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Assessment of human health risk for copper, arsenic, zinc, nickel, and mercury in marine fish species collected from Bulgarian black sea coast

Katya Peycheva, Veselina Panayotova and Mona Stancheva

Abstract
The aim of this study is to measure the levels of Cu, As, Zn, Ni and Hg found in muscle of three common fish species collected from the coast of Black Sea, Bulgaria and to determine their potential effects via calculation of the daily intake of metals and estimated weekly intake of metals. To estimate the human health risk, the target hazard quotients (THQ), was calculated. THQs for individual metals were lower than the guideline value of 1. Hazard Index of each trace element were lower than one suggesting that these pollutants perhaps pose no hazard to local residents. Target risk due to As ($3.63x10^{-5}$ – 5.47 $x10^{-5}$) and Ni ($2.65x10^{-7}$ – 5.07 $x10^{-7}$) exposure through fish consumption may not have the probability of contracting cancer over a long lifetime in future. More intensive studies are necessary in order to determine the toxic metals in fishes from this area of Black Sea.

Keywords: Black sea, fishes, health risk, health index

1. Introduction
Fish and seafood products constitute a significant and healthy part of the human diet as they are an important source of easily digestible proteins, vitamins, especially vitamins D, A and B_{12}, and minerals such as iodine and selenium [1]. The nutritional benefits of seafood consumption are mainly attributed to the effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action [2]. On the other hand, there is a safety concern related to the consumption of fish and seafood products due to the accumulation toxicology effect of some chemical contamination such as heavy metals. Heavy metals are ubiquitous in the environments, because of both natural and anthropogenic activities, and humans are exposed to them through various pathways, especially food chain [3]. Fishes are also excellent biological markers of heavy metals since they are taken up by fish and are accumulated in fish tissues. For some trace metals there is a range of intake over which their supply is adequate to the body (such as Cu 0.9 mg/day, Zn 8-11 mg/ day, Ni 0.5 mg/day) [3]. Although trace metals are, normal constituents of the marine environment and some of them are essential to marine organisms, all metals are toxic above some threshold level [4] and some negative effects are observed.
The main objective of this study is to determine the levels of five trace elements (As, Zn, Cu, Ni and Hg) by appropriate atomic absorption spectrometric determination in muscle tissues of three common fish species (bluefish, grey mullet and horse mackerel) collected from the coast of Black Sea, Bulgaria. And to use these data to calculate the estimated daily intake of metals (EDI), estimated weekly intake of metals (EWI), target hazard quotients (THQ) and target cancer risk (TR) values for all metals separately for female and male individual in order to evaluate the risk for consumers of fish from Bulgarian Black Sea.

2. Materials and methods
2.1. Sampling and sample treatment
Samples of fish were randomly acquired with the help of local fishermen from cities across the coastal waters of Bulgarian Black Sea. These fish samples were bluefish (*Pomatomus saltatrix),* gray mullet (*Mugil cephalus*) and Mediterranean horse mackerel (*T. mediterraneus ponticus*). The Black Sea is the world’s largest natural anoxic water basin below 180m in depth.

It is a closed sea with a very high degree of isolation from the world's oceans, but it receives freshwater inputs from some of the largest rivers in Europe- the Danube, the Dniester, and the Dnieper [5]. For this reason, Black Sea is considered one of the most polluted seas in the world, and the increasing concentration of nutrients in recent years have led to a higher degree of eutrophication. All the fish species were sampled in November 2010, near Nessebar, in southern part of Bulgarian Black Sea (Figure 1).
The three species (26 samples) included in this study are shown in Table 1.

Table 1: Biometrics data (mean ± SD) of fish from the coastal waters of the Bulgarian Black Sea

Sample	Habitant	N	Weight (g)± *SD*	Length (cm)± *SD*
Bluefish (*Pomatomus saltatrix*)	pelagic	11	71.1±6.8	19.3±1.0
Gray mullet (*Mugil cephalus*)	pelagic	8	335.0±1.2	32.1±0.8
Mediterranean horse mackerel (*T.mediterraneus ponticus*)	pelagic	7	10.8±5.3	9.7±1.4

Total length and weight of the sample brought to laboratory on ice after collection were measured to the nearest millimetre and gram before dissection. For small species (i.e. horse mackerel), the entire edible part of each individual was included for preparation of composite sample. However, for bigger species fillets of edible part of each individual were collected separately from the gill samples and included in the respective composite samples.
Approximately 1.0 g sample of muscle from each fish were dissected, washed with distilled water, weighted, packed in polyethylene bags and stored at -18ºC until chemical analysis.

2.2. Reagents and standard solutions
All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) generated by purified distilled water with a Millipore Milli-Q Gradient A-10 water purification system (Bedford, MA).
HNO_3 was of superb quality was purchased from Fluka (Buchs, Switzerland). All the plastic and glassware were cleaned by soaking in 2 M HNO_3 for 48 h, and rinsed five times with distilled water, and then five times with deionized water prior to use. The stock standard solutions of Cd and Pb 1000 µg/mL were Merck (Darmstadt, Germany) in 2% v/v HNO_3 and were used for preparation calibration standards.
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.

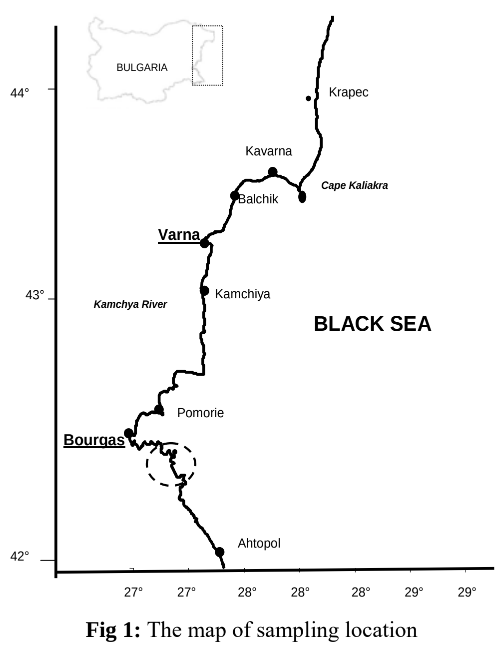

Fig 1: The map of sampling location

2.3. Sample digestion
Fish samples (whole fish body or fish fillets) were thoroughly washed with Milli-Q MQ water. The fish specimens were dissected and samples of fish fillets quickly removed and washed again with Milli-Q water. Each fish fillets (approximately 1.0 g) were analysed after homogenization in small mixer. To assess the total metal contents, microwave assisted acid digestion procedure was carried out using the parameters stated elsewhere [6].
Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) delivering a maximum power and temperature of 800 W and 300 °C, respectively, and internal temperature control, was used to assist the acid digestion process. Reactors were subjected to microwave energy at 800 W in five stages as it stated before [6].

2.4. Instrumental analysis
Determination of Cu and Zn: Flame atomic absorption spectrometric (FAAS) measurements were carried out on a Perkin Elmer (Norwalk, CT, USA) Zeeman 1100 B spectrometer (Überlingen, Germany) with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio.
Determination of As and Ni: Electrothermal atomic absorption spectrometric (ETAAS) measurements were carried out on a Perkin Elmer (Norwalk, CT, USA) Zeeman 3030 spectrometer with an HGA-600 graphite furnace. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. The spectral bandpass, the wavelengths and instrumental parameters used were as recommended by the manufacture. Only peak areas were used for qualification. Pd as $(NH_4)_2PdCl_4$ was used as modifier for ETAAS measurements of As and Cd.
Determination of the total amount of Hg was performed by Milestone DMA-80 direct Mercury Analyzer. The sample size is was between 0.020 and 0.0060 g, with drying temperature at 300 °C for 60 sec, decomposition time for -180 sec and a waiting time of 60 sec.

2.5. Statistical analysis
The whole data were subjected to a statistical analysis. Metal concentrations for all the species were calculated by application of Excel 2007 (Microsoft Inc., USA). Student's-test was employ to estimate the significance of values.

3. Results and Discussions
3.1. Cu, As, Zn, Ni and Hg levels in fish products
Copper is essential for good health but very high intake can cause adverse health problems such as liver and kidney damage [7]. The concentration of copper in the samples analyses in this study ranged between 0.34 ± 0.02 mg/kg w.w

for *Mugil cephalus* and 0.8 ± 0.1 mg/kg w.w for *Pomatomus saltatrix*. Copper in the literature range from 0.23 mg/ kg w.w to 9.49 mg/ kg for muscle of fish from Marmara Sea [8], 0.32-6.48 mg/ kg for muscle of fish from Marmara, Aegean and Mediterranean seas in Turkey [9] and 0.34-7.05 mg/ kg wet weight for fish muscle from central Aegean and Mediterranean Sea [10]. The maximum copper level permitted for sea fishes is 10 mg/kg according to Bulgarian Food Authority [11]. Comparing our observed values with those from literature, results were lower than the values from the literature

The concentration of As in fish samples from Bulgarian Black Sea were ranged between 0.73±0.05 mg/ kg wet weight to 1.1±0.1 mg/ kg wet weight. The concentration of arsenic reported in fish species from Adriatic Sea ranged of 0.56 to 10.03 mg/ kg fresh weight [12] and in Lake Kasumigaura, Japan was around 13.3 µg/g dry w.t for fish food [13]. The maximum arsenic level permitted for fishes is 5.0 mg/kg according to Bulgarian Food Standard [11]. Our results are in agreement with results reported in the literature.

Most fish contain methyl Hg stored in their protein. The concentration of mercury in muscle of fish from Black Sea, Bulgaria varied from 0.05±0.01 mg/ kg wet weight in *M.cephalus* to 0.16±0.02 mg/ kg wet weight in *T. mediterraneus ponticus*. Studies in the literature reported Hg concentration from 0.05 mg/kg in *M. cephalus* up to 0.16 mg/kg in *T. mediterraneus ponticus* for fish species from Bulgarian Black Sea [14], in the range of 0.01-0.50 µg/g in marine fishes in Malaysia [15] and 25-84 µg/kg for fishes from Black Sea [16]. The maximum Hg level permitted for fishes is 0.5 mg/kg according to Bulgarian Food Codex [11]. The levels of Hg in this study are in agreements with the guideline values.

The observed Zn concentration ranged between 5.2±0.3 mg/ kg wet weight in *M. cephalus* and 10±1 mg/kg w.w for *P. saltatrix*. In our previous studies the concentration of Zn varied between 7.3 and 11 mg/kg Zn the muscle tissues of most consumed fish species from Bulgarian Black Sea coast [6]. The concentration for zinc reported in the literature range 9.5-22.9 mg/ kg for muscle of fish from the Black Sea coast [19], 3.51-53.5 mg/ kg for species from Aegean and Mediterranean Sea [10] and 9.50-22.94 µg/ g dry weigh for fish muscle from middle Black sea [18]. The maximum zinc level permitted for fishes is 50 mg/kg according to Bulgarian Food Codex [11]. Maximum Zn level in edible parts of fish in this research was found to be below than both the Turkish permissible standards and levels reported in the literature.

Nickel levels in muscle tissues in this study were between 0.008±0.001 mg/kg w.w and 0.009±0.001 mg/kg w.w. Some Ni levels in fish species reported in the literature by other workers was in the range of 0.11-12.9 µg/g dry weight in fish species from Iskenderun Bay [19] and 0.02-3.97 µg/g in seafood from Marmara, Aegean and Mediterranean seas in Turkey [9]. The maximum nickel level permitted for marine fishes is 0.5 mg /kg according to Bulgarian Food Codex [11] thus the results from this study were below the limits sets by various health organizations and the data in the literature.

In this study, the metal concentrations were largely below the MPLs establishes by the European Union level and the guideline set by local and international agencies (FAO/WHO JECFA, USFDA, EC Directives).

3.2. Metal Intake (Estimated PTDI and PTWI)

The 'tolerable intake' is widely used to describe 'safe' levels of intake; and can be express on either a daily basis (TDI) or a weekly basis (TWI) [20]. The Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) set the tolerable intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake). PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects [21]. Estimated weekly intake was calculated by using the following formula:

$$EWI = \frac{C_{metal}\ x\ Cons\ R}{BW}$$

where EWI is estimated weekly intakes; C_{metal} is the concentration of the analyzed metal (As, Cu, Hg, Ni and Zn) in fish; ConsR is the weekly consumption of fish from Bulgarian Black Sea coast (about 95.6 g/week), and BW is the human body weight (base on 60 kg for females and 68 kg for males). The estimated tolerance daily intake (ETDI) and estimated tolerance weekly intake (ETWI) in this study were calculated and presented in Table 3. Intake estimates were expressed as per unit body weight (µg/kg b.w./day or weekly).

Table 2: Estimated dietary intake (µg/kg b.w/day or week) of Cu, As, Zn, Ni and Hg of fish species

Fish samples	Cu				As				Zn				Ni				Hg			
	EDI	EDI	EWI	EWI	EDI	EDI	EWI	EWI	EWI	EWI	EDI	EDI	EWI	EWI	EDI	EDI	EWI	EWI	EDI	EDI
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Bluefish	0.183	0.161	1.279	1.128	0.176	0.155	1.231	1.086	15.983	14.103	2.283	2.015	0.014	0.013	0.002	0.002	0.144	0.127	0.021	0.018
Gray mullet	0.078	0.069	0.543	0.480	0.251	0.222	1.758	1.551	8.311	7.334	1.187	1.048	0.014	0.013	0.002	0.002	0.080	0.071	0.011	0.010
Horse mackerel	0.128	0.113	0.895	0.790	0.167	0.147	1.167	1.030	13.586	11.988	1.941	1.713	0.013	0.011	0.002	0.002	0.256	0.226	0.037	0.032
All samples	0.129	0.114	0.906	0.799	0.198	0.175	1.385	1.222	12.627	11.141	1.804	1.592	0.014	0.037	0.002	0.005	0.160	0.141	0.023	0.020
Gudelines	700	700	3500	3500	2.14	2.14	14.98	14.98	7000	7000	1000	1000	35	35	5	5	1.61	1.61	0.23	0.23

F=Female; M=Male; Body weight for females is 60 kg; Body weight for females is 68 kg; EDI = Estimated Daily Intake; EWI=Estimated Weekly Intake; Guidelines are presented as µg/kg b.w/day or week

The Recommended Daily Allowance (RDA) of copper for adults is 0.9 milligrams (mg). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cu of 3500 µg/kg body weight/week which was equivalent to 700 µg/kg body weight/day (PTDI) [21]. Our results estimated that the EDI and EWI for copper from consumption of various fish species from Black Sea, Bulgaria was 0.129 µg/kg b.w./day (for females)/ 0.114 µg/kg b.w./day (for males) and 0.906 µg/kg b.w./week (for females)/ 0.799µg/kg b.w./week (for males) respectively. The estimated PTWI of copper in this study is far below the established PTWI.

The inorganic arsenicals are more acutely toxic than most organic forms. The JESCFA has establishes a PTWI of 14.98 µg/kg b.w./week for As, which is equivalent to 2.14 µg/kg b.w./week for inorganic arsenic [22]. The values calculated in this study were much lower than those guidelines (0.175-0.198 µg/kg b.w/day and 1.222- 1.385 µg/kg b.w/ week).

Zinc is considered to be relatively non-toxic, particularly if taken orally. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for zinc of 7000 µg/kg body weight/week which is equivalent to 1000 µg/kg body weight/day[23]. The ETDI of zinc calculated for fish consumption from this study ranged between 11.414 µg/kg b.w./day (for males) and 12.627 µg/kg b.w./day (for females) and the ETWI - 1.592 µg/kg body weight/week (for males) and 1.804 µg/kg body weight/week (for females). The estimated ETDI and ETWI of zinc in this study are below the established PTDI and PTWI indicated no health risk to the consumers.

The International Agency for Research on Cancer has determined that some nickel compounds are carcinogenic to humans and that metallic nickel may possibly be carcinogenic to humans. The JECFA has established a PTWI of 35 µg/kg b.w./week for nickel, which is equivalent to 5 µg/kg b.w./day [24]. The ETDI and ETWI in our study for Ni were 0.014 µg/kg b.w./day (for females)/0.037 µg/kg b.w./day (for males) and 0.002 µg /kg b.w./week (for females)/ 0.005 µg/kg b.w./day (for males) respectively. The observed nickel intake from fish muscle were much lower and indicates no adverse effects to the consumers based on guidelines from Joint FAO/WHO Expert Committee on Food Additives.

In June 2003, the FAO/ WHO Joint Expert Committee on Food Additives (JECFA) revised its PTWI for methylmercury to 1.6 µg/kg body weight, whereas it was previously 3.3 µg/kg body weight [23]. In a study held in Kolkata, India, the ETDI and ETWI of Hg were 0.11 µg/kg b.w./day (range, 0.07-0.13 µg/kg b.w./day) and 0.76 µg/kg b.w./week (range, 0.51-0.89 µg/kg b.w./week) from fish consumption[20]. In our study the ETDI and ETWI of Hg were 0.160 µg/kg b.w./day (for females)/ 0.141 160 µg/kg b.w./day (for males) and 0.023 µg/kg b.w./week (for females)/ 0.020 µg/kg b.w./week (for males), respectively, and therefore, were not considered to pose adverse effects to consumers according to the literature data and international standards.

3.3. Health Risk Estimation for Fish Consumption. Target Hazard Quotients (THQs) and Target Cancer Risk (TR)

Target hazard quotient (THQ) is used to estimate the human health risk from consuming metal contaminated fish [25]. THQ was determined based on the method described by Basim and Khoshnood [25] and is given by the following equation:

$$THQ = \frac{(M_c \times IR \times 10^{-3} \times EF \times ED)}{(RfD \times BWa \times ATn)}$$

where M_C is the metal concentration in muscle tissues of fish (µg/g), IR is the mean ingestion rate of fish (13.7 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 = 3×10^{-4}, Zn = 0.3, Ni= 0.02 and Hg = 3×10^{-4} 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 (HI) from THQs can be expressed as the sum of the target hazard quotients of each individual element:

$$HI = THQ_{As} + THQ_{Cu} + THQ_{Hg} + THQ_{Ni} + THQ_{Zn}$$

Target cancer risk (TR) indicates carcinogenic risks. The model for estimating TR was shown as follows:

$$TR = \frac{(M_c \times IR \times 10^{-3} \times CPSo \times EF \times ED)}{(BWa \times ATc)}$$

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 methodology for estimation of target hazard quotient (THQ) offers an indication of the risk level due to pollutant exposure. The theoretical and estimated lifetime target hazard quotients (THQs) for Cu, As, Zn, Ni and Hg; and target cancer risk (TR) for nickel and arsenic to humans due to exposure to these trough consumption of fish from Bulgarian Black Sea were calculated and presented in Table 4 and Figure 2 and 3:

Table 3: Risk values (THQ, HI and TR) of each metal contaminant in muscle of three fish species from Bulgaria Black Sea coast

Fish samples	**Target Hazard Quotient (THQ)**					**Hazard Index (HI)**	**Target risk (TR)**	
	Cu	**As**	**Zn**	**Ni**	**Hg**		**As**	**Ni**
Bluefish (*Pomatomus saltatrix*)	0.0120	0.0801	0.0010	1.4×10^{-5}	0.0094	0.1025	3.83×10^{-5}	5.07×10^{-7}
Gray mullet (*Mugil cephalus*)	0.0051	0.1144	0.0005	1.4×10^{-5}	0.0052	0.1252	5.47×10^{-5}	2.98×10^{-7}
Med. horse mackerel (*T. medit. ponticus*)	0.0084	0.0759	0.0009	1.2×10^{-5}	0.0166	0.1774	3.63×10^{-5}	2.65×10^{-7}
All samples	0.0085	0.0901	0.0008	1.4×10^{-5}	0.0104	0.1098	4.31×10^{-5}	3.57×10^{-7}

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

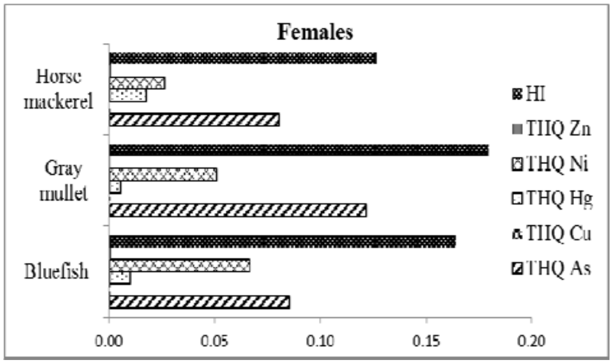

Fig 2: Various estimate target hazard quotients (THQ) for Zn, Ni, Hg, Cu and As caused by consuming fish collected from Bulgarian Black Sea coast

If the HI were greater than one as a consequence of summing several THQ, it would be appropriate to segregate to compounds by effect and by mechanism of action and to derive HI for human health.

The THQs of Cu, As, Zu, Ni and Hg were 0.0085, 0.0901, 0.0008, 1.35 x10^{-5} and 0.0104, respectively. The observed values of THQs were lower than the safe values of one [26]. The total THQ means HI was less than one for all fish species (0.1025, 0.1252, 0.1774 and 0.1098 respectively) and it demonstrated that ingestion of fish from Bulgarian Black Sea does not result in over exposure of studies metals. Thus, no adverse effect poses to the health of consumers.

Calculated average value of carcinogenic risk (TR) of bluefish, grey mullet and horse mackerel was performed for As and Ni, since only those elements from the analysed ones show carcinogenicity. The values are as follows: 4.31 x10^{-5} for As and 3.57 x10^{-7} for Ni. In the literature TR for arsenic and nickel was found to be 8.6x10^{-5} (range, 4.7x10^{-5}, *Labeo rohita* to 1.5 x10^{-4}, *Catla catla*) and 4.7 x10^{-4} (range, 3.0x10^{-4}, *Oreochromis nilotica* to 5.8 x10^{-3}, *Catla catla*), respectively for fishes from Kolkata wetland, India [20].; and between 1x10^{-6} to 1x10^{-4} for Cu, Pb, Ni, Cd and Cr except Zn (6.17 x 10^{-4}) for cultured *P. hypophthalmus* from India [28]. Comparing our values with those stated in the literature and the guidelines values, indicates that analysed fish from Bulgarian Black Sea coast are safe for human consumption

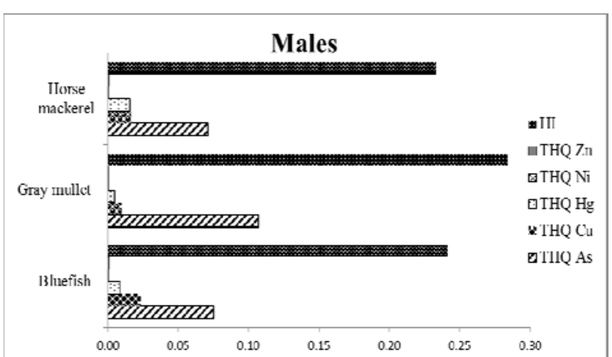

Fig 3: Various estimate target hazard quotients (THQ) for Zn, Ni, Hg, Cu and As caused by consuming fish collected from Bulgarian Black Sea coast (males)

4. Conclusion

Consumption of fish with elevated levels of heavy metals may lead to high level carcinogenic risk to human health. It is this advocated that a regular monitoring of heavy metal contamination of fish species thriving at contaminated waters must be carry out to ascertain the food safety.

The concentration of metals determined in this study did not exceed the standard recommended by different environmental agencies (such as WHO and USEPA). The values for non carcinogenic risk (THQs) showed that adverse health effects might not occur when considering different fish consumption patterns. HI of each trace element were lower than one suggesting that these pollutants perhaps pose no hazard to local residents. Target risk (TR) due to As and Ni exposure through fish consumption may not have the probability of contracting cancer over a long lifetime in future. Further studies are necessary in order to determine the metals in fishes from this area of Black Sea Bulgaria.

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