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Research Article

SELENIUM CONCENTRATION IN MUSCLE TISSUES OF *BRACHIRUS ORIENTALIS* AND *OTOLITHES RUBER* IN BUSHEHR AND ASALOUYEH SEAPORTS (PERSIAN GULF WATERS)

[*]Abdol-Rahim Pazira[1], Farshad Ghanbari[2], Saeid Moghdani[2], Narges Javad Zadeh[3]
[1]Department of Engineering, College of Natural Resources, Islamic Azad University, Bushehr Branch, Iran
[2]Young Researchers and Elite Club, Bushehr Branch, Islamic Azad University, Bushehr, Iran
[3]Department of Fisheries, Khouzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
[*]Author for Correspondence

ABSTRACT
In order to examine and compare selenium accumulation levels in muscle tissues of *Brachirus orientalis* and *Otolithes ruber* in Persian Gulf waters (Bushehr and Asalouyeh Seaports), sampling was done in both Bushehr and Asalouyeh stations during the summer 2013. After biometry, muscle tissues of the samples were separated and chemical digestion was done. Selenium accumulation levels in tissues were measured by using graphite furnace atomic absorption instrument. Based on the obtained results, mean concentrations of selenium in muscle tissues of *Brachirus orientalis* were 4.28 ± 0.84 $mgkg^{-1}$ dw in Bushehr station and 3.72 ± 0.49 $mgkg^{-1}$ dw in Asalouyeh station, and mean concentrations of selenium in muscle tissues of *Otolithes ruber* were 3.10 ± 0.55 $mgkg^{-1}$ dw in Bushehr station and 3.08 ± 0.25 $mgkg^{-1}$ dw in Asalouyeh station, and it indicated no statistically significant differences between the two stations for *Otolithes ruber* ($P>0.05$), but there were significant differences between the two stations for *Brachirus orientalis* ($P<0.05$). The obtained concentrations and analysis done indicate that based on the FAO standard, the amount of selenium was higher than the standard levels.

Keywords: *Bushehr, Asalouyeh, Brachirus Orientalis, Otolithes Ruber, Selenium, Muscle*

INTRODUCTION

The Persian Gulf is a shallow water basin in the south area of the Iranian plateau on the edge of the Indian ocean located in west-northern Oman sea. Its area is almost about 232850 km^2 and its average depth is 30 to 35 m (Al-Awadhi, 2002). This sea is considered as an important resource for accessing to the great food resources that is invaded by the various pollutants in recent years. According to the studies, the water rotation and exchange time of this sea is estimated about 3 to 5 years probably indicating the pollutants remain in the Persian Gulf for a significant period. It's clear that the effect of pollutants on aquatic environment should be more significant due to semi-closed, shallowness, water limited rotation, salinity and high temperature which are the characteristics of the north part of the Persian Gulf. In different areas of the Persian Gulf, oil pollution along with the other urban, industrial and agricultural pollutions cause the destruction of this valuable ecosystem and the valuable resources of the aquatic animals present in it are exposed to different pollution risk and have posed a threat to the aquatic animal population present in it (Pourang *et al.*, 2005). An increase in the amount of the released pollutants in marine environment have been considered in a lot of studied during the last decade. One of the main issues attracting a large number of researcher's attention is heavy metal contaminations and their influences on the environment (Yilmaz *et al.*, 2007; Henry *et al.*, 2004). Toxic elements such as heavy metals are considered as the most important environmental contaminants (Turkmen and Ciminli, 2007). Heavy metals are persistent pollutants that unlike organic compounds are not analyzed through chemical or biological processes in nature and create considerable changes in biochemical cycles and each one has an especial effect in living creature bodies. Heavy metals in marine habitats are considered due to their concentrations in waters and sediments, persistence and tendency to accumulate in tissues of marine creatures and also because of harmful effects which they have in marine creatures due to toxicity and

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bioaccumulation in food network (Harper *et al.,* 2007). Heavy metals are accumulated in tissues and organs of the fish after they entered the aquatic ecosystems and finally they enter the food chains and are considered as a potentially toxic factor for microorganisms (Chen and Chen, 1999). Changes made in industrial and agricultural areas and promotion of the human life level in recent years, have made inevitable heavy metal use in different areas. Heavy metals entered the environment through different method such as mining, melting process, fuel combustion and industrialization are carried to the aquatic environments through different ways such as rainfall, waste discharge, accidental leakage, discharging ship ballast water, discharging industrial, agricultural and domestic wastewater and soil erosion (Karadede *et al.,* 2004; Filazi *et al.,* 2003; Al–Yousuf *et al.,* 2000). Age, length, weight, sex, ecological needs, feeding habits, heavy metal concentrations in water and sediments, exposure period of the fish to the aquatic environment, fishing season, and physical and chemical properties of the water (salinity, pH, hardness and temperature) are the effective factors in heavy metal concentrations in different organs of the fish (Ghanbari *et al.,* 2014; Canli and Atli, 2003). According to this fact, all essential and non-essential metals can be toxic. This amount of bioaccumulation is usually determined by measuring the accumulated metals by living organisms which are the main purpose in biological control (Zhou *et al.,* 2008; Rainbow, 1993). The analysis of metal levels was better in living organism tissue and have more advantages than using water or sediment samples. Since some animals accumulate rare metals in high concentrations, they can provide us with strong information about the environment pollution (Laboy-Nieves and Conde, 2001). Among the aquatic animals, fish and birds have significant importance to monitor the environmental pollutions due to their place in upper levels of food chain and their food consumption (Khosravi *et al.,* 2011). From a fisheries viewpoint, *Brachirus orientalis* and *Otolithis ruber* is among commercially valuable fishes and has an important role in human food programs (Dìaz and Munroe, 1998). Selenium is a rare element and the aquatic animals especially fish are selenium–rich resources. This element in small quantities, is necessary for the body and in large quantities are very toxic. Thus, the measurement and assessment of selenium in fish are significant (Lavilla *et al.,* 2008). Therefore, studies done in the field of heavy metal contamination in aquatic ecosystems are very important from the human health and public sanitation viewpoints. On the other hand, in these studies, balance state preservation of the aquatic ecosystems is considered as a secondary objective. Since commercial and valuable fish present in the Persian Gulf form a chief part of diet of people of south area of Iran, in this research measuring the selenium concentration in muscle tissues of *Brachirus orientalis* and *Otolithis ruber* in the Persian Gulf waters (Bushehr and Asalouyeh seaports) and comparing it with the international standards have been carried out.

MATERIALS AND METHODS

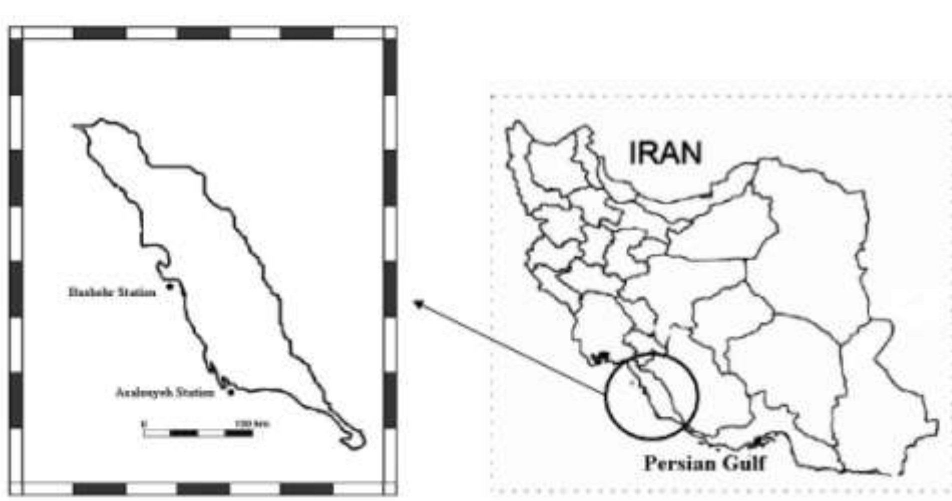

Figure 1: Location of the sampling areas

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Study Area

Bushehr is located in 28°55´19.84" N and 50°50´4.76" E of southwestern Iran and on the edge of the Persian Gulf. Asalouyeh is located in 28°28´24.48" N and 52°36´49.79" E on the edge of the Persian Gulf, 300 kms east of Bushehr and 570 kms west of Bandar Abbas and has a distance of 100 kms to the South Pars gas area located along the Persian Gulf (Figure 1).

Sampling

40 samples from *Brachirus orientalis* and *Otolithes ruber* were caught by trawl net in both regions, Bushehr and Asalouyeh seaports during summer season 2013 to do this research. Then, the samples were placed in a plastic bag and coded and were placed in an ice bucket full of ice in order to be transferred in the laboratory. The samples were transferred to Islamic Azad University Bushehr branch laboratory after fishing. The fish samples were kept at a temperature of -30° C by the analysis time in the laboratory.

Sample Preparation

First all lab dishes which were going to be used were placed in nitric acid for 24 hours and then they were washed by using distilled water and finally they were placed in an oven at a temperature of 80°C to prevent contamination. The samples were removed from the fridge. When they reached the environment temperature, biometry operation (total length, standard length, total weight) was done. Then the muscle tissues were separated and some sample muscles were transferred into the complete clean dishes (washed using nitric acid) and were placed in an oven at a temperature of 80°C for 18 hours to be dried completely. Dried samples were transferred into a mortar to be grinded completely. After grinding the samples they were placed in a desiccator to prevent them absorbing air moisture. Acid digestion is performed to release all metal connections with tissues. In this regard, 1g dried and constant tissues were transferred into a beaker and 10ml concentrated nitric acid were added to digest the dish contents and the samples were placed in the room temperature for 30 minutes until the primary digestion was done. Then the samples were heated in a heater located under the hood having a steam system in a temperature of 90°C to be dried. When the samples were cooled and reached the environment temperature, they were sieved from a 45mm Whatman filter paper and were transferred to 25ml dishes and were reached the necessary volume. Finally, the samples were transferred into lidded polyethylene dishes to be injected into the instrument (MOOPAM, 1999). A graphite furnace atomic absorption instrument was used to measure the vanadium metal levels.

Statistical Analysis

One sample Kologorov-Smirnov test in SPSS®18 was used to check the validity of the data normalization. Then, one way sample T-test was used to check interactions between heavy metals and stations. Data have been presented in diagrams as Mean±SDs with 95% of the confidence interval. Excel software was used to draw diagrams (Zar, 1999).

RESULTS AND DISCUSSION

Results

Biometric Results

Biometric results of *Brachirus orientalis* indicated that mean weight in Bushehr was higher in comparison to Asalouyeh station. Mean weight in Bushehr was 358.96g and mean weight in Asalouyeh was 212.38g.
Biometric results of *Otolithes ruber* indicated that there was no significant different between the mean weight in both stations. Mean weight in Bushehr was 376.82g and mean weight in Asalouyeh was 369.40g. Biometric results are presented in table 1 and table 2.

Table 1: Biometric results of *Brachirus orientalis* in Bushehr and Asalouyeh stations (N=20)

	Bushehr Station		Asalouyeh Station	
	Mean	**SD**	**Mean**	**SD**
Total weight	358.96	13.1	212.38	11.96
Total length	27.81	1.53	27.05	1.36
Standard length	24.57	1.28	22.79	0.96

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Table 2: Biometric results of *Otolithes ruber* in Bushehr and Asalouyeh stations (N=20)

	Bushehr Station		Asalouyeh Station	
	Mean	**SD**	**Mean**	**SD**
Total weight	376.82	14.9	369.4	4.96
Total length	34.25	1.46	33.7	0.33
Standard length	30.14	1.55	29	0.67

Table 3: Correalation between length and weight indices in *Brachirus orientalis* and *Otolithes ruber* in Bushehr and Asalouyeh

Species	Station	R	R^2	Sig.
Brachirus orientalis	Bushehr	0.982	0.964	0.000
	Asalouyeh	0.937	0.877	0.000
Otolithes ruber	Bushehr	0.948	0.898	0.000
	Asalouyeh	0.868	0.753	0.001

Selenium Concentration

The obtained results show that the lowest and the highest selenium concentration levels in muscle tissues of *Brachirus orientalis* in Bushehr station was equal to 3.3 and 6.125 mgkg^{-1} dw and in Asalouyeh was 3.125 and 4.625 mgkg^{-1} dw, respectively.

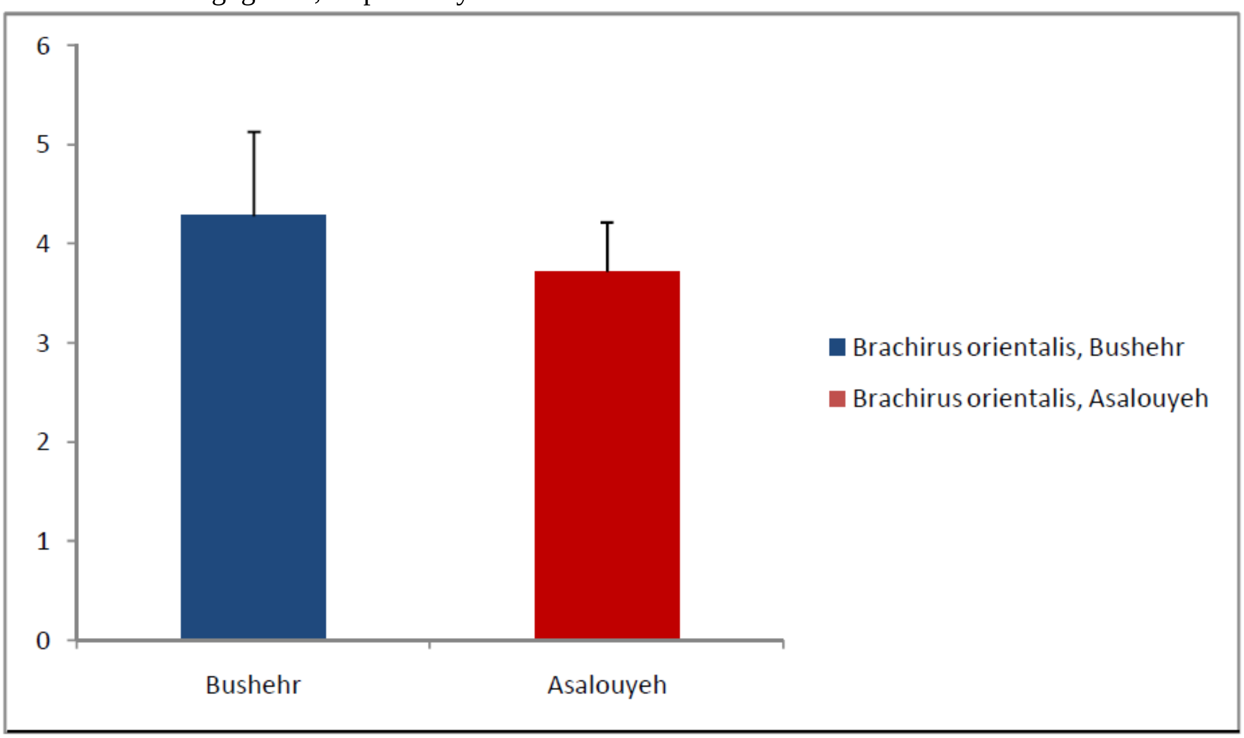

Figure 2: Comparison of selenium levels in muscle tissues of *Brachirus orientalis* in Bushehr and Asalouyeh stations

According to the obtained statistical results mean and standard deviation (SD) with the confidence interval in 95% level of selenium in Bushehr station was 4.28±0.84 mgkg^{-1} dw and in Asalouyeh station was 3.72±0.49 mgkg^{-1} dw. Based on T-test analysis, significant differences were observed between selenium levels in muscle tissues in both stations (P=0.03).

The obtained results show that the lowest and the highest selenium concentration levels in muscle tissues of *Otolithes ruber* in Bushehr station was equal to 1.625 and 3.625 mgkg^{-1} dw and in Asalouyeh was 2.7

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and 3.5 mgkg^{-1} dw, respectively. According to the obtained statistical results mean and standard deviation (SD) with the confidence interval in 95% level of selenium in Bushehr station was 3.10±0.55 mgkg^{-1} dw and in Asalouyeh station was 3.08±0.25 mgkg^{-1} dw. Based on T-test analysis, no significant differences were observed between selenium levels in muscle tissues in both stations (P=0.921). Figures 2 and 3 indicates selenium levels in muscle tissues of *Brachirus orientalis* and *Otolithes ruber* in both Bushehr and Asalouyeh stations.
Based on the obtained concentrations and comparison done it has specified that based on FAO standard, the amount of selenium in *Brachirus orientalis* and *Otolithes ruber* tissues was higher than the standard permissible levels (Table 4).

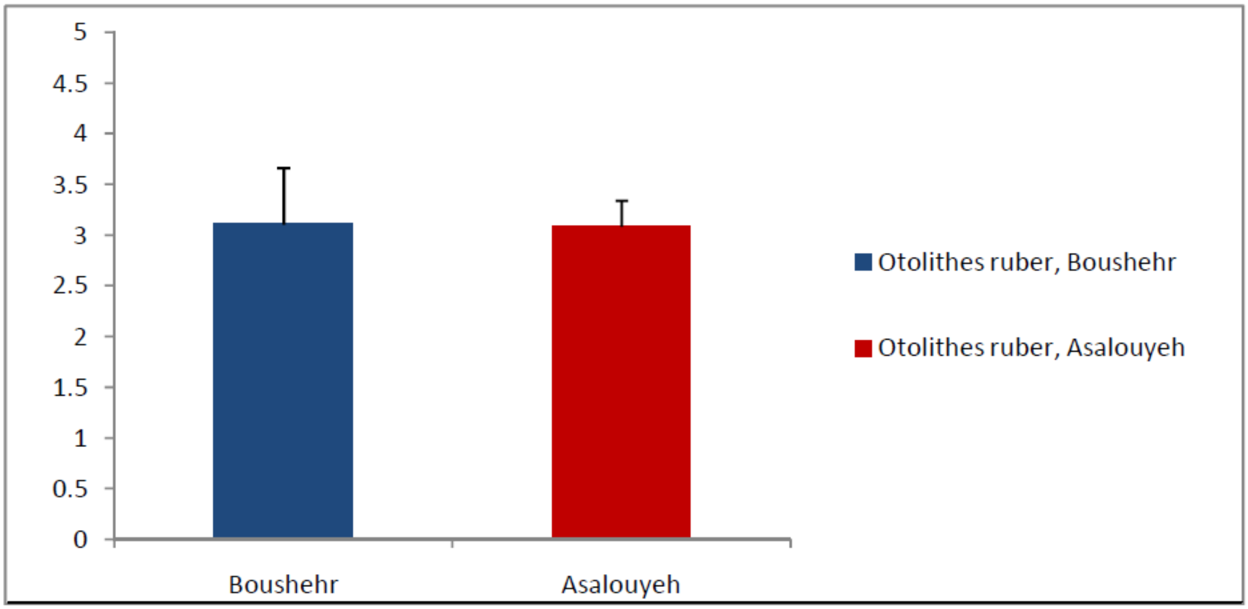

Figure 3: Comparison of selenium levels in muscle tissues of *Otolithes ruber* in Bushehr and Asalouyeh stations

Table 4: Comparison of selenium concentrations in muscle tissues of *Brachirus orientalis* and *Otolithes ruber* with FAO standard (mgkg-^{1})

Standard	Selenium
FAO (Burger and Gochfeld, 2005)	2
Brachirus orientalis, Bushehr	4.28
Brachirus orientalis, Asalouyeh	3.72
Otolithes ruber, Bushehr	3.109
Otolithes ruber, Asalouyeh	3.088

Discussion

Nowadays, the environment pollution especially aquatic resources have created a lot of problems in the environment. Entering the pollutants including a chief part of the environmental pollution into the waters due to dangers they caused to human and their accumulation in aquatic animals reveal the importance of attention to the protection of water resources and their economic value more than before. Discharging heavy metals into marine environments is a big concern all over the world. There are documents and evidence showing heavy metals have great ecological significance due to their accumulation behavior and their toxicity and can decrease diversity of marine species and ecosystems. Moreover, consumption of polluted marine food put human health in danger (Moghdani *et al.*, 2014; Al-Saleh and Shinwari, 2002).

Heavy metals are among persistent pollutants and are among pollutants having no biodegradability (De Mora *et al.,* 2004). Thus, these inorganic compounds are repeatedly accumulated in marine environments (Maanan *et al.,* 2004). In food chains, persistent pollutants including metals can transferred to upper levels of food chain. The amount of these pollutants is usually higher in aquatic animal bodies than the surrounding environment due to bioaccumulation and biomagnification and attention to this issue has great importance since a lot of marine species are eaten by humans (Ruelas-Inzunza and Osuna, 2000). Therefore, determination of heavy metals in living creatures should be a part of every assessment and monitoring program in coastal region (Usero *et al.*, 2005).

Otolithes ruber is one of the migratory and coastal fish. This species is found in coastal waters highly in regions with muddy bed. The species is Benthopleagic to the effect that they live both in bed and water surface. *Otolithis ruber* feeds on smaller fish, crustaceans like shrimps and the other invertebrates (Ield, 1985). Heavy metal accumulation in Benthopelagic species in comparison with benthic species probably has a relation with the fish diet (Boustamant *et al.,* 2003). *Brachirus orientalis* is benthic and live in shallow territorial waters on muddy and sandy beds. Benthic and sedentary species show various metal concentrations in their tissues. These differences are probably caused by different accumulation methods and food ration within them. Benthic species are exposed more to the sediments rich in metals, and interaction with benthic animals and indicate higher metal concentrations in their tissues (Huang, 2003). However, these findings can prove that mental concentrations are highly under the influence of habitat, feeding habits, mental accumulation capacity and kind of species (Agah *et al.,* 2009; Bustamante *et al.,* 2003). Bushehr seaport is one of the most important fishing and commercial seaports and the following are among its polluting factors: oil contaminations, direct discharge of coastal habitat drainage (waste waters), motor boats and fishing and cargo ship traffic fishing waste discharge into coast, the rest of the metal hull of the sunken ships, aquatic vehicle oil and fuel, direct discharge of water produced by ships and the abundant remaining waste related to fishing implements in water. Asalouyeh seaport, in addition to the presence of fishing pier has the largest world oil and gas installations (South Pars oil particular region) influencing the environment directly and indirectly.

Selenium is micronutrient element. Selenium concentration in food is affected by geochemical position. Aquatic animals especially fish are selenium-rich resources and since selenium is among rare elements and in small quantities are necessary for the body and in large quantities are very toxic, it is very important. Fish, aquatic animals as well as marine vegetables are selenium-rich resources. Generally speaking, food in terms of selenium present in it can be categorized as excellent resources of selenium including all aquatic animals especially *Scomberomorus commerson*, *Scomberomorus guttatus*, *Otolithes ruber*, shrimp, as well as mushroom and liver and very good resources of selenium including egg, chicken meat, veal, rice, yogurt, legumes, milk and soybean (Lavilla *et al.,* 2008). This element is mostly found as a compound and is less observed as pure. Selenium consumption in large quantities is toxic but in small quantities is necessary for cell activities. This element usually enter the body through breathing and eating food containing selenium. Selenium is an effective mineral in fat metabolism and also cause the improvement of immune system. On the other hand, selenium having antioxidant characteristic prevent cells being damaged by free radicals. Anti-cancer activity of the selenium is due to healing of the damaged tissues, inhibition of cancer cells, and the removal of abnormal cells. Moreover, selenium is concentrated in the active part of molecular structure of most proteins such as glutathione peroxidase which are very important to prevent cancer. Glutathione peroxidase enzyme in liver has a duty to detoxify the harmful molecules. This enzyme can do activities without selenium (Ditez *et al.*, 2003; Parkman and Hultberg, 2002). Selenium has positive effects on the health of heart, liver, pancreas, different kinds of cancers, elasticity characteristic of the muscles and most of the other diseases. Selenium rate in people not receiving enough selenium in their diet such as vegetarians, old people and pregnant and breastfeeding women decreases. Shortage and also extreme selenium rate in the body cause weakness, muscle pain, neural performance disorders (seizure, paralysis, mental retardation), nail bed turning white, cataract, hypertension, infertility, liver necrosis, kidney damages, and loss of hair melanocytes. Selenium half-life is 14.4 days. Selenium excretion is mainly through stool, urine, and exhaled breath (Muth, 1976). Skorupa

(1998) carried out a study to examine the relationship between selenium in tropical and cold water's fish. The results indicated that fish of tropical region waters have the minimum acute toxicity of selenium. Dietz *et al.,* (2003) in their research concluded that inorganic selenium is turned into organic selenium through metabolism process and its organic forms usually have less toxicity. Hamilton (2004) in his studies stated that the selenium rate in liver is higher than the edible tissues in fish. Rezayi *et al.* (2011) examined selenium in *Otolithes ruber* and *Psettodes erumei* in Khouzestan Province coasts. The results showed that selenium contents in livers of both species were higher than the edible part of that species and also there were significant differences between selenium levels between both species. Moreover, in both species no significant differences were observed between selenium levels and its length and weight. At the end of this research it was clarified that among the species, smaller species have higher selenium rate which is due to the liver role as storing organs of glutathione and is expected more selenium rate in liver is observed than the edible tissues. High levels of selenium in *Brachirus orientalis* which is a benthic species can be due to its habitat conditions since sediments absorb more selenium and benthic creatures are in touch with selenium effects in large quantities. (Hamilton, 2004)

Table 5: Comparison of selenium concentrations in the present study with the other researches (mgkg^{-1})

Refrence	**Se**	**Region**	**Species**
Rezayi *et al.*, (2011)	0.060	Persian Gulf, Khouzestan	*Otolithes ruber*
Rezayi *et al.*, (2011)	0.44	Persian Gulf, Khouzestan	*Psettodes erumei*
Alkan *et al.*, (2012)	9.83-0.02	South West Black sea	*Mullus barbatus ponticus*
Alkan *et al.*, (2012)	14.16-0.15	South West Black sea	*Merlangius merlangus euxinus*
Current study	4.28	Persian Gulf, Bushehr	*Brachirus orientalis*
Current study	3/72	Persian Gulf, Asalouyeh	*Brachirus orientalis*
Current study	3.109	Persian Gulf, Bushehr	*Otolithes ruber*
Current study	3.088	Persian Gulf, Asalouyeh	*Otolithes ruber*

According to the obtained results, the selenium concentration levels in muscle of *Brachirus orientalis* and *Otolithes ruber* have been higher than the permissible levels of FAO standard in both stations. Selenium which is considered as one of the pollutants of the aquatic ecosystems not only influences on aquatic animals and has negative effects on them but also influences on humans who are the consumers of these aquatic animals and cause some problem for humans. Therefore, Based on the high levels of selenium metal in this study it can be concluded that the use of this species in these regions are rather dangerous and will naturally have bad effects on the consumers of these products.

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