

[Research]

Heavy metals Cu, Zn, Cd and Pb in tissue, liver of *Esox lucius* and sediment from the Anzali international lagoon- Iran

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ABSTRACT

Levels of contaminants in fish are of particular interest because of the potential risk to humans consuming them. This paper examines the levels of cadmium, lead, zinc and copper in liver and muscle tissue of *Esox lucius* and in sediments from the Anzali international wetland in Iran. The wetland is a nursery and feeding habitat for fish species both from the lagoon and the Caspian Sea, which are consumed by the local inhabitants and others. Eleven main rivers discharge agricultural, industrial, urban and municipal wastes into this wetland. Twenty five *Esox lucius* specimens, with a mean weight of 804.6±121g and mean length of 430.7± 2mm were collected from the wetland by multimesh gill nets in September and December 2007.. The age was determined from scale samples according to the annual ring structure. Average concentrations of Cd, Cu, Pb and Zn accumulated in the mid-dorsal muscle tissue (filleted and skinned) of fish were 0.001, 0.21±0.02, 0.13±0.01 and 2.55±0.18 µg.g⁻¹, respectively. The concentrations of these metals in liver samples were Cd: 0.0014± Cu: 0.96± 0.61, Pb: 0.11 ± 0.04 and Zn: 2.46±1.5 µg.g⁻¹. Significant positive correlations were detected between Zn and Cu with body weight, while negative correlations were found between Pb and body weight. The concentrations of Zn, Cu and Cd were below the maximum allowable concentrations for fish proposed by WHO and MAFF (safe for human consumption) but concentration of Pb exceeded the standard levels.

Keywords: Heavy metals, *Esox lucius*, Anzali wetland, Caspian Sea.

INTRODUCTION

Aquatic environmental quality, and how humans may be affecting this, is an area which has received increasing attention in recent years. Aquatic organisms, among them fish, accumulate contaminants from the environment and therefore have been extensively used in pollution monitoring programs (e.g., ICES: OECD, 1991; UNEP, 1993; Uthe et al., 1991). Two main objectives being pursued in these programs are (i) to determine contaminant concentrations in fish muscles in order to assess the health risk for humans, and (ii) to use fish as environmental indicators of aquatic ecosystems quality (Adams, 2002). In recent years, anthropogenic factors, such as habitat destruction played a major role in the decline of commercial marine fish species. Indeed, the substantial development of urban and industrial activities result in increasing inputs of

chemical contaminants which lead to the loss or alteration of aquatic habitats. Lagoons and wetlands are economically and ecologically important because of intensive recreational and fishing activities. In recent years concentrations of contaminants were found to have increased in coastal ecosystems due to the release of industrial wastes and as a result, aquatic organisms are exposed to elevated levels of heavy metals (Kalay and Canil 2000; Sankar et al. 2006). The aquatic organisms exposed to the heavy metals from runoff water tend to accumulate these metals in their body, fishes being more commonly affected than other species (Güven et al. 1999; Henry et al. 2004). Studies carried out on different fish species have revealed that both essential and non-essential metals can produce toxic effects in fish by disturbing their growth, physiological, biochemical, reproduction activities, and mortality (Lee and Stuebing

1990; Yilmaz 2005). Hence, fishes are considered as one of the best indicators of heavy metal contamination in coastal environments (Evans et al. 1993; Rashed 2001). Since pike perch is a favorite species for sport fishing and a highly consumed fish in the region, the present study was proposed to investigate the accumulation of heavy metals in liver and muscle tissue of this species and to increase the awareness of people on health issues of the fish itself and of the lagoon.

Materials and methods:

Anzali Lagoon is located in the southwestern region of the Caspian Sea coast, at 37°28'N, 49°25'W (Fig.1). The 11 feeder rivers of this lagoons flow through large areas surrounding the lagoon and discharge huge amounts of agricultural, municipal and industrial waste materials into it. The Lagoon has long served as an important spawning and nursery ground for economically important anadromous fishes belonging to 13 families, 33 genera and 41 species. Anzali Lagoon used to be about 2-3m deep in the eastern parts and 8-11m in western regions (Vladykov, 1964; Kimball and Kimball, 1974) but due to heavy erosion, siltation and macrophyte invasion it has dropped to 1-3 m in depth which intensifies all kinds of pollution threats. Heavy metals have rarely been studied in this lagoon. In the present study Pike perch (*Esox lucius*) were collected from open water areas of the lagoon by multimesh gill nets in September and December of 2007. Concentrations of cadmium, copper, lead and zinc

concentrations accumulated in the mid-dorsal muscle tissue (filleted and skinned), and liver of fish and sediment were determined. Total body length (mm) and—to exclude the variations induced by differences in the stomach fullness—the net body weight (eviscerated) of each individual were recorded. The age was determined from scale samples according to the annual ring structure of scales (Bagenal et al., 1978). For the determination of Cd, Cu, Pb and Zn concentrations, tissue samples were subjected to wet acid digestion (Krishnamurty et al., 1976, Farkas et al., 1993). The Cd, Cu, Pb and Zn concentrations were determined with a Perkin-Elmer-5100 atomic absorption spectrophotometer. Concentrations of metals in sediments and tissues were calculated on a dry weight basis and expressed as $\mu\text{g}\cdot\text{g}^{-1}$. All samples were analyzed in batches with blanks. Analytical accuracy was determined using certified reference material of the Community Bureau of Reference, i.e. standard for trace elements in mussel tissue (CRM 278). Recoveries were within 10% of the certified values.

Condition factor (CF) was calculated according to the following formula (Bagenal and Tesch, 1978; Bolger and Connolly, 1989): $CF = W/L^b \times 100$ where W =weight (g), L =fork length (in mm). The exponent b is derived from the length-mass relationship at each site which is described by $W=a L^b$.

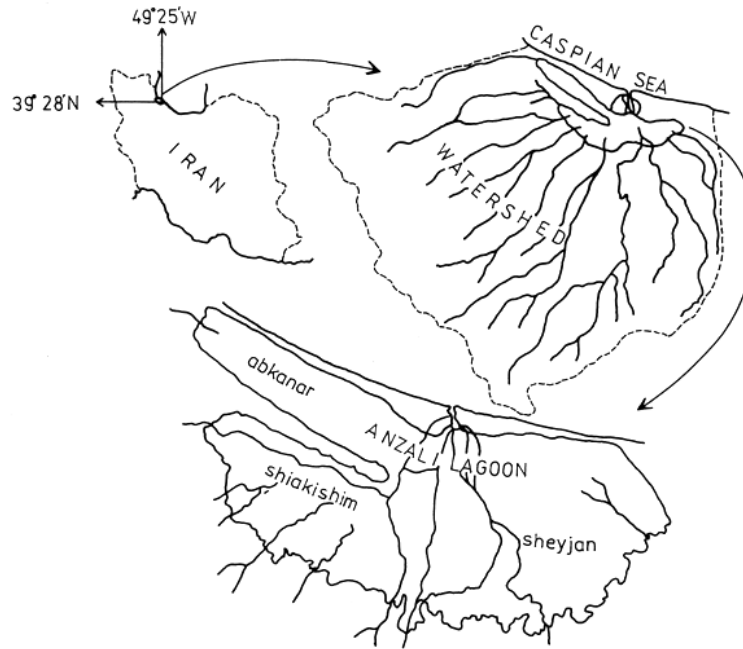


Fig 1. Anzaly lagoon.

Results:

A total of 25 fish were analyzed with a mean weight and length of 804.6 ± 121 g and 430.7 ± 2 mm, respectively (Table 1). The metal concentrations and the corresponding mean standard deviations (expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight) measured in liver and muscle tissues and also sediment samples are reported in Table 1. Zinc showed the highest concentrations in muscle, liver tissues and sediment. The mean concentration of Zn accumulated in the mid-dorsal muscle tissue (filleted and skinned) and liver were $2.55 \pm 0.18 \mu\text{g}\cdot\text{g}^{-1}$ and $2.46 \pm 1.5 \mu\text{g}\cdot\text{g}^{-1}$, respectively and $14.368 \mu\text{g}\cdot\text{g}^{-1}$ in sediment samples (Table 1). Positive correlations were recorded for Zn, Cu and Pb with body weight and negative correlations for Cd and body weight. Negative correlation was detected between condition factor and concentration of heavy

metals in liver tissues for all examined metals except for Pb ($R^2 = 0.28$). In case of liver tissue a positive correlation was detected for condition factor with Cu and Pb while a negative correlation was observed with Zn and Cd (Table 2).

The relationship between size and weight of fish and concentration of each metal was also determined (Fig. 2). In most cases in muscle and liver tissues a linear relationship best described the data though the coefficient or correlation was not very high. There were no significant correlations between metal concentrations in the liver and size of individual fish. Comparison of concentrations of Cd, Cu and Pb in liver and muscle tissues of *Esox lucius* showed that the concentration of Zn in muscle tissue was slightly higher than in the liver but concentration of Cu was significantly higher in liver than in muscle tissue (Figure 3).

Table 1. Heavy metal concentrations data of *Esox lucius* tissues and sediment collected from Anzaly lagoon

Number of samples	Elements	$\mu\text{g}\cdot\text{g}^{-1}$ dry weight average ^a (standard deviation)		
		Muscle	Liver	Sediment
25	Cd	0.004(0.001)	0.014(0.009)	1.9
	Cu	0.21(0.02)	0.95(0.6)	3.88
	Pb	0.13(0.01)	0.199(0.05)	3.706
	Zn	2.55(0.18)	3.02(1.5)	14.368

^a Arithmetical mean

Table 2. Pearson correlation coefficient (r) and levels of significance (p) for the relationship between tissue metals concentration of pike perch and their weight and condition factor

Variable	Sample count	Element	Muscle		Liver	
			r	p	r	p
			Weight	25		
		Cd	-0.694	0	-0.21	n.s
		Cu	0.419	0.01	0.12	n.s
		Pb	0.229	ns	-0.1	n.s
		Zn	0.406	0.04	-0.5	0.05
Condition factor	25					
		Cd	-0.462	0.02	-0.17	n.s
		Cu	-0.592	0.03	0.4	0.05
		Pb	-0.286	ns	0.5	0.04
		Zn	-0.492	0.04	-0.6	0.03

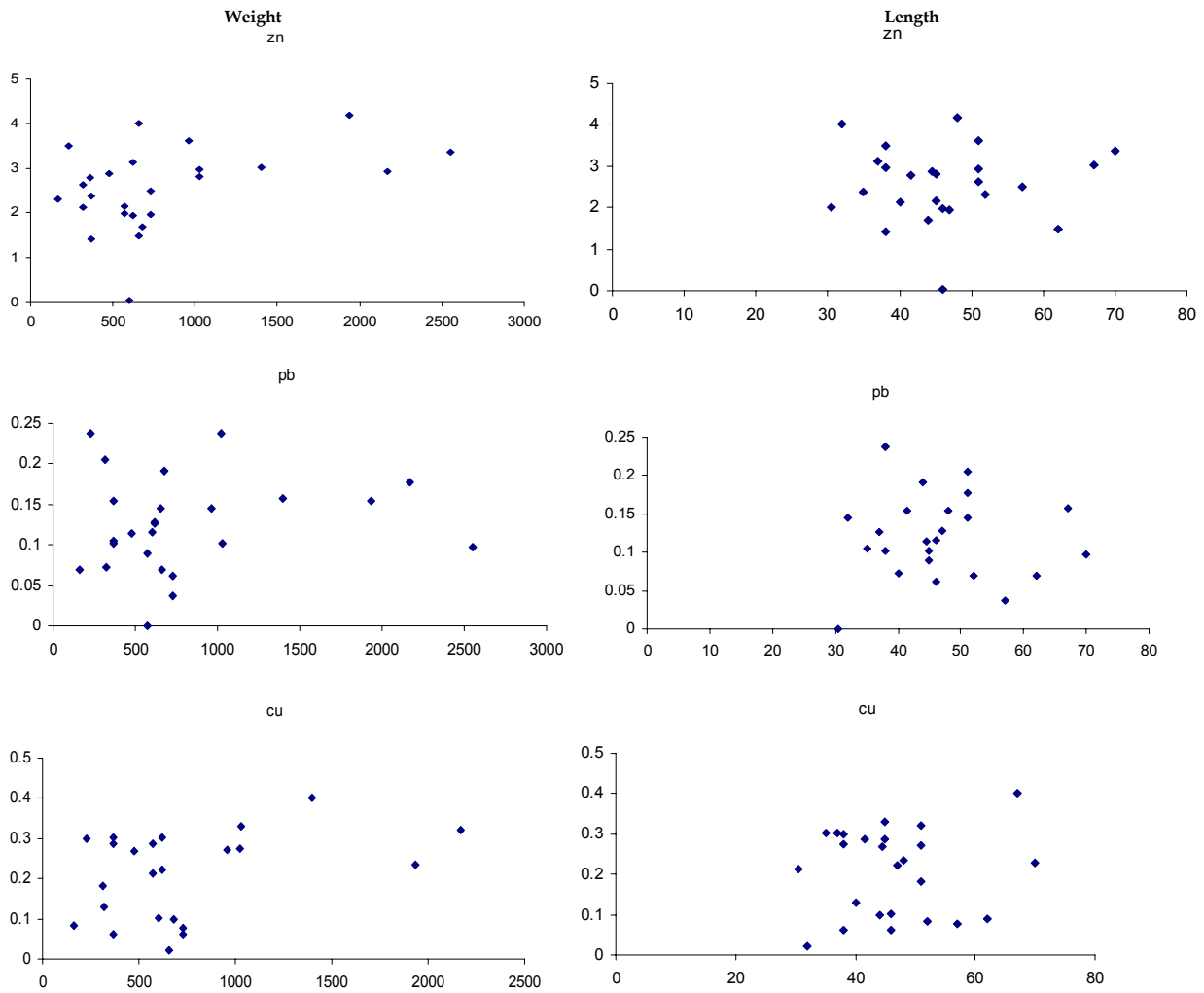


Fig 2. Concentrations of Zn, Pb and Cu in liver and muscle tissues of *Esox lucius* against fish length and weight.

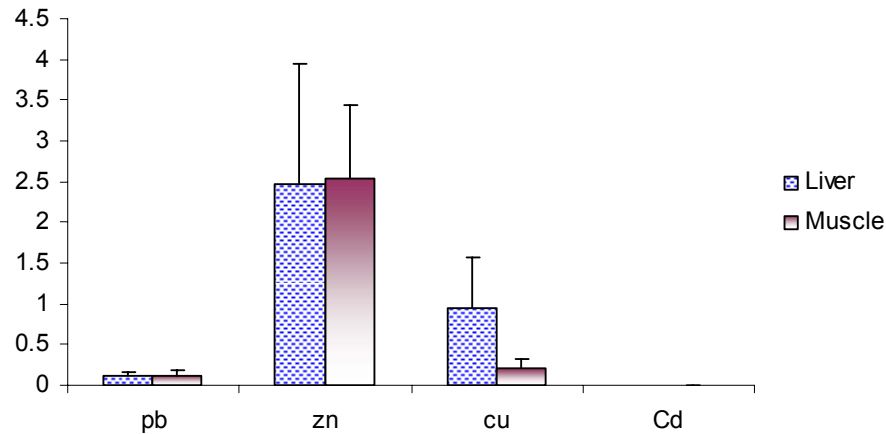


Fig 3. Mean concentrations Pb, Zn and Cu *Esox lucius* in liver and muscle tissues from the Anzali lagoon

Discussion:

The beneficial health effects of fish are based on several studies from the last 25 years, most of which have linked fish consumption to several health benefits (Kinsella et al., 1990; Oomen et al., 2000; Kris-Etherton et al., 2002). However, one potential risk of dietary fish intake is its content of heavy metals. In humans, cadmium and lead have been associated to serious health effects on adults and children and one source of exposition is the intake of fish with high content of either of these metals. This has been demonstrated by several authors; Abernathy et al. (2003); Burger and Gochfeld (2005); Andreji et al. (2006); Falcó et al. (2006); Has-Schön et al. (2006) have determined the levels of these metals in different fish species which may be out of permissible limits. Therefore the intake of fish should be regulated; information regarding the specie of fish consumed and its possible levels of content of heavy metals can be of benefit to diminish the hazard to public health (Domingo et al., 2007). *Esox lucius* was selected for this study based on background studies on its popularity and consumption by local people and others.

In this study there were positive correlations among Zn, Cu and Pb with body weight. Farkas et al., 2003 in their study on *Abramis brama* reported a negative size-related correlation for cadmium, copper, lead and zinc in the muscle and gill tissues and positive correlation for Pb which is in agreement with our findings. *Esox lucius* is a voracious, greedy predatory

species preying over rather larger preys. This increases the potential of excessive metal concentration in its tissues as compared to a benthivorous species such as bream. It is generally accepted that accumulation of heavy metals in living organisms is controlled by specific uptake, and size-specific metabolic rate of organisms [Newman and Doubet, 1989; Fagerstrom, 1997].

There were negative relationships between condition factor and concentration of heavy metals in muscle tissues for all examined metals except for Pb ($R= 0.28$). In case of liver tissues a positive correlation was detected between condition factor with Cu and Pb while a negative correlation was observed with Zn and Cd (Table 2). The negative relationship between the heavy metal concentration of organs and the condition factor of fish suggests the relative dilution effect of the lipid content of tissues. This assumption is supported well also by the fact that lipid as a percent of body weight is usually lower in younger fish, decreases during winter and spawning and reaches its peak at the end of the main feeding period (Shul'man, 1974; Weatherly and Gill, 1987). These variations may explain also the opposite correlations observed between heavy metals—size and heavy metals—condition factors of fish.

The overall levels of all metals were higher in liver than muscle tissues of all analyzed specimens. Although fish muscle is the most important part consumed by human, fish liver may also be consumed to some extent but due to less consumption of fish

liver in the region the health threat potential for human is negligible. Our study showed that the concentrations of metals in muscle and liver tissues of pike perch were generally higher than those reported for commercially important fishes from the Caspian Sea. However, they were below the recommended levels for human consumption. This does not mean that the concentration of Zn, Cu and Pb in sediment samples of the wetland may be overlooked. The presence of Cd, Cu, Hg, Pb and Zn in sediments of the Anzali lagoon is mostly due to anthropogenic sources as there are no sources of natural inputs in the region. Runoff and sanitary waste derived from urban and industrial developments are among the contamination sources that directly impact sediments of the lagoon. Particular attention must be paid to the concentration of Zn in the lagoon as a mining and refinery complex for Zn upstream of the Sephidrud River might be a huge source of Zn input in to the lagoon through irrigation canals and drainages and also other rivers. The negative impact of release of significant levels of metals from the sediment into the water column (Acevedo-Figueroa et al., 2005) needs to be evaluated in terms of bioaccumulation and toxicity, particularly with native species of this lagoon. Hence intensive studies on heavy metal concentrations in other important fish species of the Anzali lagoon and its feeding rivers are must do issues. Concentrations of Cu, Cd and Pb in this study were lower than the guidelines for food summarized by MAFF (2000) (Cu, 20 lg/g wet wt.; Cd, 0.2 lg/g wet wt.; Pb, 2.0 lg/g wet wt.). Zinc concentrations in all the specimens were also lower than the guideline value (50 lg/g wet wt.) but concentrations of Pb in this study were higher than the guidelines for food summarized by WHO (Pb, 0.0 lg/g wet wt.).

References:

- Krishnamurthy KV Andreji, J., Stránai, I., Massàyi, P., Valent, M., (2006) Accumulation of some metals in muscles of five fish species from Lower Nitra River. J. Environ. Sci. Health Part A. 41, 2607-2622.
- Bagenal TB, Tesch FW. (1978) Age and growth. In: Bagenal TB, editor. Methods for assessment of fish production in fresh waters, IBP Handbook, Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications. 3: 101-36
- Bervoets, L., Ronny Blust (2003). Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor Environmental Pollution. 126:9-19
- Burger and Gochfeld (2005). Heavy metals in commercial fish in New Jersey, Environmental Research. 99:403-412.
- Domingo, J.L., Bocio, A., Flaco, G., Llobet, J.M (2007). Benefits and risks of fish consumption. Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology. 230: 219-226.
- Evans DW, Dodoo DK, Hanson PJ (1993) .Trace element concentrations in fish livers: implications of variations with fish size in pollution monitoring. Mar Pollut Bull. 26: 329- 34.
- Fagerstrom T. (1977). Body weight, metabolic rate and tracesubstance turnover in animals. Oecologia (Berlin). 29:99-104.
- Falcó, G., Llobet, J.M., Bocio, A., Domingo, J.L.,(2006). Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. J. Agric. Food Chem. 54:6106-6112.
- Farkas, A., Sala'nki, J., Speczia' r, A., (2003). Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Research .37: 959-964.
- Farkas A. (1993). Preparation of samples for heavy metal analyses and measuring heavy metals. In: Sal!anki J, Istv!anovics V, editors. Limnological bases of lake management: 160-63.
- Güven, K., Zbay, C., Unlu, E., & Satar, A. (1999). Acute lethal toxicity and accumulation of copper in *Gammarus pulex* (L.) (Amphipoda). Turkish Journal of Biology. 23: 513-521
- Has-Schön, E., Bogut, I., Strelec, I(2006) . Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). Arch. Environ. Contam. Toxicol. 50: 545-551.
- Hatch WR, Ott WL (1968). Determination of

- submicrogram quantities of mercury by atomic absorption spectroscopy. *Anal Chem.*40:2085-7.
- Henry, F., R. Amara, L. Courcot, D. Lacouture, M.-L. Bertho (2004). Heavy metals in four fish species from the French coast of the Eastern English Channel and Southern Bight of the North Sea, *Environment International.* 30 :675- 683
- Kalay, M., Canlı, M (2000). Elimination of essential (Cu and Zn) and non-essential (Cd and Pb) metals from tissue of a freshwater fish, *Tilapia zilli*. *Tr. J. Zool.* 24: 429- 436.
- Krishnamurthy KV, Shpritt EE, Reddy MM. (1976). Trace metal extraction of soils and sediments by nitric acid-hydrogen peroxide. *Atmos Absorp Newslett.*15: 68-70.
- Kimball, K.D. and S.F.Kimball, (1974). The limnology of the Pahlavi Mordab, Iran: A study of eutrofication problems. Technical Report. Iranian Department of the environment, Tehran:43 p (Mimeo)
- Kinsella, J.E., Lokesh, B., Stone (2002). R.A Dietary *n-3* polyunsaturated fatty acids in amelioration of Kris-Etherton, P.M., Harris,W.S., Appel, L.J.,. Fish consumption, fish oil. Omega- 3 fatty acids, and cardiovascular disease. *Circulation .*106: 2747-2757.
- Lee, Y. H.,& Stuebing, R. B (1990). Heavy metal contamination in the River Toad, *Bufo juxtesper* (Inger), near a copper mine in East Malaysia. *Bulletin of Environmental Contamination and Toxicology.* 45:272-279.
- MAFF(2000). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997. *Aquatic Environment Monitoring Report No. 52.* Center for Environment, Fisheries and Aquaculture Science, Lowestoft, UK.
- Newman MC, Doubet DK. (1989). Size-dependence of mercury (II) accumulation kinetics in the mosquitofish, *Gambusia affinis* (Baird and Girard). *Arch Environ Contam Toxicol.*18:819-25.
- Oomen, C.M., Feskens, E.J., Räsänen, L., Fidanza, F., Nissinem, A.M., Menotti, A., Kok, F.J., Kromhout, D., (2000). Fish consumption and coronary heart disease mortality in Finland, Italy and The Netherlands. *Am. J. Epidemiol.* 151:999-1006.
- Perkin Elmer (1981). *Analytical Methods for Atomic Absorption Spectroscopy Using the Mercury/Hydride System.* Instrument Division, Perkin Elmer-Corporation, Norwalk, CT.
- Proceedings of the ILEC/UNEP International Training Course 24 May-5 June(1993). Tihany, Hungary, ILEC Kusatsu Shiga, Japan.
- Rashed, M. N. (2001). Monitoring of environmental heavy metals in fish from Nasser Lake. *Environment International.* 27: 27-33.
- Sankar, T. V., Zynudheen, A. A., Anandan, R., & Viswanathan Nair, P. G. (2006). Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. *Chemosphere.* 65:583-590.
- Shul'man GE.(1974) *Life cycles of fish.* New York: Wiley,.
- UNEP Guidelines for monitoring chemical contaminants in the sea using marine organisms (1993). *Reference methods for marine pollution studies, Report 6,* Athens
- Uthe, JF, Chou, CL, Misra, RK, Yeats, PA, Loring, DH, Musial, CJ (1991) .Temporal trend monitoring: introduction to the study of contaminant levels in marine biota. *ICES Techniques in Marine Environmental Sciences, Report 14,* Copenhagen;
- Vladykov, V.D(1964).*Inland fisheries resources of Iran especially of the Caspian Sea with special reference to sturgeon.* Report to the Government of Iran. *FAO Report.*1818:51 p.
- Weatherly AH, Gill HS(1987). *The biology of fish growth.* Orlando, FL: Academic Press.
- Yilmaz, A. B. (2005). Comparison of Heavy metal levels of Grey Mullet (*Mugil cephalus* L.) and Sea Bream (*Sparus aurata* L.) caught in Iskenderun Bay (Turkey). *Turkish Journal of Veterinary and Animal Sciences.* 29: 257-262.

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فلزات سنگین Cu, Zn, Cd و Pb در بافت و کبد *Esox lucius* و رسوب آنها در تالاب بین‌المللی انزلی - ایران

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چکیده

اندازه‌گیری میزان تجمع آلاینده‌ها در ماهیان، از آنجاییکه مورد مصرف انسانی قرار می‌گیرند مورد توجه قرار می‌گیرد. در این مقاله، تجمع فلزات کادمیوم، سرب، روی و مس در بافت کبد و ماهیچه اردک ماهی و رسوبات از تالاب انزلی - ایران اندازه‌گیری شده است. تالاب انزلی، زیستگاه و نوزادگاه گونه‌هایی از ماهیان بومی و دریای خزر می‌باشد. این گونه‌ها، توسط افراد محلی و سایر افراد مورد مصرف قرار می‌گیرند همچنین از طرف دیگر یازده رودخانه اصلی، مواد زاید کشاورزی و صنعتی و شهری خود را در داخل این تالاب می‌ریزند. تعداد ۲۵ نمونه اردک ماهی از تالاب انزلی توسط تور گوشگیر در مهر ماه و آذر ماه سال ۱۳۸۶ جمع‌آوری شدند. وزن و طول کل تمام ماهیان اندازه‌گیری شدند. متوسط وزن، $121 \pm 80.4/6$ گرم و متوسط طول $2 \pm 43.0/7$ میلی‌متر بودند. تعیین سن با استفاده دواپر سالانه، نمونه‌های فلس انجام شدند. متوسط غلظت کادمیوم، مس، سرب و روی در بافت عضله ماهیان به ترتیب: کادمیوم: $0.01/0.02$ ، مس: $0.21 \pm 0.01/0.01$ ، سرب: $0.13 \pm 0.01/0.01$ و روی: $2.55 \pm 0.18/0.18$ میکروگرم بر لیتر همچنین در بافت کبد به ترتیب کادمیوم: $0.014/0.014$ ، مس: $0.96 \pm 0.61/0.61$ ، سرب: $0.11 \pm 0.04/0.04$ و روی: $2.46 \pm 1.5/1.5$ میکروگرم بر لیتر می‌باشد. نتایج نشان داده است که ارتباط مثبت معنی‌داری بین فلزات مس و روی با وزن بدن و همچنین ارتباط منفی بین فلز سرب با وزن بدن وجود دارد ($p < 0.05$). مقایسه میانگین تجمع فلزات مس، روی و کادمیوم با استانداردهای جهانی (MAFF, WHO) نشان داد که پایین‌تر از حد مجاز بوده است در حالیکه میزان فلز سرب، بیشتر از حد مجاز بوده است.