Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2019, Volume 10, Issue 10, Page 948-957

http://www.jmaterenvironsci.com

Quantification of heavy metals (Cd, Pb, Fe, Mg, Cu, and Zn) in seafood (fishes and crabs) and evaluation of health risks to consumers in Limbe, Cameroon

G. Milend Mbeh [1], F. Togue Kamga [1*], A. Kouekam Kengap [2], W. Enow Atem [1], L. Oben Mbeng [1],

[1]*Institute of Fisheries and Aquatic Sciences PO Box 7236 Douala-Cameroon*
[2]*Fisheries and Oceanography Research Station Limbé PO Box 77 Limbe-Cameroon*

Received 22 Août 2019,
Revised 19 Sept 2019,
Accepted 25 Sept 2019

Keywords
- ✓ heavy metals,
- ✓ crustaceans,
- ✓ fish,
- ✓ toxic,
- ✓ populations.

kamgafulbert@yahoo.fr ;
Phone: +237690980854;
Fax: ++237243183358

Abstract

Marine and costal ecosystems are increasingly affected by human activity, which reinforce the pressure exerted on these environments. This report focuses on the quantification of heavy metals in the fish pulpit (Cd, Pb, Fe, Mg, Cu, and Zn) and the health effects of (Fako) populations. Measurements on the species show that crustaceans are more contaminated in these trace elements. The crab and fish species of Down Beach are more contaminated with metals. The most abundant metals in the pulpit are zinc and iron. The trace element distribution follows the same trend, showing slight variations between Isobe and Down Beach. The least important metals are lead and cadmium. Zinc is the most abundant after iron followed by copper and manganese respectively (451.18, 111.88, 85.96 and 48.46µg/g). Hazard quotients show us that for an adult population no metal concentration has reached the reference thresholds of toxicity for humans, but for children, zinc is likely to have a toxic risk to health. The diseases which are the most abundant, are ocular and dental diseases. It is possible that they are favored by certain metals (zinc, iron).

1. Introduction

Rapid and disorganised urban and industrial developments have contributed to the raised levels of heavy metals in the urban environment of developing countries [1, 2, 3]. Fishing activities are a source of food and employment for many people. Fish are extensively consumed, firstly because they are part of the local diet, but also because of their high protein, low saturated fat and omega fatty acids content, that are known to contribute to good health [4]. Heavy metals from natural and anthropogenic sources are discharged into aquatic ecosystems, where they cause a serious threat because of toxicity, long persistence, bioaccumulation, and biomagnification in the food chain [5,6,7]. Bonsignore et al., [8] demonstrated a highly heterogeneous anthropogenic impact in terms of heavy metals input from the industrial activity resting on land. Anthropogenic actions play a significant role in further accentuating the metals related health problems in soil plant-animal-human system [9]. Hence today, fish in the environment are inexorably exposed to chemical pollution. Heavy metals can be accumulated by marine organisms thought a variety of pathways, including respiration, adsorption and ingestion [10]. Baki et al., [11] reported some standard levels of metals in fish in (mg/kg ww) described in the literature. Although most hazardous substances are present at concentrations far below the lethal level, they may still cause serious damage to the life processes of these animals. Ginsberg and Toal, [12] reported that variety of factors such as the characteristics of the species under consideration, the exposure period, the concentration of the element, as well as abiotic factors such as temperature, salinity, pH and seasonal changes influence fish absorption of heavy metals from the surrounding environment. Hounkpatin et al., [13] showed that children's bodies absorbed more contaminants and remained unable to spread it easily. Studies on heavy metals bioaccumulation in fish is now widespread, but in recent years the risk factors calculation for the population become of great importance. Despite the beneficial aspect of aquatic food's consumption, bioaccumulation of toxic metals in fish can enhance the health risk for the consumers [14]. Sometimes, the contaminants exceed the legal limits set by European regulations for food, without represent a risk for human health. Heavy metals such as Cd and Pb have been revealed to have carcinogenic effects [15]. Maurya et al., [16] reported that highest heavy metals accumulation was found settled in the liver of some selected fish species collected from local markets in India. Many studies have been performed on the water quality of the Cameroonian coast. However few of them are devoted to the contamination of its aquatic fauna. Therefore, it is necessary to set up a monitoring network that will have an impact on the fish consuming populations of the studied areas. Hence, the purpose of this study is to contribute to the evaluation of the bioaccumulation of heavy metals in fish tissue and Crab from Down Beach and Isobe, as well as the impact of accumulated heavy metals on the health of the surrounding populations consuming these fish. Specifically, (i) quantify heavy metals in the muscle tissue of the fish species studied, (ii) compare the pollutant load to the tolerable standard, (iii) evaluate the level of toxicity of metals on the health of exposed populations.

2. Material and Methods

2.1. Presentation of the study area

LIMBE is located in the Fako Department, more precisely in the South West Cameroon Region (Figure1) between the geographic coordinates 4.001705°-4.027148° North latitude and 9.210173°-9.094025 ° East longitude (Figure 1). Limbe soil is basaltic and alluvial. The hydrographic network of the study area is dense and varied, consisting of many streams, the most are: the Mabeta River coming from the Northeast and confluence of several rivers; the Limbe river flowing inside the botanical garden etc. The coast of Limbe is dominated by an equatorial climate characterized by two seasons, a rainy season of seven months from May to November with water levels sometimes reaching 11000 mm at Debundscha, and a dry season of five months from December to April. The month of February is the hottest, it records an average temperature of 30°C. The month of August is the rainiest with an average of 308 mm of precipitation. The driest month is January, with 59 mm of rainfall. The months of July and August are the coldest with temperatures of 26.26 ° C. Wind speeds sometimes reach 18 m/s [17]. The tourist complexes, the oil exploitation and the refinery are at the base of the local and even national economic flow.

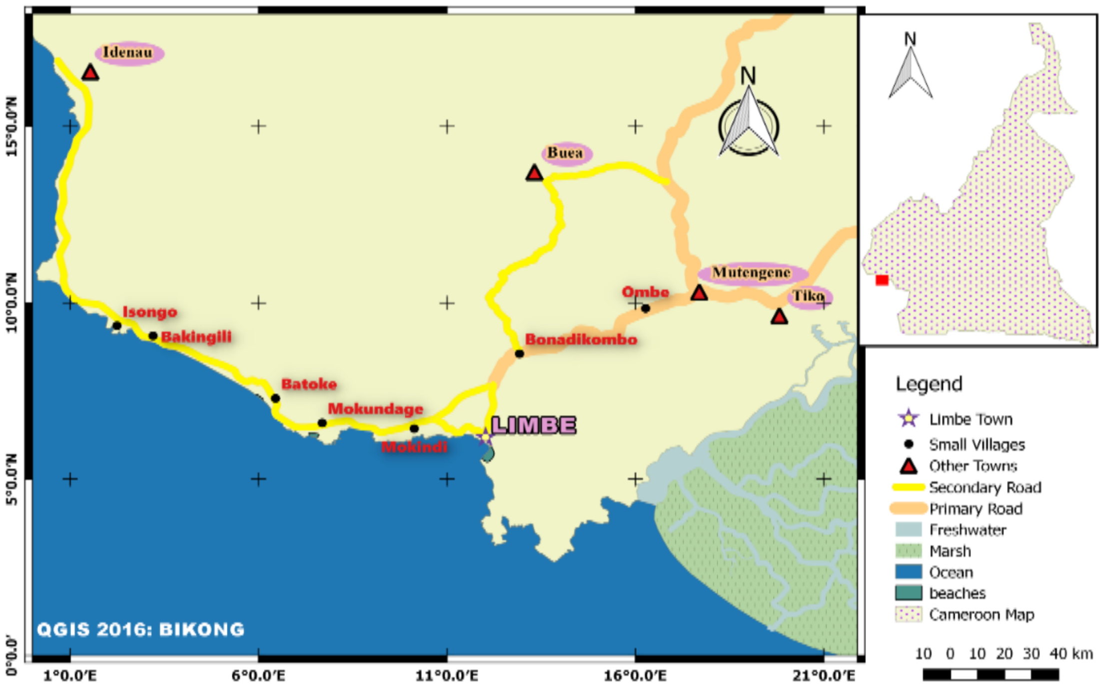


Figure 1: Location map of the Limbe area

2.2. *Criteria for the choice of studied species*

Species were purchased at two sites, namely Isobe (Idenau) and DownBeach. We rely on abundance and consumption, as well as the bio indicator capacity to choose the species we will study. Similarly, they are also based on their vertical distribution in the water column to choose the species: sole (cynoglossus monodi), bar (pseudotolithus senegalensis), crab (Carcinus maenas). Indeed the crab informs us about the bottom benthic and sole and the bar informs us about the contamination more in mezo benthic zone. These two species have been selected because they have ecological characteristics (geographical zone and depth of habitats (demersal for bar and benthic for sole), their diets (fish and invertebrates) distinct, their taxonomic remoteness and their behavioral difference (a vagile species for the bar and sedentary for the sole). After the collection of the different species they will be kept in a cooler containing the ice, then the samples are brought for cleaning and removal of the flesh of the species to be analyzed in the laboratory.

3.2.Laboratory analyzes

The heavy metals were obtained by dry grinding after drying the fish and crab pulpit for 2 days in a Memmec E0823 brand oven at 105°C. Followed a grinding to obtain the powder of the fish. 50ml of distilled water is added to the previously acidified powder (HCl/HNO_3) to obtain a liquid to be analyzed. After cooling, the liquid is left to decant after halogenation thereof. A colorless liquid is thus obtained after filtration of the homogeneous mixture. The heavy metals content will be obtained by atomic absorption using a BUCK Scientific Model 2009 HTDS Atomic Absorption Spectrometer in the laboratory, in accordance with the methodology indicated by Tahiri et al. [18] according to the standards of AFNOR [19]. Heavy metals (Cd, Pb, Fe, Cu, Mn, Zn) were read at wavelengths of 228.8nm, 283.2nm, 248.3nm, 324.8nm, 279.5nm, and 213.9nm, respectively. For a better understanding and interpretation of the data analysis results, the data were processed using the XLSTAT and SPSS Software.

3.3. *Evaluation of the daily exposure dose*

Population health data are available from the Statistical Service of the Limbe Regional Hospital. The manual count of the various diseases listed weekly in the registers was carried out in the Bureau of Statistics. Subsequently, the diseases of interest to us in our study were targeted and counted for a period of one month and for a full year. The data are taken from the weekly disease surveillance sheets each week. These cards are commissioned by the regional health delegation which targets a number of at-risk diseases. Among these data is the number of emergency consultations per month during the year 2017, in addition to the values of the abundances of diseases related to heavy metals on each month in the year 2017. In order to evaluate the level of toxicity we determined, the daily exposure dose was evaluated here according to the formula:

$$EDI = C \times Q \times F/P \quad (1)$$

Where EDI=Daily Dose Exposure to Trace Elements (mg/kg/d); C=trace element concentration of fish (mg/kg); Q = Quantity of fish ingested per day, (kg/d); F Frequency of exposure (F=1); P = Body weight of the target (kg). It is observed that the frequency equal to unity means that the average person consumes the fish once a day in Cameroon. These values are calculated by considering the following hypotheses: an adult man consumes the same quantity of fish as a child, a child consumes the same quantity of fish each day, a Cameroonian consumes an average of 15.4 kg/year of fish [20], the fish studied are consumed by the population, a child consuming this fish has a mass of 30kg [21]. The risk characterization for threshold effects is expressed by the hazard quotient (HQ). It is calculated for the oral route of exposure as follows

$$HQ = EDI / ADI \quad (2)$$

Where EDI = Daily Exposure Dose (mg/kg/d); ADI = Admitted Daily Dose (mg/kg/d). If HQ <1, the occurrence of a toxic effect is very unlikely; If QD> 1, the occurrence of a toxic effect can not be ruled out.

3. Results and discussion

3.1 *Heavy metals concentrations in the muscle tissue of fishies and crabs*

In both sites, it is observed that Pb (0.923 and 0.925µg/g) is higher than the standard (0.500 µg/g) [22] in the Isobe sole and Down Beach (Figure 2a). These results differ from the work done by Usero et al. [23] who is working on the muscles of Solea vulgaris on the southern Atlantic coast of Spain and found Pb levels (0.03-0.05) mg/kg fresh weight. This high difference is probably due to the difference in habitats between species or in the efficiency of lead assimilation between species [24]. The studied species are demersal, which is not the case of the species studied by our authors. It is also noted that lead (0.923 µg/g and 1.723 µg/g) is higher than the norm at Isobe (0,5 µg/g) [25] and Down Beach in crab.

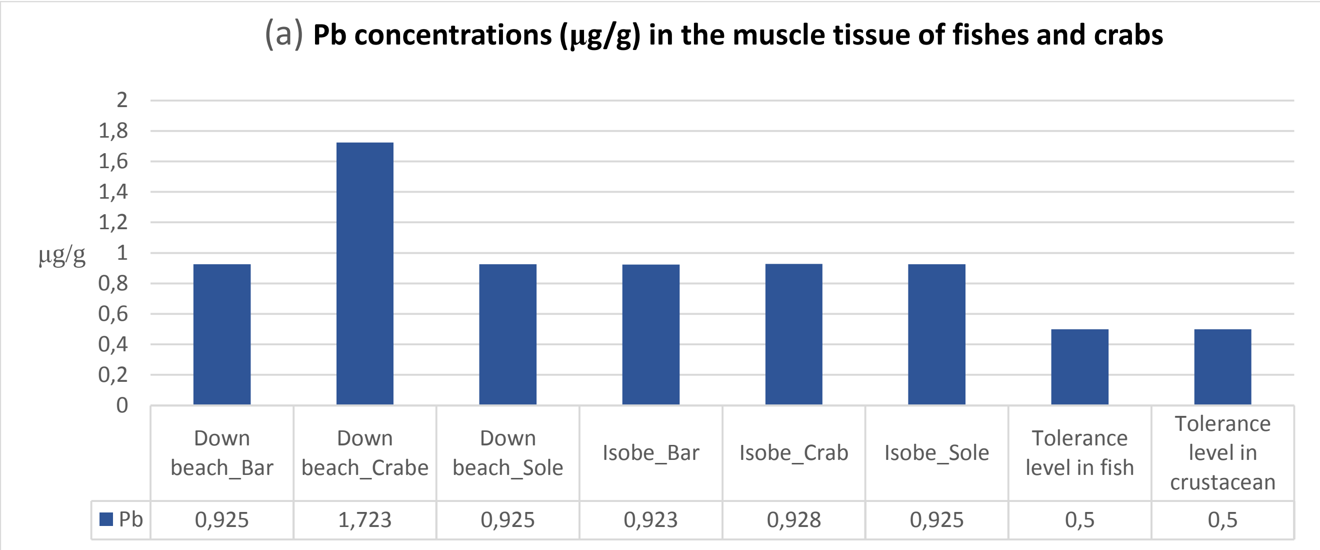
For cadmium (Figure 2b), the cadmium content (0.099 and 0.163 µg / g) of Isobe and Down Beach sole and bar is below the norm [22] prescribed for sole and bar. The cadmium content of the crab of Down Beach is equal to that of the crab of Isobe is 0,163µg/g. Similarly, the Down Beach crab and the Isobe crab have levels below the norm (0,5µg/g) [25]. The crab and the fish of down Beach are more concentrated in Cd than those of Isobe. Similarly, very low levels of Cd (0.04 mg/kg fresh weight) were recorded in fish muscle in the Moroccan Atlantic coast [26]. These low levels are probably due to a low bioavailability of the metal both in the environment where our species come from than those of our authors.

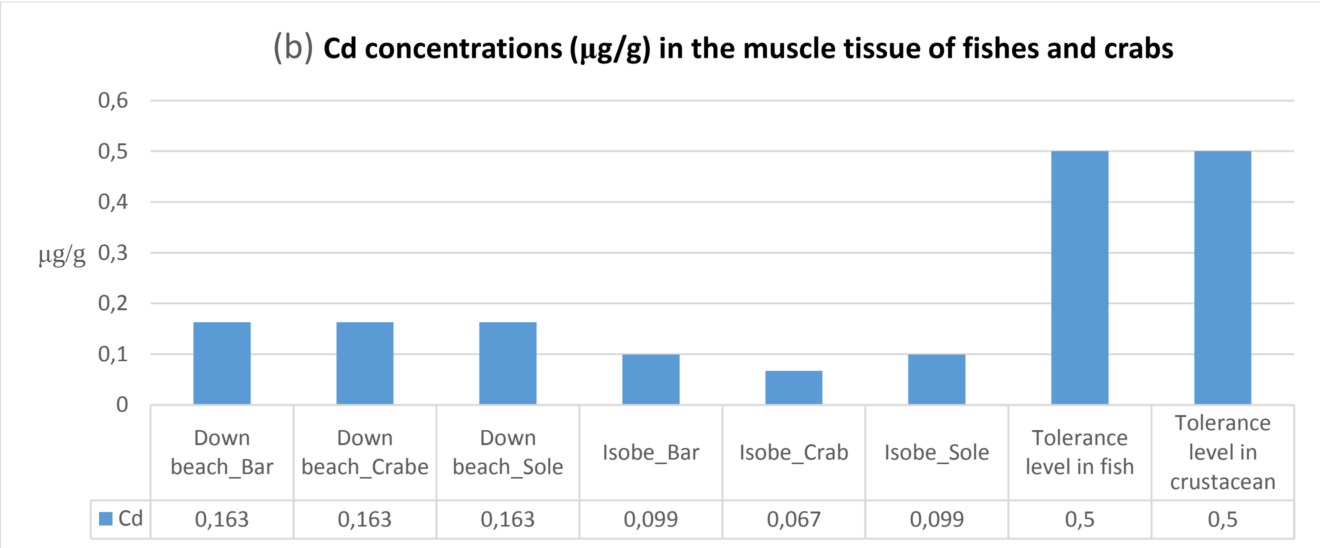
With regard to copper (3.29, 4.92 and 3.30 µg/g), both in the sole and the bar, whether at Isobe or down Beach, the values are lower than the norm (30 ug/g) [22]. And the copper value in the sole and in the Down beach bar is greater than the Isobe value (Figure 2c). Similar values (1.53 to 13.08 mg/kg dry weight) in Cu were found in Labeoumbratus muscle [27]. The copper grade value (101.16 and 141.91µg/g) in the crab of down Beach is greater than the value of Isobe. These two values are each higher than the standard (5 µg/g) [25]. These results are superior to those obtained by Sloterdijk, [24] in the English bay where the whelks and crustaceans have an average concentration of 6.6 ± 1.9 mg/kg. This difference may be due to the presence of shipyard industries that probably release copper into the environment, but it is also likely that these crabs do not have the same capacity for Cu uptake. Those of our study being more bioaccumulative than that of the estuary of the English bay.

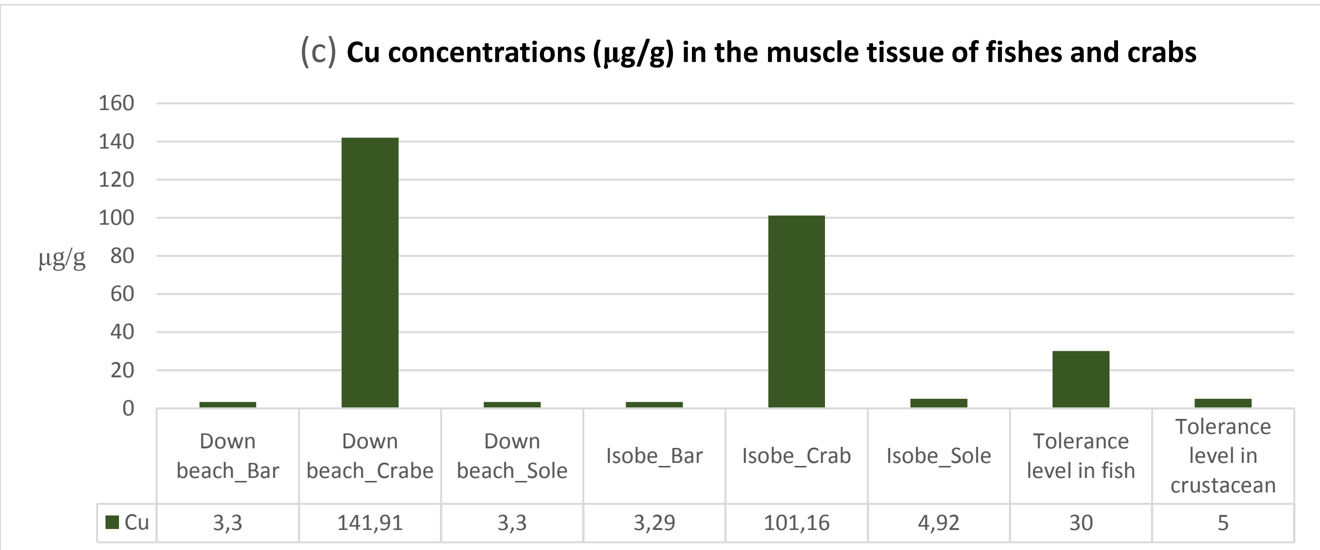
The zinc content (24.59 and 28.09 µg/g) in Isobe's sole and bar is lower than the norm (40 µg/g) [22] in effect (Figure 2d). On the other hand, low values have been mentioned in the muscle of Lisaaurata, Anguilla anguilla and Solea vulgaris (3,10-8,41, 10,1-13 and 4,17-8,52 mg/kg fresh weight, respectively) of the south Atlantic coast of Spain [23] . This difference is due to the fact that in our species the environment is demersal while the authors worked on the pelagic species, more it is possible that the available Bio part does not necessarily accumulate in the muscles of the fish. The crab Zn content (42.09 µg / g) is also lower than the norm (50 µg/g) [25]. This value is higher than the average obtained by Sloterdijk, [24] for the whelk and crustaceans of the English estuary of 12.4 ± 2.9 mg / kg. This difference would come from the inputs and source of pollution likely to produce and concentrate the larger quantities of zinc in our environment than in the English Bay.

The iron content in the sole (69.08 and 61.29 µg/g) and the down Beach and Isobe bar are above the standard (45 µg/g) [28] and the Isobe iron content (78.10 and 66, 48µg/g) is greater than the iron content of Down Beach in terms of sole and bar (Figure 2e). Different results in Fe were found in the muscle of Lisa abu and Silurustriostegus (6.88 and 6.38 mg/kg fresh weight, respectively) of Atatürk Lake by Karadede et al., [29] and in Percafluviatilis muscle (5.05) in mg/kg fresh weight of the Nitra River in Slovakia by Andreji et al., [30]. This difference may be due to the fact that our species are demersal species of marine waters. While the species of our authors are species of continental waters. In addition, the ecological zone of our species is different. There is shipyard activity that probably releases iron throughout the area. The crab iron content at down Beach and Isobe bar are above the standard (45 µg/g) [28].

The manganese in the bar and the Isobbe sole (3.58, 9.93µg/g) are lower than the standard (20 µg/g) [31], more the manganese content in Isobe is higher than that of down Beach (4.98, 10.63µg/g) for sole and bar (Figure 2f). Lower values were also obtained for herring in the English Bay Sloterdijk [24] at 0.97 ± 0.14 mg/kg. This difference is probably due to the fact that herring is a pelagic fish, its diet differs with that of our species studied hence the difference in bioaccumulation. The sites of Down Beach and Isobe are also probably more polluting in Mn than the English Bay. Anthropogenic activities are more intense at Down Beach and Isobé and the physicochemical conditions of Isbobe and Down Beach are conducive for a better bioavailability of Mn. The crab manganese at Isobbe and Down beach are also lower than the standard (20 µg/g) [31].







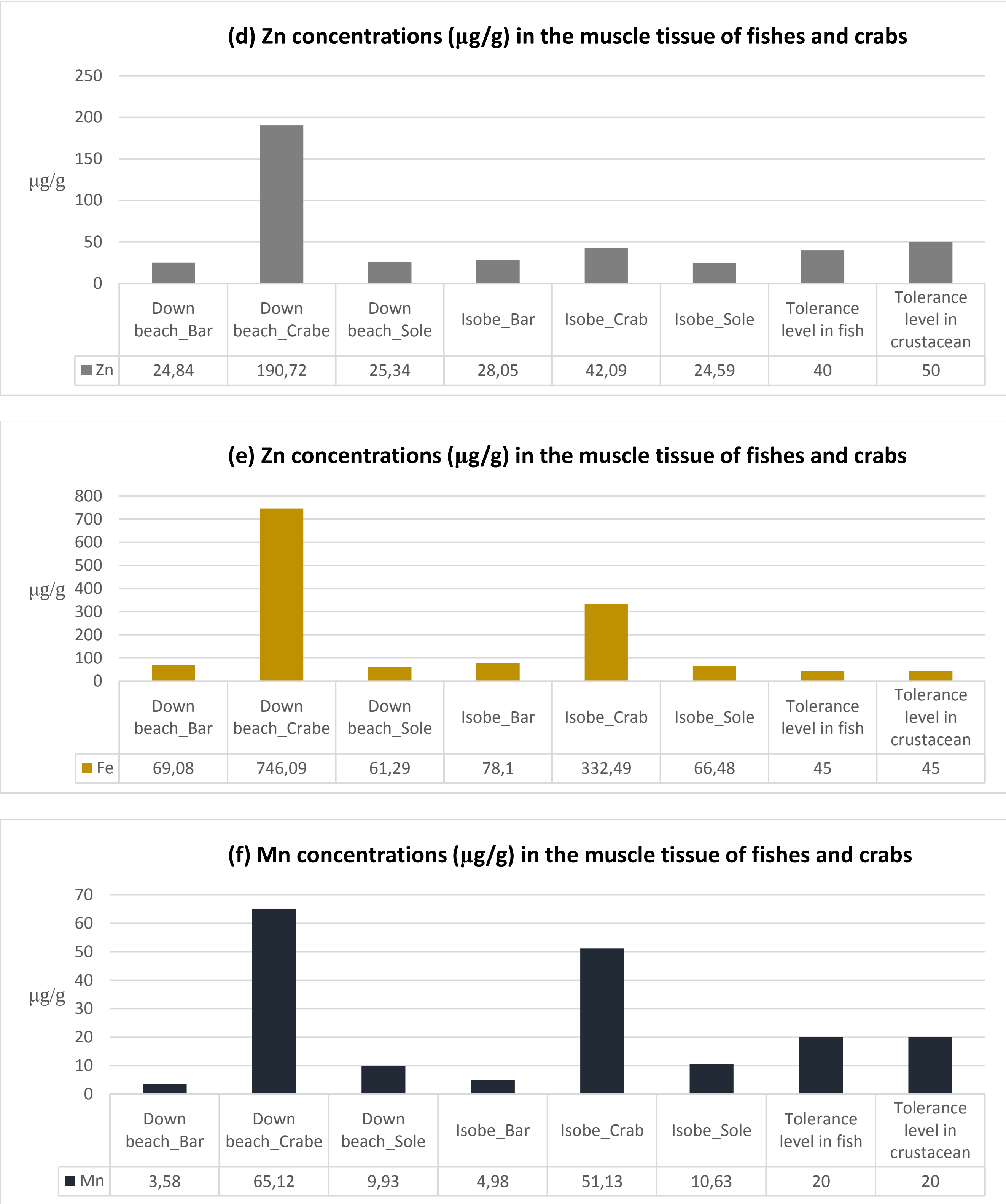


Figure 2: Concentrations of heavy metals in the muscle tissue of fishes and crabs in function of the station and their relationship to the standard.

3.2. *Evaluate the level of toxicity of metals on the health of populations*

The Table 1 below shows the average doses of exposure to various metals for an adult man. The table also presents the hazard quotients for a 60kg. It is found that all daily doses are less than 1 regardless of the species or site considered, except for the daily exposure of the crab of Down Beach and Isobe in Zinc Element (6.52 and 2.90 mg/kg/d). This is consistent with the results of Adil Chahid., [32] who finds EDI values of 0.00007, 0.00054 µg/kg/week of Cd and Pb in the skipjack landed in the Essaouira-Dakhla zone. As for the hazard quotient, all these values are less than 1 but greater than 0. The values of the high hazard quotients are those of zinc in the Isobe crab and Downbeach (0.52 and 0.23). The occurrence of a toxic effect is little likely in adults due to the consumption of the species studied. Ouro-Sama, et al, [33] found in Togo a value of HQ = 2.04 for cadmium in fish for a 60 kg adult this difference is due to the fact that cadmium and zinc the same chemical composition and moreover these metals come from different environments and from different species without forgetting that their EDI and ADI are different.

Table 1: EDI and HQ for an adult (60kg)

	Daily Exposure Dose (mg/kg/d)						Hazard quotient (HQ)					
Metals	Down beach			Isobe			Down beach			Isobe		
species	bar	sole	crab	bar	sole	crab	bar	sole	crab	bar	sole	crab
Cd(mg/Kg)	0.04347	0.04434	0.33375	0.04909	0.04303	0.07365	0.01738	0.01773	0.13350	0.01963	0.01721	0.02949
Cu (mg/kg)	0.00115	0.00115	0.04966	0.00115	0.00172	0.03540	0.00231	0.00230	0.09933	0.00230	0.00344	0.070810
Pb(mg/Kg)	0.00878	0.02432	0.15954	0.01220	0.02604	0.12527	0.00250	0.00694	0.04558	0.00348	0.00744	0.03579
Zn (mg/Kg)	0.60447	0.53630	6.52829	0.68337	0.58168	2.90932	0.04835	0.04290	0.52226	0.05467	0.04653	0.23274
Fe(mg/kg)	0.00114	0.00114	0.00114	0.00069	0.00069	0.00047	0.00011	0.00011	0.00011	0.00069	0.00069	0.00047
Mn(mg/Kg)	0.00517	0.00517	0.00964	0.00516	0.00519	0.00519	0.00064	0.00064	0.00120	0.00064	0.00064	0.00064

Table 2: EDI et HQ for a child of 30kg

	Daily Exposure Dose (mg/kg/d)						Hazard quotient (HQ)					
Metals	Down beach			Isobe			Down beach			Isobe		
espèces	bar	sole	crab	bar	sole	crab	bar	sole	crab	bar	sole	crab
Cd (mg/Kg)	0.08694	0.08869	0.66751	0.09818	0.08607	0.14731	0.03547	0.03547	0.26700	0.03927	0.03442	0.05892
Cu (mg/kg)	0.00230	0.00230	0.09933	0.00230	0.00344	0.70810	0.00461	0.00461	0.19867	0.00461	0.00689	0.14162
Pb (mg/Kg)	0.01756	0.04864	0.31900	0.02441	0.05209	0.25035	0.00501	0.01389	0.09116	0.00997	0.01488	0.07158
Zn (mg/Kg)	1.20894	1.07260	13.05658	1.36674	1.16336	1.63369	0.09671	0.08580	1.04452	0.10933	0.09306	0.46549
Fe (mg/kg)	0.00228	0.00228	0.00228	0.00138	0.00138	0.00138	0.00022	0.00022	0.00022	0.00013	0.00013	0.94184
Mn (mg/Kg)	0.01035	0.01035	0.01929	0.01033	0.01035	0.01035	0.00129	0.00129	0.00241	0.00129	0.00129	0.00129

The Table 2 above shows the average doses of exposure to various metals for a child and the daily dose assesses for a child. The table also presents the quotients of dangerosity for a child of 30 kg exposed to these studied species. This table shows us that the highest daily intake is in the Down Beach crab. This is 13.05 mg/kg/day and is followed by Isobe crab, which is 1.63369 mg/ kg/day. The lower is that of iron in Isobe crab (0.00022 mg/kg/d). The hazard quotient here is less than 1 in all station species for each metal considered, except the case of zinc in the down beach crab (1.0444 mg). Ouro-Sama, et al., [33] found a hazard of 44.11 for arsenic in a 30 kg child in fish. This variation is due to the different species exposed and to the different properties of metals. Thus children are exposed to a danger from consuming zinc in crabs down Beach.

The Histogram below (Figure 3) shows the average levels of the metals in all the species considered in µg/g versus the different metals in the species studied. It is found that the average iron is the most abundant metal in our samples is 451.18 µg/g and the least abundant metal is cadmium with an average trace element content of 0.25 µg/g. Zinc is the most abundant after iron followed by copper and manganese respectively (111.88, 85.96 and 48.46µg/g). Hence the order of general bioaccumation on unloaded fish: Fe>Zn> Cu> Mn> Pb> Cd. This classification shows that only Zn can have a significant risk for the health of populations, whereas Cd and Pb do not yet pose a risk to health. Morhit et *al*, [34] based on his work on metallic contamination of the muscles of five fish species in the lower Loukkos estuary (Moroccan Atlantic Coast) finds a classification such as: Mullet: Fe> Zn> Cu> Cr > Pb> Cd. Barbeau: Zn> Fe> Cu> Cr> Pb> Cd.

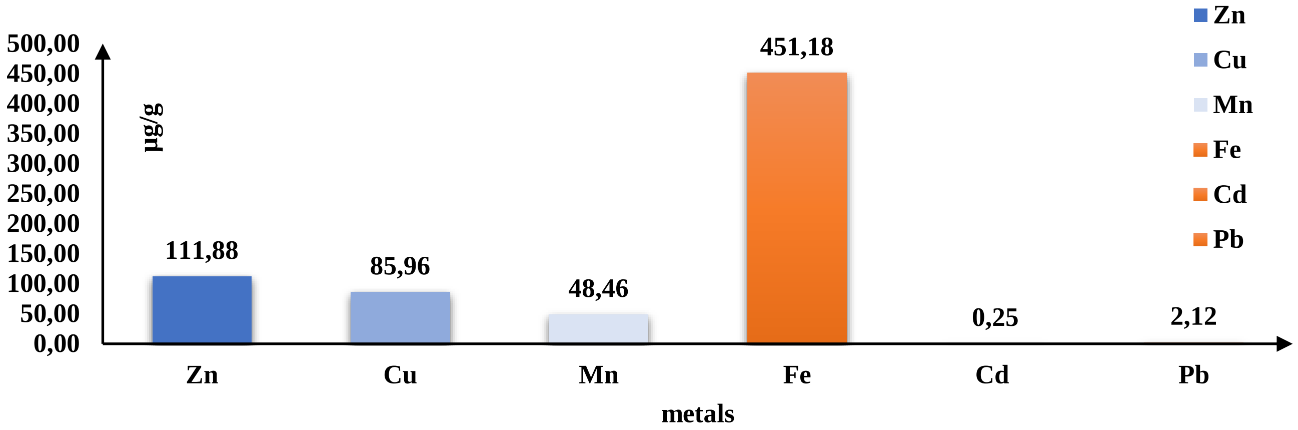


Figure 3: Average grades of metals in each species

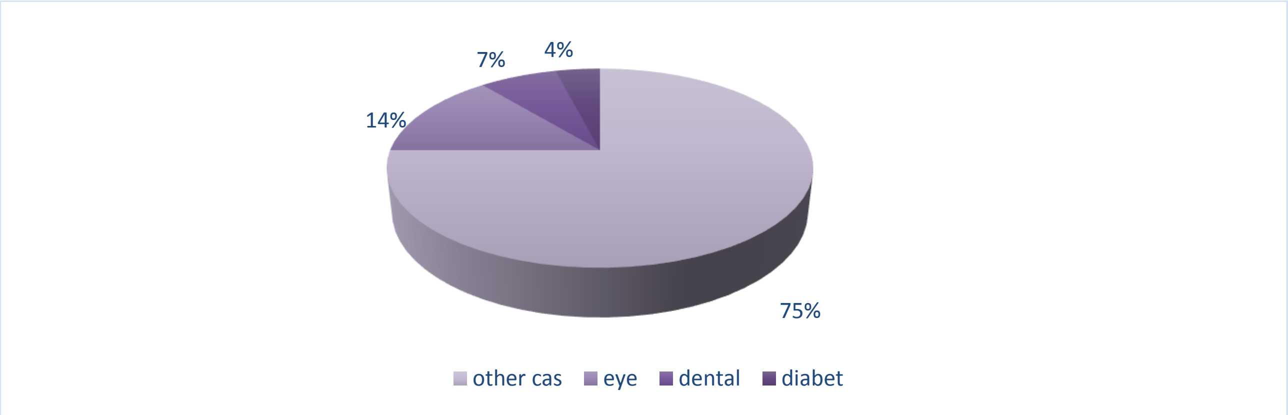


Figure 4: Suspect diseases due to metals in each species

Camembert (Figure 4) has 7% of oral and dental diseases in the limb area in 2018 (from January to May), 14% ocular and often nervous diseases and 4% of diabetes and hypertension, in the end the rest is for other pathologies (Figure 4). It is observed that 7% of diseases can be linked to zinc because diabetes is often linked to the association of zinc and copper that joins Norderg, [35] and Cohen, [36], which estimates that diseases such as diabetes are zinc-related additionally yellowish tinting of the teeth, rhinitis, occasional ulceration of the nasal septum, damage to the olfactory nerve and loss of smell are due to cadmium. It is possible that the 4% of dental patients may be due to lead and cadmium that often exceed their threshold of toxicity in the limb area.

The 14% majority eye disease among the listed diseases can be caused by iron that is at very high levels in the crabs and fish studied. Ocular siderosis is a serious chronic pathology that can lead to loss of vision of the affected eye and is due to iron when it is present in excess. It occurs also when a foreign body containing iron is found in the eye.

Conclusion

In this study the bioaccumulation of heavy metals in fish tissue and Crab from Down Beach and Isobe is evaluated. Zinc is the most abundant after iron followed by copper, manganese lead and cadmium. Hazard Quotient for Zinc Element in Crab is established to be greater than 1, therefore potential hazard quotient for children determined Down Beach fish species to be more contaminated than Isobe's Species. It has also been established that metal-related diseases are not very abundant in the Limbe area. Heavy metals were indeed present in the fish pulpit but the levels are not yet above the threshold of toxicity for humans. On the other hand, Zn presents a risk for the population of children likely to consume the crabs of down Beach and Isobe. In perspective, we are planning in the near future to conduct a comprehensive study on heavy metals in the three ecological compartments over a long period in the limbe zone.

Acknowledgements: Authors are grateful to tree anonymous reviewers for helpful and constructive revisions of this paper.

References

1. H.H. Ali Mohamed & Khairia M. Al-Qahtania; Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. *The Egyptian Journal of Aquatic Research*, 38 (2012) 31-37
2. S. Nadem, M. El Baghdadi, J. Rais & A. Barakat ; Evaluation of heavy metal contamination of sediments of the estuary of the Bouregreg (Atlantic Coast, Morocco). *J. Mater. Environ. Sci.* 6 (11) (2015) 3338-3345
3. G. Mimanne, K. Benhabib, A. Benghalem & S. Taleb; Etude de l'Adsorption des Métaux Lourds (Pb et Cd) en Solution Aqueuse sur Charbon Actif et Montmorillonite Sodée de l'Ouest Algérien (Study of the Adsorption of Heavy Metals (Pb and Cd) in Aqueous Solution on Activated Carbon and Sodium Montmorillonite from Western Algeria). *J. Mater. Environ. Sci.* 5 (4) (2014) 1298-1307
4. A. Kennedy, K. Martinez, CC. Chuang, K. LaPoint & M. McIntosh; Saturated Fatty Acid-Mediated Inflammation and Insulin Resistance in Adipose Tissue: Mechanisms of Action and Implications. *Journal of Nutrition*, 139 (2009) 1–4. https://doi.org/10.3945/jn.108.098269
5. F.Z. Kucykbay & I. Orun; Copper and zinc accumulation in tissues of the freshwater fish Cyprinus carpio L 1758 collected from the Karakaya dam lake, Malatya (Turkey). *Fresenius Environ Bull* 12 (2003) 62–66
6. N. Pourang, S. Tanabe, S. Rezvani & J.H. Dennis; Trace elements accumulation in edible tissues of five sturgeon species from the Caspian Sea. *Environ. Monit. Assess*, 100 (2005) 89–108. https://doi.org/10.1007/s10661-005-7054-7
7. F. K. Togue, G. L. O. Kuate & L.M. Oben; Physico-Chemical characterization of the surface water of Nkam River using the Principal Component Analysis. *J. Mater. Environ. Sci.*, 8 (2017) 1910-1920
8 M. Bonsignore, D. Salvagio Manta, S. Mirto, E. M. Quinci, F. Ape, Montalto, V. & M. Sprovieri; Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. *Ecotoxicology and Environmental Safety*, 162 (2018) 554–562. https://doi.org/10.1016/j.ecoenv.2018.07.044
9 R. Khanam, A. Kumar, A. K. Nayak, M. Shahid, R. Tripathi, S. Vijayakumar & H. Pathak; Metal(loid)s (As, Hg, Se, Pb and Cd) in paddy soil: Bioavailability and potential risk to human health. 134330 *Science of the Total Environment* 699 (2020) 134-330, https://doi.org/10.1016/j.ecoenv.2018.07.044
10 L. Makedonski, K. Peycheva & M. Stancheva; Determination of heavy metals in selected black sea fish species. *Food Control*, 72 (2017) 313–318. https://doi.org/10.1016/j.foodcont.2015.08.024
11. M. A. Baki, Md. M. Hossain, J. Akter, S. B. Quraishi, Md. F. H. Shojib, A.K.M. A.Ullah & Md F. Khan; Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. *Ecotoxicology and Environmental Safety*, 159 (2018) 153-163. https://doi.org/10.1016/j.ecoenv.2018.04.035
12. L. Gary Ginsberg & Brian F. Toal1; Quantitative Approach for Incorporating Methylmercury Risks and Omega-3 Fatty Acid Benefits in Developing Species-Specific Fish Consumption Advice. *Environ Health Perspect*, 117 (2009) 267–275. https://doi.org/10.1289/ehp.11368
13. A.S. Hounkpatin, A.P. Edorh, M. Sèzonlin, P. Guédénon, B. Elégbédé, G. Boni, V. Dougnon, S. Montcho, E. Kéké & M. Boko; Pollution of aquatic ecosystems by heavy metals at Ganvié's lacustrine city (Benin) . *International Research Journal of Biotechnology*, 3 (2012) 81-87
14. A. S. S. Ahmed, M. Rahman, S. Sultana, S. M. O. F. Babu, & M. S. I. Sarker; Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications. *Marine Pollution Bulletin*, 145 (2019) 436–447. https://doi.org/10.1016/j.marpolbul.2019.06.035
15. D. Trichopoulos: Cancer, Principles and Practice of Oncology, Lippincott Company, Philadelphia, (1997) 231-258
16. P. K. Maurya, D. S. Malik, K. K. Yadav, A. Kumar, S. Kumar & H. Kamyab; Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology Reports*, 6 (2019) 472–481. https://doi.org/10.1016/j.toxrep.2019.05.012

17. J. Folack & G.C Emene, Natural and anthropogenic characteristics of the cameroon coastal zone (2001)
18. L. Tahiri, L. Bennasser, L. Idrissi, M. Fekhaoui, A. El Abidi &A. Mouradi; Contamination métallique de Mytilus galloprovincialis et des sédiments au niveau de l'estuaire de Bouregreg (Maroc). *Water qual. Res. J.*, 40 (2005) 111–119. https://doi.org/10.2166/wqrj.2005.011
19. AFNOR; Qualité des sols, Recueil de normes françaises. Afnor, Paris France. (1994) 533.
20. FAO; Report of the FAO Working Group on the Assessment of Small Pelagic Fish off Northwest Africa, (2003)
21. WHO; diet, nutrition and the prevention of chronic diseases. (2003).
22. FAO/WHO; Evaluation of certain food additives and the contaminants mercury lead and cadmium, WHO technical report series 505 (1989).
23. J. Usero, J. Morillo & I. Gracia ; Heavy metals in fish (Solea vulgaris, Anguilla anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. *Environment International*, 29 (2003) 949-956.
24. H.H. Sloterdijk; Accumulation des métaux lourds et des composés organochlorés dans la chair des poissons du fleuve Saint-Laurent. Comité d'étude sur le fleuve Saint-Laurent. *Rapport technique*. 7 (1977) 181.
25. EU; Maximum levels for certain contaminants in foodstuffs, *Official Journal of the European Union*, L 364/5 (2006).
26. M. El Morhit, D. Belghity, A. EL Morhit, metallic contamination in muscle of three fish species in the southern Atlantic coast the Laâyoune (Morocco), *Larhyss Journal*, 15 (2013) 21-34
27. G. Nussey, J.H.J. van Vuren & H.H. du Preez; Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae), from Witbank Dam, Mpumalanga. *Water S A*, 26 (2000) 269-284.
28. IOM, Dietary Reference Intakes: Applications in Dietary Planning. Subcommittee on Interpretation and Uses of Dietary Reference Intakes and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Institute of Medicine of the National Academies, The National Academies Press, Washington, DC (2003) 248.
29. H. Karadede, S.A. Oymak & E. Ünlü ; Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Atatürk Dam Lake (Euphrates), *Turkey. Environ. Int.*, 30 (2004) 183–188
30. J. Andreji, Ivan Stránai, P. Massányi, V. Miroslav; Concentration of Selected Metals in Muscle of Various Fish Species. *Journal of environmental science and health*, 40 (2005) 899–912.
31. TFC; Turkish Food Codes, Official Gazette, 23 September, No: 24885 (2002).
32. A. Chahid, M. Hilali, A. Benlhachemi & T. Bouzid, Contents of cadmium, mercury and lead in fish from the Atlantic sea (Morocco) Health risks, 3rd International Summit on Toxicology and Applied Pharmacology, (2016)
33. K. Ouro-Sama, H.D. Solitoke, K. Gnandi, K.M. Afiademanyo & E.J. Bowessidjaou; Évaluation et risques sanitaires de la bioaccumulation de métaux lourds chez des espèces halieutiques du système lagunaire togolais. *VertigO*, 14 (2014). DOI:10.4000/vertigo.15093.
34. M. E. Morhit; Contamination métallique d'Anguilla anguilla au niveau de l'estuaire de Loukkos, 3ème Congrès Franco-Maghrébin de Zoologie et d'Ichtyologie, Ramdani Mohammed, Hôtel Sémiramis Marrakech, N°25 (2012)14 pages.
35. G.F. Nordberg, B.A. Fowler, M. Nordberg & L.T. Friberg, Handbook on the Toxicology of Metals (Third Edition) (2007) Pages 1-9. https://doi.org/10.1016/B978-012369413-3/50056-2
36. D. Cohen, N. Pichard, S. Tordjman, C. Baumann, L. Burglen, E. Excoffie, G. Lazar, P. Mazet, C. Pinquier, A. Verloes & D. Héron; Specific Genetic Disorders and Autism: Clinical Contribution Towards their Identification. *Journal of Autism and Developmental Disorders*, 35 (2005) 103–116

(2019); http://www.jmaterenvironsci.com