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Human exposure to some toxic and essential elements through freshwater fish consumption in Bulgaria

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Human exposure to As, Cd, Cu, Cr, Mn, Fe, Ni, Zn and Pb through consumption of freshwater fishes (carassius, freshwater bream, common roach and European crap) was estimated by evaluation of target hazard quintet (THQ), target risk (TR), and hazard (HI) indexes. The elements were determined by ICP-OES.

The results from this study show that the THQ for the studied toxic and essential microelements are less than one; signified that a daily exposure at this level is unlikely to cause any adverse effects during a person lifetime. Additionally hazard index of each microelement was also lower than one suggesting that these pollutants pose no hazard to local residents. The TR values were between 10^{-6} and 10^{-4} meaning that there is no important cancer risk associated with the consumption of the examined in the present study fish species.

Key words: *toxic/essential elements, freshwater fishes, THQ, HI, Mandra Lake*

INTRODUCTION

Fish consumption entails important potential health benefits such as lowering the risk of cardiovascular diseases. However, fish may also be a source of environmental contaminants. Exposure to these contaminants could imply health risks, especially for the more vulnerable consumer groups, such as pregnant women and children [1].

Heavy metals tends to accumulate in advanced organisms through bio-magnification effects in the food chain. Thus, they can enter into human body, and accumulate in the human tissues to pose chronic toxicity. Chronic assimilation of heavy metals is known cause of cancer [2] and can damage vital organ functions. Accumulation of heavy metals in the food web can occur either by accumulation from the surrounding medium, such as water or sediment, or by bioaccumulation from the food source [3]. In the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms where they are strongly bound with sulfhydryl groups of proteins and accumulate in their tissues. The accumulation of heavy metals in the tissues of organisms can result in chronic illness and cause potential damage to the population [4].

Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, selenium) [5]. The essential metals can also produce toxic effects when the metal intake is excessively elevated. Fish accumulates heavy metals in its tissues through absorption and human can be exposed to metals via food web. This will cause acute and chronic effect to human [6]. For the estimation of the potential risks to human health of heavy metals in fishes, several ways have been adopted such as calculating the carcinogenic and non-carcinogenic effects. Risk assessment is one of fastest method which is need to evaluate the impact of the hazards on human health and also need to determine the level of treatment which are tend to solve the environmental problem that occur in daily life [7]. Current non-cancer and cancer risk assessment methods do not provide quantitative estimate of the probability of experiencing non-cancer and cancer effects from contaminant exposure. These method are typically are based on the Target Hazard Quotients (THQ) and Hazard Index (HI).

The purpose of this study is to determine the concentration of some heavy metals such as As, Cd, Cu, Cr, Mn, Fe, Ni, Zn and Pb in four freshwater fish species habitat in Bulgarian Mandra lake, which is directly connected with Black Sea basin. By using the target quotient (THQ) and hazard index (HI), health risk associated with heavy metals in these fishes was evaluated.

MATERIALS AND METHODS

Study Sites

Mandra Lake, is a large freshwater lake in South Eastern Bulgaria, located south of Burgas. It is the southernmost of the three Burgas lakes with water surface area of about 1,300 hectares. Its length is 8 km and its maximum width is 1.3 km. It is situated

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in a well-shaped river valley, oriented perpendicular to the beach and the mouth and the dam are near the southern end of Burgas. Four rivers flow into the lake. This area was selected due to rapid growth of development which are mixed development area comprises residential, commercial, small and medium enterprises and industry.

Field sampling

The freshwater fish samples analyzed in this study are carassius *(Carassius auratus)*, freshwater bream *(Abramis brama)*, common roach *(Rutilus rutilus*) and European crap (*Cyprinus carpio*). Fish sample was collected from a single fisherman in order to assure regularity in fishing methods. The fish was collected from selected points and transported to the laboratory on the same day in the pre-cleaned polyethylene bags. Total length and weight of the sample brought to laboratory on ice after collection were measured to the nearest millimeter and gram before dissection. All samples were frozen and stored at -18ºC immediately upon returning from the field.

Sample Digestion and Instrumental Analysis

Approximately 1.0 g of homogenized miscle tissues sample was digested with 10 cm^3 HNO_3 (ultra-pure Merck ® Darmstadt, Germany) in a digestion system and diluted to final volume of 25 cm^3 with double deionised water. MARS 6 Microwave Sample Preparation System (CEM Corporation, USA) delivering a maximum power and temperature of 800 W and 200 °C, respectively, and internal temperature control, was used to assist the acid digestion process.

One reagent blank for each digestion was included as a representative standard reference, homogeneity and process efficacy in sample replicated. The digested sample was transferred to a marked flask post-cooling.

All fish samples were analyzed for As, Cd, Cu, Cr, Mn, Fe, Ni, Zn and Pb using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). ICP-OES model used in this study was Optima 8000 (Perking Elmer, USA). The instrument working parameters were as follows: plasma gas flow - 10 L/min, auxiliary gas flow - 0.4 L/min, nebulizer gas flow - 0.6 L/min, peristatic pump flow rate - 1.5 ml/min, spay chamber - cyclonic glass, nebulizer - concentric glass, MEINHARD® Type C. Results were quantified using an calibration curve generated from the responses obtained from multiple dilutions of a multi-element calibration standard prepared (Optima Family Multi-Element Standard, Matrix per Volume: 2% HNO_3). Analytical quality control included analysis of a 2 % ultrapure HNO_3 blank and a sample duplicate from the microwave digestion.

A DORM-2 (NRCC, Ottawa) certified dogfish tissue was used as the calibration verification standard. Recoveries between 90% and 108% were accepted to validate the calibration.

RESULTS AND DISCUSSION

As, Cd, Cu, Cr, Mn, Fe, Ni, Zn and Pb levels in fish species

The concentration of different metals (mg/kg wet weight) in the edible portion of fishes subject to this study are given in Table 1.

There are significant differences on the concentration presents for the heavy metals for Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn for the different fish species.

Among the heavy metals studied, Zn showed the highest level of accumulation. Tüzen observed a similar trend in his studies about some marine fishes in the Turkish part of Black Sea [8]. In a study performed in the muscle of five common Slovak fish species the lowest mean Zn concentration was detected in Wels cattish (4.61 mg/kg w.w) and the highest mean zinc

Table 1. Mean concentrations (mg/kg w.w) and standart deviation of heavy metals for each species (N is the number of analyzed fish species)

	N	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Carassius	3	n.d	0.041± 0.005	0.044± 0.004	0.17± 0.11	2.23± 1.63	0.19± 0.10	0.09± 0.05	0.27± 0.07	6.98± 0.66
Freshwater bream	3	n.d	0.020± 0.001	0.033± 0.004	0.12± 0.11	5.85± 1.97	0.32± 0.03	0.055± 0.004	0.15± 0.02	1.94± 0.16
Common roach	3	n.d	0.046± 0.007	0.05± 0.05	0.11± 0.03	1.68± 0.96	0.19± 0.16	0.11± 0.03	0.25± 0.06	4.99± 1.12
European crap	2	n.d	0.031± 0.001	0.03± 0.01	0.16± 0.01	1.92± 0.15	0.050± 0.005	0.08± 0.01	0.20± 0.02	3.27± 0.06

concentration in common carp (26.30 mg/kg w.w.)with a significant difference in the bioaccumulation of Zn among different fish species [9]. It is well established that accumulation rate of zinc is higher in omnivorous fish than predatory fish. The results from this study are in accordance with the data from literature.

The lowest mean iron concentration (1.68 mg/kg w.w) was found in common roach and highest mean iron concentration (5.85 mg/kg w.w) in freshwater beam. Similar (2.99–4.38 mg/kg w.w) to our values were found in muscle of five fish species from the Erren River [10]; in five fish species from the Candamo River (Peru) [11] and from 3.70 to 21.10 mg/kg w.w for five common Slovak fish [9].

Higher level of lead in the blood can cause kidney dysfunction and brain damage. The Pb concentration in this study varies between 0.15 mg/kg w.w for freshwater beam up to 0.27 mg/kg w.w for carassius. In a study performed in Věstonice reservoir in Czech Republic the highest lead concentrations were found in tissues of asp (0.12 mg/kg w.w) and carp (0.09 mg/kg w.w) while the lowest lead concentrations were found in pikeperch tissues (0.01 mg/kg w.w) and in pike gonads (0.03 mg/kg w.w) [12]. The mean lead concentration measured in rainbow trout in Western Anatolia, Turkey is 0.08 mg/ kg w.w which is less the values found in this study [13]. According to Bulgarian Food Codex, the maximum lead level permitted for fishes is 0.4 mg/kg [14] and our results are below this value.

The cadmium levels found in this study ranged between 0.020 mg/kg w.w in freshwater beam up to 0.046 mg/kg w.w in common roach. Cd levels in the literature varied between 0.005 mg/kg w.w in carp and pikeperch and 0.01 mg/kg w.w in catfish from Serbian part of Danube River [15]; between 0.003 mg/kg w.w and 0.005 mg/kg w.w in fish muscle in the rivers of Lithuania [16]; and around 0.03 mg/kg w.w for common carp from two ponds in Slovak Republik [9]. The Codex Alimentarius limit for cadmium content in fish muscle is 0.05 mg/kg wet weight. This limit was not exceeded.

Chromium concentrations varied from 0.03 mg/kg w.w (European crap) to 0.05 mg/kg w.w (common roach). Similar results were publishes for the muscle tissies of Europan crap from two southwestern Slovak fish farms [9]. In carp muscles from the five Bohemian ponds chromium concentrations ranged from 0.56 to 0.69 mg/kg wet weight [17]. High variations of chromium concentrations (5.57–197.12 μg/g dry mass) were found in the muscle of *Labeo umbratus* from the Witbank Dam [18]. Chromium content in fish muscle did not exceeded the limit of Codex Alimentarius (4.0 mg/kg wet weight) in any of the analysed samples.

Copper concentrations ranged from 0.11 mg/kg w.w for common roach to 0.17 mg/kg w.w for caracuss. Higher copper concentrations were reported in fish from the Atatürk Dam Lake [19, 20]. Similar mean copper concentrations to our results was found in the muscle of *Clarias fucus* from the Duy Minh Lake [21], in the muscle of three fish species from four Taimyr Peninsula Lakes [22], and in muscle of marketable carp (*Cyprinus carpio*) from five south and west Bohemian fish ponds [17]. In this study, none of the analyzed muscle samples exceeded the limit for copper allowed by Bulgarian Food Codex (10.0 mg/kg w.w) [14].

Manganese concentrations in the muscle showed a high variation and amount. Concentrations of manganese fluctuated between 0.05 mg/kg w.w. in European crap and 0.32 mg/kg w.w. in freshwater beam. Similar manganese concentrations were found in the muscle of *Lepisosteus platyrhincus* from the Okeechobee Lake (0.394 μg/g wet weight) [23], and in the muscle of *Lisa abu* and *Silurus triostegus* from the Atatürk Dam Lake (0.40 and 0.35 μg/g w.w., respectively) [19]. The limit for manganese in Codex Alimentarius is not defined but our results are in good agreement with the data found in the literature.

Our results for Ni showed relatively low nickel concentrations, fluctuating between 0.055 mg/kg w.w in freshwater beam and 0.11 mg/kg w.w in common roach. Higher levels (more than 40 times greater than our results) of nickel concentrations were found in the muscle of *Labeo umbratus* from the Witbank Dam [18]. In the muscle of *Silurus triostegus* from Atatürk Dam Lake the mean nickel concentration of 0.56 μg/g wet weight was recorded [19]. Results comparable to ours were published for the muscle of common carp (*Cyprinus carpio*) from the five south and west Bohemian ponds [17]. The Bulgarain Food Codex limit for nickel content in fish muscle is 0.5 mg/kg w.w [14]. Our values are lower than the limit.

Health risk assessment

The THQ [24] which is the ratio between the exposure and the reference dose (RfD), is used to express the risk of non-carcinogenic effects. Ratio of less than 1 signifies non-obvious risk. Conversely, an exposed population of concern will experience health risk if the dose is equal to or greater than the RfD. The method for the determination of THQ was provided in the United States EPA Region III risk-based concentration table [24]. The dose calculations were carried out using standard assumptions from an integrated United States EPA risk analysis.

A THQ below than one implies that the level of exposure is smaller than the reference dose; a daily exposure at this level is believed to cause any adverse effects during a person's lifetime. The models for estimating THQ is expressed as:

$$THQ = \frac{(M_C \, . I_R \, . 10^{-3} . EF \, . ED)}{(RfD \, . BWa \, . ATn)}$$

where M_C is the metal concentration in muscle tissues of fish (μg/g), IR is the mean ingestion rate of fish (5.205 g/day), EF is the exposure frequency (53 day/year) or number of exposure events per year of exposure, ED is the exposure duration, total for adult (70 years for females and 63 years for males), RfD is the reference dose (Cu = 0.04, As = $3x10^{-4}$, Zn = 0.3, Ni= 0.02, Cr -3 x 10^{-3}, Fe -$9x10^{-3}$, Cd -$13x10^{-3}$, Pb - $4x10^{-3}$, Mn - 0.144 μg/g day), BWa is the body weight, adult (60 kg for females and 68 kg for males kg), and ATn is the averaging time, noncarcinogens and it was calculated by multiplying exposure frequency in exposure duration over lifetime (day/year).

The hazard index [24] from THQs can be expressed as the sum of the target hazard quotients of each individual element:

HI = THQ_{As} + THQ_{Cd} + THQ_{Cr} + THQ_{Cu} + THQ_{Fe} + THQ_{Ni} + THQ_{Zn}+ THQ_{Pb} + THQ_{Mn}

In cases where cancerogenic HI did not exceed one, it was assumed that no chronic risks were likely to occur at the site.

Target cancer risk [24] indicates carcinogenic risks. The model for estimating TR was shown as follows:

$$TR = \frac{(M_C \, . I_R \, . 10^{-3} . CPSo . EF \, . ED)}{(BWa \, . ATc)}$$

where CPSo is the carcinogenic potency slope, oral (As = 1.5 and for Ni= 1.7 mg/kg bw-day); ATc is the averaging time, carcinogens (day/years) and was calculated by multiplying exposure frequency in exposure duration over lifetime. TR value for intake of As and Ni was calculated to indicate the carcinogenic risk since Cu, Hg and Zn do not cause any carcinogenic effects.

The theoretical and estimated lifetime target hazard quotients (THQs) for As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn to humans due to exposure to consumption of freshwater fishes from Mandra Lake were calculated and presented in Table 2 and 3.

As it can be seen from the Table 3 and Table 4, the THQs of As, Cd, Cr, Fe, Mn, Ni, Pb and Zn did not exceed the safe value of one.

Also the total THQ means HI was less than one for all fish species (between 0.027 and 0.061 for females and between 0.033 and 0.063 for males) and it demonstrated that ingestion of those fishes subject to this research does not result in over exposure of studies metals. Thus, no adverse effect poses to the health of consumers.

Table 2. Risk values of each metal contaminant of the freshwater fish species (females)

Females												
Fish samples	**Target hazard quotients (THQs)**								**Hazard Index (HI)**	**Target Risk (TR)**		
	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn		As	Ni	Pb
Carassius	0.00	0.003	0.001	0.0003	0.021	0.00012	0.0004	0.006	0.002	0.034	0.00	$1.33.10^{-5}$	$2.27.10^{-5}$
Freshwater bream	0.00	0.002	0.001	0.0003	0.054	0.00019	0.0002	0.003	0.001	0.061	0.00	$7.84.10^{-6}$	$1.24.10^{-5}$
Common roach	0.00	0.004	0.001	0.0002	0.016	0.00011	0.0004	0.005	0.001	0.028	0.00	$1.52.10^{-5}$	$2.10.10^{-5}$
European crap	0.00	0.003	0.001	0.0003	0.018	0.00003	0.0003	0.004	0.001	0.027	0.00	$1.14.10^{-5}$	$1.65.10^{-5}$

Table 3. Risk values of each metal contaminant of the freshwater fish species (males)

Males													
Fish samples	**Target hazard quotients (THQs)**									**Hazard Index (HI)**	**Target Risk (TR)**		
	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn		As	Ni	Pb
Carassius	0.00	0.003	0.001	0.012	0.018	0.0001	0.0003	0.005	0.002	0.042	0.00	$1.06.10^{-5}$	$1.81.10^{-5}$
Freshwater bream	0.00	0.001	0.001	0.009	0.048	0.0002	0.0002	0.003	0.001	0.063	0.00	$6.22.10^{-6}$	$9.85.10^{-6}$
Common roach	0.00	0.003	0.001	0.008	0.014	0.0001	0.0004	0.005	0.001	0.033	0.00	$1.21.10^{-5}$	$1.66.10^{-5}$
European crap	0.00	0.002	0.001	0.012	0.016	0.00003	0.0003	0.004	0.001	0.035	0.00	9.02E-06	$1.31.10^{-5}$

Calculated average value of carcinogenic risk (TR) of carassius, freshwater beam, common roach and European crap was performed for As, Pb and Ni, since only those elements from the analysed ones show carcinogenicity (Table 1 and 2). The values are as follows: between $9.02x10^{-6}$ and $1.06x10^{-5}$ for Ni for both males and females and between $9.85x10^{-6}$ and $1.24x10^{-5}$ for Pb. Since the total concentration of As is below detection limits for this toxic element TR values are equal to zero. In the literature TR for arsenic and nickel was found to be $8.6x10^{-5}$ (range, $4.7x10^{-5}$, *Labeo rohita* to $1.5x10^{-4}$, *Catla catla*) and $4.7x10^{-4}$ (range, $3.0x10^{-4}$, *Oreochromis nilotica* to 5.8 $x10^{-3}$, *Catla catla*), respectively for fishes from Kolkata wetland, India [25].; and between $1x10^{-6}$ to $1x10^{-4}$ for Cu, Pb, Ni, Cd and Cr except Zn ($6.17x10^{-4}$) for cultured *P. hypophthalmus* from India [26]. In a previous study related with Black Sea marine fishes the TR values for As and Ni are as follows: $4.3x10^{-5}$ for As and $3.57x10^{-7}$ for Ni [27]. Comparing our values with those stated in the literature and the guidelines values, indicates that analysed fish from Mandra Lake are safe for human consumption.

CONCLUSION

In this study, the selected freshwater fish individuals had metal levels below the guideline values established by different environmental agencies. The estimation of noncarcinogenic risk (THQs) conducted in this study showed that adverse health effects may not occur when considering different fish consumption patterns. Hazard index (HI) of each elements were also lower than one suggesting that these pollutants perhaps pose no hazard to local residents. The target cancer risk (TR) due to As, Pb and Ni exposure through freshwater fish consumption from Mandra Lake do not have the probability of contracting cancer over a long lifetime in future.

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ОЦЕНКА НА ПРИЕМА НА НЯКОИ ТОКСИЧНИ И ЕСЕНЦИАЛНИ ЕЛЕМЕНТИ ЧРЕЗ КОНСУМАЦИЯ НА СЛАДКОВОДНИ РИБИ В БЪЛГАРИЯ

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(Резюме)

Оценката на експозицията спрямо As, Cd, Cu, Cr, Mn, Fe, Ni, Zn и Pb е съществен елемент от количествената оценка на риска за човешкото здраве. Рискът, свързан с консумацията на някои сладководни риби, е оценен чрез пресмятане на коефицентите за неканцерогенен риск (THQ), индекс на опасност (HI) и канцерогенен риск (TR). Химичните елементи са анализирани чрез ICP-OES.

Резултатите от това изследване показват, че THQ стойностите за токсичните и есенциални микроелементи са под единица, т.е дневната експозиция при това ниво е малко вероятно да причини нежелани ефекти за целия период на човешкия живот. В допълнение, индексът на опасност за всеки един микроелемент е също под единица, което предполага, че тези замърсители не представляват опасност за местните жители. Стойностите за TR са между 10^{-6} и 10^{-4}, което показва, че не съществува риск от ракови заболявания причинени от консумацията на тези видове сладководни риби.

Ключови думи: *токсични метали, сладководни риби, THQ, HI, Езеро Мандра*.