IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 10 Ver. I (Oct. 2015), PP 38-43
www.iosrjournals.org

Heavy metals in Procambarus clarkii of Rharb Region in Morocco and Its Safety for Human consumption

Ikram. El Qoraychy[1], Mohamed. Fekhaoui[2], Abdellah. El Abidi[3], Ahmed. Yahyaoui[1]

[1] *Laboratory of Zoology and General Biology, Faculty of science, Mohammed V University, Rabat, Morocco*
[2] *Scientific Institute, Mohammed V University, Rabat, Morocco.*
[3] *National Institute of Hygiene, Rabat, Morocco.*

Abstract: *The use of Procambarus clarkii in human gastronomy is a cultural issue in the world. Since it is used in human nutrition, can potentially bioaccumulate heavy metals, it is important to quantify the toxicological risk posed by its consumption. The human consumption of crayfish could be restricted based on current criteria for Cu, Pb, and Cd. Different concentrations of metals copper, lead, and cadmium were evaluated in crayfish Procambarus clarkii tissues (carapace, flesh) for a six month period, from March to August 2013 in the Rharb of Morocco (Lagoon Merja Zerga and Nador canal). The determination of heavy metals was carried using atomic absorption spectroscopy Varian VV20. The results obtained revealed a metal's contamination of Procambarus clarkii in both sites. The concentrations differed in carapace and flesh.*

Concentrations of heavy metals examined Procambarus clarkii carapace ranged as follows: Merja Zerga (Pb 2.07 – 6.7 µg/g; Cu 2.9-9.97 µg/g) and Nador canal: (Pb 0.08-7.8 µg/g; Cu 3.40-9.3 µg/g). Concentrations of heavy metals found in flesh were as follows: Merja Zerga (Pb 0.02-5.25 µg/g; Cu 9.58-23.59 µg/g) and Nador canal (Pb 0.06-6.81 µg/g; Cu 9.5-37.20 µg/g). The distribution of those metals in Procambarus clarkii shows high levels of contamination for Lead, Copper with an absence of Cadmium in both sites (Merja Zerga and Nador canal).

Key words: *Procambarus clarkii, Heavy metals, consumption, Merja Zerga, Nador canal, Morocco.*

I. Introduction

In aquatic ecosystems, the use of indicator species seems to be a suitable way of monitoring environmental quality due to the ability of some aquatic animals to accumulate metallic ions either directly from surrounding water or indirectly through food sources [1] Procambarus clarkii species endemic to southeastern North America [2] , is well-established invasive species in many worldwide aquatic ecosystems and is responsible for several ecological and economic impacts [3], it survives in poor water quality, high and low temperatures, low oxygen concentrations and drying periods, reasons why it is able to occupy a wide variety of habitats [4]. This species has important characteristics that can increase its invasive behavior, sufficient plasticity to adapt its ecology and life cycle to changing environmental conditions, high somatic growth and reproductive output, short development time, ability to tolerate high temperatures, dry periods and low dissolved oxygen conditions [3]. Procambarus clarkii has been used as model crustacean species for a wide array of biological studies, including the evaluation of pollutant toxicity by means of toxicological bioassays [5, 6]. The tolerance to environmental pollution by heavy metals [7] these elements, no biodegradable [8] are responsible for the rapid spread of crayfish in the wild.

Procambarus clarkii is used in human nutrition, and can potentially bioaccumulate heavy metals and pesticide residues [9] are a valuable materiel for the study of aquatic pollution in fresh water ecosystems [10]. Several locations where Procambarus clarkii has been introduced are subjected to continuous chemical contamination, due to diffuse domestic pollution, industrial discharges and agricultural runoff [8] so it is important to quantify the toxicological risk posed by its consumption.

In Morocco, Merja Zerga waters are supplied by two regions, the Merja Rharb and the Loukkos. River Oued Drader supplies mostly Loukkos region while the larger area drained by the Nador canal is within the Rharb region [11].

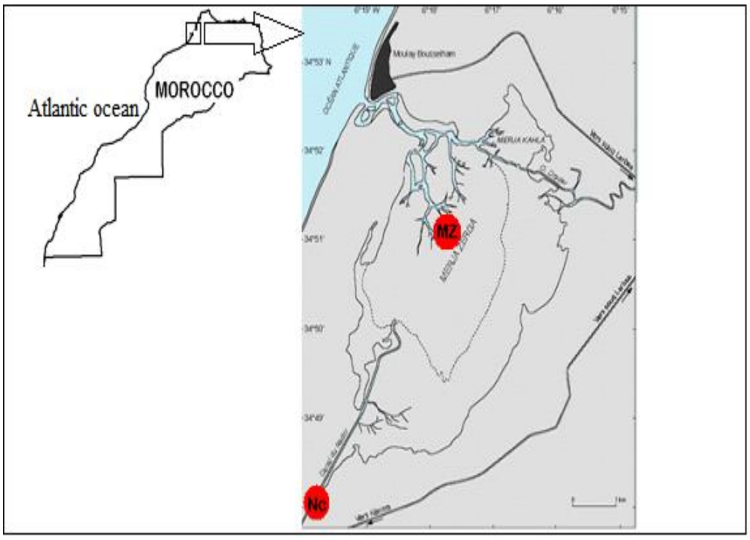

Figure 1: Map showing sampling locations of Merja Zerga (MZ) and Nador canal (NC)

Presence of heavy metals carried by the Oued Drader and Nador canal is noted especially during floods [12].

The present work aims to assess to quantify the toxicological risk posed by the consumption of Procambarus clarkii and to analyze heavy metals in red swamp crayfish in these Moroccan ecosystems. Three heavy metals (cadmium, lead and copper) were selected for examination in carapace and flesh of Procambarus clarkii, as well as a comparison between the metal concentrations detected in the tissues in both study sites. Indeed the presence of these elements traces of specific toxicity constitutes not only a problem of the environment safeguarding but also a public health problem [8].

II. Materials And Methods

2.1. Sampling sites of study

Procambarus Clarkii has been successfully established in various sites of the lagoon Moulay Bousselham in Rharb region West of Morocco. The first observation of the species in late 2008 early 2009 in Merja zerga; coincides with the introduction, in 2007, by the Spanish company ALFOCAN (company for processing and marketing wild-caught freshwater crayfish). The current distribution of P.clarkii in Morocco has been identified at swamps and rice fields of larache, the region of Rharb between Merja Zerga and souk larbaa at north dar gueddari and allal tazi in the south west of Atlantic Ocean (lake, irrigation and drainage canals, temporary merjas and rice fields).

Procambarus clarkii were collected from Merja Zerga and Nador canal.

Merja zerga 34°86’N/06°28’W a permanent biological reserve, Ramsar site(1980) is a tidal lagoon located 70km north of Kenitra on the Atlantic coast [13] , the lagoon water system is mainly supplied by the Drader River to the east and the Nador canal to the south [11]**.,** its linked to the Atlantic Ocean by a single channel (the‘‘gullet’’), that is strongly tidal and at high tide, is connected with a smaller lagoon, the Merja Kahla [14] . Merja Zerga is well known as a bird reserve and as an area of outstanding natural beauty. Its hydrology and vegetation have been studied since the 1970s and the plankton since 1986 [15, 16, 17].

The Nador canal was built in 1953; its mean annual discharge has been estimated at $150\times10^{6}m^{3}$ / year the canal drains flooded depressions (agricultural areas) of the Rharb costal [18].

2.2. Sampling and analysis

Red crayfish Procambarus clarkii samples were collected monthly from March to August 2013. The 500 grams flesh and carapace crayfish samples obtained after dissection were placed in an incubator at 65 °C for 48 h until complete drying and then ground using porcelain mortar. For this purpose, fractions of 0.5 g of the dried samples are prepared for measurement concentrations. They were placed in clean digestion bombs, to P.clarkii flesh was added 4 ml of nitric acid 65% HNO3, to the carapace samples were added 4ml of water and 4ml of regal hydrofluoric acid in the presence of boric acid (Suprapur quality, Merck). The Samples were kept overnight for predigesting, the next day they were placed in sand bath at 120 ° C for 4 h until the solution became clear. After cooling, the samples were transferred into dilution tubes of 50ml, filled up with 30ml samples and the rest with distilled water [19]. Assays metals were performed on digests diluted [19, 20, 21].

Cd, Cu and Pb were determined by atomic absorption spectrophotometer graphite furnace (Varian AA 20). The validity of the analytical methods was checked internal control by using standard samples (National research council of Canada) Bess-1 for the shells and DROM-2 for crustaceans and external control by inter calibration exercises (AIEA-MESL- 2014-01-TE).

2.2. Statistical analysis

Statistical differences between the different tissues (carapace and flesh) were determined with Excel.

III. Results

The metal concentrations analyzed in crayfish Procambarus clarkii sampled from two sites (Figure 2 and 3) summarize mean concentrations of studied metals traces (Table 1).

In ours study, Copper detected in Merja Zerga and Nador canal showed high levels in flesh and carapace (fig. 4, 5, 6 and 7). Copper concentration in flesh was considerably lower than it is in carapace.

The copper concentrations in carapace were higher in Merja Zerga than in Nador canal: levels detected are respectively 7.24µg/g dry weight and 5.70µg/g dry weight. In flesh, copper concentrations in Merja Zerga are lower than in Nador canal (17. 33 µg/g, 20, 69 µg/g dry weight) (Fig. 2 and 3).

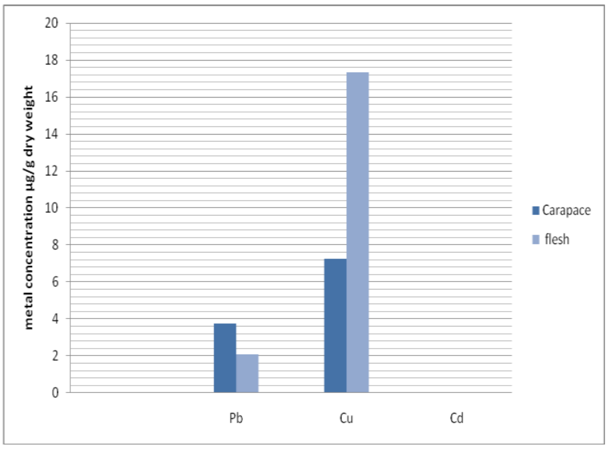

Figure 2. Distribution of Cd, Pb and Cu in Carapace and flesh of Procambarus clarkii (Merja Zerga)

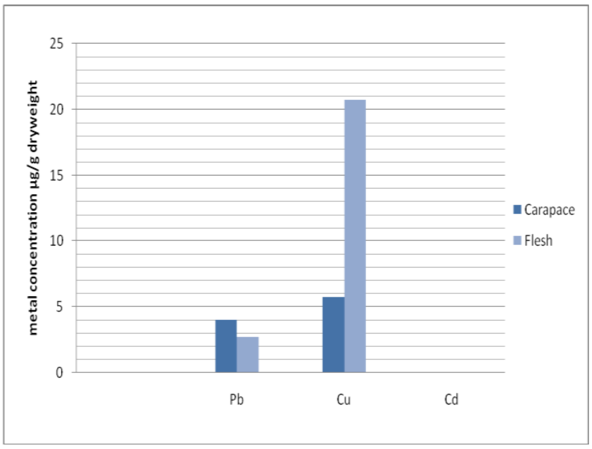

Figure 3. Distribution of Cd, Pb, and Cu in Carapace and flesh of Procambarus clarkii (Nador Canal).

The contamination of cadmium in Procambarus clarkii in both sites of Merja Zerga and Nador canal (0.0009 and 0.0002 µg/g dry weight) in carapace and in flesh (0.006 and 0.01 µg/g dry weight) (fig.2 and 3). We can say that concentrations are very low in both sites (fig. 4, 5, 6 and 7). Even though no significant differences in lead concentrations between the two sites, high concentrations of lead in both sites especially in carapace was observed (fig. 4, 5, 6, 7). Lead accumulated was mainly detected in carapace (3.74 µg/g dry weight) and in flesh (2.04 µg/g dry weight) in Procambarus clarkii from Merja Zerga. Those caught from the Nador canal contained 3.98 µg/g dry weight of lead in carapace and 2,67 µg/g dry weight in flesh (fig. 2 and 3). The mean concentration of Pb in Procambarus clarkii varied between 3.74 and 3.98 µg/g dry weights in carapace and 2.04-2.67 µg/g dry weight in flesh (fig. 2 and 3).

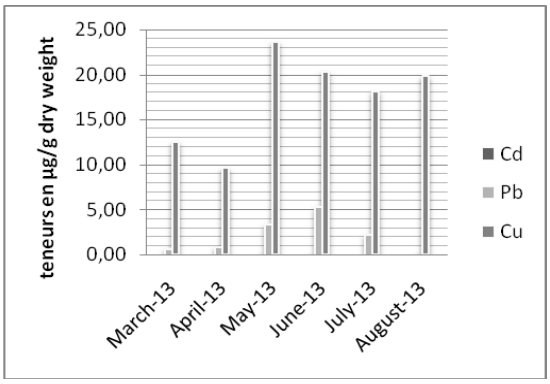

Figure 4. Distribution of Cd, Pb and Cu in flesh from March to August of Procambarus clarkii (Merja Zerga)

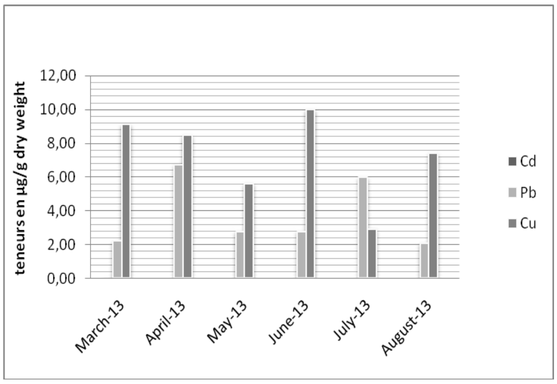

Figure 5. Distribution of Cd, Pb and Cu in carapace from March to August of Procambarus clarkii (Merja zerga).

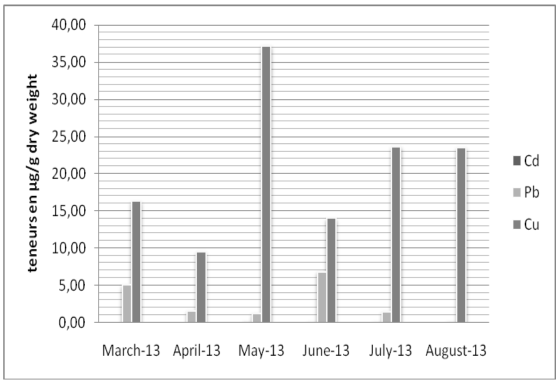

Figure 6. Distribution of Cd, Pb and Cu in flesh From March to August of Procambarus clarkii (Nador canal).

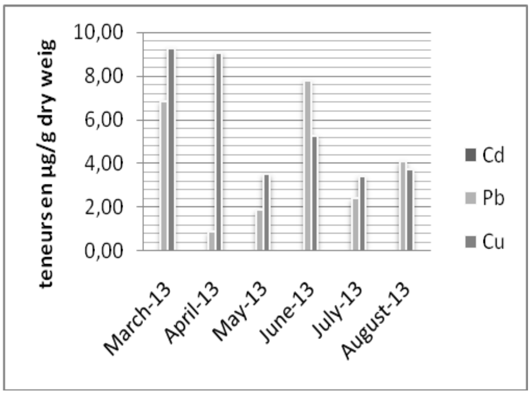

Figure 7. Distribution of Cd, Pb, Cu and Cr in Carapace from March to August of Procambarus clarkii (Nador canal).

Table 1: Mean concentrations en (µg/g dry weight) of Pb, Cd and Cu in carapace and flesh in both sites.

Morocco	Pb en (µg/g dry weight)		Cd en (µg/g dry weight)		Cu en (µg/g dry weight)	
	Carapace	Flesh	carapace	Flesh	carapace	Flesh
Merja zerga	3.74	2.04	0.0009	0.006	7.24	17. 33
Nador canal	3.98	2, 67	0.0002	0.01	5.70	20, 69

IV. Discussion

Cadmium is introduced into the bodies of water from smelting, metal plating, cadmium-nickel batteries, phosphate fertilizer, mining, pigments, stabilizers, alloy industries and sewage sludge. The harmful effects of cadmium include a number of acute and chronic disorders, such as "itai-itai" disease, renal damage, emphysema, hypertension, and testicular atrophy [29]. Department of Health and Human Services (DHHS) has determined that Cd and Cd compounds are known human carcinogens [23].

In absence of national regulatory standards for normal levels of traces elements and according to Spanish legislation, a maximum limit for heavy metal in edible wet mass of Cd is 1 µg/g (BOE 195, 15/8/1991) whereas European legislation limit is of 0, 8 µg/g (EC Regulation 466/2001) [24]. We can say that concentrations are very low and indicate that both sites are non polluted by cadmium (fig. 4, 5, 6 and 7); accumulation of environmental cadmium in crayfish tissues has been reported. Levels of environmental pollution have shown positive correlations with concentrations in tissue samples [7]. The highest concentrations of cadmium in Procambarus clarkii were found in hepatopancreas, alimentary tract, and in a less extent in blood and exoskeleton. The least affected tissues were abdominal muscles [25].

Lead has long been known to alter the hematological system by inhibiting the activities of several enzymes involved in heme biosynthesis [26], alloys are commonly found in pipes, storage batteries, weights, shot and ammunition, cable covers, and sheets used to shield us from radiation. The largest use for lead is in storage batteries in cars and other vehicles [22]. According to Spanish legislation, a maximum limit for heavy metal in edible wet mass of Pb is of 1 µg/g (BOE 195, 15/8/1991), whereas European legislation limits is of 0.8 µg/g (EC Regulation 466/2001) [24]. Our results show that lead non essential element accumulates in Procambarus clarkii caught from Merja Zerga and Nador canal due to the several paddy field (culture of rice). Rotation of rice and crawfish takes advantage of the seasonality of each crop which allows for the production and harvest of each crop in one year. Rice is grown and harvested in the summer months while crawfish grown during the fall, winter and spring in the same field in a twelve month period [8]. The metal was accumulated primarily in the hepatopancreas, carapace, and gills and reached only low concentrations in the hindgut and muscle [7].

Copper can enter the environment through releases from the mining of Cu and other metals and from factories that make or use Cu metal or Cu compounds. Copper can also enter the environment through waste dumps, domestic waste water, combustion of fossil fuels and wastes, wood production, phosphate fertilizer production, and natural sources [22]. Copper is essential micronutrient, do not accumulate in decapods crustaceans up to certain threshold levels [27]. The activities identified in the drainage areas particularly the use of copper sulfate in rice agriculture may explain this [25, 28] (Fig 4, 5, 6 and 7). Cu was higher than world average at every station along the Merja Zerga [33]. According to Spanish legislation, a maximum limit for heavy metal in edible wet mass of Cu is 20µg/g (BOE 195, 15/8/1991) [24]. Accumulation of copper was observed in tissues in the following decreasing order: gills>exoskeleton> abdominal muscle [7].

Table 2: Mean concentrations of Pb, Cd and Cu in abdominal muscle and hepatopancreas of crayfish expressed as mg kg−1dry tissue weight [7].

Country	**Pb** en (mg/kg dry tissue weight)		**Cd** en (mg/kg dry tissue weight)		**Cu** en (mg/kg dry tissue weight)	
	Hepatopancreas	Abdominal muscle	Hepatopancreas	Abdominal muscle	Hepatopancreas	Abdominal muscle
Spain	_	_	_	0.02[b]	_	_
Egypt	_	15.93	_	1.97	32.72	
	<5.0[b] 0.04[b]	<5.0[b] 0.06[b]	0.83[b]	3.55[b]	58.49[b]	6.77[b]
USA	<0.19		0.30[b] and 0.26 [b]	0.73[b] and 0.33[b]	_	44.60
			_	0.03		

[b] **Concentration from an unpolluted (or reference) locality.**

V. Conclusion

This study revealed high concentrations of Pb and Cu in Procambarus clarkii in both carapace and flesh from Merja Zerga and Nador canal, and detected the absence of cadmium in both sites, the region of Gharb has a great ecological, economical and halieutic value. Procambarus clarkii excellent bioindicator of contamination in fresh water ecosystems could indicate the toxicological risk which can be induced by its consumption. The pollution of heavy metals to our ecosystems is a warning to humankind about the importance to prevent the ongoing metals pollution [31, 32] and may exert different harmful effects that can cause a potential risk to human health [30].

Acknowledgements

This work was done in the National Institute of Hygiene, Rabat, in the Laboratory of Zoology and General Biology, Faculty of science, Rabat and in Scientific Institute, Mohammed V University. We want to thank laboratory of toxicology in National institute of Hygiene for his technical assistance.

References

[1] Devi M, Thomas DA, Barber JT, Fingerman M (1996). Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. Ecotoxicol Environ Saf, 33:38-43.

[2] Huner JV (1988). Procambarus in North America and elsewhere. In: Holdich. Eds, DM Lowery RS. Freshwater Crayfish: Biology, Management and Exploitation. Croom - Heim, London, pp. 239–261.

[3] Ronaldo S, Freitas FEP, Mota M, Nogueira AJA, Antunes C (2013). Invasive dynamics of the crayfish Procambarus clarkii (Girard, 1852) in the international section of the River Minho (NW of the Iberian Peninsula). Aquat conserv, 23:5.

[4] Huner JV, Lindqvist OV, (1995). Physiological adaptations of freshwater crayfishes that permit successful aquaculture enterprises. Amer Zool, 35: 12-19.
[5] Reddy PS, Tuberty SR, Fingerman M (1997). Effects of cadmium and mercury on ovarian maturation in the red swamp crayfish, Procambarus clarkii. Ecotoxicol Environ Saf, 37: 62-5.
[6] Naqvi SM, Howell RD. (**1993).** Toxicity of cadmium and lead to juvenile red swamp crayfish. Procambarus clarkii and effects on fecundity)**.**Bull Environ Contam Toxicol, 51(2):303-8.
[7] Antonín K, Miloš B, Pavel K (2010). Bioaccumulation and Effects of Heavy Metals in Crayfish: Review. Water Air Soil Poll, 211: 5-16.
[8] Sneddon J, Richert JC (2011). Metals in Crawfish. Aquaculture and the environment, 22.
[9] Balcão VM, Nunes BA (2009). Risk assessment of human consumption of potentially contaminated red swamp crayfish (Procambarus clarkii): a conceptual approach. Revista da Faculdade de Ciências da Saúde. Porto: edições Universidad Fernando pessoa, 6:332-342.
[10] Sharshar KM, Heiba FN, Geasa NM (2001). Accumulation of copper, lead and cadmium in some tissues of the crayfish Procambarus clarkii (Cambaridae, Crustacea). Egypt J Aqual Bio, 2:47-56.
[11] Ayache F, Thompson JR, Flower RJ, Boujarra A, Rouatbi F, Makina H (2009) . Environmental characteristics, landscape history and pressures on three coastal lagoons in the Southern Mediterranean Region: Merja Zerga (Morocco), Ghar El Melh (Tunisia) and Lake Manzala (Egypt). Hydrobiologia, 622: 15-43.
[12] Madden JD, Grodner RM, Feagley SE, Finerty MW, Andrews LS (1991) minerals and xenobiotic residues in the edible tissues of wild and pond-raised louisiana crayfish. Journal of Food Saf, 12:1-15.
[13] Madigosky SR, Alvarez-Hernandez X, Glass J (1991). Lead, cadmium, and aluminium accumulation in the red swamp crayfish Procambarus clarkii G. collected from roadside drainage ditches in Louisiana. Arch Environ Contam Toxicol, 20:253-258.
[14] Ramdani M, Elkhiati N, Flower RJ, Thompson JR, Chouba L, Kraiem MM, Ayache F, Ahmed MH (2009) .Environmental influences on the qualitative and quantitative composition of phytoplankton and zooplankton in North African coastal lagoons. Hydrobiologia, 622:113-131.
[15] Ramdani M (1988). Les eaux stagnantes du Maroc: études biotypologique et biogéographique du zooplancton. Travaux de l'Institut Scientifique Série Zoologie Rabat, 43: 40 pp.
[16] Ramdani M, Elkhiati N, Flower R J, Kraiem MM, Fathi AA, Birks HH & Patrick ST (2001). Open water zooplankton communities in North African wetland lakes: the CASSARINA Project. Aquat Ecol, 35 (3-4): 319-333.
[17] Fraikech M, Berraho A, Ramdani M, Chafik A, Serghini M, Moukrim A (2005). Evolution spatio-temporelle des dinoflagellés dans la baie d'Agadir Maroc. Mar Life, 15: 19-27.
[18] Snoussi M, Ouknine L (2006). Water, salt and nutrient budgets for Moulay Bousselham lagoon, morocco LOICZ- Biogeochemical Modelling Node, University Mohamed V, Faculty of Sciences, Department of Earth Sciences, B.P. 1014 Rabat, Morocco
[19] Auger D (1989). Méthode de dosage du plomb, du cadmium, du cuivre et du zinc dans les milieux biologiques. Direction de l'environnement et de recherché océaniques. DERO: 89-07-MR.
[20] Bouachrine M, Fekhaoui M, Bennasser L, Idrissi L (1998). Distribution of selected metals in tissue samples of fish from industrially contaminated stream (the river Sebou, Morocco). Acta hydrobiol, 40 : 173-179.
[21] Chiffoleau JF, Truquet I (1994). Nouvelles méthodes de dosage de quelques métaux traces dans les sédiments et les matières en suspension. IFREMER, 08-94.
[22] Aksu O, Adiguzel R, Numan VY, Danabas D, Seker S, Seyhaneyildiz SC, Ates M (2014) . Temporal Changes in Concentrations of Some Trace Elements in Muscle Tissue of Crayfish, Astacus leptodactylus (Eschscholtz, 1823), from Keban Dam Lake Bioinorg Chem Appl, 2014: 120-401.
[23] Agency for Toxic Substances and Disease Registry (ATSDR) (2011). Toxicological Profile for Cadmium. US Department of Health and Human Services Atlanta Ga USA
[24] Naqvi SM, Devalraju I, Naqvi NH (1998). Copper bioaccumulation and depuration by red swamp tray fish Procambarus clarki. Bull Environ Contam Toxicol, 61: 65-71.
[25] Stephen RM, Xavier AH, Jonathan G (1991). Lead, cadmium, and aluminum accumulation in the red swamp crayfish Procambarus clarkii G. collected from roadside drainage ditches in Louisiana Arch. Environ. Contam Toxicol, 20: 253-258.
[26] Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological Profile for Lead. US Department of Health and Human Services Atlanta Ga USA.
[27] Alcorlo PM, Otero M, Crehuet M, Baltanás A, Montes C (2006). The use of the red swamp crayfish (Procambarus clarkii, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). Science of the Total Environment, 366: 380-390.
[28] Kucykbay FZ, Orun I (2003). Copper and zinc accumulation in tissues of the freshwater fish Cyprinus carpio collected from the Karakaya Dam Lake, Malatya (Turkey). Fresenius Environ Bull, 12:62-66.
[29] Dianati-Tilaki RA, Mahvi AH, Shariat M, Nasseri S (2004). Study of Cadmium Removal from Environmental Water by Biofilm Covered Granular Activated Carbon. Iranian J Publ Health, 33 :(4) 43-52.
[30] Abdul wahab AS, Syed ismail SN, Praveena SM, Awang S (2014). Heavy Metals Uptake of Water Mimosa (Neptunia oleracea) and Its Safety for Human Consumption. Iranian J Publ Health, 43 :(3)103-111.
[31] Das HK, Mitra AK, Sengupta PK, Hossain A, Islam F, Rabbani GH (2004). Arsenic concentration in rice, vegetables and fish in Bangladesh: a preliminary study. Environ Int, 30 (3): 387.
[32] Dabeka RW, Mckenzie AD, Lacroix GMA, Cleroux C, Bowe S, Graham RA (1993). Survey of arsenic in total diet food composites and estimation dietary intake of arsenic by Canadian adults and children. J AOAC Int, 76: 14-25.
[33] Alaoui AM, Choura M, Maanan BZ, Marc R, Freitas MC, Cesar A, Mehdi K, Christian C (2010) . Metal fluxes to the sediments of the Moulay Bousselham lagoon, Morocco. Environ Earth Sci, 61:275-286.