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ASSESSMENT OF HEAVY METALS AND HISTAMINE IN FARMED FISH SPECIES MARKETED IN EGYPT

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ABSTRACT

Humans are exposed to certain environmental contaminants from finfish and shellfish, including heavy metals, metalloids, and biogenic amines, such as tyramine, cadaverine, and putrescine, which potentiate histamine toxicity. This study aimed to determine the extent of contamination with some heavy metals (mercury, lead, and cadmium) and levels of histamine formation in farmed fish marketed in Zagazig City, Egypt. A total of 90 fish samples were collected from farmed fish marketed in Zagazig City, Egypt. The samples represented *Mugil cephalus (M. cephalus), Oreochromis niloticus (O. niloticus), and Claris lazera (C. lazera).* 30 of each were subjected to heavy metals analysis Mercury (Hg), lead (Pb), cadmium (Cd), and histamine levels. The mean values of Hg, Pb, and Cd were 0.22 ± 0.03, 0.26 ± 0.01, and 0.09 ± mg/kg in *M. cephalus*; 0.31 ± 0.04, 0.39 ± 0.01, and 0.17 ± mg/kg in *O. niloticus; and* 0.41 ± 0.07, 0.51 ± 0.01, and 0.30 ± mg/kg in *C. lazera*, respectively. The histamine values were 10.35 ± 3.58, 14.41 ± 3.36, and 20.38 ± 4.11 mg/kg in examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively. The samples of *C. lazera* significantly contained higher contaminants ($P<0.05$) than other *M. cephalus and O. niloticus*. The effect of *L. rhamnosus* and *Bacillus polymyxa* in the control of heavy metals and histamine is considered a promising solution for human health.

Key words: Mercury, *Mugil cephalus, O. niloticus, C. lazera,* histamine.

INTRODUCTION

Fish is a healthy food due to the nutritional benefits associated with its high biological quality proteins, desirable lipid composition, valuable mineral compounds, and vitamins. Its lipid fraction is rich in essential x-3 polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),and low in cholesterol, making it an excellent primer food (Storelli, 2008). Human exposure to certain environmental contaminants, including heavy metals and metalloids, may come mostly from finfish and shellfish. These substances are dangerous because of their persistence,

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bioaccumulation, and biomagnification into the food chain (Castro-Gonzalez and Méndez-Armenta, 2008). In addition, a variety of substances, including food items, drinking water, plants, animals, petrochemical derivatives, industrial waste, and pharmaceutical compounds, have been shown to contain a variety of chemical hormones, such as estrogen, progesterone, and androgen (Gonzalez *et al., 2019*). The term "biogenic amines" refers to low-molecular-weight organic bases with biological activity produced in meals by microbial decarboxylation of the respective amino acids or by transamination of aldehydes and ketones by amino acid transaminases (Zhai *et al.,* 2012). Biogenic amines, such as tyramine, cadaverine, and putrescine, operate as potentiates of histamine toxicity, with histamine regarded as the primary cause of scombroid poisoning (Al Bulushi *et al.,* 2009; Joshi and Bhoir, 2011). *Lactobacillus rhamnosus* has been shown in a prior study by Halttunen *et al.* (2007) to be capable of chelating a variety of other harmful chemicals from the food chain. The purpose of this study was to determine the extent of contamination with some heavy metals (mercury, lead, and cadmium) and levels of histamine formation in farmed fish marketed in Zagazig City. Furthermore, the study extended to control Pb and Cd using *L. rhamnosus* and histamine by using *Bacillus polymyxa* in artificially inoculated fish fillets.

MATERIALS AND METHODS

Handling of fish and all practical protocols were approved by the Ethics Committee of the Veterinary Medicine Faculty, Benha University, with ethical no. BUFVTM 47-09-23

1. Collection of fish samples

A total of 90 random samples of farmed fish represented by *M. cephalus, O. niloticus, and C. lazera* (30 of each) were collected from a certain fish market in Zagazig City, Egypt. The collected samples were kept in a separate sterile plastic bag, labeled, and preserved in an icebox, then transferred to the laboratory to evaluate heavy metals (mercury, lead, and cadmium) and levels of histamine.

2. Detection of heavy metals

2.1. sample preparation and digestion for heavy metal analysis

The preparation and digestion of samples, according to Shibamoto and Bjeldanes (1994). Briefly, a sterile stainless-steel knife and forceps were used to aseptically remove 1 g of a targeted part of the dorsal muscle from each fish, starting from the area slightly behind the nape and moving toward the fish tail. After being cut into pieces with a ceramic knife, the muscle was then put into clean, previously washed 20-mL screw-capped tubes that contained a mixture of 2 mL of concentrated perchloric acid (70%) and 4 mL of concentrated nitric acid (65%) in a ratio of 2:4. The screw-capped tubes were then sealed and left in a water bath at 53 °C overnight to allow thorough digestion. The digest was diluted with water and filtered through Whatman filter paper No. 42 into a clean glass beaker after coming to room temperature. The filtrate was then further diluted with deionized water to a final concentration of up to 50 mL. The filtrate was diluted, then put into clean screw-capped bottles, labeled with the quantity, season, and type of fish, and kept at room temperature until it was analyzed for the presence of heavy metals. The same process was used to generate a blank digest, which was examined for any remaining metals after the sample was removed.

2.2. Validation of heavy metals

According to the recommended concentrations for the calibration curve set by AOAC (2015) (https://brooksapplied.com/wp-content/uploads/2015/07/AOAC-Method-2015.01.pdf). By diluting a stock solution of 1000 mg/L of the examined components with acidified ultrapure water (5% v/v HNO_3), standard solutions for the calibration curves were created. Standard solutions with concentrations of 0.005, 0.01, 0.01, 0.05, 0.25, 2.5, and 10 g/L were used for Pb, whereas solutions with values of 0.01, 0.05, 0.1, 0.5, 2, and 5 g/L were used for Hg. The accuracy of the digestion method used to assess homogenized fish tissues and the recovery percentage of the certified reference material, in which the spiked fish samples were digested and analyzed using the same analytical process as the fish samples.

2.3. Heavy metal analysis

The heavy metal (Hg, Pb, and Cd) residues (µg/g wet weight) from all samples were determined by using a "Buck Scientific USA 210 VGP Atomic Absorption Spectrophotometer equipped with an oxidizing air acetylene flame" (Norwalk, CT, USA) at the central laboratory in the Faculty of Veterinary Medicine, Benha Univ., Egypt. The apparatus can work at wavelengths of 283.3, 253.7, and 228.8 nm for Pb, Hg, and Cd, respectively.

3. Determination of histamine by ELISA

According to **Leszczynskai *et al.* (2004)** and the manufacturer's instructions, an ELISA was used to determine histamine using the RIDASCREEN_ histamine kit (R-Bipharm AG, Germany). Fresh fish have a detection limit of 2.5 ppm. The ELISA plate reader calculated the optical density to be 450 nm. The optical density and complex quantity bound to the plate were inversely related to the amount of histamine present in the fish sample.

4. Effect of Lactobacillus rhamnosus on concentrations of lead and cadmium experimentally inoculated into fish fillet samples

Preparation of bacterial suspension Lactobacillus rhamnosus strain was enriched in Brain Heart Infusion (BHI) broth 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 for min), and the bacterial pellets were washed twice with deionized water (Halttunen *et al.,* 2008). The bacterial strain was suspended in 1 kg of meat fillets. The mixture was adjusted to reach a final concentration of either $1×10^7$ bacteria plus 50 mg/kg ionic mercury standard solution, $1×10^7$ bacteria plus 30 mg/kg ionic lead standard solution, $1×10^7$ bacteria and 10 mg/kg ionic cadmium standard solution, or $1×10^7$ bacteria plus 10 mg/kg ionic arsenic standard solution according to Halttunen *et al.* (2008) with some modification. The fish fillets with bacterial strain and metal solution were incubated for 24 hours on a Fine Mixer SH2000 Orbital Shaker (FINEPCR, Seoul, Korea) with soft agitation. Accordingly, the fish fillets contaminated with metals were served as a control assay. However, the test groups represented the fish fillets contaminated with heavy metals and *Lactobacillus rhamnosus that* were served as treated groups. The samples were acidified with ultrapure HNO_3 and examined at zero, 6, 12, 18, and 24-hour time points for measuring each free metal by flame atomic absorption spectrophotometer.

5. Effect of *Bacillus polymyxa* on concentration of histamine experimentally inoculated into fish fillets:

The effect of *Bacillus polymyxa* as a biological trial for reducing the concentrations of histamine experimentally inoculated into fish fillets was demonstrated as follows: According to Eom *et al.* (2015), the *Bacillus polymyxa* strain was cultivated in Brain Heart Infusion (BHI) Broth (Fluka, Sigma-Aldrich Chemie GmbH) for 24 hours 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) (Merck, Darmstadt, Germany). Accordingly, the viable count of *Bacillus polymyxa* strain was carried out according to the plate count method (a volume of the culture broth corresponding to approximately $1×10^7$ bacteria was centrifuged (500 rpm, 15 minutes at 5ºC), and the bacterial pellets were washed twice with deionized water. In the binding assay, briefly, bacterial pellets were suspended in 1 kg of fresh fish fillets. The mixture was adjusted to reach a final concentration of 1×10^7 bacteria and 50 mg/kg histamine level. Bacterial pellets and histamine standard solution were vortexed for 5 seconds (Stuart, Staffordshire, U.K.) and incubated for 24 hours on a fine mixer SH2000 orbital shaker (Finepcr, Seoul, Korea) with soft agitation. Accordingly, the tested fish fillets contaminated with histamine (without *Bacillus polymyxa*) were served as a control assay. However, the test group represented fish fillets contaminated with histamine, as well as treated with *Bacillus polymyxa, which* were served as the treated group. The samples were acidified with ultrapure HNO_3 and examined at zero, 8, 16, and 24-hour time points for measuring their histamine (Halttunen *et al.,* 2008).

6. Statistical Analysis:
According to Feldman *et al. (2003),* the Analysis of Variance (ANOVA) test was used to statistically evaluate the results for each parameter.

RESULTS

1. Heavy metal residues in fish
The heavy metal concentrations (mg/kg wet weight) in the examined samples of the three fish species from the Zagazig fish market in the present study are shown in Table (1). Mercury was detected in all samples at concentrations ranging from 0.06 to 0.64, 0.08 to 0.71, and 0.13 to 1.03, with mean values of 0.22 ± 0.03, 0.31 ± 0.04, and 0.41 ± 0.07 mg/kg in M. *cephalus*, *O. niloticus*, and C. lazera, respectively.

The residual concentrations of Pb ranged from 0.06 to 0.57, 0.11 to 0.83, and 0.15 to 0.98, with mean values of 0.26±0.01, 0.39±0.01, and 0.51 ± mg/kg in examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively (Table 1). By comparing our findings of Pb to the MRL (Figure 1), the obtained result showed 26 (86.6%), 23 (76.6%), and 22 (73.3%) were within the MRL. Meanwhile, 4 (13.3%), 7 (23.3%), and 8 (26.7%) of examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively, exceeded the established limit of 0.3 ppm permitted by ES (2010).

The level of Cd residues ranged from 0.01 to 0.24, 0.02 to 0.37, and 0.02 to 0.56, with mean values of 0.09 ± 0.01, 0.17 ± 0.01, and 0.30 ± mg/kg in examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively (Table 1). The Egyptian standard (2010) determined the MRL of Cd as 0.05 mg/kg. According to the MRL, 28 (93.3%), 25 (83.3%), and 23 (76.7%) were accepted. Meanwhile, 2 (6.7%), 5 (16.7%), and 7 (23.3%) of examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively.

Table 1: Statistical analytical results of mercury, lead, and cadmium residues in mg/kg (ppm) in examined fish species (n=30).

	Fish species	Minimum	Maximum	Mean ± SE
Hg	*M. cephalus*	0.06	0.64	0.22 ± 0.03^b
	O. niloticus	0.08	0.71	0.31 ± 0.04^b
	C. lazera	0.13	1.03	0.41 ± 0.07^a
Pb	*M. cephalus*	0.06	0.57	0.26 ± 0.01^c
	O. niloticus	0.11	0.83	0.39 ± 0.01^b
	C. lazera	0.15	0.98	0.51 ± 0.01^a
Cd	*M. cephalus*	0.01	0.24	0.09 ± 0.01^c
	O. niloticus	0.02	0.37	0.17 ± 0.01^b
	C. lazera	0.02	0.56	0.30 ± 0.01^c

Means with different letters in the same column are significantly different ($P<0.05$)

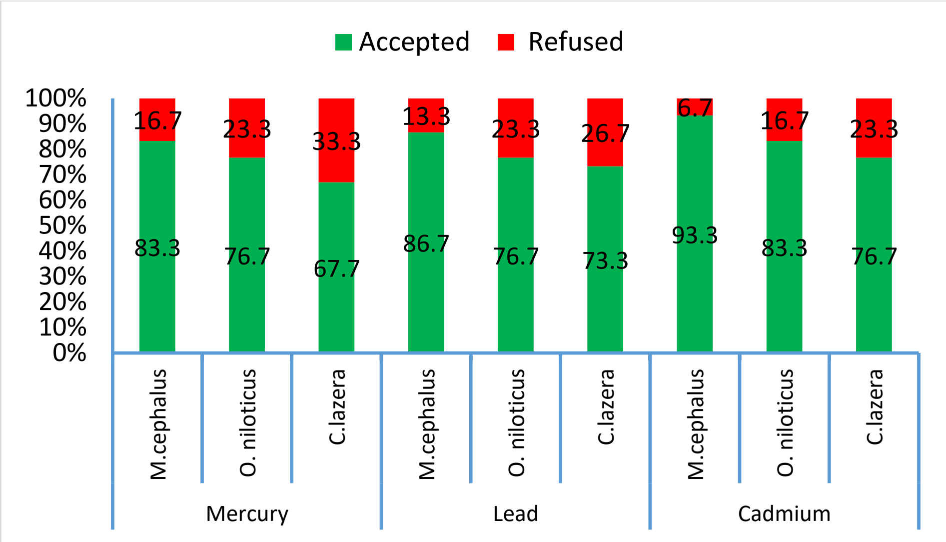

Figure 1. Percentages of acceptability among examined fish samples according to Egyptian standard 2010.

2. Histamine level in fish

The results in Table (2) declared that histamine ranged from 0.97 to 22.26, 1.45 to 29.66, and 1.98 to 37.66, with mean values of 10.35 ± 3.58, 14.41 ± 3.36, and 20.38 ± 4.11 mg/kg in examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively. The acceptability of the examined samples based on their levels of histamine, according to ES (2010), which stated their maximum permissible limit of histamine was 20 mg%. Therefore, the number of accepted samples was 27 (90%) in *M. cephalus*, 25 (83.33%) in *O. niloticus,* and 21 (70%) in C. lazera, while the number of unaccepted samples of marine fish was 3 (10%), 5 (16.67%), and 9 (30%) in the examined *M. cephalus*, *O. niloticus,* and *C. lazera*, respectively.

Table 2: Analytical results of histamine (mg/kg) in the examined fish samples, compared with the permissible limit.

	Min	Max	Mean ± SE	Accepted samples	Unaccepted samples
Mugil cephalus	0.97	22.26	10.35 ± 3.58^{c}	27 (90%)	3 (10 %)
Oreochromis niloticus	1.45	29.66	14.41 ± 3.36^{b}	25 (83.33%)	5 (16.67%)
Clarias lazera	1.98	37.66	20.38 ± 4.11^{a}	21 (70%)	9 (30%)

Means with different letters in the same column are significantly different (P<0.05).
Maximum Residual Limit of histamine (20 mg %) stipulated by Egyptian Organization of Standardization "ES" (2010).

3. Effect of *L. rhamnosus* on the levels of lead, cadmium, and histamine experimentally inoculated into fish fillets

The results in Table (3) declared that the level of Pb reduced from 30 mg/kg to 21.8, 14.5, 9.3, and 5.6 mg/kg, with a reduction of 27.3%, 51.7%, 69.0%, and 81.3% after dipping the inoculated fish fillet for 6, 12, 18, and 24 hours, respectively. Also, Cd reduced from 10 mg/kg to 6.9, 5.4, 3.7, and 2.5 mg/kg, with reductions 31%, 46%, 63%, and 75%, after dipping the inoculated fish fillet for 6, 12, 18, and 24 hours, respectively, (Table 4).

4. Effect of Bacillus polymyxa on the levels of histamine experimentally inoculated into fish fillets

The results in Table (5) declared that the level of histamine reduced from 50 mg/kg to 29.7, 22.1, and 17.9 mg/kg, with a reduction 40.6%, 55.8%, and 64.2% after dipping the inoculated fish fillet for 8, 16, and 24 hours, respectively.

Table 3: Effect of *L. rhamnosus* culture ($2x10^7$) on the concentration of lead experimentally inoculated to fish fillets (30 mg/Kg).

Treatment / Storage time	Control mg/Kg	*L.rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	30	30	-------
6 hours	30	21.8	27.3
12 hours	30	14.5	51.7
18 hours	30	9.3	69.0
24 hours	30	5.6	81.3

Table 4: Effect of *L. rhamnosus* culture ($2x10^7$) on the concentration of cadmium experimentally inoculated to fish fillets (10 mg/Kg).

Treatment / Storage time	Control mg/Kg	*L.rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	10	10	-------
6 hours	10	6.9	31.0
12 hours	10	5.4	46.0
18 hours	10	3.7	63.0
24 hours	10	2.5	75.0

Table 5. Effect of *B. polymyxa* culture ($1x10^7$) on the levels of histamine experimentally inoculated to fish fillets (50 mg/Kg).

Group / Storage time	Control mg/Kg	*B. polymyxa* Treated group (mg/Kg)	Reduction %
Zero time	50	50	-------
8 hours	50	29.7	40.6
16 hours	50	22.1	55.8
24 hours	50	17.9	64.2

DISCUSSION

1. Heavy metal residues in fish

The disposal of agricultural and industrial waste into the ocean or brackish water, where it becomes harmful for many aquatic animals, is a severe problem in many nations. Heavy metals, in particular Hg, Pb, and Cd, are carcinogenic substances that can have negative biological effects on children's and adults' mental and cognitive development, as well as kidney and reproductive malfunction (Uluozlu *et al.,* 2007).

The heavy metal concentrations (mg/kg wet weight) in the examined samples of the three fish species from Zagazig fish market in the present study are shown in Table (1).

The obtained values in this study were comparatively lower than that obtained by Hussein *et al.,* (2011) and Hamada *et al.,* (2018), where they detected 1.87±0.17 and 0.94±0.10 mg/kg, respectively, from fish samples in Egypt. Also, a higher mean level of 3.154 mg/kg for Hg has been reported in Iran (Fard *et al.,* 2015). On the other hand, the obtained results were agreed with that recorded by Hassan and Salem (2003), who found that mean concentration was 0.45±0.03 mg/kg. The obtained results are relatively higher than that reported by Hashim *et al. (2008),* who reported Hg concentration as 0.013±0.001 mg/kg, and Marzouk *et al.* (2016), who noted that mean concentration was 0.105±0.005 ppm. Mercury toxicity is linked to adult nervous system damage and newborns' and children's poor neurological growth. Mercury that has been consumed may bioaccumulate, gradually increasing the loads on the body. This paper discusses the systemic pathophysiology of specific organ systems linked to mercury poisoning. The toxicological effects of mercury on cells, the heart, the blood, the lungs, the kidneys, the immune system, the nervous system, the endocrine system, the reproductive system, and the embryo are significant (Rice *et al.,* 2014). The acceptability of examined samples according to the Hg 25 (83.3%), 23 (76.6%) and 20 (67.7%) in *M. cephalus*, *O. niloticus* and *C. lazera*, respectively, according to the ES (2010), which set the MRL to 0.5 ppm. Meanwhile,5 (16. content was 7%), 7 (23.3%) and 10 (33.3%) of examined *M. cephalus*, *O. niloticus* and *C. lazera*, respectively, exceeded the established limit (Figure 1). The most well-known mercury compounds in the environment are the soluble salts, mono-methyl and dimethyl mercury, which anaerobic bacteria generate from inorganic mercury in sediment and then release into natural water (Manahan, 1989). According to Bishop and Neary (1974), methyl mercury, which is lipid soluble and quickly absorbed and disseminated through biological systems, comprised an average of 88.9% of the total mercury found in fish meat.

Nearly similar Pb values were detected in fish species from the Black and Aegean seas: 0.33 to 0.93 mg/kg (Uluozlu *et al.,* 2007), 0.33 to 0.86 mg/kg in fish samples collected from the Marmara, Aegean, and Mediterranean seas (Turkmen, *et al.,* 2008) and 0.34 to 0.54 mg/kg in fish samples collected from Menoufia Governorate, Egypt (Hamada *et al.,* 2018). Lower Pb values 0.014 to 0.037 in fish samples collected from Portuguese waters (Vieira *et al.,* 2011) and 0.11 to 0.16 mg/kg in freshwater fish from Egypt (Madiha, 2009). Meanwhile, much higher Pb values 6.5 and 10.01 mg/kg in fish samples from Fayoum and Manzala Egypt (Ali and Abdel-Satar 2005; Saeed and Shaker 2008). Twenty to seventy percent of ingested lead absorbed, and increased blood Pb levels have an impact on behavior, cognitive function, postnatal growth, delay puberty, and lower hearing ability in infants and kids. Pb damages the central nervous system, the kidneys, the heart, and the reproductive system in adults. Pb can impair fetal growth in the early stages of pregnancy (Kumar *et al.,* 2020).

The Cd residues detected in the current study were similar to those previously obtained, 0.10 to 0.15 mg/kg in fish samples collected from Menoufia Governorate, Egypt (Hamada *et al.,* 2018). Lower Cd values (0.03 mg/kg) and (0.006-0.024mg/kg) in fish from Lake Manzala, Egypt (El-Moselhy, 1999: Sallam *et al.,* 2019) and 0.003 to 0.021µg/g in fish from Taihu Lake of China (Chi *et al.,* 2007). Much higher Cd value10.36 mg/kg was recorded, in fish from Manzala Lake of Egypt (Saeed & Shaker, 2008).

The edible tissues of the species under study contained the lowest concentrations of cadmium. It is known that Cd only exists in tiny amounts in marine life and aquatic species. However, it adversely affects several organs, including the brain, placenta, kidney, lung, bones, and central nervous system (Castro-Gonzalez and Méndez-Armenta, 2008). The edible tissues of the species under study contained the lowest concentrations of cadmium. It is known that Cd only exists in very small amounts in marine life and aquatic species. The central nervous system, brain, kidney, lung, bones, placenta, and hepatic, hematological, and immunological reactions are all adversely impacted (ATSDR, 2008). Numerous studies (Castro-González and Méndez-Armenta, 2008; Storelli et al., 2005) show that Cd preferentially accumulates in internal organs such as the liver and kidney, but not in muscles, where concentrations are often relatively low.

The levels of contamination with Hg, Pb, and Cd among the examined species were in the following order: *C. lazera* > *O. niloticus* > *M. cephalus*. Furthermore, analysis of variance indicated significant differences ($P<0.05$) between Hg, Pb and Cd concentration among the three fish species analyzed. Different fish habitats, their needs, how they process food, and their eating habits might explain why heavy metal levels in muscle tissue vary among fish species (Chi *et al.,* 2007), along with factors like their feeding levels, location, size, age, and the ability of metals to build up in food chains (Wei *et al.,* 2014). Additionally, ecological requirements, body metabolic rate, water salinity, and temperature all affect how well fish absorb heavy metals from contaminated water and food. The ability of fish to absorb heavy metals from contaminated water and food is further influenced by ecological requirements, body metabolic rate, water salinity, and temperature (Satheeshkumar and Kumar, 2011).

2. Histamine level in fish

Histamine levels indicate spoilage potential for scombroid poisoning in the fish. Histidine may be converted to histamine during fish spoilage in the presence of decarboxylases, especially if the temperature rises above 10 ^{O}C (Koutsoumanis *et al.,* 1999). The results in Table (2), are nearly similar to results obtained by Ibrahim and Amin (2017), who reported 2.8 to 41.5 with an average of 22.68 ±1.96 for *O. niloticus*, 4.0 to 62.1 with an average of 37.14 ± 2.79 for *C. lazera*. Also, Helmy *et al.* (2018), who recorded histamine values as 21.59 ±1.72; 18.31±1.45 and 11.64 ±1.19 mg/kg for *C. gariepinus, O. niloticus* and *M. cephalus,* respectively. Meanwhile, lower values as 0.6 ± 0.06, 0.76 ± 0.08 and 0.96 ± 0.08 mg/kg in examined *C. lazera*, *O. niloticus* and *M.cephalus*, respectively (Hussein *et al.,* 2019). The samples of *C. lazera* contained significantly higher histamine ($P<0.05$) than other examined samples, which may be attributed to higher bacterial load, which led to higher decarboxylase activity (Koutsoumanis *et al.,* 1999).

The European Food Safety Agency has reported a maximum daily intake of histamine (50 mg) in foods for healthy adult individuals (EFSA 2011). Acute illness known as scombroid fish poisoning is caused when humans consume fish with high levels of histamine. This illness is marked by facial flushing, sweating, rash, diarrhea, and abdominal cramps and typically goes away on its own after several hours. However, serious symptoms including difficulty breathing, tongue swelling, and blurred vision must call for medical attention (CDC, 2007).

4.3. Effect of *L. rhamnosus* on the levels of lead, cadmium and histamine experimentally inoculated to fish fillets

The results in Table (3) and (4) recorded a reduction in the level of Hg, Pb and Cd within 24 hours of incubation of inoculated fish with *L. rhamnosus*. Samir *et al.* (2021) noticed a reduction in the level of Pb and Cd as 84.3 and 72%, respectively, within 24 hours of incubation of inoculated fish with *L. rhamnosus*. The mechanism of action may be attributed to that heavy metal attached to the cell wall matrix of the *L. rhamnosus* (Zoghi *et al.,* 2014).

4. Effect of Bacillus polymyxa on the levels of histamine experimentally inoculated to fish fillets

The results in Table 5 indicated that the level of histamine had decreased. *Bacillus polymyxa* is a biological trial for reduction of the concentrations of histamine (Eom *et al.*, 2015).

Conflict of Interest: All authors confirm that they have no conflict of interest related to the submitted manuscript

CONCLUSION

The examined fish contained heavy metal residues in varying amounts, indicating contamination during the culture period. Also, histamine was determined in the samples, indicating the presence of microbes that convert the amino acid histidine to histamine. Therefore, the correct principles must be followed in raising fish in a healthy environment, as well as effective preservation after fishing and in sales outlets to reduce risks to the consumer. Furthermore, *L. rhamnosus* shown a promising decreasing approach to reduce the buildup of heavy metals in fish tissues.

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تقييم المعادن الثقيلة والهستامين في أنواع الأسماك المستزرعة المباعة في مصر

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يتعرض البشر لبعض الملوثات البيئية من الأسماك الزعنفية والمحاريات، بما في ذلك المعادن الثقيلة، وأشباه الفلزات، والأمينات الحيوية، مثل التيرامين، والكادافيرين، والبوتريسين، والتي تزيد من سمية الهيستامين. هدفت هذه الدراسة إلى تحديد مدى التلوث ببعض المعادن الثقيلة (الزئبق، والرصاص، والكادميوم) ومستويات تكوين الهيستامين في الأسماك المستزرعة المُسوّقة في مدينة الزقازيق، مصر.

تم جمع ٩٠ عينة من الأسماك المستزرعة من السوق في مدينة الزقازيق، مصر. العينات الممثلة بـ البوري والبلطي(و القرموط(تم إخضاع ٣٠ منها لتحليل المعادن الثقيلة الزئبق ((Hg، الرصاص ((Pb، الكادميوم (Cd).) ومستويات الهستامين. كانت القيم المتوسطة للزئبق والرصاص والكادميوم ٠,٢٢ ± ٠,٠٣ و٠,٢٦ ± ٠,٠١ و٠,٠٩ ± ٠,٠١ ملجم/كجم في البوري؛ ٠,٣١ ± ٠,٠٤، ٠,٣٩ ± ٠,٠١ و ٠,١٧ ± ٠,٠١ ملغم/كغم في البلطي؛ ٠,٤١ ± ٠,٠٧، ٠,٥١ ± ٠,٠١ و ٠,٣٠ ± ٠,٠١ ملغم / كغم في القرموط، على التوالي. كانت قيم الهستامين ١٠,٣٥ ± ٣,٥٨،١٤,٤١ ± ٣,٣٦ و ٢٠,٣٨ ± ٤,١١ ملغم / كغم في البوري والبلطي و والقرموط على التوالي. تحتوي عينات القرموط على ملوثات أعلى بشكل ملحوظ (P <٠,٠٥) مقارنة بأنواع البوريو البلطي الأخرى. يعتبر تأثير بكتيريا L. rhamnosus و Bacillus polymyxa في السيطرة على المعادن الثقيلة والهستامين حلاً واعداً لصحة الإنسان.