

ORIGINAL ARTICLE

Measuring the concentrations of the heavy metals Nickel, Lead, and Cadmium in the muscle tissue of the *Epinephelus coioides* in Asaluyeh port

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ABSTRACT

The present research was carried out with the aim of measuring the levels of the heavy metals nickel, lead and cadmium in the muscle tissues of the *Epinephelus coioides* in Asaluyeh port in Bushehr Province. To this end, 20 *Epinephelus coioides* with an average weight of 660.68 and 598.62 gr were captured in winter and spring of 2016, respectively. The samples were washed with distilled water and a portion of the pure muscle tissues was isolated. After dehydrating the tissues and discharging the water in the intertextual space, 1 gr of the powdered tissue sample was isolated and the acid digestion of the samples was carried out using the Moopam method. To measure the levels of the heavy metals nickel, lead and cadmium a (PG AA 500) atomic absorption spectrophotometer was employed. The results of measuring the concentrations of the heavy metals in the muscle tissues of the *Epinephelus coioides* suggested that the mean and standard deviation values of nickel, lead and cadmium were (31.38±0.14, 46.04±0.22 and 22.24±0.31) during winter, respectively. In spring, the mean and standard deviation values of nickel, lead and cadmium were (18.82±0.28, 17.59±0.64 and 13.92±0.43) μ g/kg dry weight respectively. These figures show a significant difference between the results of winter and spring ($P<0.05$). The results of measuring heavy metals in the study area were lower than the international WHO, FAO, NHMRC, and UK(MAFF) standards and therefore these concentrations pose no threat to humans.

Keywords; nickel, lead, cadmium, Asaluyeh port, *Epinephelus coioides*

Received 30.05.2018

Revised 11.07.2018

Accepted 17.08.2018

How to cite this article:

H Z Nourbakhsh, A Rahim Pazira, R Heidari, R Obeidi. Measuring the concentrations of the heavy metals Nickel, Lead, and Cadmium in the muscle tissue of the *Epinephelus coioides* in Asaluyeh port. Adv. Biores. Vol 9 [5] September 2018: 140-145.

INTRODUCTION

Aquatic animals are extremely important economic and nutritional sources for humans. Fish are aquatic creatures that are known as a valuable source of protein for many humans and it is estimated that 15 to 20% of animal proteins are supplied from water resources. Heavy chemical elements accumulate in the organs and tissues of the fish and enter the food chain in their biological cycle. Therefore, these elements are potential toxic threats to microorganisms and are eventually transferred to humans [1,2]. Hence, the assessment and control of heavy metals is an important issue to nutritionists, medical scientists, and environmentalists [3,4], because heavy metals are stable pollutants that are not decomposed through chemical or biological processes in the nature [5]. Moreover, heavy metals play a major role in the biological systems and excessive concentrations of these metals in the environment have toxic effects[6].

In the classification of heavy metals by toxicity, the heavy metals nickel, lead and cadmium are classified as highly toxic metals, which are largely scattered in the environment [7]. These elements enter the environment through the wastewater of plating factories, output of water incineration factories, fossil fuels, effluents of refineries and oil fields, urban and industrial wastewater, passage of ships, vessels, and launches, and the use of mud and fertilizers for agricultural purposes [8]. Nickel causes headaches, sleeplessness, low patience, nausea, vertigo, and vomit. Some of the pathological signs of contamination with nickel are hemorrhage and cell disorders [7]. Impaired biosynthesis of hemoglobin, anemia,

hypertension, kidney damage, abortion, premature birth, nervous system disorders, brain damage, male infertility, reduced learning, and behavioral disorders in children are some of the negative side effects of the increase in the concentration of lead in the human body [9]. The signs of cadmium poisoning include proteinized urine, increased urine glucose, increased urea amino acid, prostate cancer, lung cancer, diarrhea, nausea, vomit, shortness of breath, headaches, fever, kidney failure, hypertension, increased calcium and potassium excretion, and cardiac diseases [10].

With an average depth of 30 to 35 m, the Persian Gulf is located in the south of the Iranian Plateau on the banks of the Indian Ocean in the Northeast of the Sea of Oman. This sea is a half-closed sea with a relatively long water retention time. Therefore, any contamination of this sea may last long and result in excess contamination [11]. The Asaluyeh port in Bushehr Province and on the banks of the Persian Gulf is 300 km from the east of Bushehr port and is located approximately 100 km from the South Pars Gas Field. This port is extremely important to the oil, gas and petrochemical sector on the national, regional, and global levels [12].

The *Epinephelus coioides* belongs to the Serranide family and is considered to be of nutritional value. This fish plays a significant role in the human diet. The *Epinephelus coioides* is a demersal fish in salty water and is found in soft river beds and coasts with hard textures and inside rubbles. This fish is present at a maximum depth of 100 m and may enter the estuaries of rivers [13,14,15].

Many studies have been conducted to measure and study the concentrations of heavy metals in the aquatic animals, especially the fish, and some of these studies included the research on the concentrations of the heavy metals Ni, Pb and Cd in the muscle of *Epinephelus coioides* in the north of the Persian Gulf that was carried out by Pourang *et al.*, (2005) these studies showed values higher than the WHO and FAO standards. In addition, Biswas *et al.*, (2012) examined the concentrations of the heavy metals Cu, Mn, Zn, Fe, Cr and Pb in the muscle tissue of 9 species of valuable edible fish in the southwest of India and reported that the concentrations of metals posed no threat to humans. In the same year Alkan *et al.*, compared the concentrations of the heavy metals Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Hg and Pb in the muscle tissues of two species of fish from the southwest of the Black Sea. The measured calculations were smaller than the WHO limits. In 2015 Obeidi *et al.*, stated that the concentration of lead in the muscles of *Pomadasys kaakan* in Bushehr port was higher than the WHO and FAO standards. The present research was carried out to measure the levels of the heavy metals nickel, lead and cadmium in the muscle tissue of the *Epinephelus coioides* in the coasts of Asaluyeh port and to compare the results to the global standards.

MATERIAL AND METHODS

Study area

The study area was Asaluyeh port which is at the 27° ,28' ,24.48" N and the 52° ,36' ,49.79" E latitude and longitude, respectively [12] (Figure 1).

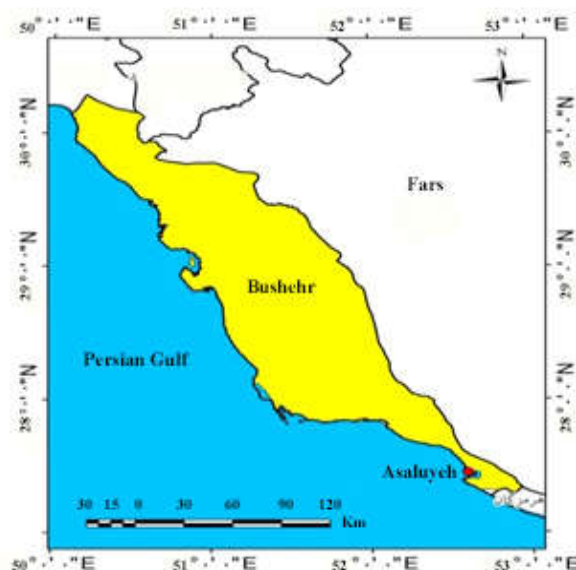


Figure 1: Location of the study area

A total of 20 *Epinephelus coioides* were randomly captured during the spring and winter of 2016 from the Asaluyeh port using fish traps and hooks. The samples were coded, put in an icebox full of ice and transferred to the laboratory [16]. The samples were washed with distilled water and a biometric analysis was carried out (to measure the total length and total weight) [17]. After separating the tissues using a polyethylene knife a portion of the isolates was put in a completely clean container (and was washed with nitric acid). The samples were put in an oven at the 80 °C temperature for 18 hours to dry up, and the dehydrated samples were completely powdered by a grinder. Afterwards, the samples were put in a desiccator to prevent the absorption of humidity. To provide for the chemical and acid digestion of the samples, first 1 gr of the powdered tissues was isolated and put in a beaker. Next, 10 ml of concentrated HNO₃ was added to the beaker and the samples were stored at the laboratory temperature for 30 minutes to allow for the primary digestion of the samples. The digestion process continued by placing the containers on a thermal plate with a temperature of 90 °C under a hood and after removal of the white vapor, the resulting solution was filtered using a 42-micron Whatman filter paper. Finally, using double-distilled water the volume of each sample was reduced to 25 ml. The digested samples were also stored in polyethylene before the analysis [18]. To measure the concentrations of nickel, lead and cadmium in the samples a PG AA500 atomic absorption spectrophotometer was used. In addition, data analysis was carried out in SPSS,20 and the dispersion of the resulting data was normalized using the one-sample Kolmogorov-Smirnov test. Afterwards, using the one-way analysis of variance (ANOVA), Tukey's and t-test methods the statistical analysis of the research data was performed at the 95% confidence level. Finally, the related diagrams of the research results were obtained using Excel 2013.

RESULTS

In this research, the muscle tissues of 20 *Epinephelus coioides* with an average weight of 660.68±2.32 gr in winter and 598.62±1.95 gr in spring and a mean length of 32.62±0.92 cm in winter and 27.18±0.53 cm in spring were isolated to measure the levels of the heavy metals nickel, lead and cadmium (Table 1). The investigation results revealed that the highest and lowest levels of nickel in the muscle tissue of *Epinephelus coioides* were (31.57 , 23.60) and (31.10 , 12.60) μ g/kg dry weight during winter and spring, respectively. In addition, the highest and lowest levels of lead measured during winter and spring were (46.36 , 21.40) and (45.60 , 12.50) μ g/kg dry weight, respectively. Finally, highest and lowest levels of cadmium in the muscle tissues of *Epinephelus coioides* were (22.85 , 14.60) and (21.80 , 13.10) μ g/kg dry weight during winter and spring, respectively. Hence, it was found that the mean concentrations of the heavy metals nickel, lead and cadmium were higher during winter than spring. Furthermore, there was a significant difference between the concentrations of heavy metals during spring and winter based on the t-test results (P<0.05) (Figure 2).

Table 1: The average weight and length of the *Epinephelus coioides* during spring and winter (N=20)

Average	Season	
	Spring	Winter
Weight (gr)	598.62±1.95	660.68±2.32
Length (cm)	27.18±0.53	32.62±0.92

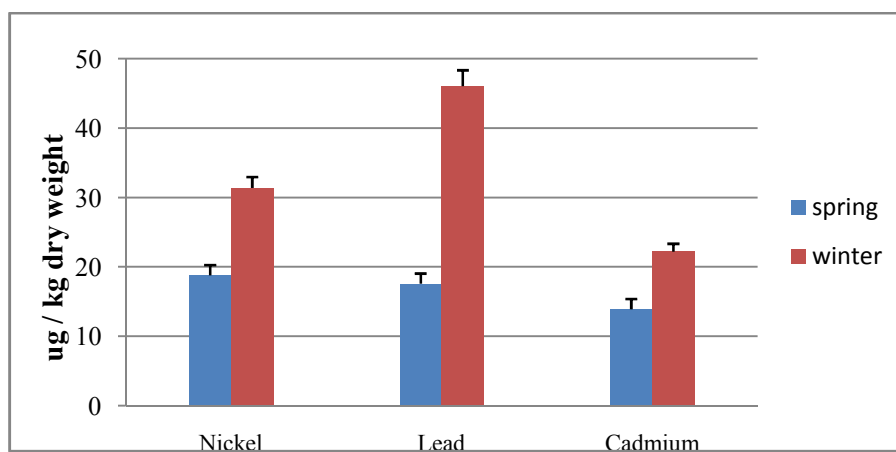


Figure 2: The mean and SD values of the concentration of nickel, lead and cadmium during spring and winter (μ g/kg dry weight)

DISCUSSION AND CONCLUSION

The investigation results showed that the pattern of accumulation of heavy metals was as follows: lead > nickel > cadmium.

Ibigoni Clinton *et al.*, [31] measured the concentrations of the heavy metals nickel and cadmium in the muscle of *Typanotonus fuscatus* in the mangrove regions of Nigeria during November 2001 and December 2002. They reported that the concentration of lead was higher than cadmium during these months and the concentrations of both elements were higher than the global standards. Fatihfidan *et al.*, [30] measured the levels of the heavy metals nickel and lead in the muscle of *Carassius carassiu* and in the samples collected from Lake Eber (Turkey). Their results revealed that the measured concentrations of these metals were lower than the levels approved in Turkey. Hence, these metals pose no threat to the consumers.

Abdolahpour Monikh *et al.*, [24] determined the concentrations of the heavy metals nickel, lead and cadmium in the muscle tissue of three commercial fish species (*Euryglossa orientalis*, *Cynoglossus arel*, *Johnius belangerii*) in three estuaries in the north of the Persian Gulf. Of the species under study, *E.orientalis* had the highest level of nickel, whereas *C.arel* and *J.belangerii* had the highest concentrations of lead. Tapia *et al.*, [39] measured the concentrations of the heavy metals lead and cadmium in the muscle of *Micropogonias manni* in Lake Budi and found that the concentrations of these metals were low in the muscle tissues of this fish. The average recorded concentrations of metals in the edible tissue of this fish were in the ranges reported by the authors of similar studies and were lower than the limits specified by WHO and FAO. Therefore, these metals pose no threat to the consumers of this fish. Petkovsek *et al.*, [37] measured the concentrations of the heavy metals lead and cadmium in the tissues of the following 10 fish species in Lake Salk (Turkey): *Abramis brama danubii*, *Alburnus alburnus alburnus*, *Barbus meridionalis petenyi*, *Carassius auratus gibelio*, *Cyprinus carpio*, *Lepomis gibossus*, *Leuciscius cephalus cephalus*, *Perca fluviatilis fluviatilis*, *Rutilus rutilus*, and *Scardinius erythropthalmus*. The average concentrations of lead and cadmium were 0.01-0.48 and 0.01-0.19 mg/kg dry weight, respectively. These levels are lower than the WHO and FAO standard limits and pose no risk to the consumers of these species. Moghdani *et al.*, [33] measured the concentrations of the heavy metals nickel and lead in the muscle tissue of *Brachirus orientalis* in Bushehr and Asaluyeh ports and realized that the mean concentrations of the heavy metals lead and nickel were 1.459 and 1.378 mg/kg dry weight in Bushehr Port, respectively. Moreover, the mean concentrations of the heavy metals lead and nickel were 3.166 and 2.208 mg/kg dry weight in Asaluyeh port, respectively. The concentrations of lead and nickel were higher than the WHO and FAO international standards in both stations, and this finding does not comply with the results of the present study. According to the report by Obeidi *et al.*, [35] the concentration of lead in the muscle tissue of *Pomadasys kaakan* in Bushehr port is 6.01 mg/kg dry weight, which is higher than the international WHO and FAO standards. These findings are not in line with the results of the above research. Ghanbari *et al.*, [12] studied the concentrations of nickel and lead in the muscle tissue of *Otolithes ruber* in Bushehr and Asaluyeh ports. They reported that the concentrations of nickel and lead were (0.262 and 0.293) mg/kg dry weight and (0.335 and 2.684) mg/kg dry weight in Bushehr and Asaluyeh ports, respectively. Therefore, the levels of nickel were higher than the global WHO standard. Moreover, the concentrations of lead were lower than the WHO, FAO, NHMRC and UK (MAFF) standards in Bushehr port but were higher in Asaluyeh port. The levels of the metals measured in this region are consistent with our findings.

The difference between the concentrations of heavy metals in different studies is caused by ecological, biological and metabolic differences as well as the inhabitance, nutritional behavior, food level, age, durability of heavy metals and hemostasis regulating activities of the fish [19]. The method of measuring heavy metals and the devices used for this purpose may also influence the results.

Investigation results revealed that the highest concentration of nickel and cadmium were found in the gills, flakes and bones in the order mentioned [20]. Other examinations also proved that nickel and cadmium are stored in different organs, but they are mainly stored in the skin, gill and bones of aquatic animals in addition to the kidneys and livers [21]. This may be the main cause of the low levels of these metals in this research. It is also worth stating that cadmium and nickel are two of the elements that can be regulated automatically in the bodies of the fish. According to Bremner (1979) the excretion of cadmium and nickel occurs in the gills and through the liver-bile duct in the intestine. Moreover, the secretion of mucus is an important factor in reducing the concentration of these elements in the fish [22,23].

The results are research activities are valid if they are compared to the international standard. The results of comparing the measured concentrations of the heavy metals under study (nickel, lead and cadmium) to the WHO, FAO, NGMRC and UK(MAFF) international standards indicated that the

concentrations of nickel, lead and cadmium were lower than the international standard limits and thus these metals pose no threat to the consumers. However, the contamination of the muscle tissues of *Epinephelus coioides* may have been caused by the flow of the effluent of the petrochemical, oil and gas industries in Asaluyeh to the sea. Hence, it is necessary to monitor all of the environmental pollutants in the water, sediments and aquatic animals of the Persian Gulf and ensure the health of these animals by identifying and controlling the exact sources of pollution.

Table 2: Comparison of the concentration of Ni, Pb and Cd in muscle tissues of *Epinephelus coioides* with standards (mg kg⁻¹ dry weight).

Standard	Ni	Pb	Cd
WHO (Madany <i>et al.</i> , 1996)	0.2	0.5	0.2
FAO (Dural <i>et al.</i> , 2007)	-	0.5	0.3
NHMRC (Darmono & Denton, 1990)	-	1.5	0.05
UK(MAFF) (Mormede & Davies, 2001)	-	2	0.2
<i>Epinephelus coioides</i> (Spring, present study)	0.018	0.017	0.013
<i>Epinephelus coioides</i> (Winter, present study)	0.031	0.046	0.022

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