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STUDY THE EFFECT OF CONTAMINATION IN HEAVY METALS AND MICROBIAL LOAD IN IRAQI FISH

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Abstract

This study was conducted to investigation of heavy metals contamination as well as microbial load in fish samples from Baghdad's Tigris River. A 60 local carp fish samples in total were collected from different markets in Baghdad province. Bacterial isolate identification was confirmed by Viteck2, while heavy metals (HM) concentrations were detected by Atomic Absorption Spectrophotometer. The zoonotic bacterial isolation shows (highest – lowest) *E. coli, Staph aureus, Aeromonas spp, Klebsiella spp, Enterobacter spp, Citrobacter spp, Proteus spp, Yersinia enterocolitica.* While, HM Lead (Pb), Cadmium (Cd), Copper (Cu), Mercury (Hg) and Nikle (Ni) average counts results for the local river, lake and marsh fish meat (ppm) were exceeding their limits according to FAO, EC, WHO. Therefore, the researchers and consumers should be aware of the risk of ingestion contaminant fresh-fish meats with zoonotic bacteria and HM as well, which will be affecting their health status as a result. Thus, further studies for other fresh-fish types are urgently needed.

Keywords: heavy metals, microbial load, river, lake, marsh fish, AAS, vitek.

Introduction

Pollution including the ordinary destruction condition of the environment via human activities (1, 2). In recent years, a considerable attention to the environmental contamination's issues through a widespread range of chemical pollutants, such as the heavy metals (HM), globally in general and in Iraq in particular (3,4,5). HM are attributed to the set of metalloids as well as metals which have an atomic density higher than 4 g/cm^3 (trace elements) (6, 7). Metals in minerals as well as rocks were generally harmless; however, it became potentially toxic when they dissolved in water (8, 9). It is known that some trace metals were essential for biological processes and were needed for biota to complete as well as developing their life cycle; nevertheless, it could be toxic when their concentrations exceed their required for an exact nutritional response (10,11, 9), due to HM are not metabolized, therefore, it persistent and bio accumulated (12,13). The pollution of aquatic environments by HM, especially rivers, has become a worldwide issue of extreme and scientific concern since it is non-degradable and majority of them have such a toxic effect on organisms causing hazard to humans' fishery resources ingesting (14, 15, 4).

In Iraq, (16) demonstrated that the main bacterial isolates from variety organs of fresh water fish of Tigris-River samples including *Enterobacter spp. Staphylococcus spp., E. coli, Citrobacter spp.*

and Proteus spp. respectively, they also reported the heavy metals (Hg, Pb, Cd) concentrations exceed their limits according to the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) and World Health Organization (WHO) recommended.

(17) demonstrated the histopathological effect of accumulated Pb and Cd on the gills, muscles, renal and hepatic tissues of Tigris river's fish (*Luciobarbus xanthopterus*), which exceeded the maximum levels referred by WHO, therefore, it suggested that this kind of fish could be used as a remarkable bio-monitoring in assessing metal pollution in numerous aquatic environments due to their ability to bio-accumulate some heavy metals in their tissues with various levels and so it could be used to detect the contamination level.

Furthermore, In Baghdad, (18) demonstrated the evaluation (ppm) of Cd, Mn and Zn in filtered (0.004, 0.007 and 0,023) and the Tigris River sediments (1.38, 231.4 and 86) as well as the Ghattan tissues (muscles, gills and intestine), While Al-Hammam, (19) reported Cd concentration (ppm) only in Muscle (0.014), gills (0.012) and intestine (0.011).

(17) revealed marked differences ($P < 0.05$) in the aqueous metals (mg/L) content; i.e., the maximum means were analyzed in two out of three sites that the 3rd site were shown Pb (1.25) > Cd (0.06) followed by 2nd site; Pb (1.04) > Cd (0.05), her metal accumulation results in fish *(Luciobarbus xanthopterus*) tissues were detected in muscles, livers, gills, kidneys.

In Baghdad (21, 22) revealed that most poultry or Buffalo milk samples showed the highest toxic heavy metals concentration (Pb, Cd) which exceed the limit of FAO/WHO, EC, CN, as well as some zoonotic bacterial isolates (*E. Coli*, *Salmonella* Spp., *Enterobacter Spp.*, *E. Proteus* spp., *E. coli* O_{157}:H_7, *Pseudomonas* Spp., *Staph. aureus*, *Klebsiella* Spp., and *Yersinia enterocolitica* were reported, thus, the food is non-safe to be consumed by humans As a result, the current study was designed to focus on fresh-river fish meat and its contents in Baghdad.

Materials and methods

Sample collection

Sixty samples of local carp fish (river, lake and marsh) each sample purchased from markets in Baghdad and collected in labeled sterile plastic bags then sent in cool box to the Microbiology laboratory for isolation and identification of bacteria. Also, for detection of heavy metal (Pb, Cd, Cu, Hg, and Ni).

Isolation and identification of bacterial contamination

In polyethylene sac (250) gram of raw fish meat (without bone) then mixed well in the stomacher, each meat sample was inoculated into peptone broth with (25gr/225ml) then incubated for (24-48) hours at (37°C). Following incubation, 0.1ml of the inoculated broth was sub-cultured onto Nutrient agar, Blood agar, Eosin methylene blue (EMB), MacConkey agar, Xylose lysine deoxychocolate agar (XLD), Mannitol salt agar, and CIN agar plates. Gram staining is followed by biochemical analysis on the Vitek 2 system.

Heavy Metals Analysis

According to (23, 24), 10 grams of meat were baked at 80°C for 48 hours. Or until completely dry. The heavy metals analysis was carried out by incorporating concentrated HNO3 into the

dried fish meat. (0.5 gr of dried meat and 5 mL of concentrated HNO3) were added to the digestion flask. The mixture was then heated at 80-90 °C for 10 mints by placing it on a hot plate for digestion before being raised to 100 °C and more acid was added up to 3-5 mL until a clear solution was obtained. The samples were cooled to room temperature, filtered through filter paper, and the volume was increased to 25 ml using non- ionized water. A blank sample was also prepared. The Atomic Absorption Spectrophotometer was invented. was used to identify the presence of heavy metals in meat samples (FAAS, ShimadzuAA-7000F) (23, 15, 7). SAS, 2010, was used for statistical analysis of the samples.

Results

The results of this study showed heavy metals concentrations in local river, lake and marsh fish meat samples were tested in table (1). The average counts of **Pb** concentrations were 1.87 ppm, 2.0 ppm and 1.98 ppm in local river, lake and marsh fish meat samples, respectively (Fig.1). Mean concentrations of **Cd** were 0.61 ppm in local river samples and 0.58 ppm in lake meat samples and 0.54 ppm in local marsh samples (Fig.2).

The average counts of **Cu** concentrations were 33 ppm and 41 ppm and 36 ppm in local river, lake and marsh fish meat samples, respectively (Fig.3). Mean of total **Hg** count were 0.32 ppm in local river fish samples and 0.41 ppm in local lake fish samples and 0.51 ppm in local marsh fish (Fig.4). Mean of total **Ni** count were 45 ppm in local river fish samples and 40 ppm in local lake fish samples and 49 ppm in local marsh fish (Fig.5).

Table (1): The average and standard error of the heavy metals' concentration in local river, lake and marsh fish sample

Heavy Metal	Local river fish	Local lake fish	Local marsh fish	Permissible upper limits		
				FAO	EC	WHO
Lead (Pb)	1.87 ± 0.37	2.0 ± 0.41	1.98 ± 4.5	2	1	0.5
Cadmium (Cd)	0.61 ± 0.09	0.58 ± 0.12	0.54 ± 0.11	0.5	0.5	0.5
Copper (Cu)	33	41	36	30	30	-
Mercury (Hg)	0.32 ± 0.12	0.41 ± 0.15	0.51 ± 0.11	0.5	0.5	0.5
Nickel (Ni)	45	40	49	55	40	30

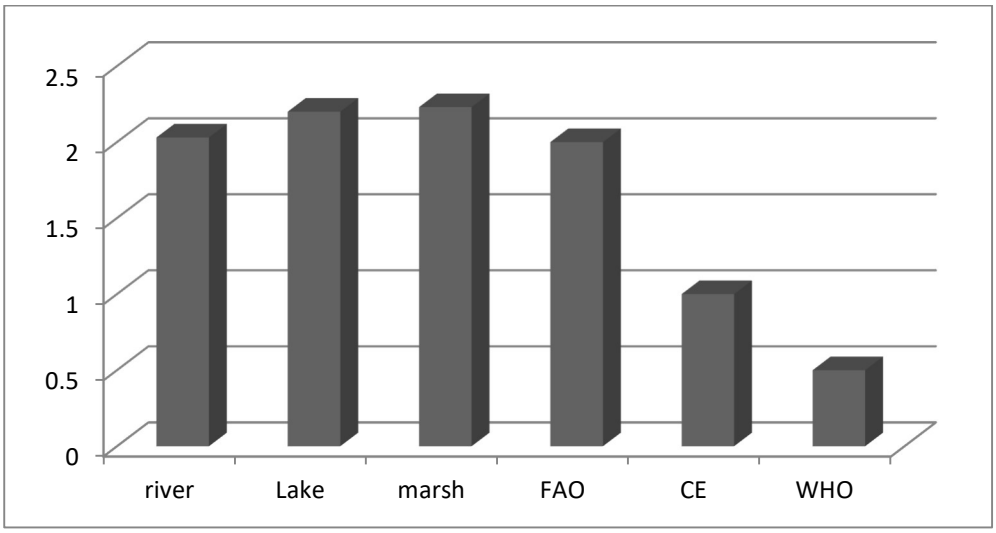

Fig. (1): The average of Pb in local river, lake and marsh fish sample

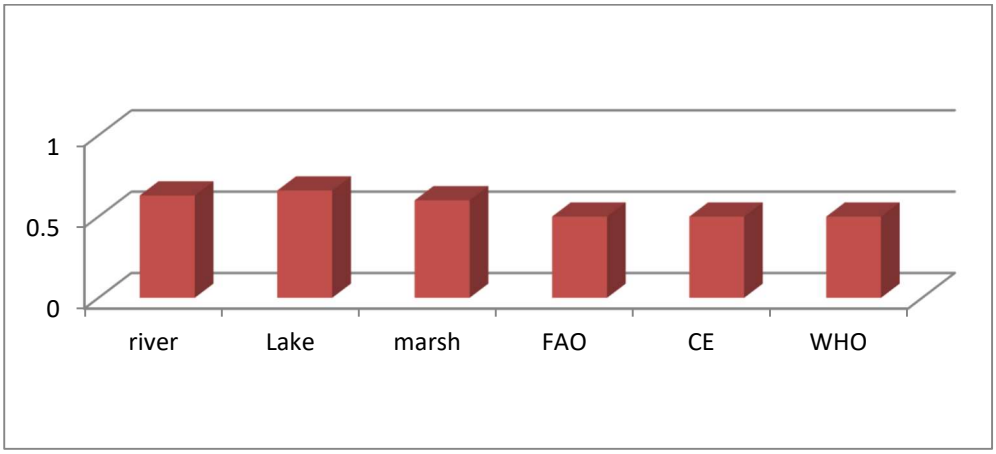

Fig. (2): The average of Cd in local river, lake and marsh fish sample

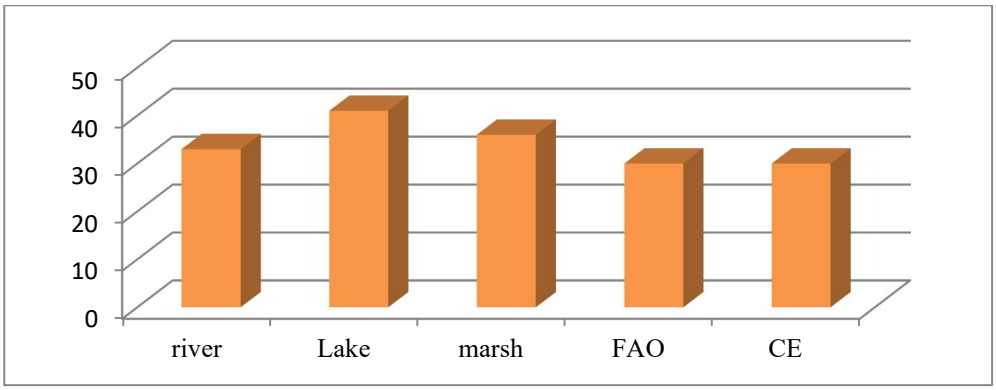

Fig. (3): The average of Cu in local river, lake and marsh fish sample

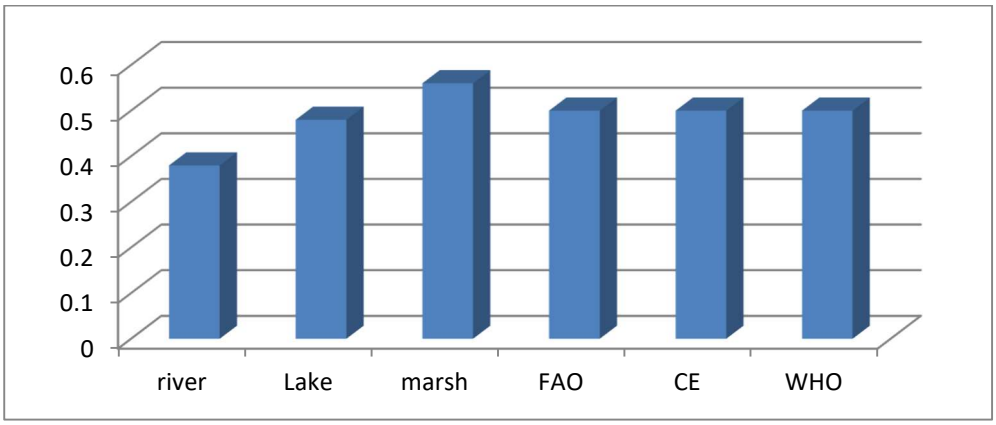

Fig. (4): The average of Hg in local river, lake and marsh fish sample

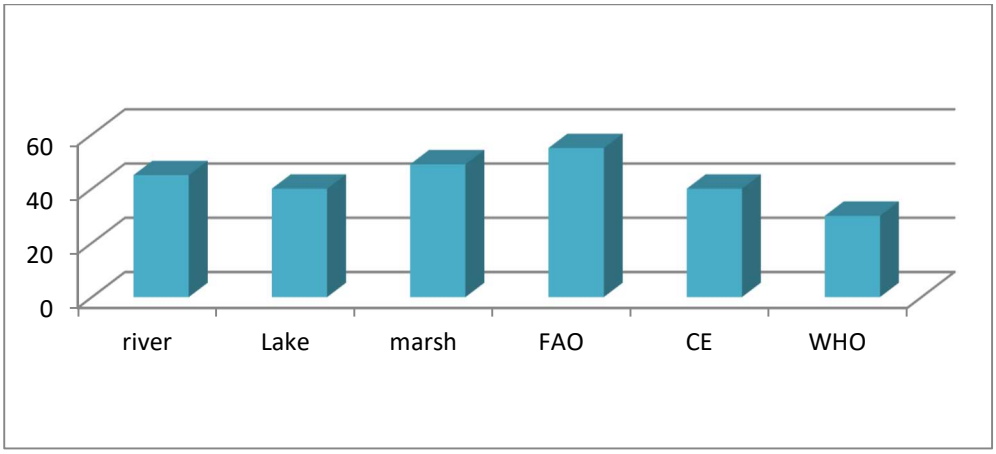

Fig. (5): The average of Ni in local river, lake and marsh fish sample

The results of this study showed the percentage of bacterial isolates in local river, lake and marsh fish meat samples were tested in Table (2). The results of bacterial isolation showed the presence of 30 isolates of local river fish meat samples where many samples contained mixed infection classified into 9 types includes *E.Coli* (60%), *Staph. aureus* (30%), *Pseudomonas auregenosia* (10%), *Aeromonas* Spp. (15%), *Klebsiella* Spp. and *Enterobacter* Spp. (10%), *Proteus* Spp., *Citrobacter* Spp. and *Yersinia enterocolitica* (5%).

Also, the results of the bacterial isolation of local lake fish samples showed the existence of 38 isolates divided into 9 types involve *E.Coli* (70%), *Staph. aureus* (30%), *Pseudomonas auregenosia* (15%), *Klebsiella* Spp. and *Enterobacter* Spp. (15%), *Aeromonas Spp.* (20%), *Proteus* Spp. (5%), *Citrobacter* Spp. and *Yersinia enterocolitica* (10%). while, the results of the bacterial isolation of local marsh fish meat samples showed the existence of 25 isolates divided into 9 types involve *E.coli* (45%),*Staph. aureus* (35%), *Pseudomonas auregenosia* (5%), *Klebsiella* Spp. and *Citrobacter Spp.* (5%), *Aeromonas spp. and* Enterobacter Spp. (15%), *Proteus* Spp. and *Yersinia enterocolitica* (0%).

Table (2): The percentage of isolated bacteria from local river, lake and marsh fish

Isolated bacteria	River fish 20 sample	lake fish 20 sample	Marsh fish 20 sample
E. Coli	12 60%	14 70%	9 45%
Staph aureus	6 30%	6 30%	7 35%
Pseudomonas auregenosia	2 10%	3 15%	1 5%
Aeromonas Sp.	3 15%	4 20%	3 15%
Klebsiella Sp.	2 10%	3 15%	1 5%
Enterobacter Sp.	2 10%	3 15%	3 15%
Proteus Sp.	1 5%	1 5%	0
Citrobacter Sp.	1 5%	2 10%	1 5%
Yersinia enterocolitica	1 5%	2 10%	0

Discussion:

To date, the awareness in which water and its normal creatures, such as fish would be considered as one of the most life-threatening ordinary resources is increasing globally (17). The bacterial and HM contaminant in the current study findings were agreed with (16) who revealed that bacterial isolates from Tigris-River-fresh-water-fish samples were *Proteus Spp, Enterobacter Spp, E. coli, Staphylococcus Spp, and Citrobacter Spp.,* however, the current study shows other isolates such as *Aeromonas Spp, Klebsiella Spp ,Yersinia enterocolitica* and *Pseudomonas auregenosia.* In addition, their research group also revealed that Hg, Pb, Cd concentrations exceed their limits according to the CEFAS and WHO recommended which also agreed with the current study which is also reports that Ni level is exceeding the limit of Food and Agriculture Organization (FAO) and WHO. In Baghdad, the current study findings are in agreement with (19, 18, 17) including the HM (Cd, Pb), but not in Hg, Ni, Cu. While in Duhok city markets, during two seasons, (4) reported that the HM determination in three trading fish species' muscles were Fe, Al, Mn, Cu, Zn within their lowest limits, while Ni, Pb, Co, Cd and Cr were non-detectable. However, (17) was focusing on the effect of HM on histopathological changes in different tissues of fresh river meat, while, (18) reported these HM contaminants in the river sediments and in the Ghattan tissues (muscles, gills and intestine) as well. While, (19) Cd levels in the Al-Gharraf River ranged from 6.0 to 10.1 mg/l., and (20) reported Cd concentration (ppm) only in Muscle, gills and intestine. (21) Was also agreed with the current study results considering the HM, and zoonotic bacteria contamination, however their study including chicken meat.

The present research agreed with (26), who largely isolated Enterobacteriaceae from ear infections in humans and dogs, which attributed them to the use of contaminated food and water, as it is one of the opportunistic germs that are widespread in nature and when they enter the body, causes various infections. Furthermore, (26) isolated *Pseudomonas auregenosia* from the human ear, indicated that this bacterium prefers to grow in humid and warm places, so swimming pools are the ideal environment for its growth, causing ear infections, and it's called swimmers ear.

The fundamental reasons for fresh water rivers biological contamination were detailed by (25) who observed the Gram positive bacteria predominance among Cr-tolerant strains obtained from sewage sources within their studied metals' concentrations, in addition to their demonstration of Gram-negative-bacteria in which it tended to be extra-tolerant to HM than the Gram positive, Thus, the term tolerance seemed to be more appropriate to be referred to the bacterial strain growth ability within the high metal concentrations presence. Therefore, we suggested that HMs could be useful tool as a remarkable monitoring for bacteria pollution assessing in abundant aquatic environments according to their ability of producing visible bacterial colonies within the 24 hours within the chosen HM concentrations presence i.e. it could be used as a detector for the contamination level. For all these studies findings which suggested that more awareness and precautions must be followed and further studies are urgently needed including a discovery of rapid kit and natural alternatives that might be used to reduce or eliminate the HM and zoonotic bacteria effects in fish meat.

Conclusion:

The current study revealed that the zoonotic bacterial isolation from the highest – lowest shows *E. coli, Staph aureus, Klebsiella Spp, Aeromonas Spp, Enterobacter Spp, Citrobacter Spp, Proteus Spp, Yersinia enterocolitica and Pseudomonas auregenosia.* While, HM average counts results of local river, lake and marsh fish meat (ppm) show: Lead (Pb): 1.87, 2.0, 1.98; Cadmium (Cd): 0.61, 0.58, 0.54; Copper (Cu): 33, 41, 36; Mercury (Hg): 0.32, 0.41, 0.51; Ni: 45, 40, 49 ppm, respectively.

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