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Non-Carcinogenic Risk Estimation of Cr, Cd, Pbin Human to Fish Consumption from Anzali Wetland

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Abstract: The presence of heavy metals in our environment has been of great concern because of theirtoxicity when their concentration is more than the permissible level. These metals enter in theenvironment by different ways like industrial and agricultural activitiesetc. This investigation quantified chemical contaminants in fish muscle tissue samples and followed methods of the U.S. Environmental Protection Agency (EPA) to estimate the hazard indices with consuming fish caught in the Anzali wetland. The fish samples *Abramisbrama, Cyprinuscarpio, Esoxlucius, Chaleaiburnuschaleoide* and *Carassiuscarassius* were collected from Anzali wetland. In this study we have used Spectroscopy technique for determination of Cd (Cadmium), Cr (Chromium), Pb (Lead) in different species of fish. The concentrations of Pb are high as compared with other metals as these metals were not in the maximum permissible. Pb concentration in *Cyprinuscarpio* and *Esoxlucius* species was higher than WHO standard level. Hazard index < 1 suggests in study and consumption limit of fish was acceptable dairy intake of human. Although the heavy metals analyzed in fishes from Anzali wetland did not pose any immediate health risk to humans but due to the bioaccumulation and magnification of these heavy metals in humans, it is essential to safeguard levels of the metals in the environment.

Key words: Concentration · Heavy Metals · Bioaccumulation · Hazard Index · Spectrophotometery

INTRODUCTION

Anzali international wetland is among valuable wetland of southwest Caspian Sea which has especial importance due to specific ecological, economical, socialconditions and diversity on one hand, it receives water of 19 rivers and on the other hand it transmits this water through two outflows. These rivers by passing through forest, urban and rural regions carry all kinds of minerals, organics, sedimentary materials, industrial area of Anzali and given the long periodof time of arresting input river's water to this pond as properties of water entering to Caspian Sea through itaswell as high diversity and number of plant and animal outflow [1]. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio-accumulate in aquatic ecosystems, although some of pollutants are present at trace levels, they can induce adverse effects on humans and wildlife [2]. Metals can enter into the food web through direct consumption of water or organisms, or through uptake processes and be potentially accumulated in edible fish, there is a growing concern that metals accumulated in fish muscle tissues may represent a higher health risk than a health benefit, especially for populations with high fish consumption rates [3]. Even at low aqueous concentrations, the metals can damage the nervous system [4] and disrupt the immune system [5]. Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals [6]. Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments,

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soil erosion and runoff, air depositions of dust and aerosol and discharges of waste water [7]. Fish constitute an important source ofprotein for many people throughout the world and fish consumption has increased in importance among human because it provides a healthy, low cholesterol source ofprotein and other nutrients, Fish provide omega-3 (n-3) fatty acids that reduce cholesterol levels and the incidence often art disease, stroke and preterm delivery [8]. Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from the water [9]. Factors such as high population growth accompanied by intensive urbanization, increase in industrial activities and higher exploitation of natural resources including cultivable land have caused pollution increase. Heavy metals have the tendency to accumulate in various organs fish, which in turn may enter into the human metabolism through consumption causing serious health hazards. Reports from literature suggest that these toxicants are responsible of hazardous effects on human health [3-4-9] unfortunately; few studies have been conducted to investigate the health risk induced by the metals in the Anzali wetland. The main objective being pursued in this study is to determine contaminant contractions in fish muscles in order to assess non-carcinogenic risk forconsumers of fish in Anzali city. The results obtained might be used as abenchmark by the authorities that regulate and control thedischarge of metals.

MATERIALA AND METHODS

Anzali wetland is located in thesouthwestern region of the Caspian Sea coast, at 37°28'N, 49°25'W. The Anzali wetland used to beabout 2-3m deep in the eastern parts and 8-11m in western regions but due toheavy erosion, siltation and macrophyteinvasion it has dropped to 1-3 m in depthwhich intensifies all kinds of pollutionthreats (Fig. 1).

Determination of Heavy Metal in Samples: A total of 44 specimens, comprising five different fish Species, the collected species were *Esoxlucius* (n=10), *Cyprinuscarpio* (n=10) *Chaleaiburnuschaleoide* (n=10), *Abramisbrama* (n =8) and*Carassiuscarassius* (n = 6) were captured using fish traps. The samples were collected from different parts in wetland randomly and kept in the laboratory deep freezer (-18°C). For the determinationof Cd, Pb and Cr concentrations, tissuesamples were subjected to wet aciddigestion and then heavy metals concentrations were determined with a Shimadzu AA/680 atomic absorption spectrophotometer. Concentrations of metals tissues were calculated on a dry weight basis and expressed as µg/g. These are commercially important for species (Table 2).

Health Risk Estimation: The current study's risk assessment is based on EPA's[10] Guidance for Assessing Chemical Contaminant Data for Use in Fish

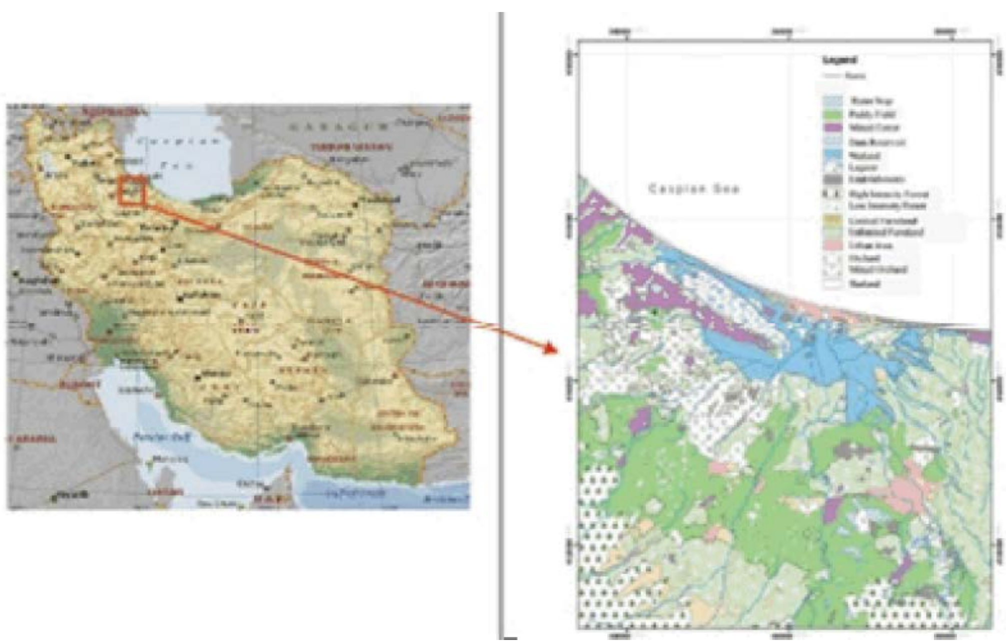

Fig. 1: Map of the AnzaliWetland

Advisories: Vol. 2. Risk Assessment. The level of exposure resulting from the consumption of a particular chemical in fish tissue, whether muscle tissue can be expressed by an estimation of daily intake levels in the following equation:

Average Daily Dose (mg/kg-day) = (CxIRxEFxED)/ (BWxAT)

where:

C = Mean total metal concentration in muscle tissue of fish

IR = Mean ingestion rate of fish = 0.0312 kg/day

EF = Exposure frequency = 365 days/year

ED = Exposure duration (years) over lifetime = 70 years 90th percentile length of time an individual stays at one residence

BW = Body weight = 70 kg

AT = Averaging time (days): ED.EF (non-carcinogen)

Potential Risk for chemicals were calculated for this study and the equation listed above is the most commonly used intake equation. For non-carcinogens the average daily dose, or ADD, is calculated which expresses the average daily intake of a specific chemical over a certain period of time.Estimating risk levels, the mean a maximum total chemical concentrations, were determined from the data from the five fish tissue samples. The Exposure frequency (EF) represents the average percapita number of meals by the population based on a long term average contact rate [11]. For all calculations, an average adult body weight (BW) of 70, standard exposure duration (ED) of 70 years and averaging time (AT) of 365 days were assumed, to keep with the default values provided in EPA's [12] Exposure Factor's Handbook.

Non-carcinogenic risks are quantified by the calculation of a Hazard quotient (HQ). The Hazard quotient is a ratio of the intakedose (I) divided by the reference dose (RfD) of the chemical of concernThe HQ is an estimate of the risk level (noncarcinogenic) due to pollutant exposure. The oral route is the route of exposure observed in this study; therefore, oral RfD values from the EPA's [13] IRIS database were used. Reference dose for assessing non-carcinogenic health effects was for Cadmium 1.0E-03, Chromium 3.0E-03 and lead 3.0E-02. Table 4 shows the results of estimated average metal intake doses (µg/kg/day) and hazard quotients (HQ) caused by consuming different fish of Anzali Wetland.

The hazard index (HI) from HQ can be expressed asthe sum of the hazard quotients.When the intake exceeds the reference dose and thus the HQ is greater than or equal to one it is probably that non-carcinogenic adverse health effects will be observed.

Data obtained in this study were analyzed using Excel 2007. The least significant differences test was used to measure the difference of the metal levels and health risks among the five fish.

RESULTS AND DISCUSSION

Table 1 is showing the lists studied fish species, the numbers of fishes sampled, mean of thelength and weight ranges with standard deviation. The fish samples under study belonged to the 2 to 5-year age group. They ranged in length from 143 to 466 mm and in net weightfrom 26 to 889 g. the highest length and weight related to *Esoxlucius* and the lowest to*Chaleaiburnuschaleoide* in this study.

The results indicated that the metal concentrations were different among fish muscle tissues, concentrations cadmium, lead and chromium are presented in Table 2 with means and SD. the mean concentration varied from 0.52 to 1.24 for Cr,0.10 to 0.23 for Cd, from 0.89 to 2.56 for Pb µg/g dry wight in different species.

Cadmium tended to be the least concentrated in the all fish as compared to other elements measured. In all studied fishesconcentrationsof Pbwerehigh. The higher metal concentrations were found in the *Esoxlucius*. The sequence of order of the heavy metals measured in the fish samples observed from the AnzaliWetland was as follows: Cd< Cr<Pb, respectively.

The relationship between size of fish and metal accumulation is subject to variable opinions from different authorities. In this study concentration of metals Cr, Cd, Pb as size of fish increased *Abramisbrama* and *Carassiuscarassius* (Table 3).

In this study, metal concentrations were regressed with fish total lengths statistically significant strong positive correlations were observed for lead and cadmium in *Carassiuscarassius*, while were observed for *Esoxlucius, Cyprinuscarpio* and *Chaleaiburnuschaleoide* weak correlation total lengths and weight with concentration of metals. Also metal concentrations were regressed with fish weight statistically significant strong positive correlations were observed for lead, chromium and cadmium in *Abramisbrama* and *Carassiuscarassius.* A l-Yousuf *et al.*

Table 1: Average fish length and weight ± standard error of fish species collected in AnzaliWetland

		Weight (g)		Length (mm)	
Fish	N	Mean	Range	Mean	Range
Esoxlucius	10	557.90±190.44	377-889	416.10±30.86	362-466
Cyprinuscarpio	10	214.9±118.90	45-336	240.3±56.95	145-305
Abramisbrama	8	179.87±99.98	63-302	229.25±36.74	147-270
Carassiuscarassius	6	226.33±13.66	206-237	234.5±3.88	230-240
Chaleaiburnuschaleoide	10	32.4±4.55	26-39	154.6±7.30	143-168

Table 2: The mean of heavy metals(Pb,Cr,Cd) in fish samples collected from Anzali wetland(Guilan) (µg/g dry wight)

		Sex		Cr		Cd		Pb	
Fish	N	Nf	Nm	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Esoxlucius	10	5	5	1.24±0.11	1.07-1.38	0.23±0.04	0.2-0.31	2.56±0.06	1.98-3.64
Cyprinuscarpio	10	8	2	0.83±0.11	0.65-0.96	0.16±0.04	0.12-0.23	1.56±0.44	1.06-2.12
Chaleaiburnuschaleoide	10	9	1	0.52±0.20	0.27-0.87	0.10±0.29	0.07.0.15	1.28±0.30	0.98-1.87
Abramisbrama	8	8	-	0.88±0.09	0.79-1.02	0.19±0.02	0.16-0.24	1.27±0.38	0.95-1.87
Carassiuscarassius	6	4	2	0.88±0.13	0.72-1.05	0.17±0.03	0.13-0.22	0.89±0.11	076-1.03

*Nf: number of female,Nm: number of male

Table 3: Correlation between heavy metal concentrations in muscle tissue (µg/g dry wight) and length (mm), body weight (g) of different fish in Anzali wetland

Species	Parameter	Length	Weight
Esoxlucius	Pb	0.4424	0.6421
	Cd	0.3021	0.5642
	Cr	0.3316	0.0709
Cyprinuscarpio	Pb	0.1134	0.0358
	Cd	0.2805	0.2212
	Cr	0.5332	0.5926
Chaleaiburnuschaleoide	Pb	0.3610	0.3433
	Cd	0.4208	0.2425
	Cr	0.4872	0.1712
Abramisbrama	Pb	0.8512*	0.9624*
	Cd	0.7683*	0.8718*
	Cr	0.8812*	0.9772*
Carassiuscarassius	Pb	0.9865*	0.8541*
	Cd	0.9624*	0.8712*
	Cr	0.7217*	0.5532

* Values >0.5 or < - 0.5 are significantly correlated

[14] found that the concentration of Cd in *Lethrinuslentjan*a positive correlation with fish length and weight. Guo [15] found that for *Branchiostomabelcheri*, a positive correlation existed between concentrations of Cd with fish length however, no significant relationship was found between Pb and fish length and weight. Some research have shown negative relationships between fish size and the metal concentrations found in fish [16, 17]. Despite these studies, there are no definite or established relationships between heavy metal concentration and fish size. Metal accumulation in fish has been found to reach a steady state after a certain age [18].

Samples collected from Anzali wetland (Guilan), the concentrations of metals have been expressed on a dry weight basis (µg/g dry wight). A wet weight-dry weight conversion factor of 0.2 can be assumed [19].

Contamination levels of heavy metal in fish are normally compared to the permissible limits recommended by Food and Agriculture Organization (FAO) and World Health Organization (WHO) [20] and other standards. According to the WHO maximum permissible concentrations are 0.2 µg /g for Cd and the European Community proposed threshold values of metal concentrations in fish muscle of only 0.05µg/g [21]. In this study, Cd concentration for all studied fish species was lower than WHO standard. Cadmium can be accumulated with metallothioneins and uptake of 3-330mg/day is toxic and 1.5-9 mg/day is lethal to humans [22]. Cadmium injures kidneys and cause symptoms of chronic toxicity, including impairment of kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction [23].

That toxic metals represent at high concentrations in fish is of particular importance in relation to the FAO/WHO [20]. The maximum permissible doses for an adult are 3 mg Pb per week, but the recommended doses

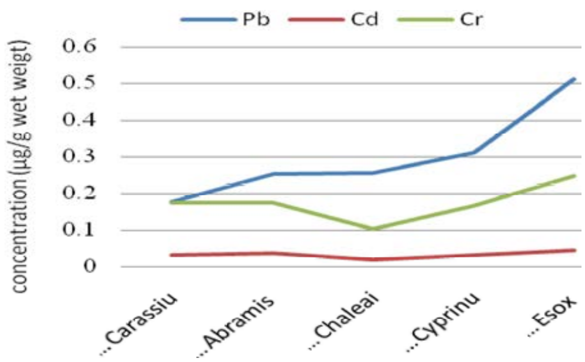

Fig. 2: Comparison ofaverage heavy metal concentrations (µg/g dry wight) in different fish species from anzali wetland

are only one-fifth of those quantities [20]. Chinese food standards [24] are 1µg/g. Turkish acceptable limits and EU limits are 0.4µg/g. The range of international standards for Pb in fish is 0.5-10µg/g [21]. Lead causes renal failure and liver damage in humans [4, 5],Gorell *et al*. [25] reported and suggested that chronic exposures to copper, mercury, zinc and lead, etc., were associated with Parkinson's disease and that they might act alone or together over time to help produce the disease. Also states WHOmaximum permissible concentrations are 0.3 µg/ g for Pb [20]. In this study, Pb concentration for *Esoxlucius* and *Cyprinuscarpio* showed higher concentration than those of WHO standard and Turkish acceptable limits but it lower than Chinese food standards and was in the range of international standards (Fig. 2).

So far, few studies have investigated the non-carcinogenic effects induced by the metals in Anzali wetland and limited information is availableabout the toxicity effects caused by these metals. On abasis of the experimental results, we performed a non-carcinogenicrisk assessment on the metals in the five fish that are consumption of human.Fish have been identified as a significant source of human exposure to various compounds [26]. We also discuss this in the context of overall health risk assessment.

The development of food consumption databases such as those that have been developed in countries such as the United States of America and Canada would make risk assessment easier and faster [26].

This study focused on adult fish consumers. However fish consumption rates could vary in different subpopulations, children may consume larger quantities compared to their body masses than adults, prenatal exposure may occur through pregnant women; these subpopulations are considered as potential high risk groups [27]. Fish consumption guidelines would reduce the risk to fish consumers by providing information that would lead to the voluntary restriction of fish consumption to levels that pose limited, if any risk [28]. Unlikely adverse health effects whereas HI> 1 suggests the probability of adverse health effects. This hazard index calculation yields a number less than 1.0, indicating that adverse health effects are not likely to occur due to ingestion allowable limit fish per day. Calculations of the hazard index obvious that consumption of heavy metal from fish species in the present study was not harmful for human health. Therefore, the daily intake of Cd, Cr and Pb for regular of the five fish species in the present study did not have any hazardous effect on human health. Moreover, daily intake values of heavy metals from fish were determined in many previous studies in several countries as Taiwan, Egypt and Saudi Arabia. For instance In a study performed by Huang, the maximum daily intake of Zn, Cd, Cu and Pb from consumption fishes from eastern Taiwan were 241, 32, 9.6 and 11.9 µg/kg per day, respectively [29], that is higher than daily intake in our study for all species.

Hazardindex for cadmium metal in *Esoxlucius* is higher than other samples (0.054) but Hazardindex < 1 suggests for all of the samples that is unlikely adverse health effects (Figure 3).

Table 4: Hazard Indices and Risk range calculated for the elements Cd, Cr and Pb of consumption fish in human(sustainable adults with intake rate 30g/day and average life expectancy of the general public 70 year)

	ADD (µg/kg/day)			HQ (Hazard quotients)			
Species	Pb	Cr	Cd	Pb	Cr	Cd	HI (Hazard index)
Esoxlucius	0.219	0.106	0.019	0.0007	0.035	0.019	0.054
Cyprinuscarpio	0.133	0.071	0.036	0.0004	0.023	0.013	0.036
Chaleaiburnuschaleoide	0.109	0.044	0.008	0.0003	0.014	0.008	0.020
Abramisbrama	0.108	0.075	0.016	0.0003	0.025	0.016	0.041
Carassiuscarassius	0.076	0.075	0.014	0.0002	0.025	0.014	0.039

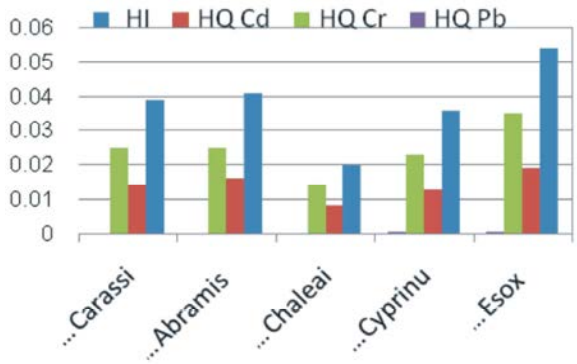

Fig. 3: Various estimated hazard quotients (HQ) for metals caused by consuming fish collected from different Anzali Wetland

In order to have a clear judgment about the level of pollution in tissue of certain types of fish consumed by people, the obtained data should be compared with some other literature data belongs to comparable studies. The concentrations of metals in fish species were generally comparable to values reported in other studies. Kalay *et al.* found that muscle tissues in *Mugilcephalus* and *Mullusbarbatus* collected from Northeast Mediterranean Sea contained cadmium, chromium, lead at 1.17-0.96, 1.53-1.30, 6.79-6.24 µg/g species, respectively [30]. in this study, we found lower concentrations of metals than Kalay *et al*. It should be noted that the assessment methods used in this study contain some possible uncertainties. Ebrahimpour *et al*. [31] reported cadmium, chromium and lead in the muscle tissues of *Carassiusgibelio* collected from the Anzali wetland, Iran at concentrations of 0.25 µg/g, 0.7 µg/g and 1.2 µg/g, respectively. In our study, Pb was observed at higher concentrations and Cd was observed at lower concentrationsthan the Ebrahimpour et al. studyfor all species.

Serious health concerns arising from consumption of contaminated fish have resulted in the establishment of various fish consumption advisories to protect human health across the USA, including most of its territories. The methodology developed by USEPAwas applied to derive advisory consumption recommendations for minimizing the risk of both cancer and noncancerendpoints due to consumption of the fish species analyzed in this study. This methodology has also been used by other researchers to develop fish advisories for specific locations and species of fish [32].

Differences in consumer age and exposure conditions might result in different risks [32]. Systematic and comprehensive investigations on the potential health risks induced by trace metals in source water are necessary to provide sufficient information for local governments to properly regulate and control the discharge of metals into the wetlands. Although all results of heavy metal concentrations studied showed that regular consumption of the fish species did not cause any harm effect on human health, recommendations should be taken into consideration. Similar studies may be performed to check contamination with other toxic heavy metals such as mercury, cobalt and arsenic in commercial fish, periodical monitoring heavy metals level in commercial fish is needful, especially for endangered species that are important forsurvival of ecosystem. Also to improve the estimates of risk from fish consumption, it is important to conduct as speciation analyses in edible fish from AnzaliWetland. Furthermore, it is well known that fish may contain a variety of bio-accumulative chemical contaminantsthat are a health concern [2-29].

CONCLUSION

In this study, the levels of three metals including Cd, Cr and Pb of five specieswere determined in Anzali Wetland and the potential non-carcinogenicrisk induced by these metals was assessed. Among the metals analyzed, Pb was the one with the highest concentration, while Cd had the lowest level. The concentrations of metals determined in this study the exception Pb concentration of *Esoxlucius* and *Cyprinuscarpio* did not exceed the standards recommended by the WHO and HI of each element were lower than one, the non-carcinogenic hazard index, suggesting that these pollutants perhaps pose little hazard to local residents. Future efforts should focus on health risks posed by metal contamination in the other biological factor along the AnzaliWetland.

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