Research article

Measurement of some heavy metals in tissues infect by intestinal worms of two kind of fishes in Al-Qadisiyah Province

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Abstract

The present study has been conducted in four parts of AL-Qadisiyah (Al-Diwaniyah center, Shamiya, Hamza and Afak). A sample of 945 fish of Barbus grypus and Liza abu has been examined. It was found that 205 of the Barbus grypus fish and 344 of the Liza abu were infected with parasitic intestinal worms. Some parasitic intestinal worms have been detected such as the Neoechinorynchus iraqensis in both fish types. The female paulisentus, Bothriocephalus acheilognathi were found in both Barbus grypus, Liza abu and the larva of Contracaecum spp was seen in the Liza abu and Barbus grypus type. Four heavy metals were measured. They included Cadmium, Lead, Zinc ,Nickel, by means of an atomic absorption spectrophotometer issues from the a fore mentioned types of fish were measured for these heavy metals these tissues were from the liver, muscles, gills, from four districts of Al-Diwaniyah throughout the year, Cadmium registered high concentrations in autumn (12.67) Mg/ gm in the liver of the Barbus grypus. Lower concentrations of Cadmium were registered in winter (1.30) Mg/ gm in the liver of Liza abu. The Barbus grypus livers had high concentrations of lead in autumn (21.03) Mg/ gm in Hamza rivers, whereas gills of the Liza abu registered lower concentrations (6.43) Mg/gm in spring. The livers of the Barbus grypus registered high concentrations of zine in autumn which where (32.27)Mg/ gm in Hamza river while Shamiya river they dropped to 21.17 Mg/ gm in spring, It was observed that Nickel had high concentrations in the livers, muscles, gills, of both fish types, The highest of which was in autumn, the Hamza river had the highest concentrations in autumn which were (29.17,28.63,27.67) Mg/g in Barbus grypus , in spring the nickel concentrations were low in the gills of the Liza abu11.10 Mg/ gm in Shamiya river

Keywords: Fish, Intestinal worms, Heavy metals.

Introduction

Fish are exposed to many diseases, as are any organisms. Recently, these diseases have attracted the attention of many researchers. Parasites are a major cause of disease due to large economic losses either directly or indirectly. The various members of the fish acted as bad breath or could lead to the introduction of many other pathogens or lead to a decrease in growth rates among fish (1) . The problem of pollution is one of the most important environmental problems in the

modern world because of its great impact on humans, animals and plants as it leads to a decline in the GDP for the industrial and agricultural sector and then to the decline in per capita income and then the low standard of living and environmental pollution causes economic costs, Or human losses due to the deterioration of plant and animal production (2). Hazardous pollutants are the accumulation of heavy metals in the body of fish. Methods of accumulation and loss of

heavy metals vary depending on the species. Although there are common general methods that involve many species, heavy metals enter the fish bodies through either the skin (3) or gastrointestinal tract (4) or the surface of the respiratory tract such as the gills (5). Heavy metals are significantly increased when sewage and industrial and agricultural wastes are thrown into the water (6). Heavy metals are dangerous because they are difficult to observe when accumulate in the environment, unlike other contaminants such as oil that can be seen and accumulate directly in aquatic organisms and lead to increases their risk (7). Some metals are necessary for living organisms, including humans, but they become poisonous when they are over-limits (8). Some are characterized by poisonous until it found low concentrations, and in different studies of the biologic importance of some heavy metals, For exposure to these metals, such as chromium which caused cancer diseases, as well as for lead, which is the most dangerous metal toxicity to high ability to inhibit enzymes and has a clear effects on the nervous system (9). As for the cadmium metal, recent studies have shown that this metal is very dangerous in destroying the cellular structure of the organism (10). It effects on the nitrogen fixation process and has the potential to replace calcium and zinc in the body of the organism (11). The concentration of lead, cadmium, zinc and nickel was measured in 10 species of fish collected during 2008 from Lake Madivala in Bangalore, India, and the liver record was highest in comparison with gills and muscle (12). Some heavy metals such as iron, zinc, copper, manganese, cadmium and lead were also measured in liver, gills and fish muscles *oreochromis niloticus* were collected from the northern delta lakes of Egypt and the liver record high concentrations of metals compared to the muscles (13). In a study to measure some heavy metals in the *Barbus grypus* tissue in Nassiryah city, it was observed that the liver had the highest

concentration of copper followed by muscle (14).

Materials and Methods

Fish collection:-

The fish were collected from the beginning of March 2012 and the end of February 2013, bringing together 945 fish, including 611 *Liza abu* and 334 *Barbus grypus* from four districts of Qadisiyah governorate, including Shamiya, Afak, Hamza and Diwaniyah, fishing with the help of fishermen using cinnamon nets, By means of plastic containers containing a quantity of water from the area from which the samples were taken and transferred to the laboratory and examined directly.

Isolation of intestinal worms:

The fish were explained according to the method (15) and (16) to detect the internal parasites as the opening of the body of the fish from the inner side by making a longitudinal incision from the opening aperture forward to the mouth opening and when the internal organs revealed cut the gastrointestinal tract of pharynx and Petri dish containing the normal saline solution either the digestive tract was opened longitudinally and emptied its contents in a Petri dish and examined the anatomy microscope in search of parasites in the cavity or wall and then isolated the parasites found in the test tubes container to a normal saline and shake calmly to get rid of the lingering waste.

Fixation, preservation and pigmentation: Cestoda

The tapeworms were stabilized and stored in ethyl alcohol at a concentration of 70% and then transferred to acetocarmine dye stain for no more than 5 minutes for small worms and no more than 10 minutes for large worms. The drying of parasite models was placed in graduated concentrations of ethyl alcohol, 10 minutes in 70% concentration and then transferred to alcohol at a

concentration of 80% for 10-5 minutes after being transferred to alcohol at a concentration of 90% for five minutes and then to absolute alcohol 100% for one minute and then to absolute alcohol and Xylol 1:1 for 1 minute and then transferred to the cell to illustrate the internal structures of the parasite and finally attended the slides to the parasite by mounting it onto a slide using Canada balsam (17).

Nematodes:

It was fixed by using hot alcohol with 70% concentration and was stored in alcoholic calycerine and clearing by the use of lactophenol and for the use of slides mounted by gelatin (18).

Acanthocephali

Worms were washed with a normal saline solution and placed for 12 hour in Petri dish in the refrigerator to appear the proboscis, then stabilized in an ethyl alcohol solution with 70% concentration (19).

Diagnosis:

The parasites were diagnosed according to base on a variety of taxonomic sources

specialized in the diagnosis of parasites (20, 21, 22, 23).

Heavy metals:

Extraction of heavy metals in fish tissues:-

Take one gram of fish tissue (liver, muscle and gills) were mixed with 5 ml of 65% nitric acid and 1 ml of hydrogen peroxide in the vial of digestion and place in a microwave oven at 170 ° C and press 100 ° C for 20 min then leave in The room temperature, then transferred to a 25 ml vial and supplemented with distilled water (24).

Measurement of heavy metal ions:

Heavy metals ions were measured in the study samples using the atomic absorption spectrophotometer (pye-unicam). and the Hollow cathode bulbs were used for each metal.

Statistical analysis:

The full randomized design (CRD) Complete Randomized Design was used for one-way ANOVA, and the mean was measured using the least significant difference (LSD) at the probability level (P <0.05) (25)

Results

Cadmium:

Liver and muscle of *Barbus grypus* were record to high concentrations of 12.67 and 12.20 microgram /g in the Hamzah River during autumn. It was noted during the summer that concentrations of cadmium in the fish tissue of the river Hamzah recorded high concentrations in the liver, muscles and gills of fishes of *Barbus grypus* and *Liza abu* followed by the fish Diwaniyah River with concentrations of 9.53, 8.70, 7.80, 8.83, 8.27

and 7.37 microgram /g Respectively. In the Shamiya fish tissue, there was a low concentration of 8.47, 7.90, 6.93, 7.93, 7.37 and 6.57 microgram / g, respectively, in both *Barbus grypus* and *Liza abu*. For winter, Fish tissue recorded low concentrations compared to other seasons. The Afak River recorded low concentrations of 1.87, 1.37 and 0.70 microgram /g respectively, in the *Liza abu* fish liver and its muscles and gills.

Table (1): Cadmium concentrations in *Barbus grypus* and *Liza abu* fish tissue by season and area of study (microgram / g)

Spring season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
2.70	2.97	3.27	3.07	3.40	3.77	Diwaniyah River
2.80	2.53	2.77	2.60	3.00	3.23	Shamiya River
2.87	3.30	3.60	3.27	3.73	4.23	Hamzah River
2.50	2.73	3.03	2.87	3.23	3.50	Afak River
L.S.D. _{0.05} = 0.75						
Summer season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
7.37	8.27	8.83	7.80	8.70	9.53	Diwaniyah River
6.57	7.37	7.93	6.93	7.90	8.47	Shamiya River
7.80	8.60	9.10	8.27	9.10	9.87	Hamzah River
6.90	7.73	8.37	7.37	8.23	8.97	Afak River
L.S.D. _{0.05} = N.S.						
Autumn Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
9.90	10.90	11.37	10.80	11.77	12.23	Diwaniyah River
8.77	9.87	10.50	9.60	10.80	11.20	Shamiya River
10.43	11.40	11.83	11.40	12.20	12.67	Hamzah River
9.33	10.43	10.97	10.20	11.27	11.73	Afak River
L.S.D. _{0.05} = N.S.						
Winter Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
0.97	1.97	2.40	1.53	2.60	3.07	Diwaniyah River
0.43	0.93	1.30	0.63	1.53	2.03	Shamiya River
1.53	2.43	2.93	2.13	3.07	3.63	Hamzah River
0.70	1.37	1.87	0.97	2.07	2.60	Afak River
L.S.D. _{0.05} = 2.24						
N.S.= Non-significant						

4-2-Lead:

The lead metal recorded different concentrations during the seasons in *Barbus grypus* and *Liza abu* tissues in four rivers in Qadisiyah Governorate (Table 2). It was observed that the spring season recorded high concentrations of lead in both *Barbus grypus* and *Liza abu* tissues collected from the

Hamzah and Diwaniyah rivers; muscles and gills of *Liza abu* fish had a lower concentration of 14.70 and 13.80 microgram /g respectively in the Shamiya River during the summer. The Afak River recorded high concentrations in muscles both *Barbus grypus* and *Liza abu* for 19.40 and 18.37 microgram / g during the autumn season. As

for winter, livers of *Barbus grypus* and *Liza abu* tend to have higher cumulative concentrations in the study areas than in the spring season, which recorded highest concentration in the Hamzah River at 12.27 and 11.50 microgram / g, respectively.

Table (2): Concentrations of lead in *Barbus grypus* and *Liza abu* fish distributed by season and area of study (microgram / g).

Spring season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
7.30	8.77	10.13	8.00	9.70	11.10	Diwaniyah River
6.43	8.00	9.33	7.17	8.77	10.27	Shamiya River
7.53	9.23	10.53	8.50	10.23	11.60	Hamzah River
6.80	8.37	9.70	7.53	9.20	10.60	Afak River
L.S.D. _{0.05} = 1.49						
Summer season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
14.77	15.60	16.10	15.90	16.93	17.43	Diwaniyah River
13.80	14.70	15.17	14.77	16.10	16.63	Shamiya River
15.23	15.97	16.47	16.33	17.30	17.87	Hamzah River
14.30	15.10	15.67	15.20	16.57	17.00	Afak River
L.S.D. _{0.05} = N.S.						
Autumn Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
17.90	18.93	19.57	18.83	19.93	20.47	Diwaniyah River
16.60	17.90	18.43	17.60	18.77	19.47	Shamiya River
18.37	19.50	20.20	19.63	20.57	21.03	Hamzah River
17.20	18.37	19.03	18.30	19.40	20.00	Afak River
L.S.D. _{0.05} = 2.90						
Winter Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
9.23	10.37	10.90	10.00	11.10	11.60	Diwaniyah River
8.20	9.33	9.93	8.90	10.07	10.57	Shamiya River
9.90	10.97	11.50	10.70	11.73	12.27	Hamzah River
8.57	9.77	10.30	9.27	10.43	10.97	Afak River
L.S.D. _{0.05} = 3.26						
N.S.= Non-significant						

4-3- Zinc:

The concentration of zinc is high in muscles and gills of the *Barbus grypus* fish compared to *Liza abu* fish, which record 20.93 and 14.50 microgram / g respectively in Diwaniyah River while in the Shamiya River it decreased to 20.03 and 13.27 microgram / g respectively during the spring. The autumn and summer Concentrations of the zinc element compared to the other seasons, the highest concentration of the element was observed in livers of *Barbus*

grypus and *Liza abu* fish, which was 32.27 and 31.40 microgram / g respectively in the Hamzah river during autumn. It was noted that the winter recorded high concentrations in livers of *Barbus grypus* and *Liza abu* fish and decreased in gills of both fishes. As Hamzah and Diwaniyah River, record high concentrations of 22.67 and 22.27 micrograms / g, respectively, in the livers of *Barbus grypus* 21.80 and 21.43 micrograms / g, respectively, in the livers of the *Liza abu* fish.

Table (3): Concentrations of zinc in *Barbus grypus* and *Liza abu* fish distributed by season and area of study (micrograms / g)

Spring season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
12.63	19.53	21.13	14.50	20.93	22.03	Diwaniyah River
11.87	18.60	20.13	13.27	20.03	21.33	Shamiya River
13.07	19.97	21.57	15.07	21.33	22.40	Hamzah River
12.30	19.10	20.67	13.97	20.40	21.70	Afak River
L.S.D. _{0.05} = 2.54						
Summer season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
26.13	26.83	27.47	27.07	28.03	28.73	Diwaniyah River
25.20	26.10	26.67	26.13	27.20	27.83	Shamiya River
26.57	27.37	27.97	27.53	28.47	29.17	Hamzah River
25.73	26.40	27.03	26.57	27.63	28.23	Afak River
L.S.D. _{0.05} = N.S.						
Autumn Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
29.30	30.53	31.00	30.33	31.33	31.83	Diwaniyah River
28.23	29.33	29.93	28.90	30.20	30.73	Shamiya River
29.80	30.87	31.40	30.93	31.77	32.27	Hamzah River
28.83	29.90	30.47	29.70	30.77	31.30	Afak River
L.S.D. _{0.05} = 3.43						
Winter Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
19.77	20.90	21.43	20.63	21.67	22.27	Diwaniyah River
18.30	19.60	20.30	19.37	20.40	21.17	Shamiya River
20.33	21.27	21.80	21.13	22.07	22.67	Hamzah River
18.97	20.23	20.83	19.90	21.07	21.57	Afak River
L.S.D. _{0.05} = 3.20						
N.S.= Non-significant						

Nickel:

The nickel component, as shown in Table (4), showed low cumulative accumulation in spring in fish tissue compared to other seasons. The lowest concentration of nickel was recorded in muscle and gills in *Barbus grypus*, and was 16.00, 14.67 and 11.80 micrograms / g respectively, with concentrations of 15.47, 13.50 and 11.10 micrograms / g respectively in Shamiya fish. The summer-recorded concentration of nickel was 23.53 and 22.50 micrograms / g respectively in muscles of the *Barbus grypus*

and *Liza abu* of the Afak River. These concentrations increased to 24.03 and 22.93 micrograms / g respectively in the Diwaniyah River. Winter did not register any significant change, Nickel 17.90 and 17.13 micrograms / g respectively in livers of the *Barbus grypus* and *Liza abu* fish in the Diwaniyah River, which are high concentrations compared to the spring season. The gills of *Barbus grypus* fish recorded a high concentration of 17.10 micrograms / g compared to the *Liza abu* fish in the Hamzah River.

Table (4): Concentrations of nickel in *Barbus grypus* and *Liza abu* fish distributed by season and area of study (micrograms / g).

Spring season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
11.73	14.23	16.37	12.67	15.60	16.73	Diwaniyah River
11.10	13.50	15.47	11.80	14.67	16.00	Shamiya River
12.27	14.60	16.77	13.13	16.03	17.13	Hamzah River
11.50	13.87	15.97	12.23	15.10	16.33	Afak River
L.S.D. _{0.05} = 2.21						
Summer season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
21.97	22.93	23.43	23.07	24.03	24.67	Diwaniyah River
21.20	22.03	22.60	22.07	23.07	23.63	Shamiya River
22.43	23.30	23.93	23.47	24.47	25.17	Hamzah River
21.53	22.50	23.00	22.53	23.53	24.13	Afak River
L.S.D. _{0.05} = N.S.						
Autumn Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
26.20	27.20	27.80	27.03	27.97	28.57	Diwaniyah River
25.13	26.20	26.80	25.87	27.00	27.53	Shamiya River
26.93	27.87	28.47	27.67	28.63	29.17	Hamzah River
25.57	26.70	27.30	26.40	27.53	28.00	Afak River
L.S.D. _{0.05} = 3.50						
Winter Season						
<i>Liza abu</i> fish tissues			<i>Barbus grypus</i> fish tissues			Region
Gills	Muscles	Liver	Gills	Muscles	Liver	
15.50	16.60	17.13	16.37	17.33	17.90	Diwaniyah River
14.43	15.67	16.23	15.37	16.47	16.97	Shamiya River
16.37	17.23	17.80	17.10	17.83	18.43	Hamzah River
15.00	16.13	16.70	15.93	16.93	17.47	Afak River
L.S.D. _{0.05} = N.S.						
N.S.= Non-significant						

Discussion

Intestinal worms in *Barbus grypus* and *Liza abu* fish in the present study:

The worms were found in the intestines of freshwater fish in Qadisiyah governorate. The worm *N. iraqensis* was recorded in the intestines of *Barbus grypus* and *Liza abu*. This worm was recorded in the *Liza abu* fish in the Al-Mabazal network in Al-Madaen area south of Baghdad (26). The *Paulisentus* sp was found in the current study in the *Barbus grypus* and *Liza abu* habitats. this worm found also in Fish examined in the waters of Salah al-Din (27). The current study recorded larva worm Nematodes *Contracaecum* sp and recorded also in Bahr Najaf in *Barbus grypus* (28), the present study indicated that the injured *Barbus*

grypus and *Liza abu* fish by one of Cestoda called *B. acheilognathi* worms and also (29) record this worm in the shilluk fish, because of the similarity of species recorded in the current study with some previous studies in Iraq result to similar types of fish examined and similar the nature of the feed and rivers water sources .

Concentration of heavy metals in fish tissues:

The present study showed that the concentration of cadmium in the studied fish tissue, namely the *Barbus grypus* and the *Liza abu* fish, was highest in the liver. The highest concentration was observed in both species during the spring season in the Hamza River, after the Diwaniyah River.

This is consistent with (30) that the liver contains the highest concentration of cadmium from the host muscles. also the present study agrees with the findings of (31). Noting that cadmium was 75% more concentrated in the liver compared with the other organs and this may be due to the liver is the first capsule of the accumulation of toxins, and on the whole, the rise of concentrations of heavy metals in the river Shamiya may be due to the use of chemical fertilizers for agricultural purposes and flow from farmland to rivers and their water and then transmission to aquatic organism through food chain and increased of these heavy metal concentrations (32) can be explained. The increase in cadmium concentration in *Barbus grypus* tissues compared with *Liza abu* fish tissue can be explained by the larger *Barbus grypus* compared to the *Liza abu*, which is consistent with (33). The reason for the increase in concentrations of metals in both Al-Hamzah and Afak rivers may be due to the dumping of industrial and human contaminants directly into the river without treatment for the absence of treatment plants. The increase in the Diwaniyah River is also due to the dumping of human and industrial waste such as hospitals, And the treatment plant has a low capacity and has not been maintained over the previous years and cannot cope with the steady increase in population numbers and activities (34). As for the lead metal, it has the highest concentration in all study regions through the autumn season followed the summer season. The high concentration of the lead metal, especially in the hot seasons, may be due to the consumption of leaded fuel, which is associated with the increase in daylight hours and the increase in the hours of power cut, which results in an increase in the operating hours of the electric generating devices. In the environment, which eventually reaches the aquatic environment with the dust falling in the rivers and this is consistent with (35). And

that the high concentration of lead in the city of Diwaniyah and Hamzah may be due to the traffic momentum due to the increase in the population according to (36), which in turn leads to an increase in the number of cars and other means of transport still using gasoline added to the tetraethyl lead, causing serious problems for the environment and this agree with (37). The reason for the high concentration of zinc in the studied fish tissue may be due to its absorption in large quantities of fish because it is necessary in the conservation of manna and is necessary for the metabolism of living organisms (38) Also, the high concentration in the city of Diwaniyah river may be due for being a big city features a number of large population is an increase in the human and industrial activities and increased human waste and lack of vegetation is important in reducing the damage of heavy metals in the environment (39). The current study recorded the lowest concentration of nickel during spring and winter while the highest concentration was in autumn and summer. The higher concentration of nickel in *Barbus grypus* tissues compared to *Liza abu* fish may be due to the fact that *Barbus grypus* fish have a greater tendency to accumulate heavy metals in their tissues. Increasing the concentration of heavy metals in fish tissues is strongly correlated with increased concentration in the aquatic environment (40).

Conclusion

1-The ability of fish to accumulate some heavy metals in their tissues at high concentrations special in the summer and autumn.

2-There is a close relationship between parasitism and pollution, the greater the percentage of river pollution increased the concentration of heavy metals in the tissues of fish infected with parasites.

3-The study showed that the most heavy metals concentrated in fish tissue is the liver and muscle.

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