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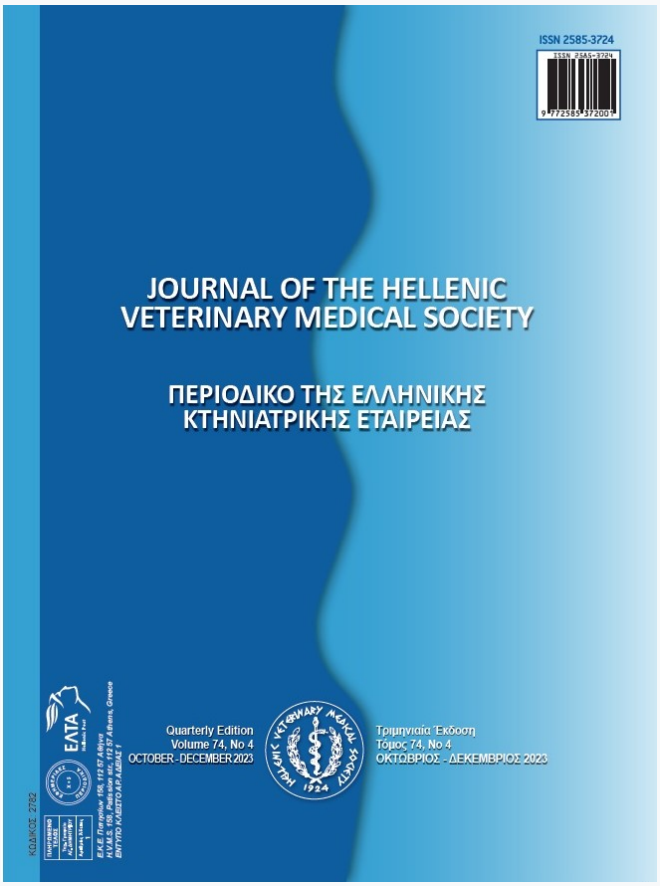

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Heavy Metal Residues and Microbial Status of Farmed and Channeled Cat Fish

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ABSTRACT: The purpose of the study was to look into the bioaccumulation of Cu, Cd, Pb, Mn, Fe and Zn in the muscles and livers of *Clariasgariepinus* catfish from farms and channels sources of River Nile in Kafr El-Sheikh province, Egypt, with microbiological evaluation of fish quality, and detection of Shiga toxin genes (stx1, stx2), and metal resistant genes (MRGs) in isolated and identified Shiga toxin-producing *E. coli* (STEC) from fish muscles to predict the crucial hazards on consumers' health from fish of both sources. The results showed increased metal levels in the tissues and liver of farmed fish than in the channeled. Lead (Pb) was detected by very high concentrations (16.39±1.26 and 11.90±1.68) mg/kg in 20% of examined muscles and liver samples of farmed fish, all detected levels of Pb were exceeded the permissible limits (PLs). Pb was not detected in the channel source. Mn, Fe and Zn were detected in all samples of both sources by (100%), but Mn and Fe only exceeded the PLs, while Zinc was with values lower than PLs. The (TBC), (TCC), Contamination rates with *E.coli*, STEC, and *E.coli* with (nccA and czcD) were found also significantly higher ($p < 0.05$) in examined muscle samples from farmed than channeled source.Stx2, nccA, and czcD genes were detected by 10%, 30%, and 20% respectively from identified STEC samples. In conclusion, examined farmed catfish was with higher levels of heavy metals residue and bacterial pollution than channel catfish. The metal resistant bacteria may be considered an indicator of heavy metal contamination in the aquatic ecosystem.

Keywords: *Clariasgariepinus*; Heavy metals; Bacterial count; *E.coli*; STEC; MRGs.

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INTRODUCTION

Fish play an important role in food security in both low and middle-income countries, while it considered one of the main sources of the national income in Egypt, conversely, they are prone to contamination with chemical and biological hazards which affect the consumer health and decrease the request for consuming fish (Eltholth et al., 2018). Aquaculture products differ according to the culture system in aquatic food safety hazards that may involve contamination by toxic metals and foodborne pathogens due to pathogenic bacteria that are introduced into the aquatic environment through contamination by human or animal feces or otherwise (Reilly and Kaferstein, 1997).Due to the bio-accumulative potential of heavy metals in tissues of aquatic organisms and the difficulty of breaking down, it considered major pollutants in aquatic environments, heavy metals such as lead(Pb), cadmium(Cd), and copper(Cu) modify the host's immune system to increase susceptibility to auto-immune diseases and infections (Paschoalini et al., 2019).Heavy metals pollution in the environment originates from anthropogenic sources such as mining, power stations, and the use of pesticides containing metal, fertilizer, and sewage sludgeand finally are expanded through the food chain, giving rise to serious ecological and health problems (Nriagu and Pacyna, 1988).The toxicological effect of heavy metals on human beings and other living organisms like plants, animals, and many microorganisms gives heavy metals a special concern (Atlas and Bartha, 1993).Most of them are characterized by the accumulation in tissues, leading to the poisoning of fish. So, fish are playing an important role in monitoring heavy metals pollution levels in the aquatic systemas bio-indicator (Yarsan and Yipel, 2013). Some metals, like Cd, Pb, etc., may be poisonous, whereas others, including Cu, Zn, Fe, and Mn, are necessary (Biswas et al., 2011). These can also invoke toxic effects when excessive intake of the metal occurs.

Contamination by toxic metals such as lead could be derived from dissimilar sources, along with discharge from sewage, agriculture, and industrial effluent, unexpected chemical waste releases and fishing boat gasoline leaks (Kolappan and Satheesh, 2011). Fish ingest heavy metals through their feeding, and the silt around them keeps a lot of heavy metals at the bottom sediment (Burgos & Rainbow, 2001).

The consumption of fish carrying heavy metals in their flesh and liver for long period may cause both immediate and long-term degenerative consequences, particularly to the neurological system, liver, and kidneys (Ibrahim et al., 2006). In addition, the creation of free radicals causes DNA damage as a result of the carcinogenic and teratogenic effects (Valko et al., 2005).

Heavy metals also affect the growth, morphology, and biochemical activities of the microbial population, resulting in diminished biomass as well as diverseness. High concentrations of metals are bactericidal, so the exposed bacteria accordingly promote resistance to tolerate the effect of the metals through efflux, or reduction of metal ions (Redaktor and Cecilie, 2021).Bacteria that have resistance to metals have a critical role in the biogeochemical cycling of ions of those metals (Asif et al., 2012).Many bacterial strains contain genetic determinants often found on their plasmids and transposons to resist heavy metals such as Pb, Cd, Cu, and others (Silver and Misra, 1988).

Escherichia coli, a gram-negative bacteria, is one of the most reliable thermo tolerant indicator species used to monitor fecal pollution in aquatic environments which indicates the area is polluted with feces of animal or human origin (Terzi, 2018). It is also known to carry heavy metal resistance genes (MutluNisa et al., 2005; Ture et al., 2020; Yang et al., 2020)

One of the most prevalent fish-related bacteria, *E. coli*, is known to lead to food-borne diseases in both industrialized and poor nations (Bakhiet and Gebrel 2020). Food- and water-borne infections called Shiga toxin-producing *E. coli* (STEC) also cause mild to serious diseases in humans through more than 70 different serotypes. From moderate to bloody diarrhoea, hemorrhagic colitis (HC), and finally hemolytic uremic syndrome (HUS), there are several illnesses (Ali et al., 2014; El-Gamal et al., 2016)

In Egypt, fish are harvested either from wild sources or from the nation's many aquaculture plants. According to Bayomy et al. (2015), *Clariasgariepinus*, also known as quarmote in Egypt, is one of the most significant freshwater fish species there. It makes up around 17.5% of Egypt's total Nile catch (Abdel-Hafez and El-Caryony, 2009). These fish species are plentiful all year long. They provide valuable and affordable animal protein sources (Hagras et al., 2017).

Little information concentrates on the freshwater fish contamination from channel branches of the riv-

er Nile which is a significant source for the popular fisheries in Egypt, African catfish are most frequently caught from channels and branches of the river Nile, in addition to aquaculture farm sources (Hagras et al., 2018).

Therefore, the primary goal of this study was to evaluate the quality of channeled catfish from the Meet Yazeed channel, which originates from the Damietta branch of the River Nile and empties into Lake El-Burullus, in kafrelsheikh province. chemically and microbiologically through determining, Copper(Cu), Cadmium (Cd), Lead (Pb), Manganese (Mn), Iron (Fe), and Zinc (Zn) levels in relation to microbial status using total bacterial count (TBC) and total coliform count (TCC), and also detection of pathogenic *E. coli* which carrying heavy metal resistant genes (MRGs) in catfish from both sources, considering the isolation of such strains as a marker for heavy metals contamination (Garai et al., 2021), and then assess the level of hazard and risk from both sources on the public health.

MATERIALS AND METHODS

Samples collection and preparation

Fifty alive *Clariasgariepinus* catfish (25 of each source, farmed and channeled) were collected singly with nearly the same weight from Kafr-el sheikh farms and Meet Yazeed channel which comes from the Damietta branch of River Nile. Each fish is considered one sample. The collected fish were washed to remove loosely held particles with water before they was packed in tight sterile and labeled polyethylene bags each sample were packed and conserved in an insulated icebox to the laboratory of Animal Health Research Institute.

The samples were handled in the laboratory within 2 hours. Each sample was disinfected by using 70% ethyl alcohol solution on the body surface of the fish, then prepared by cutting out, with sterile instruments, then the fish fillet was divided into two parts for chemical and bacteriological examination (AOAC, 2005).The liver as a whole was taken for heavy metal estimation.

Heavy metals estimation

Heavy metals concentrations were estimated in prepared samples of fish fillet and liver using the atomic absorption spectrophotometry (Model-analyst 200, PerkinElmer). Digestion of the samples was done in a microwave digestion system (Speed-wave four, Bergh of, GmbH, Germany) using 5 ml. Nitric acid ($HN0_3$) 65% with 2 ml. hydrogen peroxide (H_2O_2) 30% and diluted with deionized water to 25 ml according to the technique described by AOAC official method 999.11 (AOAC, 2005). The data were expressed as "µg/g wet weight (ppm)" and contrasted with Egyptian standards (EOS) from 2005, 2010, and other years.

Bacteriological analysis

Total Bacterial Count (TBC)

For counting all bacteria (Maturin and Peeler, 2001). Fish fillet samples (25 g) were homogenized with sterile buffered peptone water (225 mL) to create the initial tenfold serial dilution from which plates on standard plate count agar (Oxoid Ltd, UK) were made, then incubation at 37 °C for 48 ± 2 h.

Total Coliform Count(TCC)

According to APHA, MacConkey broth (Oxoid, UK) was inoculated with serially diluted samples and incubated at 37°C for 48 hours (2005). The production of acid and gas was recorded. The relative MPN index of coliform organisms was obtained according to APHA, (1998).

Isolation, and identification of *E. coli*

From MPN positive tubes, a loopful was streaked on EMB agar (LABM, UK) (Eosin Methylene Blue). Typicalcolonies with metallic sheen were isolated (FDA, 2001)and confirmed by biochemical examination (APHA, 2001)

.Serological identification

According to Kok et al., (1996) and Quinn et al., (1994), biochemically verified *E.coli* were serologically identified for Shiga toxin-producing *E. coli* (STEC) using quick diagnostic antisera kits (DENKA SEIKEN Co., Japan) (2002).

Polymerase chain reaction (PCR)

Following the manufacturer's instructions, DNA was extracted from bacteria using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). From the sample suspension, 200 µl were incubated with proteinase K (10 µl) plus lysis buffer (200 µl) for 10 min. at 56**°C**then, 100% ethanol (200 µl) was added to the lysate after incubation. The sample was then washed and centrifuged. Nucleic acid was flushed with elution buffer (100 µl).

Oligonucleotide Primers used were from Metabion (Germany), are listed in Supplementary table 1.

Amplification of PCR

Duplex PCR for *stx1* and *stx2* genes, and multiplex PCR for *pbrA*, *nccA*, and *czcD* genes were performed. Briefly, 50 µl of total PCR reaction volume was utilized containing 25 µl of Master Mix Emerald Amp Max PCR (Takara, Japan), 1 µl of each forward and reverse primer (20 pmol), 15 µl of free water, and 6 µl DNA template. Reaction in an applied bio-system 2720 thermal cycler was performed.

Analysis of PCR products

PCR products were separated on 1% agarose gel (Applichem, Germany, GmbH) using gradients of 5V/cm in gel electrophoresis, (40 µl) of PCR products were loaded in each gel slot. To determine the fragment sizes, Gene ruler 100 bp ladder (Fermentas, Germany), and Gene direx 100-3000bp DNA ladder H3 RTU (Gene direx, Taiwan) were used. Using a gel documentation device, the gel was captured on camera (Alpha Innotech, Biometra). Software was used to analyze the data.

Analytical Statistics

Using the SPSS statistical programmer version 20, measured data were analyzed using ANOVA and Duncan's multiple comparison test to evaluate various parameters between the two study sources. The results were expressed as means and standard errors of the means (SEM). When taken into account, a p-value ≤ 0.05 is statistically significant

RESULTS

The quality of farm and channel catfish has been evaluated chemically by determining, Copper (Cu), Cadmium (Cd), Lead (Pb), Manganese (Mn), Iron (Fe), and Zinc (Zn) levels in relation to microbial status using total bacterial count (TBC) and total coliform count (TCC), and also detection of pathogenic *E. coli* which carrying heavy metal resistant genes (MRGs) in catfish from both sources. As shown in table 2, the concentrations of detected heavy metals in farmed catfish samples, either in muscle or liver, were significantly higher than those in channel catfish samples (Figure 3). Briefly, Pb showed a high mean level in farmed catfish samples, followed by Fe, Mn, and Zn, while in the channeled catfish samples the order was Mn, followed by Fe, then Zn, while Pb was not detected. Moreover, Cu and Cd in different edible (muscles) and non-edible (liver) fish tissues collected from both studied sources were not detected. The total bacterial count, coliform count (Table 3), Contamination rates with *E.coli*, and the occurrence of Shiga toxin producing *E.coli* (Table 4) with shiga toxins genes *(stx1*and *stx2*) and heavy metal resistance genes (MRGs) *(nccA), (czcD)* (Table 5, Fig 1, and Fig 2) were significantly higher in examined catfish muscle samples from farm source than from channel source.

DISCUSSION

Heavy metals in samples collected from the two sources.

The results of Pb, Mn, Fe, and Zn accumulation are similar to those reported by Elnimr (2011) and Afshan

Table 1. Lists of the target genes' primer sequences, amplicon sizes, and cycle conditions of *stx1, stx2, nccA, pbrA,* and *czcD*genes *in E. coli.*

Target gene	Primers sequences(5'-3')	Amplified segment (bp.)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Stx1	F/ ACACTGGATGATCTCAGTGG R/ CTGAATCCCCCTCCATTATG	614	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	Dipineto et al.., 2006
Stx2	F/ CCATGACAACGGACAGCAGTT R/ CCTGTCAACTGAGCAGCACTTTG	779						
nccA (Ni, Cr, Cd resistance)	F/ ACGCCGGACATCACGAACAAG R/ CCAGCGCACCGAGACTCATCA	1141						
pbrA (Pb resistance)	F/ ATGAGCGAATGTGGCTCGAAG R/ TCATCGACGCAACAGCCTCAA	2396	94°C 5 min.	94°C 30 sec.	57°C 1 min.	72°C 2 min.	72°C 10 min.	Mustafa et al., 2021
czcD (Cr, Zn, cd resistance)	F/ CAGGTCACTGACACGACCAT R/ CATGCTGATGAGATTGATGATC	398						

F= forward
R= Reverse

Table 2. Concentrations of heavy metals (mean ± SE) in wet weight muscles and liver of cultured (farmed) and wild (channeled) catfish (25 samples for each).

catfish type	sample type	Cu mg/kg	Cd mg/kg	Pb mg/kg		Mn mg/kg		Fe mg/kg		Zn mg/kg	
				+ve samples		+ve samples		+ve samples		+ve samples	
				No (%)	Means ±SE	No (%)	Means ±SE	No (%)	Means ±SE	No (%)	Means ±SE
farmed cat fish	Muscle	ND	ND	5(20)	16.39±1.26**	25(100)	4.14±2.37*	25(100)	37.54±29.63*	25(100)	11.17±9.61
	liver	ND	ND	5(20)	11.90±1.68**	25(100)	4.32±2.79*	25(100)	39.86±30.17*	25(100)	12.79±10.83
channeled cat fish	Muscle	ND	ND	0(0)	0	25(100)	3.62±2.47*	25(100)	35.53±28.63*	25(100)	9.32±8.11
	liver	ND	ND	0(0)	0	25(100)	3.95±2.31*	25(100)	36.57±29.96*	25(100)	10.34±7.88
Samples exceed pl. (farmed)	Muscle	0	0	5(20)		9(36)		7(28)		0(0)	
	liver	0	0	5(20)		15(60)		12(48)		0(0)	
Samples exceed pl. (channeled)	Muscle	0	0	0(0)		4(16)		3(12)		0(0)	
	liver	0	0	0(0)		10(40)		5(20)		0(0)	
MPL.	mg/kg	20*	0.05***	2.0*		2.5**		30		40*	

Cu= copper, Cd= Cadmium, Pb= Lead, Mn= Manganese, Fe= Iron, Zn= Zinc
(P≤0.05)* Means significant, (P≤0.01) ** means highly significant.
MPL= maximum permissible limits for metals in fish
MPL* acc.to: Abdalhamid et al., (2013), Pb= 2, Zn= 40, Cu= 20 mg/kg.
MPL** acc.to: WHO, (1993) and Badr et al., (2014) Mn= 2.5 mg/kg wet weight in tissues.
MPL*** acc.to: EOS, (2010) = Egyptian Standard ES.7136, (2010): Pb=0.1, Cd = 0.05, Fe=30 (mg/kg wet weight)
EU, (2006): Pb= 0.3
ND, Not-Detected
SE= Standard error of mean
pl = permissible limit

Table 3. Mean total bacterial count (mean ± SE) (CFU/g), total coliform count (log10 CFU/g), and rate of isolation of *E.coli* for cultured (Farmed) and wild (Channeled) catfish muscles. n=25 for each sample.

Type of analysis	farmed cat fish (25 samples)					channeled cat fish (25 samples)					*Egyptian Standard CFU/g
	Positive samples					Positive samples					
	No/%	Min	Max	Mean	Samples exceed EOS No (%)	No/%	Min	Max	Mean	Samples exceed EOS No (%)	
TBC (CFU/g)	25 (10%)	3×10^2	7×10^6	$4.2\times10^4 \pm 1.2\times10^2$	10 (40%)	25 (100%)	2×10^2	5×10^6	$2.7\times10^4 \pm 0.6\times10^2$	4 (16%)	1×10^6
TCC (CFU/g)	18 (72%)	0.3×10	4.9×10^4	$7.3\times10^2 \pm 1.1\times10$	6 (24%)	13 (52)	0.3×10	3.9×10^3	$1.6\times10^2 \pm 0.2\times10$	2 (8%)	1×10^2
E.coli isolation rate	14/25 (56%)					9/25 (36%)					-
Shiga toxin producing *E.coli* (STEC)	6/25 (24%)					4/25 (16%)					-
E.coli with HMRGs	3/5 (60%)					2/5 (40%)					-

*MPL stated by Egyptian Standard (EOS, 2005) for fresh chilled fish
TBC= Total Bacterial Count
TCC= Total Coliform Count
HMRGs= Heavy Metal Resistant Gene
CFU/g= Colony Forming Unit/Gram

Table 4. Serological identification of Shiga toxin producing *E.coli* (STEC) isolated from Farmedand Channeled catfish muscles (n=25) for each type.

Serodiagnosis	No.	%	Farmed catfish (25)	Channeled catfish (25)
O20	1/50	2	1	0
O26	1/50	2	1	0
O91	1/50	2	1	0
O111	2/50	4	1	1
O117	1/50	2	0	1
O128	1/50	2	0	1
O146	2/50	4	1	1
O163	1/50	2	1	0

Table 5. Results of duplex PCR for Shiga toxins genes *(stx1* and *stx2*), and multiplex PCR for Heavy metals resistance genes (*nccA*), (*pbrA*), (*czcD*) of 10 isolates randomly taken from total 23 isolates of *E. coli*.

source	Sample	*Stx1*	*Stx2*	*pbrA*	*nccA*	*czcD*
Farms	1	-	-	-	-	-
	2	-	-	-	-	+
	3	-	-	-	-	-
	4	-	+	-	+	-
	5	-	-	-	+	-
Channels	6	-	-	-	-	-
	7	-	-	-	+	+
	8	-	-	-	-	-
	9	-	-	-	-	-
	10	-	-	-	-	-
Total	10	0	1	0	3	2
%		0	10	0	30	20

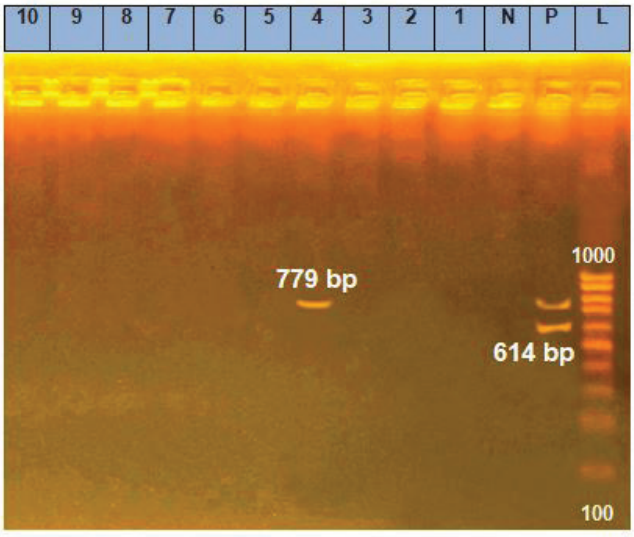

Fig 1. Agarose gel electrophoresis of duplex PCR for Shiga toxins genes *stx1* (614bp.), *stx2* (779bp) of *E. coli.*

Lane L: 100bp ladder as molecular size DNA marker .Lane P: Control positive genes. Lane N: Control negative. Lanes 4: Positive for *stx2* gene. Lanes 1 to 10 except 4: negative for *stx2* gene. Lanes 1 to 10: negative for *stx1* gene.

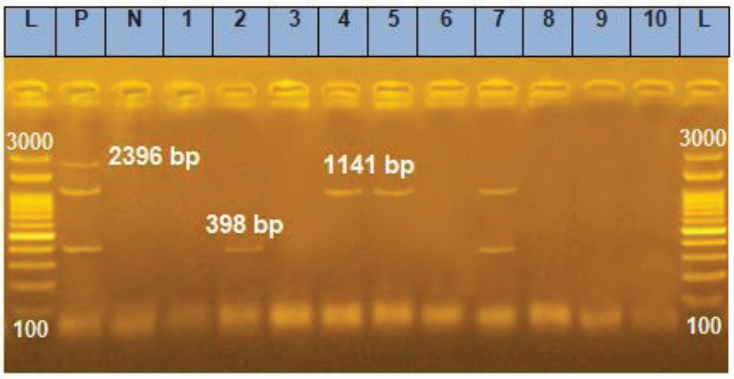

Fig 2. Agarose gel electrophoresis of multiplex PCR for and Heavy metals resistance genes *nccA* (1141bp), *pbrA* (2396bp), *czcD* (398bp) multiplex PCR of *E. coli*.

Lane L: 100bp. ladder as molecular size DNA marker. Lane P: Control positive genes. Lane N: Control negative. Lanes 4, 5, 7: positive for *nccA* gene. Lanes 1, 2, 3, 6, 8, 9, 10: negative for *nccA* gene. Lanes 2, 7: positive for *czcD* gene. Lanes 1, 3, 4, 5, 6, 8, 9, 10: negative for *czcD* gene.

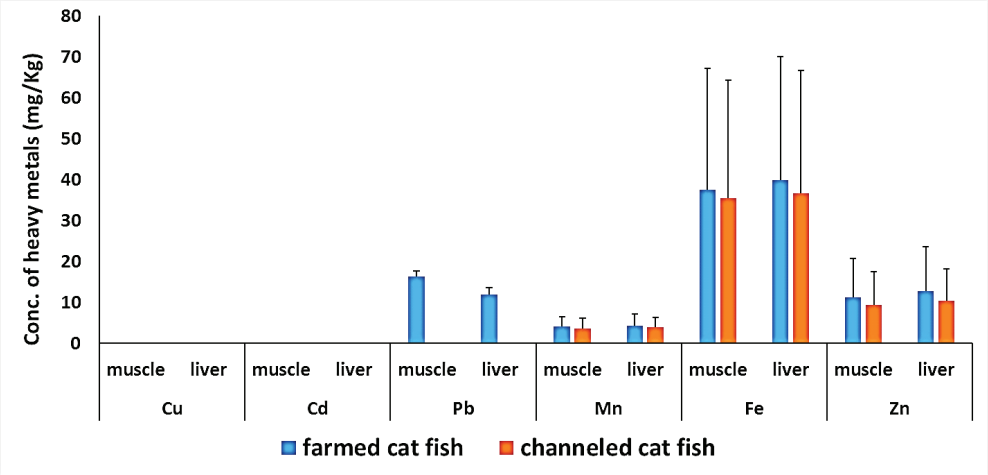

Fig 3. Concentrations of heavy metals mg/kg in wet weight muscles and liver of cultured (Farmed) and wild (Channeled) catfish

et al., (2014). Pb levels in tested fish tissues and livers from farmed sources exceeded the permissible limit by a highly significant margin. Kolappan and Satheesh's detection of Pb leaking from gasoline sources in the analyzed site of farmed catfish may be responsible for these extremely high quantities of Pb (2011).

Additionally, there were differences in the prevalence of Mn between the muscles and the liver, with the concentration in the liver being considerably greater ($p < 0.05$) than in the muscle with the liver having samples that surpassed permissible limit (PL).

Fe demonstrated no significant difference between the two sources; it was detected in 100% of all samples, with higher values in the liver than in the muscles.

Between the two sources, there was a significantly significant difference in Pb concentration, but there was a significant difference in Mn and Fe concentration between the two sources; those exceeded the PL of Mn in livers of the cultured type (60%) compared to the wild source (40%), similar to Rizkalla et al. (2012).

There was no significant difference between the two sources in Zn concentration; it was detected in 100% of samples from both sources, and all detected values didn't exceed PL.

The only highly significant ($P < 0.01$) value between both sources was shown in Pb in farmed catfish, with mean values of (16.39±1.26) and (18.90±1.68) mg/kg wet weight muscles and liver, respectively. On the other hand, Pb couldn't be detected in channeled source samples. This was agreed with Bahnasawy et al. (2019),who detected Pb with a highly significant increase in aquaculture catfish farms located in the Damietta Governorate (6.56±0.37 mg/kg); conversely, with us, they detected Pb in a wild channeled source with a highly significant increase (55.23±5.13 mg/kg). Bayomy et al. (2015) discovered highly toxic heavy metal Pb in wild catfish from the Nile Rosetta branch in Behira, Egypt, in concentrations ranging from 13.05 to 19.89 g/Kg on a wet basis for catfish; this means that our wild source in Kafr el-Sheikh governorate is safer than some other sources throughout the country.

The calculated lead levels in cultured (farmed) catfish samples substantially surpassed the 0.1 and 0.3 mg/kg, respectively, recommended safety limits set forth by Egyptian regulations ES No. 7136 (2010) and EU (2006). Referring to our examined, farmed *Clariasgariepinus's* high possible health concerns. Our Pb results were better than what Elawady et al. had discovered (2019). However, Osman and Kloas (2010) found that catfish from the Kena, Damietta, and Rosetta peaches, respectively, had greater lead residue levels (7.48, 14.51, and 14.10 mg/kg). Salam et al. (2020)determined heavy metal residues in catfish from wild sources at values of 0.64 and 0.020 µg/g for Pb and Cd, respectively. These variations in environmental pollution may be the cause of the Pb concentration variances from other research (Hashemi, 2018). Lead primarily affects the neurological system, nephropathy, hypertension, gastrointestinal tract, and reproduction systems (Rubio et al., 2005). As well as cancer, toxicity caused by Pb is also a potential issue (Bahnasawy et al., 2019).Mn, Fe, and Zn concentrations in the various organs of *C. gariepinus* followed the order of liver > muscles; these findings were in agreement with those of Abdel-Baky (2001) and Al-Nagaaway et al (2009). The Fe and Zn detection limits were in agreement with those found in

the liver by Rizkalla et al. (2012), but not with his Mn findings. Additionally, he noted that the higher Fe level in the sediment caused high Fe levels in fish through the gills while *C. gariepinus* was bottom feeding, elevating Fe in organs as a result of the gills' constant interaction with blood (blood-borne Fe). Mn and Fe were found in higher concentrations than those recommended by the FAO/WHO. Significant lower values of Zn were detected in catfish muscles and livers of both sources ($P < 0.05$).

The accumulation of heavy metals in catfish varied according to, the metabolism of fish, and the degree of metal pollution in sediment, water, and food (Burger and Gochfeld, 2005).Heavy metal limits found higher in farmed catfish than in channel cat fish because in the farms, the water is more stagnant and polluted, in addition due to the large number of exhausts from the irrigation machines used to stir the water and the boats used in the farm fishing.

Bacteriological evaluation and detection

The mean result of TBC was $4.2\times10^4 \pm1.2\times10^2$and $2.7\times10^4 \pm 0.6\times10^2$ CFU/g respectively for both sources, and samples that exceeded EOS No (%) were 10(40%) and 4(16%). For coliform count, the mean results were$7.3\times10^2 \pm1.1\times10$ and$1.6\times10^2 \pm0.2\times10$. Samples that exceeded EOS were 6(24%) and 2(8%) for both sources respectively. This difference in contamination rates in both sources could be related to the bacteriological quality of aquaculture (FAO, 2010). TBC results were consistent with those reported by Bahnasawy et al., (2019)for both farmed and wild catfish. While the TCC in all examined samples from both sources in his study, was highly significant increased ($p<0.0001$).

Contamination rates with *E.coli* were also significantly increased in examined catfish muscle samples from cultured source when compared to that of wild source 14(56%) and 9(36%) respectively. Approximately related findings were declared byBahnasawy et al., (2019). And conversely, findings were recorded byMona et al., (2017).

The main sources of pathogens such as *E.coli* in waters are agriculture pollution, and storm-water runoffwith municipal sewage **(**Bahnasawy et al., 2019; Redaktor and Cecilie, 2021).

Serological identification for Shiga toxin-producing *E. coli*(STEC)

Ten out of 23 isolates were serotyped as illustrated in table 4, the rate of isolation of (STEC) from each source (No. =25) was 6 (24%) and 4 (16%) Which means the higher percentage was in farmed fish including (O20, O26, O91, O111, O146, and O163), while in channeled fish O111, O117, O128, O146 were detected. Similar results were recorded by Ayulo et al., (1994). Isolated serotypes represents a potential hazard, as well as an indication for the presence of other entero-pathogens (Ladan and Reza 2006)

Molecular characterization

Table (5), figures 1 and 2 revealed respectively the occurrence of Shiga toxins genes *(stx1*and *stx2*) and heavy metal resistance genes (MRGs) *(nccA), (pbrA), (czcD)* in 10 *E.coli* bacteria isolates randomly taken from total 23 isolates positive for *E. coli* (5 from each source). Only the *stx2* gene was detected in farm fish isolate samples by 10%, while not detected in channeled isolates ones.Shiga toxins are key virulence factors for the pathogenesis of enteropathogenic*E.coli* strains (Gyles, 2007). The Centers for Disease Control and Prevention estimates STEC are responsible for 112,000 illnesses in the United States every year (Scallan et al.,2011).

Shiga toxin-producing *E. coli* (STEC) have emerged as an important enteric food born zoonotic pathogens of considerable public health significance in Egypt and worldwide (Ahmed and Shimamoto, 2015; Brooks et al., 2005). Shiga toxins (*stx1* and *stx2*) can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in human (Grant et al., 2011).

MRGs *(nccA and czcD)* were detected by 30%, and 20% respectively in both *E. coli* isolates from farmed and channeled fish samples. Bacteria gain resistance to toxic levels of metals through growing an efflux-based system to remove each element(Kaci et al., 2014). Although (*nccA*) is encoded for Cd and (*czcD*) is encoded for Zn and Cd, in our study the zinc level was detected at 100% in fish muscle but not at toxic levels, and moreover, Cd was not detected in fish samples, as well as MRG *(pbrA),* which is for Pb, was not found in any of the isolates from either source, despite having significant amounts in the farmed source's muscle and liver. This suggests that the presence of genes in bacteria is unaffected by an elevated metal content in the muscle. Therefore, there may be an unintentional increase in metal in farmed fish muscle or meat contamination from bacteria that have previously carried various MRGs. The

Pb level in the muscle and the presence of *pbrA* gene in the bacteria were not found to be correlated in our investigation. While a significant positive correlation (≤ 0.05) was found between the occurrence of MRGs, coliform count, and *E. coli* isolation rate, *E. coli* that harbors metal resistance genes is regarded as a difficulty for aquaculture and the aquatic ecology as a whole. The accumulation of MRGs in bacteria in ecosystems may result from the medicinal and preventative use of metal ions (Ture et al., 2018). Additionally, numerous studies have linked MRGs to antibiotic resistance; as a result, the prevalence of MRGs may potentially encourage antibiotic resistance, which is an issue in aquaculture (Knapp et al., 2011).

The currency of the metal resistance gene for a particular heavy metal, which could be for a different metal, and the concentration of that metal in fish muscle have not been linked. While the existence of bacteria with the metal resistance gene in fish may be due to contamination of fish with this strain carrying MRG, the high level of heavy metal may be caused by unintentional contamination by this metal and vice versa.

CONCLUSIONS

Our findings showed that farmed catfish had higher levels of heavy metals and bacterial pollution than channel catfish. The *E. coli* isolates from both sources of catfish included some MRGs. A public health risk was found through heavy metal and bacteriological investigation, but the study's channel catfish are less dangerous than those raised in farms. Therefore, we suggested that fish farms could reduce or prevent this hazard by using optimal management practices and a hazard analysis critical control point system (HACCP). Our findings can also be applied to the HACCP system and used to evaluate sanitary practices throughout the farmed catfish production chain.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

REFERENCES

Abdelhamid AM, Gomaa AH, El-Sayed HGM (2013) Studies on some heavy metals in the River Nile water and fish at Helwan area, Egypt. Egypt. J. Aquat. Biol. & Fish. 17 (2): 105- 126. ISSN 1110 - 6131 www.ejabf.eg.net

Abd El-Baky TE (2001) Heavy metals concentrations in the catfish, *Clariasgariepinus* (Burchell, 1822) from River Nile, El-Salam Canal and Lake Manzala and their impacts on cortisol and thyroid hormones. Egypt. J. Aquati. Biol. & Fish. 5(1): 79-98.

Abdel-Hafez SM, El-Caryony IA (2009) An economic study on the production of catfish in the Egyptian fisheries. J. Arab. Aqua. Soc 4.1: 19-34.

Afshan S, Ali S, Ameen U S, Farid M, Bharwana SA, Hannan F, Ahmad R (2014) Effect of Different Heavy Metal Pollution on Fish. Res. J. Chem. Env. Sci. 2(1) 74-79. ISSN 2321-1040. http://www.aelsindia.com

Ahmed AM, Shimamoto T (2015) Molecular analysis of multidrug resistance in Shiga toxin-producing *Escherichia coli* O157:H7 isolated from meat and dairy products. Int. J. Food. Microbiol. 193:68-73.

Ali MM, Ahmed SF, Klena JD, Mohamed ZK, Moussa TA, Ghenghesh KS (2014) Enteroaggregative*Escherichia coli* in diarrheic children in Egypt: molecular characterization and antimicrobial susceptibility. J. Infect. Dev. Ctries. 14 (5): 589-596.

Al-Nagaaway AM, Shehata MN, Dawah AM, Mansour EM (2009) Evaluation of some heavy metals residues in different water sources and Nile Tilapia in Sharkia Governorate, Egypt. Egypt. J. Agric. Res. 87 (2): 591-600.

AOAC (2005) Official Methods of Analysis of AOAC International, 18th ed., 33.2.43AOAC International, Maryland, USA.

APHA (2005) Enumeration of heterotrophs, fecal coliforms and *Escherichia coli* in water: comparison of 3M™ Petrifilm™ plates with standard plating procedures. J.microbiological methods. 60(3): 335-342.

APHA (1998) Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association, Washington, D.C.

APHA (2001) Compendiums of methods for microbiological examination of foods. 4th ed. 365-366. 800.1st, NW Washington DC.2000. 1-3710.

Asif L, Anurup M (2012) An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain *Bacillus sp*. isolated from municipal waste. J. Microbiol. Biotech. Res. 2 (1):178-189. http://scholarsresearchlibrary.com/archive.html

Atlas RM, Bartha R (1993) Microbial ecology: fundamentals and applications / Ronald M. Atlas, Richard Bartha-book. ASIN: ⁊0805306536; Publisher: Benjamin-Cummings Pub Co; 3rd Ed.

Ayulo AMR, Machado RA, Scussel VM (1994) Enterotoxigenic*Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. International J. of Food Microbiology, 24(1-2): 171-178.

Badr AM, Mahana NA, Eissa A (2014)Assessment of Heavy Metal Levels in Water and Their Toxicity in Some Tissues of Nile Tilapia (*Oreochromisniloticus*) in River Nile Basin at Greater Cairo, Egypt. Global Veterinaria 13 (4): 432-443.

Bahnasawy M, Saad EA, Maroof HA, AbouShehatta SA (2019) Bioaccumulation of some heavy metals in water and tissues of naturally infected *Oreochromisniloticus* from two polluted sites in Egypt, with reference to related oxidative stress.Effects of Heavy Metals and Pathogenic Bacteria on the Antioxidant Enzyme Activities and Immune Response of *Clariasgariepinusin* Egypt. IJSER j. 10(1):610-615. Project: Ph.D. Research., ISSN 2229-5518. IJSER © 2019 http://www.ijser.org.

Bakhiet HHA, Gebrel WME (2020) Quality evaluation of chilled fish (orechromisnilotcus and bagrusbayad) at al-damer fish market-Sudan. Advances in Tissue Engineering & Regenerative Medicine j. 6 (1)

Bayomy HM, Rozan MA, Ziena HM (2015)Lead and Cadmium Contents in Nile Water, Tilapia and Catfish from Rosetta Branch, River Nile, Egypt. J. Food and Dairy Sci., Mansoura Univ. 6 (4): 253- 262

Biswas S, Prabhu RK, Hussain KJ, Selvanayagam M, Satpathy KK (2011) Heavy metals concentration in edible fishes from coastal region of Kalpakkam, south eastern part of India. Environ. Monit. Assess. http://dx.doi.org/10.1007/s10661-011-2325-y.

Brooks JT, Sowers EG, Wells JG (2005) Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J. Infec.t Dis. 192:1422-1429.

Burger J, Gochfeld M (2005) Heavy metals in commercial fish in New Jersey. Environmental Research. 99: 403 - 412.

Burgos MG, Rainbow PS (2001) Availability of cadmium and zinc from sewage sludge to the flounder, Platichthysflesus, via a marine food chain. Marine environmental research. 51.5: 417-439.

Dipineto L, Santaniello A, Fontanella M, Lagos K, Fioretti A, Menna LF (2006) Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. Letters in Applied Microbiology 43 (2006) 293-295.

El-Gamal AM, Abdou MS, Salem NIE, Ebied NA (2016) Isolation and Molecular Characterization of Shiga Toxin-Producing *Escherichia Coli* in Beef Retail Markets. Alexandria J. of Veterinary Sciences**.** Jan. 48 (1): 50-56 ISSN 1110-2047**. www.alexjvs.com**. DOI: 10.5455/ajvs.205072

Elnimr T (2011) Evaluation of some heavy metals in Pangasiushypothalmus and Tilapia nilotica and the role of acetic acid in lowering their levels. International J. of Fisheries and Aquaculture. 3(8): 151-157.

Eltholth M, Kimberly FK, Grace D, Rushton J, Häsler B (2018)Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh fish markets. J. gfs. 17:14-20. https://doi.org/10.1016/j.gfs.2018.03.003

EOS (2005) Egyptian Organization for Standardization and Quality, Chilled fish. ES No.3494.

EOS (2010) Egyptian Organization for Standardization and Quality, Maximum level for heavy metal contaminants in food. ESNO.7136/2010. Cairo, Egypt.

EU (2006) Commission Regulation setting maximum levels for certain contaminants in foodstuffs. Official J. of the European Union 1881/2006. (12) (PDF) Determination of selected heavy metals in tuna fish (Thunnusthynnus). Available from: https://www.researchgate.net/publication/275094647_Determination_of_selected_heavy_metals_in_tuna_fish_Thunnus_thynnus.accessed Jan. 20/ 2022.

FAO (2010) "Food and Agriculture Organization"Expert workshop on the application of biosecurity measures to control Salmonella contamination in sustainable aquaculture FAO Fisheries and Aquaculture Report No. 937 ISSN 2070-6987, Mangalore, India, 19-21 Jan.

FDA (2001) "Food and Drug Administration," Bacteriological Analytical Manual, *Staphylococcus aureus*, Bennett, R.W. and G.A. Lancette, Ch. 12, Rev. Jan. 8th Ed., Rev. A. AOAC, International, Gaithersburg, MD.

Garai P, Banerjee P, Mondal P, Saha NC (2021) Effect of heavy metals on fishes: Toxicity and bioaccumulation. J ClinToxicol. S, 18.

Grant MA, Hedberg C, Johnson R(2011) Executive summary. The significance of non-O157 Shiga toxin-producing *Escherichia coli* in food. Food Prot. Trends. 33-42.

Gyles, CL (2007) Shiga toxin-producing *Escherichia coli*: An overview. J. Anim. Sci. 66: E45-E62.

Hagras AE, Elbaghdady HM, Gouda AMR (2018) Assessment of Water Quality and Heavy Metals in Water, Sediments, and Some Organs of African Catfish (Clariasgariepinus) in El-Serw drain, Nile Delta,Egypt. International J. of Environment. 7 (4): 124-141

Hagras AE, El-Gammal MI, Abdrabouh E, El-Bahnasy HT (2017) Environmental Impact Assessment of Heavy Metals on African Catfish (*Clariasgariepinus*) of some Drains in Dakahlia Governorate, Egypt. J. of Environmental Science, Toxicology and Food Technology. 39-51.

Hashemi M (2018) Heavy metal concentrations in bovine tissues (muscle, liver and kidney) and their relationship with heavy metal contents in consumed feed. Ecotoxicol Environ Saf. 154: 263-267.

Ibrahim D, Froberg B, Wolf A, Rusyniak DE (2006) Heavy metal poisoning: clinical presentations and pathophysiology. Clinics in laboratory medicine. 26(1): 67-97.

Kaci AF, Petit P, Lesueur D, Boust A Vrel, Berthe T (2014) Distinct diversity of the *czcA*gene in two sedimentary horizons from a contaminated estuarine core. Environ. Sci. Pollut. Res. 21: 10787-10802. https://doi.org/10.1007/s11356-014-3029-y

Kok T, Worswich D, Gowans E (1996) some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee J, Fraser A., Marmion B, and Simmons A., eds.) 14th ed., Edinburgh. Churchill Livingstone. UK.

Kolappan A, Satheesh S (2011) Efficacy of UV treatment in the management of bacterial adhesion on hard surfaces. Polish Journal of Microbiology 60.2. 119.

Ladan MN, Reza G (2006)A study on enteropathogenic*Escherichia coli* isolated from domestic Iranian soft cheese; Vet arhiv76 (6), 531-536.

Maturin L, Peeler JT (2001) BAM: Aerobic plate count. US Food and Drug Administration: Silver Spring, MD, USA.

Mona TR, Naser GA, Amany LF, Abdallah MIM (2017) Risk evaluation of some fish aquaculture". Journal of Egyptian veterinary medical association. 77(2) 313-336.

Mustafa GR, Zhao K, He X, Chen S, Liu S, Mustafa A, Zou L (2021) Heavy Metal Resistance in *Salmonella Typhimurium* and Its Association with Disinfectant and Antibiotic Resistance. *Frontiers in microbiology*. Nriagu, J.O. and Pacyna, J.M. Quantitative Assessment of Worldwide Contamination of Air, Water and Soils by Trace Metals. Nature, 333(6169): 134-199. DOI: 10.1038/333134a0

MutluNisa ÜC, Hatice K, Burhan A, Gökhan C (2005) Plasmid mediated heavy metal resistances in *Enterobacter spp*. isolated from Sofulu landfill, in Adana, Turkey. Annals of Microbiology. 55 (3): 175-179.

Osman AG, Kloas W (2010) Water quality and heavy metal monitoring in water, sediments and tissues of the African catfish *Claris gariepinus* (Burchell, 1822) from the River Nile. Egypt. J. of Environ. Prot.1 (4):389-400.

Paschoalini AL, Savassi LA, Arantes FP, Rizzo E, Bazzoli N (2019) Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus* from a polluted neotropical river. Ecotoxicol Environ Saf.169:539-550.

Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC, Maguire D (2002) VeterinaryMicrobiology and Microbial Disease.Published by Blackwell. PP. 113-116.

Rubio C, Gonzalez-Iglesias T, Revert C, Reguera JI, Gutierrez AJ, Hardisson A

(2005)Lead dietary intake in a Spanish population (Canary Islands). J Agric. Food

Chem. 53 (16): 6543-6549.

Redaktor ER, Cecilie HI (2021) Screening for Antimicrobial- And Heavy Metal Resistant Bacteria in Copper Contaminated Areas. Rapport Fra, Havforsknin Gen. NR. 18. ISSN: 1893-4536

Reilly A, Käferstein F (1997)Food safety hazards and the application of principles of the Hazard Analysis and Critical Control Point (HACCP) system for their control 10.1046/j.1365-2109.1997.00939.x.

Rizkalla EH, Haleem HH, Yassa VF (2012)Variability of Heavy Metals in Wild Catfish *Clariasgariepinus*Collected South and North Cairo Area. Egypt. J. Aquat. Biol. & Fish16 (4): 17-42 ISSN 1110 -1131 www.ejabf.eg.net

Salam MA, Paul SC, Zain RAMM, Bhowmik S, Nath MR, Siddiqua SA, Amin MFM(2020) Trace metals contamination potential and health risk assessment of commonly consumed fish of Perak River, Malaysia. Plos one, 15(10). e0241320.

Scallan E, Hoekstra RM, Angulo FJ (2011) Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17: 7-15.

Silver S, Misra TK (1988)Plasmid-Mediated Heavy Metal Resistances. Annual Review of Microbiology. 42:717-743 https://doi.org/10.1146/annurev.mi.42.100188.003441

Terzi, E. 2018. Antimicrobial resistance profiles and tetracycline resistance genes of *Escherichia coli* in Mediterranean mussel and sea snails collected from Black Sea, Turkey. Alinteri. JAS. 33(1): 43-49. https://doi.org/10.28955/alinterizbd.355019.

Ture M, Altinok I, Alp H (2018) Effects of cage farming on antimicrobial and heavy metal resistance of *Escherichia coli*, *Enterococcus faecium*, and *Lactococcusgarvieae*. Microb. Drug Resist 24(9):1422-1430. https://doi.org/10.1089/mdr.2018.004

Ture M, Kilic MB, Altinok I (2020) Relationship between heavy metal accumulation in fish muscle and heavy metal resistance genes in bacteria isolated from fish. Biological Trace Element Research, https://doi.org/10.1007/s12011-020-02246-0.

Valko MMHCM, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. Current medicinal chemistry. 12.10: 1161-1208.

WHO (1993) Guidelines for drinking-water quality, 2ndedn, Vol 1.Recommendations, Geneva.

Yang S, Deng W, Liu S, Yu X, Mustafa GR, Chen S, He L, Ao X, Yang Y, Zhou K, Li B, Han X, Xu X, Zou L (2020) Presence of heavy metal resistance genes in *Escherichia coli* and *Salmonella*, and analysis of resistance gene structure in *E. coli* E308. J. of Global Antimicrobial Resistance. 21: 420-426. https://doi.org/https://doi.org/10.1016/j.jgar.2020.01.00 9 Mar. Sci. Tech. Bull. (2021) 10(1): 42-53 e-ISSN: 2147-9666 info@masteb.com dergipark.org.tr/en/pub/

Yarsan E, Yipel M (2013) The important terms of marine pollution Biomarkers and biomonitoring, bioaccumulation, bioconcentration, biomagnification. J. MolBiomarkDiagn S1.