0.24**a**	0.14 ± 0.06**b**	0.33 ± 0.08**a**
Zinc	5.55 ± 2.78**a**	5.60 ± 4.00 **a**	5.82 ± 4.80**a**	5.33 ± 2.57**a**	5.20 ± 2.12**a**	5.23 ± 2.49**a**	4.77 ± 1.80**a**
Iron	1.80 ± 1.59**a**	4.63 ± 1.76**a**	7.10 ± 3.99**a**	3.38 ± 0.20**a**	4.53 ± 0.65**a**	2.66 ± 1.90**a**	4.40 ± 0.83**a**
Lead	0.54 ± 0.12**b**	1.21 ± 0.43**a**	4.74 ± 3.82**a**	1.88 ± 1.21**a**	0.72 ± 0.22**a**	1.09 ± 0.59**a**	0.82 ± 0.23**a**

Note: Unequal letters in horizontal form, show that there are significant statistical differences between mean concentrations ($p \geq 0.05$).

Comparing the mean levels of lead in trout muscles, the registered values exceeded the PML established by EU Regulation (CE) N° 1881/2006 for fish meat (0.3 µg/g). In CP1, 1.8; CP2, 4.03; CP3, 15.8; CP4' 6.27; CP5, 2.4; CP6, 3.63 and CP7, 2.73 times, respectively. Likewise, the registered values exceeded the PML of the Mexican official standard, NOM-027-SSA1-1993, for fresh, chilled and frozen fish products (1.0 µg / g): CP2, 1.21; CP4, 4.74; CP4, 1.88 and CP6, 1.09 times, respectively. Production centers CP1, CP5 and CP7 did not exceed the PML (Figure 2).

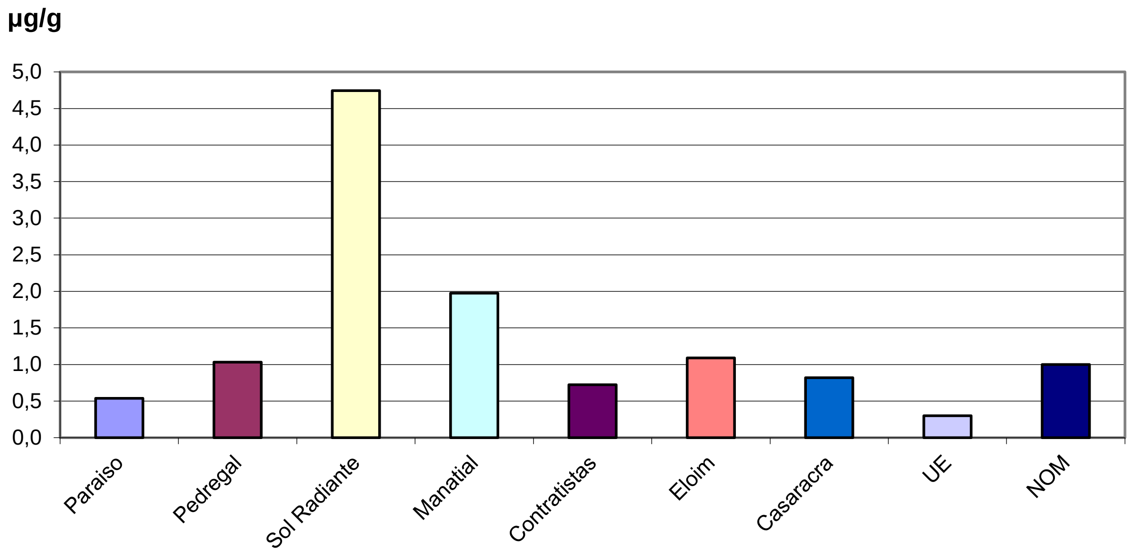

Figure 2. Comparison of the mean concentrations of lead in trout muscles and PML of UE and NOM for fish meat.

3.5. Concentration of heavy metals in water used for the production of trout

Little is known about the level of pollutants in water used for the production of trout for export in the central highlands of the Peruvian Mantaro River Watershed, which is greatly influenced by the mining and metallurgical industries. Nevertheless, in the last two decades the Peruvian government has promoted the farming of fish for export purposes in this part of Peru.

The results obtained show significant levels of copper, zinc, iron and lead in the water used by the production centers included in the study compared with the levels reported by MINAM (Peru, 2008) for waters from rivers in this watershed. This reveals the strong anthropogenic pressure that these aquatic ecosystems are undergoing. These results are supported by Rizzo et al. (2010), who reported that heavy metals that exceed the normal concentration threshold become potentially toxic. However, even low concentrations of metals can threaten the health of aquatic and terrestrial organisms, including man (Sarmiento et al., 2011).

The toxicity of heavy metals also depends on environmental factors such as temperature, dissolved oxygen and water pH. In the study, the behavior of the physical-chemical factors of water was similar in the Huari and Tishgo Rivers (Table 4). However, the highest temperature and pH value was recorded at the CP6 production center and the highest dissolved oxygen at the CP7 production plant.

In aquatic environments, pH facilitates the bioavailability and toxicity of most of these metals by affecting the balance between metal speciation, complexation, solubility and ion exchange (Janssen, 2003). In fish, these factors favor the bioavailability of heavy metals during the gill respiration process (De Schamphelaere and Janssen, 2004). The alkaline pH values registered in the water of fish production centers suggest that heavy metals are less available. However, Hg Cd and Pb that exceeded the maximum permissible limits in water for human consumption are possibly associated with particles suspended from the water, which, like As, Mo, Se and Cr, tend to be less available at alkaline pH (Manzione and Merrill, 1989).

Table 4. Physicochemical factors of water.

Production Center	River	Temperature (ºC)	Dissolved oxygen (ppm)	pH
CP1	Huari	10.3	7.6	7.86
CP2	Huari	10.4	7.4	7.79
CP3	Tishgo	11.2	7.8	7.93
CP4	Tishgo	11.0	7.8	8.09
CP5	Tishgo	11.3	7.7	8.14
CP6	Tishgo	11.6	7.9	8.25
CP7	Tishgo	10.4	8.1	8.03

Regarding copper concentration, the results showed a similar concentration behavior in six fish production centers, elevated only in the CP5 production center, at 0.0399 mg/L, a result that is higher than those reported by MINSA (Peru, 2006) for the Huari River, with copper concentrations ranging from <0.005 to 0.022 mg/L, and for the Tishgo River, with concentrations of 0.005 mg/L. These results agree with some studies carried out in the rivers of northern Peru, which present similar environmental and anthropogenic characteristics (Huaranga et al., 2012). The highest average concentration of copper was registered at the CP4 production center, at 0.0515 mg/L. The values above this concentration become toxic to fish and other aquatic organisms, even more so if they act synergistically with zinc (Lombardi et al., 2010; Sakulsak, 2012) (Table 5).

The mean concentrations of copper, with the exception of CP5, exceeded the environmental quality standard (EQS) for river water in the sierra, established by the Ministry of the Environment of Peru, which is 0.02 mg/L (Peru, 2008), and the standard of quality for aquatic life in freshwater of the US Environmental Protection Agency (USEPA, 1994), which is 0.012 mg/L, and the standard for copper in surface water of the World Health Organization (WHO), which is 0.003 mg/L (Pittaluga and Suvires, 2006) (Figure 3). However, they did not exceed the quality standard of water for trout farming of the European Union, which states that the maximum total copper concentration should not exceed 0.1 mg/L, nor the quality standard for fish and trout farming of < 0.3 mg/L reported by Klontz (1991).

The mean concentration of zinc in all production centers under study exceeded the EQS for the rivers of the highlands, established by MINAM, which is 0.03 mg/L (Peru, 2008), the quality standard for aquatic life in fresh water of the US EPA, which is 0.032 mg/L, the standard for zinc surface in surface water of WHO, which is 0.02 mg/L (Pittaluga and Suvires, 2006) (Figure 4), and the quality standard for the fish and trout farming reported by Klontz (1991) for fish and trout farming, which is < 0.04 mg/L.

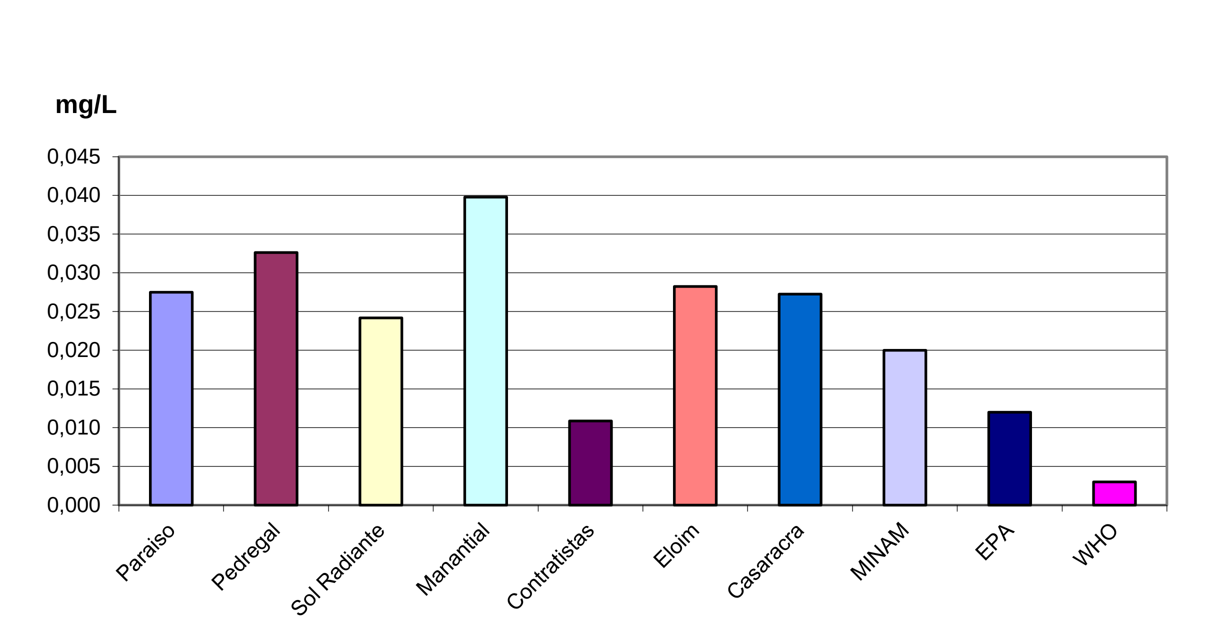

Figure 3. Comparison of the mean concentration of total copper of water with EQS of MINAM and PML of the EPA and WHO.

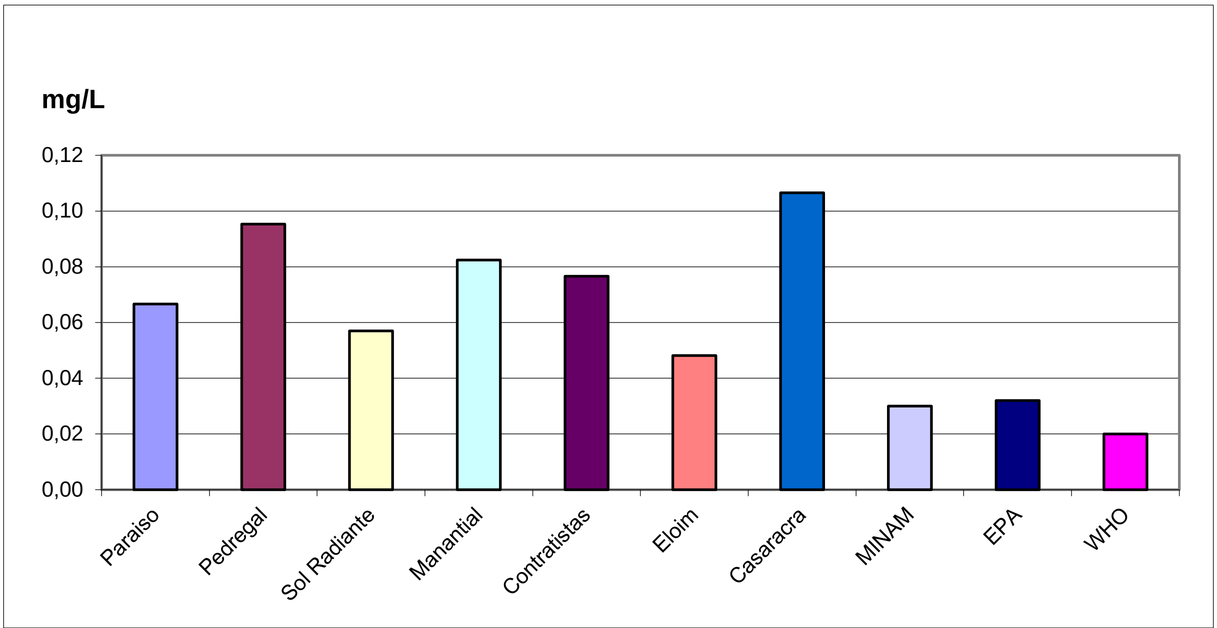

Figure 4. Comparison of the mean concentration of total zinc in water with EQS of MINAM and PML of the EPA and WHO.

Studies conducted by MINSA (Peru, 2006) in Yauli-Junín reported concentrations of zinc from < 0.052 to 0.308 mg/L for the Huari River and concentrations of < 0.087 to 0.179 mg / L for the Tishgo River. There are studies carried out in other countries at similar altitudes, such as Pittaluga and Suvires (2006), which reported a zinc concentration of 0.001 mg/L, and Alcalde et al. (2000), which report a Zinc concentration of 0.02 mg/L for the Colorado River. These results are much lower than those found in this research.

Pérez et al. (2002), who studied the pollution of the Copper River, reported a zinc concentration from 0.010 to 1.040 mg/L, and Gómez-Álvarez et al. (2004) reported a zinc concentration from 0.03 to 2.45 mg/L for surface water of the San Pedro River; these results are higher than those found in this work.

The concentration levels for iron did not exceed the quality standard reported by Klontz (1991), which is < 1.0 mg/L for fish and trout farming. They did not reach lethal levels, since according to Garcia (2002), concentrations of 0.9 mg / L and pH 6.5 - 7.5 lead to the death of fish.

This study found higher results than those reported by MINSA (Peru, 2006) in Yauli-Junín, which found iron concentrations of < 0.164 to 0.2759 mg/L for the Huari River and of < 0.289 to 0.681 mg/L for the Tishgo River. But the results are low in relation to those found by Pérez et al. (2002), who reported a high iron concentration from 0.001 to 0.25 mg/L in Copper River water, and by Gómez-Álvarez et al. (2004) who reported a high iron concentration of 0.26 to 15.23 mg/L for the San Pedro River.

The mean concentrations of lead exceeded the EQS, established by the MINAM for the rivers of the Peruvian highlands, which is 0.001 mg/L (Peru, 2008)), the standard of quality for aquatic life in fresh water of the US EPA, which is 0.0032 mg/L, the standard for lead in surface water of WHO, which is 0.003 mg/L (Pittaluga and Suvires, 2006) and that of the EU for trout farming, which is 0.03 mg/L. The exception was CP7, which in this case did not reach lethal levels (Figure 5).

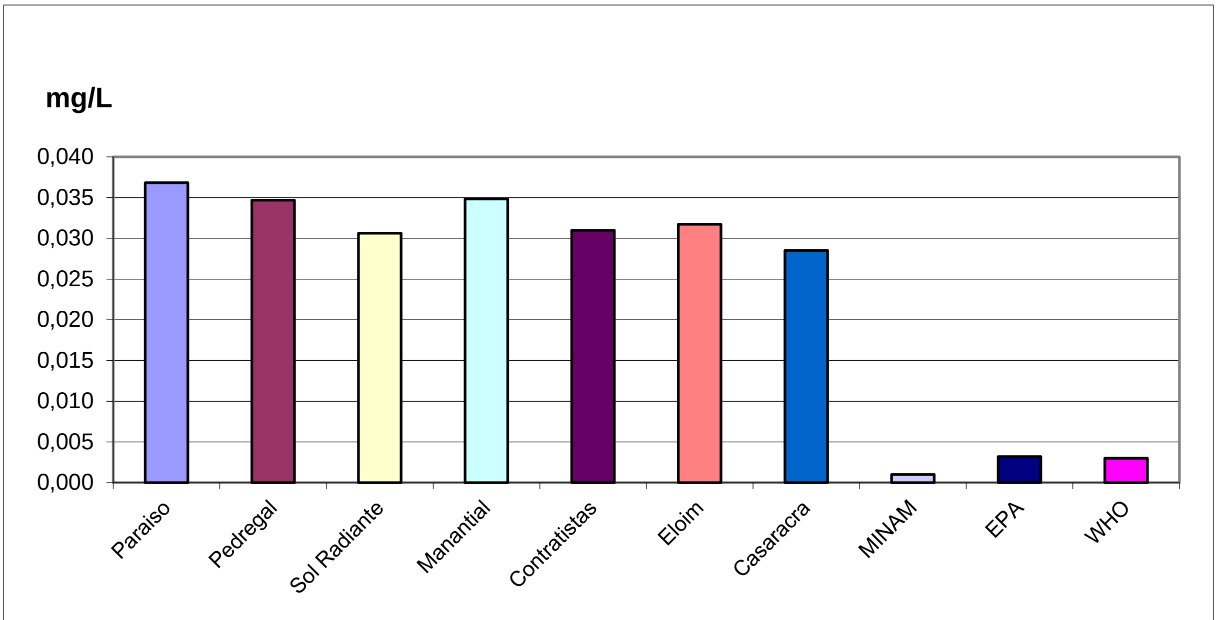

Figure 5. Comparison of the mean concentration of total lead of water with EQS del MINAM y PML de la EPA y WHO.

The results of lead concentrations found in this study are higher than those reported by MINSA (Peru, 2006) in Yauli-Junín, which found that lead concentrations ranged from 0.025 to 0.079 mg/L in the Huari River and concentrations of <0.025 mg/L for the Tishgo River. Pitaluga and Suvires (2006) found a variation in lead concentration from 0.001 to 0.012 mg/L in the Castaño River and Alcalde et al. (2000) found a lead concentration of 0.007 mg/L in the Colorado River. However, the results of the study are lower than those found by Gómez-Álvarez et al. (2004), who reported concentrations ranging from below the detection limit to 0.3 mg/L in the water of the San Pedro River.

Table 5. Mean concentration of total heavy metals in water.

Metals (mg/L)	Production centers						
	CP1	CP2	CP3	CP4	CP5	CP6	CP7
Copper	0.0275 ± 0.0422**a**	0.0326 ± 0.0361**a**	0.0242 ± 0.0438**a**	0.0399 ± 0.0515**a**	0.0109 ± 0.0131**a**	0.0282 ± 0.0420**a**	0.0273 ± 0.0383**a**
Zinc	0.0666 ± 0.0258**a**	0.0953 ± 0.0494**a**	0.0570 ± 0.0236**a**	0.0824 ± 0.0410**a**	0.0766 ± 0.0357**a**	0.0481 ± 0.0200**a**	0.1064 ± 0.0938**a**
Iron	0.1359 ± 0.0925**a**	0.1288 ± 0.0640**a**	0.1163 ± 0.1361**a**	0.1109 ± 0.0615**a**	0.0977 ± 0.0486**a**	0.0773 ± 0.0562**a**	0.1060 ± 0.0902**a**
Lead	0.0368 ± 0.0241**a**	0.0347 ± 0.0113**a**	0.0306 ± 0.0202**a**	0.0347 ± 0.0089**a**	0.0310 ± 0.0098**a**	0.0317 ± 0.0181**a**	0.0285 ± 0.0156**a**

Note: Unequal letters in horizontal form show that there are significant statistical differences between mean concentrations ($p \geq 0.05$).

The correlation analysis between water metals and trout tissues at a significance level of 0.05 showed a low and non-significant correlation between the concentration levels of copper, zinc, iron and lead in the water and the concentration levels of these metals in the livers, kidneys and muscles, with the exception of copper, which had a significant correlation (Table 6).

Table 6. Correlation coefficients between the concentration level of heavy metals of water and trout tissues.

Heavy metals of water	Trout tissues		
	Livers	Kidneys	Muscles
Copper	0.179 n.s.	0.024 n.s.	0.389 *
Zinc	0.018 n.s.	0.236 n.s.	0.047 n.s.
Iron	0.183 n.s.	0.276 n.s.	0.01 n.s.
Lead	0.349 n.s.	0.194 n.s.	0.101 n.s.

***** = Significant ($p \leq 0{,}05$).
n.s. = Non-significant.

4. CONCLUSIONS

The greatest bioaccumulation of heavy metals in the trout tissues of the Yauli-Junín production centers occurs in livers, followed by kidneys and is much smaller in muscles.

The metal that bioaccumulates most in the livers is iron, followed by zinc, copper and lead. The metal that bioaccumulates most in the kidneys is iron, followed by zinc, lead and copper. The metal that most bioaccumulates in the muscles is zinc, followed by iron, lead and copper.

The concentration levels of copper, zinc, iron and lead in the livers followed the same pattern of accumulation (Fe > Zn > Pb > Cu) obtained by other authors for rainbow trout and other fish in natural environments. The kidneys and muscles did not follow the same pattern of accumulation.

The concentrations of copper, zinc, iron and lead in the muscles, is greater than the results obtained by other authors for rainbow trout and other fish.

The concentrations of lead in muscles exceeded the maximum allowable limit established by EU Regulation (CE) N°. 1881/2006 for the meat of fish in all production centers and by the Mexican official standard NOM-0127-SSA1-1993 for fresh, chilled and frozen fishery products, with the exception of Paraíso, Contratistas Véliz, and Casaracra-UNCP.

The concentrations of copper, zinc iron and lead in the waters of the Yauli-Junín trout production centers exceeded national environmental quality standards for water established by the Ministry of the Environment of Peru for the rivers of the coast and highlands.

The lead concentrations were higher than the maximum permissible limit established by the European Union for trout farming.

The correlation between the concentrations of copper, zinc, iron and lead in water and the concentrations of these metals in the livers, kidneys and muscles, is low and not significant, except for copper, which had a significant and average correlation.

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