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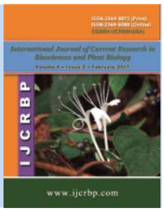

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Assessment of Some Heavy Metals in the Economic Fishes in Hail Market, Kingdom of Saudi Arabia

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Abstract

Makarona fish contains the highest crude protein, followed by Bagha, Basa and Denise; whereas the lowest was recorded in the Sardine Mabroom and Bori. Bagha fish contains the lowest total fat, followed by Filet National, Makarona, Morgan, Basa, but the highest was found in the Sardine Mabroom, followed by Bori. However, crude protein correlated negatively with total fat and ash; whereas ash correlated positively with total fat. Generally, and from the nutritive value point of view, Makarona, Bagha, Basa, and Denise fish are the best since they are the richest animal crude protein sources containing the lowest total fat percentages and moderate ash contents in the Saudi markets. Sardine contains the significantly highest concentration of Zn, Fe and Cu in Sardine, and the highest level of Cd in Mackerel. There was a significant positive correlation between Zn and Cu.

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Introduction

Although fish is not truly a staple of the Saudi diet, demand for fresh fish is climbing up. Annual fish consumption per capita increased from 3 kg in 1977 to 11.5 kg in 2010 (FAO, 2012). The Kingdom of Saudi Arabia occupies 80% of the Arabian Peninsula land surface, with the length of its coastal belt along the Red Sea and the Gulf exceeding 2 400 km. Aquaculture development is available along the Red Sea in the west and the Arabian Gulf in the east. This makes the country a rich source of a wide range of fish suitable for commercial exploitation. Scarcity of freshwater confirms that mariculture is the future for fulfilling Saudi's future fish-food demand.

Many fish species are successfully cultured in Saudi Arabia, e.g. local Hamoors (Malabar grouper *Epinephelus malabaricus*, brown-marbled grouper *E. fuscoguttatus*, and camouflage grouper *E. polyphekadion*). The other marine fish is the marbled spinefoot (*Siganus rivulatus*), known locally as safi or sigan, which has been identified as one of the local species with commercial potential in the Kingdom, having a big demand in the local market. The culture of Asian seabass is now in the process of being adopted by several marine fish farms. Gilthead seabream (*Sparus aurata*) though considered an exotic species was bioinvasive in the Red Sea vicinity of the opened Suez Canal and established successful (FAO, 2017).

In the last decades, contamination of aquatic systems by heavy metals has become a global problem. The concentrations of heavy metals in fish have been extensively enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbor activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, 2009). The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factors posing serious threat to the survival of aquatic organisms including fish.The growing human population has increased the need for food supply. Because they are good protein sources, the demand for fish products has increased. Worldwide, people obtain about 25% of their animal protein from fish (Bahnasawy et al., 2009). In 2004, about 75% (105.6 million tons) of estimated world fish production was used for direct human consumption (FAO, 2007). It has been predicted that fish consumption in developing countries will increase by 57%, from 62.7 million tons in 1997 to 98.6 million in 2020 (Retnam and Zakaria, 2010). The real importance of fish in human diet is not only in its content of high-quality protein, but also to the two kinds of omega-3 polyunsaturated fatty acids, i.e. eicosapentenoic acid (EPA) and docosahexenoic acid (DHA). Omega-3 (n-3) fatty acids are very important for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Burger and Gochfeld, 2005; Al Bader, 2008). Fish also contain vitamins and minerals which play essential role in human health. Therefore, the present research aimed in giving light on the chemical composition and the status of some heavy metals (Zn, Fe, Cu, Pb and Cd) in Saudi-market's fish including frozen fish, imported fish and marine fish.

Materials and methods

Collection of fish samples

One hundred samples of fish were collected from Hail Market, Hail, Saudi Arabia from March – June 2016, where 10 samples per each fish species were collected. These fish species were namely frozen fish [Makarona (Brushtooth lizard fish, *Saurida undosquamis*) and Mackerel, (*Scomber scombrus*)], imported frozen fish [Filet (National fishes) of Elephant-snout fish (*Mormyrus kannume*) and Basa (Basa fish, *Pangasius bocourti*)], and marine fish [Sardine mabroom (Slender rainbow sardine, *Dussumieria elopsoides*), Morgan (Red porgy, *Pagrus pagrus*), Denise (Giltheassea bream, *Sparus aurata*), Bagha (Blue runner, *Caranx crysos*), Bori (Mullet, *Mugil cephalus*), and Striped red mullet (Surmullet, *Mullus surmuletus*)]. The collected samples were packed in a sterile polyethylene bags, sealed and cooled in an insulated box contained crushed ice, then immediately transferred to the laboratory without delay, where subjected to examination. Each fish sample was wrapped separately in polyethylene bag, given an identification number and the collection site and date of collection were recorded on it. The fish were kept in ice box until the samples were prepared for analysis.

Chemical analysis

The tested fish-muscles' samples were stored frozen at -2°C till be chemically analyzed in the chemistry department's lab. (IDAC, MERIEUX, NutriSciences, P.O. Box 7133 Kharj 11942, Kingdom of Saudi Arabia). Moisture was determined using the method reference: AOAC - 925.09, crude protein: FOSS - AN – 300, total fat: NEN-ISO 6492, and ash: AOAC - 940.26; with detection limits of 0.01%.

Heavy metals determination

For heavy metals determination (in Central Laboratory, Faculty of Science University of Hail, Kingdom of Saudi Arabia), the head and external skeleton of each sample were removed. The edible (internal) muscles were transferred to a clean, dry container and then minced to obtain a fine mince representing the whole flesh. The samples of fish were prepared according to Greig et al. (1982). Where, the muscles were homogenized in a porcelain mortar. Three grams of each homogenized samples were transferred into acid washed screw-capped digestion tube with a polyethylene stopper. Ten ml of pure Analar concentrated nitric acid (99.9% - Merck) were added and thoroughly mixed then kept overnight at room temperature. The mixture was heated in a shaking water bath at 65-70°C for 6 hrs. The mixture was cooled at room temperature then few drops of 30% hydrogen peroxide were added. The tubes were reheated with the same previous manner but for 3 hrs only. After cooling, 10 ml of deionized water were added, to dilute the digesta. The diluted digesta were filtered and the clear filtrates were kept in screw-capped tubes in a refrigerator. One-hundred ml of each collected water samples were transferred into clean glass bottles with 0.3 ml of pure concentrated nitric acid (99.9% - Merck) according to Polprasert (1982).

Statistical analysis

Numerical data collected were statistically analyzed for analysis of variance and least significant difference as well as Pearson correlation using SAS (2001) System, GLM Procedure, Class Level Information and Duncan (1955).

Results and discussion

Chemical analysis

It is clear from the data given in Table 1 that the moisture content widely varied by ca. 34.83 % (62.29-88.56%) and consequently also the dry matter sharply varied by *ca.*106.88% (11.44-37.71%); therefore, to precisely compare among the tested fish samples it is important to convert the data on dry matter basis as given in Table 2.

Table 1. Proximate analysis of the tested fish samples (% fresh weight basis).

Fish sample	Moisture	Crude protein	Total fat	Ash
Mackerel	78.20	18.42	0.69	1.76
Denise	78.96	19.02	0.82	1.17
Bori	70.39	19.89	6.61	2.21
Makarona	80.29	18.33	0.47	0.85
Morgan	79.91	17.36	0.52	2.18
Sardine Mabroom	62.29	17.66	14.36	4.47
Bagha	78.35	19.94	0.36	1.24
Striped Red Mullet	76.82	18.47	2.12	2.58
Filet National	88.49	10.24	0.23	0.46
Basa	88.56	10.40	0.31	0.72

On dry matter basis, Makarona fish contains the highest crude protein (CP), being 93 %, followed by Bagha (92.1 %), Basa (90.91 %) and Denise (90.4 %); whereas the lowest was recorded in the Sardine Mabroom (46.83 %) and Bori (67.17 %). Bagha fish contains the lowest total fat (TF) being 1.66 %, followed by Filet National fish (2.00 %), Makarona fish (2.38 %), Morgan fish (2.59 %), Basa (2.71 %), but the highest TF was found in the Sardine Mabroom fish (38.08 %), followed by the Bori (22.32 %). However, CP correlated negatively with TF and ash; whereas ash correlated positively with TF. Generally, and from the nutritive value point of view, Makarona, Bagha, Basa, and Denise fish are the best since they are the richest animal CP sources containing the lowest TF percentages and moderate ash contents in the Saudi markets.

Table 2. Proximate analysis (%) of the tested fish samples.

Fish sample	Dry matter	Dry matter basis		
		Crude protein	Total fat	Ash
Mackerel	21.8	84.50	3.17	8.07
Denise	21.04	90.40	3.90	5.56
Bori	29.61	67.17	22.32	7.46
Makarona	19.71	93.00	2.38	4.31
Morgan	20.09	86.41	2.59	10.85
Sardine Mabroom	37.71	46.83	38.08	11.85
Bagha	21.65	92.10	1.66	5.73
Striped Red Mullet	23.18	79.68	9.15	11.13
Filet National	11.51	88.97	2.00	4.00
Basa	11.44	90.91	2.71	6.29

Abdelhamid et al. (2013) analyzed of Nile tilapia and mullet fish as percent on dry matter basis showed significant ($p \leq 0.0001$) effects of each of sampling seasons and stations as well as their interactions and fish species also on the chemical analysis of the tested fish. Mullet fish contained higher DM and EE and lower ash than tilapia. Actually, sometimes there were positive relationships between DM on one side and each of CP, EE, and ash percentages on the other side. Also, there were negative relationships between CP on one hand

and either EE or ash percentages on the other hand. These relationships were reported too by many authors (El-Ebiary and Zaki, 2003; Abdelhamid et al., 2005). However, in this concern, there is a negative relationship between CP and TF in the chemical composition of fish, on one hand (Abdelhamid et al., 1997, 1998, 2002 and 2007; El-Ebiary and Zaki, 2003) and a positive correlation between CP and ash contents of fish, on the other hand (Abdelhamid et al., 1995, 1997, 1998 and 2007). A negative relationship was noticed between CP and TF contents of fish body but a position relationship between CP and ash contents was recorded too (Abdelhamid et al., 1999, 2000 and El-Saidy and Gaber, 2002). Yet, Eid (1995), Kobeisy and Hussein (1995), Gaber (1996), El-Saidy and Gaber (1998 and 2002) and El-Saidy et al. (1999)found that there was a positive correlation between crude protein and fat contents of the fish, but Goda (2002) and Magouz et al. (2002a,b) found a negative correlation between protein and fat contents of the fish. There were significant effects of sampling locations and seasons on the chemical analysis of mullet flesh. Elevated protein content in winter may be due to the lower ($p<0.05$) heavy metals content (Pb and Fe) in fish flesh during this season than in summer (Abdelhamid et al., 2007).

Heavy metals determination

Table 3 shows that the highest ($p\leq 0.05$) Zn concentration was found in Sardine and Bagha, Fe in Sardine and Filet, Cu in Sardine, Cd in Mackerel; whereas there were no significant ($p\geq 0.05$) differences among fish species in Pb levels. The overall mean concentrations of the tested metals took the descending order Fe > Zn > Cu >Pb> Cd. This may be due to the source of the tested fish (local or imported, marine or freshwater fish).Sardine contains lowest crude protein, highest total fat, therefore contains the significantly highest level of Zn, Fe, and Cu. In accordance with Noaman et al. (1992) who found that crude protein content was decreased in Nile tilapia grown in water polluted with cadmium, copper and lead as compared with the untreated fish. Also, Magouz et al. (1996) who found that water pollution with Cd and Cu reduced crude protein content in tilapia (*Oreochromis aureus*) after 8 weeks, but ether extract increased by the treatment. Residues of Cd and Cu in the fish were proportional to their levels in the rearing water. Chromosomal examination showed that the number of abnormal cells were higher than in the control. Divided cells and mitotic index in the treated fish were significantly lower than in the control. Moreover, the dietary supplementation of Fe for 6 weeks led to significant decrease of tilapia and catfish muscular protein content. On the other hand, muscular dry matter and ash percentage increased significantly, whereas the muscular ether extract percentage increased insignificantly (Abdelhamid et al., 2000). In addition, levels of heavy metals are correlated with salinity changes due to the discharge of water (Radwan, 2000). The highest concentration of lead in fish musculature was 1.78 ppm in Assiut area. The examined muscle samples for lead showed values less than the permissible, which is 2.26 ppm. The highest concentration of copper in fish musculature was 15.93 ppm in Manqabad area. Muscle samples collected from Manqabad, Bany-Qurra and Assiut exceeded the permissible limit of WHO, which is 10 ppm Pb (Sayed et al., 2000). Moreover, fish muscles area decreased at each pollutant (Cu, Cd, Pb) compared with the control (Salem, 2003).

Table 3. Heavy metal concentration (ppm) in the tested fish samples.

Fish species	Zn	Fe	Cu	Pb	Cd
Bagha	4.7722^{a}	6.7993^{bcd}	0.8070^{b}	0.1966	0.0284^{e}
Basa	2.1707^{b}	8.2186^{abc}	0.4485^{d}	0.4530	0.0394^{cd}
Bori	3.7356^{ab}	8.4029^{ab}	0.6830^{bc}	0.5505	0.0426^{bc}
Denise	2.0193^{b}	3.6716^{ef}	0.6694^{bc}	0.3032	0.0402^{cd}
Filet	2.3277^{b}	9.5412^{a}	0.8411^{ab}	0.2001	0.0317^{de}
Makarona	3.7142^{ab}	5.5150^{de}	0.5279^{cd}	0.1791	0.0450^{bc}
Mackerel	2.1926^{b}	6.1072^{cd}	0.7255^{bc}	0.3283	0.0569^{a}
Morgan	2.5501^{b}	2.8968^{f}	0.7863^{b}	0.1868	0.0366^{cde}
Sardine	4.9005^{a}	9.7665^{a}	1.0047^{a}	0.4646	0.0492^{ab}
S.R.Mullet	2.9040^{b}	3.2122^{f}	0.7628^{b}	0.1441	0.0335^{de}
SEM	0.5515	0.7382	0.0650	0.1170	0.0028

a-f: Means with the same letter in each column are not significantly different at $p\leq 0.05$.

Abdelhamid (2006) reviewed the heavy metals status in the Egyptian water bodies and cited that thirty two danger diseaseare caused by water pollution. However, rearing catfish (*Clarias lazera*) in lead contaminated water for 6 weeks led to deaths among fish exposed to the inorganic pollutant ($PbCl_2$) but not with the organic lead acetate. Fish protein content decreased (but ether extract content increased) gradually by increasing the contamination levels (10, 50 and 100 ppm). Fish muscles content of Ca, Mg, and Pb increased but Na, K and P decreased in the treated fish (Abdelhamid and El-Ayouty, 1991). Moreover, there were variations in the levels of the heavy metals due to sampling locations, seasons, and fish species. The elements' concentrations in the fishes were much higher than the corresponding values in the water, particularly for iron. Lead and cadmium levels in fish muscles were over the permissible levels, recommended by the national authorities. Zinc, lead, and cadmium were concentrated more in fish, while iron was highest in sediments followed by fish tissues. *Mugil cephalus* samples were more frequently contaminated than *Liza ramada* and *Sparus aurata* (Abdelhamid et al., 1997). Zn, Mn, Cu, Pb, and Fe concentrations varied in the different fish tissues throughout the year months. Pollution of water is reflected in the form of heavy metal accumulation in different fish tissues. The lowest bioaccumulation factors were calculated in fish muscles, therefore muscles only are suitable for human consumption. The bioconcentration of iron was higher than that of zinc > lead > copper in fish muscles (Abdelhamid et al., 2000). Anyhow, there was a significant positive correlation between Zn and Cu (Table 4). Lead can be absorbed by the body and take the place of calcium or zinc (Ayyat et al., 2003). Moreover, A survey study was conducted on some heavy metals (Pb, Cd, Cu, Zn, and Fe) in fish samples from Ashtoum El-Gamil protected area, Egypt. Data obtained revealed that there were significant ($p \leq 0.0001$) differences among sampling seasons and stations as well as their interactions concerning the levels of heavy metals tested in fish collected from this protected area. The elements level took the descending order Fe ≥ Pb ≥ Zn ≥ Cu ≥ Cd in the fish body samples (Abdelhamid et al., 2013).

Iron, copper, and zinc are among the essential elements to life. However, only small amounts are needed for good nutrition, and higher doses can have adverse effects (Marquita, 1997). Generally, cadmium and cadmium compounds are known to be human carcinogens (prostate, renal and bladder cancers) (van der Gulden et al., 1995; Mandel et al., 1995). Metals suggested being carcinogenic including cadmium and lead (El-Batanouni and Abo El-Ata, 1996). El-Nady (1996) found that the industrial runoff is mostly responsible for the contribution of heavy metals than the municipal wastes. Heavy metals (Pb, Fe, Cd and Zn) were accumulated in tilapia fish at levels higher than those found in their rearing water. Yet, their concentrations were low in the fish edible muscles relative to levels in other fish tissues. The level of each element depends also on the sampling season and location (Ibrahim et al., 2000).

Table 4. Pearson correlation between the tested heavy metals in the fish muscles regardless to the tested fish species and significance level (*p*-value) between brackets.

	Zn	**Fe**	**Cu**	**Pb**
Fe	0.101 (0.315)			
Cu	0.264 (0.008)	0.15 (0.135)		
Pb	0.016 (0.876)	0.153 (0.129)	0.004 (0.972)	
Cd	0.06 (0.553)	0.104 (0.304)	-0.034 (0.738)	0.192 (0.056)

Siam (2001) found Cd level was generally lower than that of Pb in fish muscles. Most of the fish generally showed levels of Cd close to that of the recommended standard (2.0 mg/kg) of the National Health and Medical Council in Australia. Staniskiene et al. (2005) found high concentrations of Fe in 15 fish species as a direct result of water contamination with heavy metals. Metal concentrations were found to be influenced by fish type. Cadmium-binding protein level in the cells of the intestine was increased after exposure to Cd, so it appears that this protein is synthesized as a response to Cd exposure. Summer cadmium and lead concentrations in mullet (*Mugil* spp.) were higher than in winter (Joyeux et al., 2004).

Although there was no difference between fish of natural resources and those of aquaculture concerning metals pollution (Abdelhamid et al., 2007); recently, apolyculture system (Nile tilapia, silver carp, common carp, and African catfish at a rate 1: 1: 1: 1) for 102 days reflected that catfish body contained the highest level of Cu but silver carp body contained the highest Pb and Cd levels. The interaction effect (dietary treatment × fish species) was significant, except for Cu. Silver carp contained significantly higher Cu and Cd and tilapia contained the highest level of Pb in the muscles comparing with the other fish species. Silver and common carps were more tolerant than Nile tilapia and catfish (Abdelhamid et al., 2014).

Heavy metals in the edible parts of tilapia were within the safety permissible level for human use (Abdel-Baki et al., 2011). Since Abdelhakeem et al. (2002) gave the following permissible levels in ppm in fish: Cd-0.50, Pb-2.00, Cu-20.0, Fe-30.0, and Zn-40.0 ppm fresh weight basis. However, Saeed and Mohammed (2012) found the concentrations of Cu, Fe, and Zn in edible part of fish below the notified toxic limits. The commission regulation setting maximum Pb level for muscles of fish, released from the European communities, as 0.2 mg/kg wet weight. Yet, the Egyptians' standards are 0.1 ppm Pb and Cd in food fish (ES, 1993).

Generally, *Mugil cephalus* showed higher levels of Fe and Pb concentrations than *Sparus aurata* (Yilmaz, 2005). This may be due to the store tissue of each metal in the fish, i.e., Pb is probably an external pollutant (Rashed and Awadallah, 1994), whereas, Fe and Cd were internal pollutants. Since Kirby et al. (2001) mentioned that mullet are directly exposed to trace metal concentrations as a result of feeding and the ingestion of contaminated sediment and detritus. Also, there were remarkable effects on microelements of fish muscles as well as their bioaccumulation factors due to sampling seasons and locations and fish species (Abdelhamid and El-Zareef, 1996).

However, Abdelhamid et al. (1997) registered significant variations in heavy metals concentrations due to different fish species from the natural fisheries and to sampling locations too. Lead and cadmium levels in fish muscles were concentrated more in fish, while iron was highest in sediments followed by fish tissues. *Mugil cephalus* samples were more frequently contaminated than *Liza ramada* and *Sparus aurata*. The effects of varying sampling locations and fish parts on the heavy metal level or presence were also reported by Abdelhamid et al. (2000). Siam (2001) found high level of accumulation of Cd, Fe and Pb in the different organs (gills, liver, stomach and brain) of Alexandria coast fish, with respect to their corresponding in the muscle tissues. He added that the accumulation factors for these metals were higher in the herbivorous fish (*Siganus rivulatus*) than in the carnivorous ones (*Mugil capito*). Fe was the more pronounced one reflecting increase the trophic level of the fish.

Samples of mullet (*Mugil cephalus*) from Mediterranean Sea, Lake Manzalah and fish farms as well as of tilapia (*Oreochromis niloticus*) from the River Nile and fish farms were analyzed for lead. The obtained results revealed that there were no significant ($p \geq 0.05$) differences in lead concentrations in the tilapia fish muscles due to sampling locations. However, the samples contained higher Pb levels in Nile fish than in the farm fish. Significant differences were calculated among Pb concentrations in mugil flesh due to sampling locations. Marine samples contained significantly higher Pb than farm and lake samples. There were significant differences in Pb concentrations in fish muscles due to fish species and fish sampling locations, since tilapia reflected higher levels than mugil and samples from farm, marine and Nile contained more Pb than from lake. The Nile samples were more contaminated with Pb (0.205 ppm) than the farm fresh (0.109 ppm) than the marine fresh samples (0.086 ppm), respectively. Pb levels varied from 0.00 ppm to 0.502 ppm (Abdelhamid et al., 2011). So, from the above mentioned data, there is no contamination with either of the tested metals, i.e., the available fishes in Hail market are safe for human consumption.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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