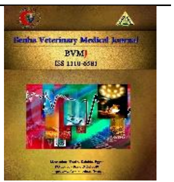
Benha Veterinary Medical Journal 40 (2021) 132-136

Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/



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Original Paper

Degradation effect of *Lactobacillus rhamnosus* on some heavy metals experimentally inoculated in fish fillet model

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ARTICLE INFO

Keywords

Heavy metals

Fish

Shellfish

Lactobacillus rhamnosus

Received 17/05/2021
Accepted 17/06/2021
Available On-Line
01/10/2021

ABSTRACT

A total of 120 samples of fish and shellfish samples represented by *Oreochromis niloticus, Mullus surmuletus*, silver carp, shrimp, crab and oyster (20 of each) were collected from different markets at different localities in Qalubiya Governorate, Egypt. The collected samples were examined for their levels of mercury, lead, and cadmium by atomic spectrophotometer. The obtained results revealed that *O. niloticus* was the highest contaminated within fish samples with mean values of 1.91, 1.05, and 0.37 mg/kg; while oyster was the highest within shellfish samples with mean values of 1.53, 0.84, and 0.29 mg/kg for mercury, lead, and cadmium, respectively. Advanced experimental work was conducted aimed to assess the degradation effect of one of *Lactobacillus rhamnosus* (10^7 CFU/ml) on lead and cadmium levels in experimentally inoculated fish fillet along of 24 hours. Results revealed promising rapid reductions in lead and cadmium levels within 24 hrs of interaction with *L. rhamnosus*, where lead and cadmium were reduced by 84.3 and 72.0%, respectively. Accordingly, regular investigation of heavy metals levels in aquatic environment and creatures is recommended, with strictly recommendation to safe disposal of factory wastes. Furthermore, *L. rhamnosus* showed promising diminishing technique to decrease heavy metal accumulation in fish tissues, where advanced research on its effect on the aquatic environment is recommended.

1. INTRODUCTION

Seafoods have been globally consumed for their nutritional value, along with its high-quality proteins, omega-3, and vitamin B-complex (Özden *et al.*, 2018). In contrast, fish and fish products may cause a serious health hazard along with its consumption because of its high affinity to heavy metals accumulation in their muscle after taking from surrounding environment along their life cycle (Li *et al.*, 2017); marine and fresh water heavy metals contamination can occur through various urbanization, agricultural and industrial unhygienic disposal of their wastes.

Referring to the nature of fish feeding systems, fishes can accumulate heavy metals in their tissues more than surrounding environment as they feed mainly on organic matters, algae and other small organisms which were previously uptake surrounding environmental pollution by heavy metals (Olaifa *et al.*, 2004). The prominent sources of heavy metal contamination are agricultural inputs, domestic and commercial sewage, untreated or partially treated effluents from small and large scale industrial units (Morais *et al.*, 2012); metal electroplating, manufacture and disposal of batteries (Chen *et al.* 2012), circuit boards, car repair, motor workshops (Singh *et al.* 2018).

Seafoods have many elements, which are required for human life at low concentrations, which will be toxic in higher concentrations such as manganese, zinc, and magnesium. Other elements such as mercury (Hg), cadmium (Cd) and lead (Pb) have unknown essential function in life and were toxic even at low concentrations when ingested over a long period; therefore, many health authorities regarded any presence of these elements in foods as a health hazard (Oehlenschläger, 2005).

In the aquatic environment heavy metals are assimilated by fish, since fish is great bioaccumulator, assimilation can occur by ingestion, ion exchange across gills or membrane surface and adsorption by tissues of the fish (Ahmed *et al.*, 2014). It happens in different parts of different fish species due to the varying soluble nature of metals in water, bioavailability and the different habitats, life cycles, nature of feeding, ecology and physiological nature of fish (Anandkumar *et al.*, 2017). High bioaccumulation of heavy metals in the aquatic body can lead to genotoxic damage of aquatic species and high concentrations of heavy metals can have cytotoxic, mutagenic and genotoxic effects on the consumers (Matos *et al.*, 2017).

Numerous studies indicated that lactic acid bacteria (LAB) play an essential role in the body's immunity, detoxification, and elimination of heavy metal residues (Rao, 2009), because of their ability to adhere to- or bind different

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metallic molecules. Previous report by Halttunen *et al.* (2007) recorded ability of *Lactobacillus rhamnosus* to chelate many other toxic substances, such as aflatoxin B1, and mutagens from food.

Therefore, many experimental trials have been conducted to eliminate or decrease heavy metal concentrations in food chain to save consumer's health. Consequently, the present study aimed to detect the concentrations of some heavy metals (mercury, lead and cadmium) in the examined fishes and shellfish samples. In addition, assessment of *Lactobacillus rhamnosus* diminishing effect was performed.

2. MATERIAL AND METHODS

2.1. Collection of Samples:

A total of 120 samples of *Oreochromis niloticus* (*O. niloticus*), *Mullus surmuletus* (*M. surmuletus*), and silver carb (20 of each) as fishes samples; moreover, shrimp, crab and oyster (20 of each) were collected as shellfishes (20 of each) from different fish markets at different localities in Qalubiya Governorate, Egypt. The collected samples were kept separately in sterile bags and transferred to the laboratory without undue delay.

2.2. Determination of heavy metals:

2.2.1. Washing procedures (Lars, 2003)

Glass wares and vessels were washed by deionized water and soaked in hot diluted HNO_3 (10%) for 24h, and rinsed several times with deionized water, then dried. Accordingly, all containers were thoroughly washed with deionized water and air-dried in incubator.

2.2.2. Digestion technique (Staniskiene et al., 2006)

Accurately, one gram of each sample was macerated and digested by 10 ml of digestion mixture (60ml of 65% Nitric acid, and 40ml of 70% perchloric acid) in screw capped tube, followed by vigorous shaking and heated for 4h in water bath at 110°C to ensure complete digestion of the samples. Each tube was diluted with deionized water till reach 25 ml, then the digest was filtered with Whattman filter paper. The filtrates were collected and kept at room temperature until analyzed for their mercury, lead and cadmium concentrations.

2.2.3. Analysis:

The digest, blanks and standard solutions were aspirated by Atomic Absorption Spectrophotometer and analyzed for their concentrations of such elements.

2.3. Experimental part (effect of Lactobacillus rhamnosus on concentrations of lead and cadmium experimentally inoculated into fish fillets samples):

2.3.1. 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 15min), and the bacterial pellets were washed twice with deionized water (Halttunen *et al.,* 2007).

2.3.2. Binding assay

The bacterial pellet (10^7 CFU/ml), 30 mg/Kg ionic lead, and 10 mg/Kg ionic cadmium solutions were mixed with fish fillet samples according to Halttunen *et al.* (2008).

Experimental design

Fish fillet contaminated with metals was served as a control positive group (G1), the test groups represented fish fillets inoculated with *L. rhamnosus* (10^7 CFU/g) and lead (30 mg/kg) (G2), fish fillets inoculated with *L. rhamnosus* (10^7 CFU/g) and cadmium (10 mg/kg) (G3), were served as treated groups. The samples were acidified with ultrapure HNO_3 (Lars, 2003), and examined at zero, 8-, 16-, and 24-hour time points for measuring the free metal by atomic absorption spectrophotometer.

2.4. Statistical analysis:

the obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman *et al.* (2003).

Reduction rate (%) = $\frac{B-A}{A} x100$

B = mean value of the next heavy metal level.

A = mean value of the previous heavy metal level.

Table 1 Operating analyses of different heavy metals conditions

Condition	Mercury	Lead	Cadmium
Lamp wavelength (nm)	253.7	217.0	228.8
Lamp current (mA)	4	5	4
Slit width (nm)	0.5	1.0	0.5
Used gas	AC/N_2O	AC/A	AC/A

AC/N2O= Acetylene / Nitrous oxide, AC/A= Acetylene / Air

3. RESULTS

It is evident from the results recorded in Table (2) that among the examined fish samples, *O. niloticus* samples revealed the highest contamination with mercury, lead, and cadmium levels (mg/kg), followed by silver carb and *Mullus surmuletus*, respectively. Regarding with the examined shellfish, oyster samples were the highest contamination levels, followed by crab, and shrimp, respectively.

Table 2 mean values of different heavy metals levels "mg/Kg" in the examined fish and shellfish samples (n=20).

Fish and shellfish	Mercury	Lead	Cadmium
Mullus surmuletus	1.05 ± 0.09^c	0.49 ± 0.05^c	0.15 ± 0.01^c
Silver Carb	1.49 ± 0.21^b	0.72 ± 0.06^b	0.24 ± 0.03^b
Oreochromis niloticus	1.91 ± 0.27^a	1.05 ± 0.11^a	0.37 ± 0.04^a
Shrimp	0.87 ± 0.06^d	0.36 ± 0.02^d	0.09 ± 0.01^d
Crab	1.22 ± 0.18^c	0.58 ± 0.04^c	0.20 ± 0.02^c
Oyster	1.53 ± 0.22^b	0.84 ± 0.07^b	0.29 ± 0.02^b

Mean values with different superscripts were significantly different ($p<0.05$)

Referring to the Egyptian standards for the maximum residual limits as mentioned in Tables (3 to 5), 42.5, 38.3, and 29.2% of the total examined samples were regarded unfit for human consumption because of exceeding MRL recommended by the Egyptian authorities.

Table 3 Edibility of the examined samples of fish and shellfish based on their levels of mercury (n=20).

Fish and shellfish	MRL (mg/Kg)*	Accepted Samples		Unaccepted Samples	
		No.	%	No.	%
Mullus surmuletus	0.50	13	65	7	35
Silver Carb		11	55	9	45
Oreochromis niloticus		8	40	12	60
Shrimp		15	75	5	25
Crab		12	60	8	40
Oyster		10	50	10	50
Total		69	57.5	51	42.5

* Maximum Residual Limit of Egyptian Standards ES" (2010).

Table 4 Edibility of the examined samples of fish and shellfish based on their levels of lead (n=20).

Fish species	MRL (mg/Kg)*	Accepted Samples		Unaccepted Samples	
		No.	%	No.	%
Mullus surmuletus	0.3	14	70	6	30
Silver Carb		11	55	9	45
Oreochromis niloticus		9	45	11	55
Shrimp		15	75	5	25
Crab		13	65	7	35
Oyster		12	6	8	4
Total		74	61.7	46	38.3

* Maximum Residual Limit of Egyptian Standards ES" (2010).

Table5 Edibility of the examined samples of fish and shellfish based on their levels of cadmium (n=20).

Fish species	MRL (mg/Kg)*	Accepted Samples		Unaccepted Samples	
		No.	%	No.	%
Mullus surmuletus	0.05	16	80	4	20
Silver Carb		13	65	7	35
Oreochromis niloticus		11	55	9	45
Shrimp		17	85	3	15
Crab		15	75	5	25
Oyster		13	65	7	35
Total		85	70.8	35	29.2

* Maximum Residual Limit of Egyptian Standards ES" (2010).

Referring to the demonstrated results in Tables (6 and 7), addition of *L. rhamnosus* culture (10^7 CFU/ml) revealed rapid promising diminishing effect on lead and cadmium levels as they were reduced by 84.3 and 72% within 24h of the incubation; furthermore, higher degradation level on lead were recorded than cadmium levels in experimentally inoculated fish fillet model.

Table 6 Effect of *L. rhamnosus* culture (10^7 CFU/g) on the levels of lead experimentally inoculated to fish fillets (30 mg/Kg).

Storage time	Control (mg/Kg)	*L. rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	30	30	-------
8 hours	30	9.2	69.3
16 hours	30	5.8	80.7
24 hours	30	4.7	84.3

Table 7 Effect of *L. rhamnosus* culture (10^7 CFU/g) on the levels of cadmium experimentally inoculated to fish fillets (10 mg/Kg).

Storage time	Control (mg/Kg)	*L. rhamnosus* Treated group (mg/Kg)	Reduction %
Zero time	10	10	-------
8 hours	10	4.9	51.0
16 hours	10	3.6	64.0
24 hours	10	2.8	72.0

4. DISCUSSION

Heavy metals are hazardous contaminants in food and environment. These are non-biodegradable with long biological half-lives (Heidarieh *et al.,* 2013). So, the present study was carried out to survey and investigate the concentrations (mg/kg) of mercury, lead and cadmium in some commercially present fish and shellfishes in the Egyptian markets represented by (*Mullus surmuletu*s, silver carb, *O. niloticus*, shrimp, crab, and oyster).

Regarding Table (2), the obtained results of mercury were higher than those obtained by El-Nahas (2015) (0.46 mg/kg in *O. niloticus*), Fatema *et al.* (2015) (<0.03 mg/kg of the examined shrimp samples), El-Said (2016) (0.037 mg/kg in *O. niloticus*), and Helmy *et al.* (2018) (1.29, 0.95, 1.37, 1.14, and 0.73 mg/kg of *O. niloticus*, *M. cephalus*, oyster, shrimp, and crab samples, respectively).

Mercury can accumulate in human body through consumption of fish and other marine creatures. Neurotoxic effect of mercury toxicity, particularly in developing organisms, fetuses, infants, and young children generally are more sensitive than adults to the neurological effects (Agusa *et al.,* 2005).

In regard to the obtained results of lead concentrations, they were nearly similar with those recorded by Fatema *et al.* (2015) (ranged from 0.108 to 0.87 mg/kg in shrimp samples collected from Kobadak river, Bangladish), while higher than those recorded by El-Said (2016) (0.24 mg/kg in *O. niloticus* samples), Hadeed *et al.* (2017) (0.18 mg/kg *Pagrus bayad*), Helmy *et al.* (2018) (0.53, 0.56, 0.48, and 0.4 mg/kg of *O. niloticus,* oyster, shrimp, and crab samples, respectively); but also, they were lower than those recorded by Ilgar (2016) (was 5.57mg/kg in *S. pilchardus*), and Mehouel *et al.* (2019) (2.13 mg/kg in sardine).

High doses of lead causes chronic toxicity that includes renal, GIT, hematological and neuromuscular disorders. The main sings of lead intoxication in children are disability of learning and behavior abnormalities. In contrast, the principal sings of lead intoxication in adults are abdominal pain, kidney damage, headache, loss of memory, weakness, male reproductive impairment and tremors in extremities. Severe problems as dysfunction in nerve, joints and muscles, loss of memory, heart, bone and renal disorders occur due to the exposure to highly doses of lead than permissible limits (Environmental Working Group, 2010).

Referring to the documented results of cadmium levels, nearly similar results were recorded by Sohsah (2009) (0.26 mg/kg in *O. niloticus*). Lower results were recorded by Fatema *et al.* (2015) (ranged from ND to 0.04 mg/kg of the examined shrimp samples), El-Said (2016) (0.051 mg/kg in *O. niloticus*), Helmy *et al.* (2018) (0.19, 0.22, 0.15, and 0.12 mg/kg of *O. niloticus,* oyster, shrimp, and crab samples, respectively). and Gawish and Hosni (2017) (0.0492 and 0.0366 mg/kg in sardine and morgan, respectively). While higher results were recorded by Mehouel *et al.* (2019) (0.55 mg/kg in sardine samples), and Hadeed *et al.* (2017) (0.14 mg/kg in *Pagrus bayad* samples). Moreover, Omobepade *et al.* (2020) (0.003 and 0.025 mg/kg for cadmium and lead concentrations in shrimp samples, respectively).

Cadmium is a heavy metal that accumulates in the body resulting in brain damage, astroglial toxicity (Im *et al.*, 2006). Chronic toxicity of cadmium induces bone abnormalities and kidney impairment, while acute toxicity results in vomiting, gastritis, and diarrhea. Furthermore, lung cancer is reported due to inhalation of cadmium (ATSDR, 2008).

Statistical analyses of the recorded results revealed significant differences ($P \geq 0.05$) between the examined results; where *O. niloticus* and oyster were the predominant highly contaminated samples in the examined fishes and shellfishes, respectively that may be caused by the highly contaminated surrounding environment and/or bioaccumulation process based on the age of the collected samples.

The recorded variations between the obtained results and the other records may be attributed to the difference in the localities of sample's collection, age of fishes as a significant factor in bioaccumulation process, and types of the examined fishes.

An experimental study was conducted to investigate the lead and cadmium levels degradation under the effect of *L. rhamnosus* in fish fillet model. Results as tabulated in Tables (6 and 7) showed a great diminishing effect in lead and cadmium levels, respectively.

Generally, potential significant reduction in the prevalence of genotoxicity and hepatotoxicity rat model by cadmium after inoculation of *L. rhamnosus* (Jama *et al.*, 2012). The diminishing interaction effect of LAB with heavy metals can be attributed to its ability to bind with the metals intra- or extracellular by biosorption the passive no metabolically mediated binding process of metal ions to the LAB cell wall, so preventing any harmful interaction in the host cell (Mrvcic *et al.*, 2012).

5. CONCLUSION

As conclusion, *O. niloticus* and oyster were the predominantly contaminated samples with heavy metals residues within the examined fish and shellfish samples, respectively. Detection of heavy metals in such toxic concentrations in the examined samples possess a potential risk to the consumers, so regular investigation of heavy metals levels in aquatic environment and creatures is recommended, with strictly recommendation to safe disposal of factory wastes. Furthermore, *L. rhamnosus* showed promising diminishing technique to decrease heavy metal accumulation in fish tissues, where advanced research on its effect on the aquatic environment us recommended.

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