Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Overviews of heavy metals residues in some frozen fish and its biocontrol by probiotic

Asmaa, E. Hassan*, Amany, M. Salama. and Amira, A. Elokle ***

**Food Hygiene Department* and
**Chemistry department *Animal Health Research Institute, Agriculture Research center, Dokki, Egypt*

Received in 7/8/2024
Received in revised from 1/9/2024
Accepted in 10/9/2024

Keywords:

heavy metals
frozen fish
probiotics.

ABSTRACT

Heavy metals are the most significant pollutants in aquatic networks due to their toxicity, accumulation, and bio-magnification by marine organisms. Environmental pollution is the universal problem. So, A total of 100 random samples of frozen fishes which include Mackrel (*Scomber*), Horse mackerel (*Trachurus trachurus*) (30 of each) and Hiring (*Clupea harengus*) (40 samples) were collected from different fish markets in Cairo governorate, Egypt, for determination of (Lead, Cadmium, Copper, Zinc, Iron, Arsenic, Nickel and tin) levels by atomic spectrophotometer. The obtained results revealed that all elements were within the permissible limit.

High level exploratory work was led expected to survey the corruption impact of one of *Lactobacillus rhamnosus* (10^7 CFU/ml) on lead and cadmium levels in experimentally inoculated fish for zero, 8, 16 and 24 hours. Within 24 hours of interacting with *L. rhamnosus*, the levels of lead and cadmium were reduced by 83.6 and 71.1 percent, respectively. Appropriately, normal examination of heavy metals levels in aquatic environment and creatures is recommended public and worldwide, with stringently proposal to safe removal of plant squanders.

INTRODUCTION:

Fish is viewed as a fundamental wellspring of protein due to its high natural worth, polyunsaturated unsaturated fats, nutrients, and minerals including calcium and phosphorus. Because red meat is so scarce and fish is cheaper than chicken and meat, Egypt is becoming a nation that eats more fish **(Morshdy et al. 2019).**

Fish is significant piece of a solid eating routine because of numerous nourishing advantages as they contain great protein, low cholesterol level, omega-3 unsaturated fats, minerals as well as nutrients. Additionally, their lower costs potentiate their nutritive qualities. An even eating routine incorporates an assortment of fish and shellfish can add to

Corresponding author: Asmaa, E. Hassan, Food Hygiene Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt.
E-mail: semsemaezat@gmail.com
DOI: 10.21608/ejah.2024.381657

heart wellbeing and youngsters' development and improvement. Weighty metals are normally happening components despite the fact that their various modern, horticultural, drug and mechanical applications have prompted their wide circulation in the climate, raising worries over their likely impacts on environments and human wellbeing **(Tchounwon et al. 2012)**.

The contamination of sea-going climate with heavy metals is a general wellbeing peril during ongoing years. Food residues have been linked to immune suppression, chemical hypersensitivity, and breast cancer, as demonstrated by scientific researchers; decrease sperm count and fruitlessness **(Sharp, 1999)**.

Heavy metals are dissolved and easily accumulate in the various parts of aquatic living organisms, including fish, once they enter the aquatic system. Consumers of contaminated fish then take in these heavy metals. **(Authman, 2015).**

Copper, cadmium, and lead, among other heavy metals, may be highly toxic to aquatic life when introduced into the water supply **(Ambreen et al. 2015).**

Moreover, fish are situated toward the finish of the sea-going order of things and may aggregate metals and pass them to people through food causing constant or intense infections **(Al- Yousul et al. 2000).**

Lead has hepatotoxic effects and raises liver function test parameters significantly **(Adeyemi et al. 2009)**.

Cadmium is toxic to the kidneys, initially causing damage to the kidney tubules. It can also damage bones, either directly by affecting bone tissue or indirectly through renal dysfunction **(Wang et al. 2008)**.

Copper is a fundamental trace element that, when ingested in large quantities, is extremely harmful to organisms and organs. Toxic levels of copper sulfate in the liver cause oxidative damage in the form of granular degeneration, hepatocyte necrosis, and impairment of the cell lining of the remark cords. These biochemical changes in malondialdehyde and glutathione levels are evidence of this **(Emin et al. 2010)**.

Zinc is remarkable component that is minimal fundamental for human wellbeing. An excess of Zn can cause conspicuous medical conditions, for example, skin disturbances, stomach cramps, sickliness, retching and queasiness. Arteriosclerosis is brought on by high levels of zinc, which damage the pancreas and disrupt protein metabolism (**Afshan et al. 2014).**

Iron is the primary driver of malignant growth because of iron inebriation is through the age of free revolutionaries which assault DNA bringing about cell harm, change and threatening change which thusly cause a variety of sicknesses **(Grazuleviciene et al. 2009).**

Arsenic acts to coagulate protein, structures edifices with coenzymes and represses the creation of adenosine tri phosphate (ATP) during breath **(INECAR, 2000)**. It is conceivably cancer-causing in mixtures of all its oxidation states and significant level openness can cause passing **(USDOL, 2004).**

Nickel can cause respiratory problems and is a carcinogen **(Anonymous 2003; Agency for Toxic Substance and Disease Registry, 2004).**

Tin could cause unusual digestion of the fundamental components like zinc, copper and iron. Furthermore it diminishes calcium content in bone and prompts organ harm especially in the kidney **(Rader, 1991)** and was carcinogenic at doses up to 2000 mg in mice with increased incidence of hepatocellular adenomas (**ATSDR, 2003**).

Such countless preliminaries were completed to control these components and their difficult issue, one of these preliminaries is probiotics. Probiotics—literally, "for life"—are bacteria or yeasts that are thought to benefit the host organism's health. Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus, and Bacillus are the six different genera of probiotic organisms used in interventions. The goal of the review was to compile all of the information that was known about the safety of such interventions. Alone or in mix, used to lessen the gamble of, forestall, or treat sickness in research studies **(Hempel et al. 2011).**

The decrease of the harmful perils might be credited to that the metal particles tie to the cell wall and extracellular polysaccharides by suitable or latent cells including adsorption, particle trade, complexation, chelation and microprecipitation **(Veglio et al. 1997)**.

Probiotic strains of lactobacillus can bind to heavy metals, making them useful for heavy metal detoxification **(Milatovic et al. 2017)**. These days, probiotics are viewed as a promising age to mitigate weighty metal harmfulness **(Giri et al. 2018)**.

Consequently, the point of this study is to determine a heavy metals residue (Lead, Cadmium, Copper, Zinc, Iron, Arsenic, Nickel and tin) in frozen fish as Mackerel, Horse mackerel and hiring moreover its biocontrol by the probiotic. The general wellbeing significance and the perilous harmful impacts of these heavy metals were talked about as well as the intriguing suggestions to limit fish contamination with these heavy metals were referenced.

2. MATERIALS and METHODS: -

2.1. Collection of samples:

A sum of 100 random samples of frozen fish nearly similar in the weight and size which include Mackrel (*Scomber*), Horse mackerel (*Trachurus trachurus*) (30 of each) and Hiring (*Clupea harengus*) (40 samples) were collected from different fish markets in Cairo governorate, Egypt, for determination of (Lead, Cadmium, Copper, Zinc, Iron, Arsenic, Nickel and tin) residue levels.

2.2. Preparation of fish samples for residual detection of heavy metals:

All the fish samples were separately kept at -20 °C then allowed to thaw, washed with running water, then placed on an aluminum foil to avoid contact with the working bench. The samples dissected with sterile scissors to get muscles. The muscles were transferred into sterile sample beakers, labeled for digestion and analysis of heavy metals.

2.3. Procedures for detection of heavy metals residue:

Every fish homogenized then one gram of the muscle tissue was digested with 10 ml of nitric / sulfuric / perchloric acids (8: 1: 1).

Kept for 4 hours at room temperature then heated in water bath at 40-45°C for one hour then the temperature was increased to 75°C until complete digestion of the tissue.

Allow samples to cool at room temperature, and then dilute the digest to 20 ml with deionized water and filtered through 0.45 µl Whatman filter paper.

The clear filtrate of each sample can be kept in refrigerator till analysis using atomic absorption spectrometry following the guidelines of **AOAC (2002) Official Method 999.10.**

The digest, blanks and standard solutions were aspirated by Atomic Absorption Spectrophotometer (AAS) and analyzed for their concentrations of such elements. The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the conditions recommended by the instrument instructions.

2.4. Quantitative determination of heavy metals residue:

Absorbency of elements was directly recorded from the digital scale and their concentrations were calculated according to the following equation: **C=R x (D/W).**

Where, **C**= Concentration of the element (wet weight)

R= Reading of digital scale of AAS.

D= Dilution of the prepared sample.

W= Weight of the sample.

N. B. The concentration of each element in the blank solution was also calculated and subtracted from each analyzed sample.

2.5. Experimental part:

The effect of *Lactobacillus rhamnosus* on the concentrations of lead and cadmium in fish samples experimentally inoculated with.

2.5.1. Preparation of bacterial suspension:

Lactobacillus rhamnosus strain (from National Research center) was enriched in Brain

Heart Infusion (BHI) broth for 24hr at 37ºC to prepare an overnight culture. One mL of the cultivated bacterial suspension was decimally diluted in sterile peptone water (0.1%, w/v) and counted by spread cultivation on BHI agar. A volume of the culture broth corresponding to approximately 1×10^7 CFU/ml bacteria was centrifuged (500 rpm / 15min) and the bacterial pellets were washed twice with deionized water **(Halttunen et al. 2007)**.

2.5.2. Binding assay:

The bacterial pellet (10^7CFU/ml), 30 mg/Kg ionic lead, and 10 mg/Kg ionic cadmium solutions were mixed with fish samples free from heavy metal residues as indicated by **Halttunen et al. (2008).**

2.5.3. Experimental design:

Metal-contaminated fish served as the control positive group (G1). Fish inoculated with *L. rhamnosus* (10^7 CFU/g) and lead (30 mg/kg) (G2), and fish inoculated with *L. rhamnosus* (10^7 CFU/g) and cadmium (10 mg/kg) (G3). (G2) and (G3) were used as the treated groups. The samples were acidified with ultrapure HNO3 **(Lars, 2003)**, and examined at zero, 8-, 16-, and 24-hour time points for measuring the free metal by atomic absorption spectrophotometer.

2.6 Statistical analysis:

Statistical analysis was assessed using the SPSS (v.13, SPSS Inc., Chicago, IL). Results were recorded as mean ± standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance. One-Way ANOVA test was applied to compare differences among means. Paired samples t-test was used to compare samples before and after adding probiotic strain.

RESULTS

Table 1. Heavy Metals concentrations (ppm "mg/kg") in the examined frozen fish samples:

Examined samples (No. of samples analyzed)	Mackerel (*Scomber*) (30)			Horse Mackerel (*Trachurus trachurus*) (30)			Hiring (*Clupea harengus*) (40)		
Heavy Metals concentrations	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE
lead (pb)	0.04	0.12	0.074 ± 0.004^{a}	0.07	0.13	0.082 ± 0.004^{b}	0.05	0.26	0.12 ± 0.01^{ab}
Cadmium (cd)	0.032	0.048	0.041 ± 0.001^{a}	0	0.041	0.015 ± 0.003^{a}	0.01	0.049	0.031 ± 0.003^{a}
Cupper(Cu)	0	0.18	0.06 ± 0.02	0	0.92	0.14 ± 0.06	0.04	0.31	0.13 ± 0.02
Tin (Sn)	1.6	10.6	4.92 ± 0.7^{a}	2.38	7.81	4.66 ± 0.4^{b}	0	2.57	0.92 ± 0.2^{ab}
Arsenic (Ar)	0	0.04	0.003 ± 0.002^{abc}	0	0.5	0.12 ± 0.05^{ab}	0	0.44	0.16 ± 0.04^{ac}
Nickel (Ni)	0	0.06	0.034 ± 0.006^{a}	0	0.13	0.05 ± 0.008^{b}	0	0.28	0.09 ± 0.02^{ab}
Zinc (zn)	0.06	0.35	0.22 ± 0.02^{a}	0.27	0.51	0.38 ± 0.02^{a}	0.14	0.5	0.3 ± 0.02^{a}
Iron (Fe)	0	0.9	0.44 ± 0.06^{abc}	0.37	0.83	0.6 ± 0.03^{ab}	0.36	1.04	0.7 ± 0.05^{ac}

There were significance P ≤0.05 between the same letters in the same row.

Table 2. The residual levels of heavy metal (ppm "mg/kg") in analyzed fish samples as compared with the permissible limit

Metal	Fish species	Maximum and standard levels in mg/kg	Samples below & within permissible limits	Samples above permissible limits
lead (pb)	Mackerel *(Scomber)*	0.5 mg/kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO(1983)and FAO/ WHO (1989)	30(100%)	0
	Hiring *(Clupea harengus)*	3.0 mg/kg (JECFA, 2000) 0.30 mg/Kg EOS (2010)	40(100%)	0
Cadmium (cd)	Mackerel *(Scomber)*	mg/kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO (1983),EU (2001) and WHO (2004)	30(100%)	0
	Hiring *(Clupea harengus)*	0.05 mg/Kg EOS (2010)	40(100%)	0
Cupper(Cu)	Mackerel *(Scomber)*	70 mg/ kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO (1983) 30 mg/ kg	30(100%)	0
	Hiring *(Clupea harengus)*	FAO/WHO (1989) and MAFF(1995) 10 mg/ kg EU (2001)	40(100%)	0
Tin (Sn)	Mackerel *(Scomber)*	250 mg/kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	WHO (JECFA, 2006) 200 mg/kg	30(100%)	0
	Hiring *(Clupea harengus)*	(EU, 2006)	40(100%)	0
Arsenic (Ar)	Mackerel *(Scomber)*	mg/ kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO (1983) and (CEPA) (1995-97)	30(100%)	0
	Hiring *(Clupea harengus)*		40(100%)	0
Nickel (Ni)	Mackerel *(Scomber)*	0.5–0.6 mg/kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	WHO(1985) 8.97 mg/ kg	30(100%)	0
	Hiring *(Clupea harengus)*	FAO/WHO (1989)	40(100%)	0
Zinc (zn)	Mackerel *(Scomber)*	100 mg/ kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO/WHO (1989) 30 mg/ kg	30(100%)	0
	Hiring *(Clupea harengus)*	FAO (1983) and EU (2001)	40(100%)	0
Iron (Fe)	Mackerel *(Scomber)*	186 mg/ kg	30(100%)	0
	Horse Mackerel *(Trachurus trachurus)*	FAO/WHO (1989)	30(100%)	0
	Hiring *(Clupea harengus)*		40(100%)	0

Table 3. Impact of *L. rhamnosus* culture (10^7 CFU/g) on the degrees of lead experimentally inoculated to fish (30 mg/Kg).

Storage time	Control (mg/Kg)	*L. rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	30	30	-------
8 hours	30	10.4	65.3
16 hours	30	7.2	76
24 hours	30	4.9	83.6

Table 4. Impact of *L. rhamnosus* culture (10^7 CFU/g) on the degrees of Cadmium experimentally inoculated to fish (10 mg/Kg).

Storage time	Control (mg/Kg)	*L. rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	10	10	-------
8 hours	10	4.3	57
16 hours	10	3.2	68
24 hours	10	2.9	71

DISCUSSION: -

Heavy metals are environmental and food-safe contaminants. These have long biological half-lives and are not biodegradable **(Heidarieh et al. 2013)**. As a result, the purpose of the current study was to survey and investigate the concentrations (ppm or mg/kg) of various heavy metals in some frozen fish that was sold commercially in Egyptian markets represented by mackerel fish *(Scomber)***,** Horse Mackerel *(Trachurus trachurus)* and Hiring *(Clupea harengus)*.

Regarding, the results in table (1) revealed that the mean value of lead levels were 0.074± 0.004 , 0.082±0.004 and 0.12±0.01 ppm with minimum values of 0.04, 0.07 and 0.05ppm while the maximum values were 0.12, 0.13 and 0.26 ppm in mackerel fish*(Scomber)***,** Horse Mackerel *(Trachurus trachurus)* and Hiring*(Clupea harengus)*, respectively. There were significant differences between mackerel *(Scomber)* and Hiring *(Clupea harengus)* also between Horse Mackerel *(Trachurus trachurus)* and Hiring *(Clupea harengus)*.

In table (2) there were no samples exceed the permissible limit as indicated by **FAO, (1983)** is 0.5 mg kg, and Joint Expert Committee on Food Additives **(JECFA, 2000)** allowed up to 3.0 mg kg. Moreover, the maximum Residual Limit of lead (0.30 mg/Kg) stipulated by Egyptian Organization of Standardization "**EOS" (2010).**

Heavy metals are a class of elements with a high density and are poisonous at low concentrations **(Koller and Saleh 2018)**. Metals, once consumed, can gather in the body of a living creature to levels adequate to become poisonous **(Ojuederie and Babalola 2017)**.

Low results were recorded by **Sireli et al. (2006)** and **Ekpo et al. (2008)**. Nearly similar results obtained by **Shokr et al. (2019)**, while higher results by **Abubakar et al. (2015)**, **Ali et al. (2016)** and **Ahmed et al. (2019)** estimated Pb ranged between 2.76 and 4.63 ppm for commercial marine fish species .

Moreover table (1) revealed that the mean cadmium levels were 0.041±0.001 ppm, 0.015 ± 0.003 ppm and 0.031± 0.003 ppm with minimum values of 0.032, 0, 0.01 ppm while the maximum values were 0.048, 0.041, 0.049 ppm in mackerel fish*(Scomber)***,** Horse Mackerel *(Trachurus trachurus)* and Hiring *(Clupea harengus)*, respectively. There were significant differences between the three fish species.

These results are almost identical to those obtained by **Ayeloja et al. (2014)**. However, **Ekpo et al. (2008)** and **Badr et al. (2014)** recorded higher results.

An acceptance limits for Cd 0.1 mg kg was set up by **FAO, (1983), EU, (2001)** and **WHO, (2004)**.

Moreover the maximum residual limit of Cd (0.05 mg/Kg) stipulated by Egyptian Organization of Standardization **"EOS" (2010). (Table 2)** according to that all samples not exceed this permissible limit.

In addition to table (1) mentioned that the mean copper residual levels were 0.06± 0.02, 0.14± 0.06 and 0.13±0.02 ppm with minimum value of 0, 0 and 0.04 ppm. However the maximum values were 0.18, 0.92 and 0.31ppm in mackerel *(Scomber*)**,** Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*), respectively. There were no significant differences between any fish species.

According to table (2) none of the examined muscle samples exceeded the limit of **FAO**, (**1983)** 70 mg kg, **FAO/WHO, (1989)** and **(MAFF** (Ministry of Agriculture, Fisheries and Food), **1995)** which expressed that the permissible limit of copper should not be in excess of 30 mg/kg. Moreover **EU, (2001)** mentioned that the permissible limit of copper 10 mg/kg.

Higher results obtained by **Kaoud and EL -Dahshan (2010)** and **Ali et al. (2016)**.

The level of Tin residues were 4.92± 0.7, 4.66± 0.4 and 0.92± 0.2 ppm as mean value with minimum values of 1.6 , 2.38 ppm and 0 (not detectable) while the maximum values were 10.6, 7.81 and 2.57 ppm in mackerel *(Scomber*), Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*), respectively. These differences in the accumulation levels may contribute to the sample origin, feeding habit and the age of fish. There were significant differences between mackerel *(Scomber*) and Hiring *(Clupea harengus*) also between Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*).

In table (2) the permissible limit of tin 250 mg/kg and 200 mg/kg by **WHO (JECFA, 2006)** and **(EU, 2006)**, respectively. So, all samples were accepted.

Lower results were obtained by Morshdy et al. (2013) and Morshdy et al. (2021).In table (1) the mean values of **Arsenic** residues **(Ar)** were 0.003± 0.002, 0.12± 0.05 and 0.16 ± 0.04 ppm with minimum values of 0 (not detectable) while the maximum values were 0.04, 0.5 and 0.44 ppm in mackerel *(Scomber*), Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*), respectively. There were significant differences between mackerel *(Scomber*), Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*), also between Horse Mackerel *(Trachurus trachurus*) and mackerel *(Scomber*), moreover between Hiring *(Clupea harengus*) and mackerel *(Scomber*).

In table (2) the Permissible limit for Arsenic was set up at 1.0 mg kg by **FAO, (1983)** and California Environmental Protection Agency **(CEPA) (1995-97)**, so there weren't samples exceed these Permissible limit

In a study which was conducted by **Javaheri Babooli and Velayatzadeh (2013**) the mean concentrations of Arsenic in fish samples 0.117±0.07 ppm which was similar to our results.

In another study which was conducted by **Heidarieh et al. (2013)** arsenic concentrations in fish samples was 21.38±3.31 ppm which was higher than our results.

Moreover in table (1) the **Nickel (Ni)** mean values were 0.034± 0.006 , 0.05 ±0.008 and 0.09±0.02 ppm with no detectable minimum values and 0.06, 0.13 and 0.28 ppm as maximum values ppm in mackerel (Scomber), Horse Mackerel (Trachurus trachurus) and Hiring (Clupea harengus), respectively. There were significant differences between mackerel *(Scomber*) and Hiring *(Clupea harengus*) also between Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*).

However in table (2) there weren't any

sample exceed the permissible limit which 0.5 –0.6 mg/kg set by the **WHO, (1985)** and 186 mg/kg by **FAO/WHO, (1989)**.

Ni is an accretive body toxin and its concentration in the environment should remain as low as possible.

Higher concentrations of Ni have been detected to be 1.2-3.4 mg/kg by **Mendil et al. (2005)** and 3.40 mg/kg by **Nisbet et al. (2010).**

Like other living things, fish need zinc to survive; and numerous enzyme structures contain it. Also, it is recognized that it has critical capabilities in safeguarding film structure. Since it is a fundamental component, fish have a capacity of enduring zinc **(Eisler, 1981)**.

The mean values of **Zinc (zn)** in table (1) were 0.22± 0.02, 0.38± 0.02 and 0.3± 0.02 ppm with minimum values of 0.06, 0.27 and 0.14 ppm while the maximum values were 0.35, 0.51 and 0.5 ppm in mackerel (Scomber), Horse Mackerel (Trachurus trachurus) and Hiring (Clupea harengus), respectively. There were significant differences between the three fish species.

The maximum permissible limit of Zn in fish and fish product was 100 mg/ kg proposed by the **FAO/WHO, (1989)** while **FAO, (1983)** and **EU, (2001)** 30 mg/ kg. None of the examined muscle samples exceeded the limit in table (2).

The higher zinc concentrations obtained were (19.55 mg/ kg) by **Nisbet et al. (2010)** but **Tüzen, (2003)** found that concentrations of zinc were 9.50 mg/ kg.

Eventually in table (1) the mean values of **Iron (Fe)** residues were 0.44± 0.06, 0.6± 0.03 and 0.7± 0.05 ppm with minimum values of 0, 0.37 and 0.36 ppm while the maximum values were 0.9, 0.83 and 1.04 ppm in mackerel (Scomber), Horse Mackerel (Trachurus trachurus) and Hiring (Clupea harengus), respectively. There were significant differences between mackerel *(Scomber*), Horse Mackerel *(Trachurus trachurus*) and Hiring *(Clupea harengus*), also between Horse Mackerel *(Trachurus trachurus*) and mackerel *(Scomber*), moreover between Hiring *(Clupea harengus*) and mackerel *(Scomber*).

So, the whole samples accepted according to **FAO/WHO, (1989), (Table 2)**

Higher concentrations of Fe ranged from 21.17 to 33.78 mg/kg obtained by **Nisbet et al. (2010).**

The recorded varieties between the got results and different records might be credited to the distinction in the territories of test's assortment, period of fishes as a huge consider bioaccumulation cycle, and kinds of the analyzed fishes.

Biocontrol of heavy metals by probiotic:

Impact of *L. rhamnosus* culture (10^7 CFU/ g) on the degrees of lead and cadmium experimentally inoculated to fish:

The toxic nature of heavy metals (bioaccumulation and bio magnification) in food chain, so the tainting of the climate with these serious poisons, related straightforwardly to general wellbeing **(Hussain et al. 2012)**.The cadmium and lead are reported amongst the top ten toxic metals in the Priority List of Hazardous Substances **(ATSDR, 2007)** for causing a grossly biological impact by bioconcentration, bioaccumulation phenomena. Certain species of lactic acid bacteria (LAB), as well as other microorganisms, can bind metal ions to their cells surface or transport and store them inside the cell. As a result, interactions between metal ions and LAB have been extensively studied over the past few years to develop their use in new biotechnology processes in addition to their health benefits and probiotic properties. LAB has a high potential for absorption of toxic and essential metal ions in model aqueous solutions, which can be used to improve food quality and safety **(Mrvčić et al. 2012).**

An exploratory review was led to examine the lead and cadmium levels debasement under the impact of *L. rhamnosus* in fish model. Results as classified in Tables (3 and 4) showed an extraordinary reducing impact in lead and cadmium levels, separately. Within

24 hours of the incubation, they were reduced by 83.6 and 71%; besides, higher corruption level on lead was recorded than cadmium levels in experimentally inoculated fish.

Nearly similar results obtained by **Samir et al. (2021)** who recorded reduction in lead and cadmium levels experimentally inoculated in fish fillet 84.3 and 72% within 24h of the incubation**.**

CONCLUSION: -

When the results of this study were compared to fish standards set by the United States and other countries, it was found that none of the frozen fish samples examined went above what was allowed by those standards. In addition, L. rhamnosus demonstrated a promising method for decreasing the accumulation of heavy metals (lead and cadmium) in fish tissues.

Because of the medical advantages and extraordinary significance of fish in human eating routine, consideration ought to be given to their pollution with harmful or possibly poisonous ecological synthetics like weighty metals. Utilization of fish ought to be directed particularly in weak populaces, for example, kids and pregnant ladies as well as in exorbitant fish-eating populaces.

REFERENCES

Abubakar A, Adamu Uzairu A, Ekwumemgbo AP, Okunola JO. 2015. Risk Assessment of Heavy Metals in Imported Frozen Fish Scomber scombrus Species Sold in Nigeria: A Case Study in Zaria Metropolis. Hindawi Publishing Corporation Advances in Toxicology. Article ID 303245, 11 pages http://dx.doi.org/10.1155/2015/303245

Adeyemi O, Ajayi JO, Olajuyin AM, Oloyede OB, Oladiji AT, Oluba OM, Adeyemi O, Ololade IA, Adebayo EA. 2009. Toxicological evaluation of the effect of water contaminated with lead, phenol and benzene on liver, Kidney and colon of Albeno rats. Food chem. Toxicol., 47(4):885-887.

Afshan S, Ameen SU, Farid M, Bharwana AS, Hannan F, Rehan Ahmad, R. 2014. Effect of Different Heavy Metal Pollution on Fish. Res. J. Chem. Env. Sci., Volume 2 Issue 1 February (2014): 74-79 Online ISSN 2321-1040

Agency for Toxic Substance and Disease Registry. (ATSDR) 2003. Toxicological Profile for Mercury, U.S. Department of Health and Humans Services, Public Health Service, Centers for Disease Control, Atlanta, GA.

Agency for Toxic Substances and Disease Registry "ATSDR" 2007. CERCLA Priority List of Hazardous Substances. Atlanta, GA: Public Health Service, U.S. Department of Health and Human Services. http://www.atsdr.cdc.gov/ cercla/07list.html.

Ahmed ASS, Rahman M, Sultan S, Babu SMOF, Sarker MSI. 2019. Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications. Mar Poll Bull (145):436–447

Ali MHF, Abdel-Attya SN, ouf MMJ, Moustafa SAM. 2016. Heavy metal residues in local and imported fish in Egypt. Journal of veterinary medical research 2016, 23 (1): 71 – 76.

Al- Yousul MH, El- Shahawi MS, Al- Ghais SM. 2000. trace metals in liver, skin and muscle of lethrinus lentjan fish species in relation to body length and sex. Sci. total Environ. (256): 87- 94.

Ambreen F, Javed M, Batool U. 2015. Tissue specific heavy metals uptake in economically important fish, Cyprinuscarpio at acute exposure of metals mixtures. Pakistan J. Zool., (47): 399-407.

Anonymous. 2009. Mercury, Lead, Cadmium, Tin and Arsenic in Food. Toxicol Fact Sheet Series (1): 1-13.

Anonymous. 2003. Dietary reference intakes: Applications in dietary planning. Subcommittee on interpretation and uses of dietary reference intakes and the standing committee on the scientific evaluation of dietary reference intakes. Institute of Medicine of the National Academies, the National

Academies Press, Washington D.C. p 248.

Anonymous. 2004. Agency for Toxic Substances and Disease Registry, Division of Toxicology, Clifton Road NE, Atlanta GA.

AOAC. 2002. AOAC Official method 999.10 Lead, Cadmium, Zinc, Copper and Iron in foods. Atomic Absorption Spectrophotometry after microwave digestion. First Action1999- NMLK- AOAC Method.

Authman MM. 2015. Use of fish as Bioindector of the effects of heavy metal pollution. J. Aqac. Res. Dev. (6): 1-13

Ayeloja AA, George FOA, Shorinmade AY, Jimoh WA, Afolabi QO, Olawepo KD. 2014. Heavy metal concentration in selected fish species from Eleyele reservoir Ibadan, Oyo state south-western Nigeria. Afr. J. Environ. Sci and Techno, 8(7): 422-427.

Badr AM, Mahana NA, Eissa A. 2014. Assessment of Heavy metals levels in water and their toxicity in some tissues of Nile Tilapia (Oreochromis niloticus) in River Nile Basin at Greater Cairo, Egypt. Global. Vet. 13(4): 432-443.

CEPA. 1995-97. California Environmental Protection Agency, State Water Resources Eisler R. 1981. Trace metal concentrations in marine organisms. Pergamon Press, New York, pp. 685.

Ekpo KE, Asia IO, Amayo KO, Jegede DA. 2008. Determination of lead, cadmium and mercury in surrounding water and organs of some species of fish From Ikpoba river in Benin city, Nigeria. Inter. J. Phys. Sci, 3 (11): 289-292.

Emin OO, Hayati Y, Yasar E, Cevik TA, Gunfer T. 2010. The Effects of Copper Sulfate on Liver Histology and Biochemical Parameters of Term Ross Broiler Chicks. Biological Trace Element Research, 133 (3):335-341.

EOS" Egyptian Organization for Standardization "2010. Maximum Levels for certain contaminants in foodstuffs. No 7136/2010. Egyptian Standards, Ministry of Industry, Egypt

EU. 2001. Commission regulation n. 221/2002 of the 6 February 2002 amending regulation (EC) n. 466/2002 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Communities. Brussels (2002).

EU. 2006. Commission r-Regulation setting maximum levels for certain contaminants in food stuffs (EC) No 1881/2006.

FAO. 1983. Compilation of legal limits for hazardous substances in fish and fishery product. 746. Library (Lond) 104.

FAO/WHO. 1989. Evaluation of certain food additives and the contaminants mercury, lead and cadmium, WHO Technical Report, Series No. 505.

Giri SS, Yun S, Jun JW, Kim HJ, Kim SG, Kang JW, Kim SW, Han SJ, Sukumaran V, Park SC. 2018. Therapeutic effect of intestinal autochthonous Lactobacillus reuteri P16 against waterborne lead toxicity in Cyprinus carpio. Front Immunol (9):1824. https://doi. org/10.3389/ fimmu.2018.01824.

Grazuleviciene R, Nadisauskiene R, Buinauskiene, J, Grazulevicius T. 2009. Effects of elevated levels of Manganese and iron in drinking water on birth outcomes. Polish J of Environ Stud. 18 (5):819-825.

Halttunen T, Collado M, El-Nezami H, Meriluoto J, Salminen S. 2008. Combining strains of lactic acid bacteria and heavy metal removal efficiency from aqueous solution. Letters Appl. Microbiol. (46):160 –165.

Halttunen T, Salminen S, Tahvonen R. 2007. Rapid removal of lead and cadmium from water by specific lactic acid bacteria. Inter. J. Food Microbiol., (114): 30-35.

Heidarieh M, Ghannadi Maragheh M, Azizi Shamami M, Behgar M, Ziaei F, Akbari Z. 2013. Evaluate of heavy metal concentration in shrimp *(Penaeus semisulcatus)* and crab *(Portunus pelagicus)* with INAA method. *Springer Plus*, 2, 1-5, 2013.DOI: 10.1186/2193-1801(2):72-76.

Hempel S, Newberry S, Ruelaz A, Wang Z, Miles JNV. 2011. Safety of probiotics to reduce risk and prevent or treat disease. Agency for Healthcare Research and Quality (AHRQ) publication No. 11-E007.

Hussain RT, Ebraheem MK, Moker HM. 2012. Assessment of Heavy Metals (Cd, Pb and Zn) contents in Livers of Chicken available in the local markets of Basrah City, Iraq. Bas.J.Vet. Res., 11(1): 43–51.

Institute of Environmental Conservation and Research INECAR. 2000. Position Paper Against Mining in Rapu-Rapu, Published by INECAR, Ateneo de Naga University, Philippines (www.adnu.edu.ph/Institutes/Inecar/pospaper1.asp)

Javaheri Baboli M, Velayatzadeh M. 2013. Determination of heavy metals and trace elements in the muscles of marine shrimp, fenneropenaeus merguiensis from Persian Gulf, Iran. *J Anim Plant Sci,* 23 (3): 786-791.

JECFA. 2000. Evaluation of certain food additives and contaminants. Fifty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. (WHO Technical Report Series, No. 896).World Health Organization, Geneva

Kaoud HA, El-Dahshan AR. 2010. Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish, Nature and Science. 8(4):147-156.

Koller M, Saleh HM. 2018. Introductory chapter: Introducing heavy metals, heavy metals. https: //doi. Org/ 10.5772/ intechopen.97198.

Lars J. 2003. Hazards of heavy metal contamination. British Med. Bull (68): 167-182.

MAFF. 1995. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1993. Aquatic Environment Monitoring Report No. 44. Directorate of Fisheries Research, Lowestoft

Mendil D, Uluözlü OD, Hasdemir E, Tüzen M, Sari H, Suiçmez M. 2005. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chem, 90 (1-2): 175179.

Milatovic D, Gupta RC, Yin Z, Zaja-Milatovic S, Aschner M. 2017. Manganese in reproductive and developmental toxicology: 567–581. https://doi.org/10.1016/B978-0-12-804239-7.00032-9

Morshdy AE, Hafez AE, Darwish WS, Hussein MA, Tharwat AE. 2013. Heavy metal residues in canned fishes in Egypt. *Jpn. J. Vet.* (61):S54–S57.

Morshdy AEMA, Darwish WS, Daoud JRM, Sebak MAM. 2019. Estimation of metal residues in *Oreochromis niloticus* and *Mugil cephalus* intended for human consumption in Egypt: a health risk assessment study with some reduction trials. *J. Consum. Protect. Food Saf.* ; (14):81–91.

Morshdy AE, Hussein MA, Darwish WS, Yousef RE, Tharwat AE. 2021. residual contents of selected heavy metals in commercial canned fish in Egypt. Dietary intakes and health risk assessment. Solv. Vet. Res. 58(suppl. 24): 101-7.

Mrvcic J, Stanzer D, Solic E, Stehlik-Tomas V. 2012. Interaction of lactic acid bacteria with metal ions. Opportunities for improving food safety and quality. World J. of Microbiol. and Biotechnol., 28 (9): 2771-2782.

Nisbet C, Terzi G, Osman Pilgir O, Sarac N. 2010. Determination of Heavy Metal Levels in Fish Samples Collected from the Middle Black Sea. Kafkas. Univ. Vet. Fak. Derg. 16 (1): 119-125, 2010 DOI:10.9775/kvfd. 2009.982.

Ojuederie OB, Babalola OO. 2017. Microbial and plant – assisted bioremediation of heavy metal polluted environments: a review. Int. J. Environ. Res. Public Health 14(12): 1504. https://doi. Org/10.3396/; Jerph 14121504.

Rader JI. 1991. Anti- nutritive effects of dietary tin. Adv. Exp. Med. Biol. (289):509-24.doi: 10.1007/ 978- 1- 4899- 2626.5- 34.

Samir MYO, Edris MA, Edris NS, Gamal Heikal IG. 2021. Degradation effect of *Lactobacillus rhamnosus* on some heavy metals experimentally inoculated in fish fillet model. Benha Veterinary Medical Journal (40): 132-136. Journal homepage: https://bvmj.journals.ekb.eg

Sharp M. 1999. Towards sustainable pesticides. J. Environ. Monit. (1): 33-36.

Shokr AL, Mohamed Ahmed Hassan AM, Elbahy FE. 2019. Heavy Metals Residues (Mercury and lead) Contaminating Nile and Marine Fishes. Benha veterinary medical journal, vol. (36): no. (2):40-48

Sireli UT, Goncuoglu M, Yildirim Y, Gucukoglu A, Cakmak O. 2006. Assessment of heavy metals (cadmium and lead) in vacuum packaged smoked fish species (Mackerel, Salmosalar and Oncorhynhusmykiss) marketed in Ankara (Turkey). E.U Fisheries&Aquat. Sci. J.23, (3-4): 353 -356.

Tchounwon PB, yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. In luch, A.(eds) Molecular, clinical and environmental toxicology. Experientia supplementum. Vol. 101.Springer: Pp 133- 104.

Tüzen M. 2003. Determination of heavy metals in fish samples of middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. Food Chem, (80): 119-123.

United States Department of Labor (USDOL) 2004. Occupational Safety and Health Administration (OSHA); Safety and Health Topics: Heavy metals, USDOL Publication, Washington, D.C. (www.osha.gov/SLTC/metalsheavy/index.html)

Veglio F, Beolchini F, Gasbarro A. 1997. Biosorption of toxic metals: an equilibrium study using free cells of Arthrobacter sp. Process Biochemistry, 32(2): 99-105.

WHO. JECFA 2006. Evaluation of Certain Food Contaminants: Sixty-Fourth Report of the Joint FAO/WHO Expert Committee on Food Addi-Tives. WHO Technical Report Series No. 930.

Wang S, Xing TR, Tang ML, Yong W, Li CC Chen L, Wang HL, Tang JL, Ruan DY. 2008. Effects of Cd2+ on transient outward and delayed rectifier potassium currents in acutely isolated rat hippocampal CA1 neurons. Naunyn Schmiedebergs Arch Pharmacol., 377(3): 245-53.

WHO. 2004. Guidelines for drinking water quality, 3rd edn. World Health Organization, Geneva.

World Health Organization (WHO) 1985. Guidelines for drinking water quality (ii): Health criteria and supporting information. Geneva: WHO; 1985. p.130.