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Title

Sara H shaban1, Abd Elrahman M.Elbagoury2, Mohammed A.Hassan3 and Marwa A.Saad 4[1]
Affiliation 1; e-mail@e-mail.com
[2] Affiliation 2; e-mail@e-mail.com

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***Correspondence to**
sarahamdey264@gmail.com

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ABSTRACT

Heavy metals are considered highly toxic and their presence in foods may constitute human health hazard.

Accordingly, 90 random samples of frozen fish fillets, including Oreochromis niloticus, Lates niloticus and imported Basa(30 of each) were gathered from fish markets in Menoufia governorate,Egypt. The collected samples were examined for detection of their contamination with certain heavy metals (mercury, lead and cadmium) using Atomic Absorption spectrophotometer (AAS). Regarding mercury the mean value of Oreochromisniloticus (1.02+0.01mg/kg),Latesniloticus(0.67+0.01mg/kg) and importedbasa(0.45+0.01mg/kg) ,while the lead mean value of Oreochromis niloticus (0.65+0.0mg /kg),lates niloticus(0.41+0.01mg/kg)and imported Basa (0.24+0.01mg/kg), Finally cadmium mean value of Oreochromis niloticus(0.36+0.01mg/kg) ,lates niloticus(0.22 + 0.01 mg/kg)andimported Basa (0.10+0.01 mg/kg).

The obtained results indicated that the highest levels of mercury, lead and cadmium were recorded in Oreochromis niloticus, however the lower levels present in imported basa.It is evident thatthe maximum allowable limits of Egyptian regulations were surpassed by the amounts of heavy metals in certain fish samples.

The ability of Lactobacillus rhamnosus and chitosan to decrease mercury،lead and cadmium contamination were also studied. Thepublic health significance and some recommendations to produce fish product safe for human consumption were discussed.

1. INTRODUCTION

Fish is good diet of great health benefits, as it contain high-quality protein, low cholesterol, high levels of omega-3 , poly saturated fatty acids, vital minerals and vitamins [1].

Fish and fish products may be contaminated with some environmental pollutants of heavy metals, which mainly accumulated in fish through gills and digestive tract.The heavy metals in aquatic environment comeprimarily from the industry, agricultural, sewage and ship wastes that pollute [2]. Fish have a greater capacity than thesurrounding environment to accumulate heavy metals in their tissues, due to the nature of their feeding systems [3].

Accumulatedmercury in Aquatic media is considered highly toxic residues and constitute hazard to human health [4].Theorganic form of mercury that formed due to action of microorganism in ocean is considered dangerous toxin [5] has adverse effect in pregnant women and children [6]

Lead has a low rate of elimination, so it is regarded as a highly hazardous metal that can accumulate in humanbodies. Moreoverlead poisoning symptoms include encephalopathy, anemia, abdominal pain, and colic. Lead is also regarded as one of the immunesuppressive substances in humans [7].

Cadmium through the food chain enters humans and readily accumulates in plants and animals, affecting numerous organs and leading to severe ailments[8].

Therefore, the current study assessed the level of mercury, lead and cadmium contaminating the fish fillets and their control using probiotic and chitosan.

2. MATERIALS AND METHODS

2.1 Heading Title:

2.1. Samples collection:

Ninety random samples of frozen fish filets, consisting of thirty pieces each of Oreochromis niloticus, Lates niloticus and imported Basa were gathered from several fish markets in Menoufia governorate,Egypt. The collected samples were analyzedtodetermine residues using Atomic Absorption Spectrophotometer. Also, the application of biological trials either by using Lactobacillusrhamnosus culture or chitosan to reduce such toxic residues in fish fillets was performed.

2.2. Digestion technique [9].

Accurately, 1 g of each sample was macerated by sharp scalpel and digested by 10ml of digestion mixture (60ml of 65% Nitric acid and 40ml of 70% per chloric acid) in screw capped tube after maceration for determination of lead and cadmium residues, in regard to mercury, 0.5 g of macerated sample was digested in 10 ml of concentrated H_2SO_4/ HNO_3 solution (1:1)

The Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS) inhaled all standard, digestand blank solutions before being examined for mercury, lead and cadmium.

3. Effect of Lactobacillus rhamnosuson heavy metals inoculated into fish fillets.

3.1. Preparation of bacteria suspension [10]:

To create an overnight culture, a strain of Lactobacillus rhamnosus was cultured for 24 hours at 37ºC in Brain Heart Infusion (BHI) Broth (Fluka, Sigma-Aldrich Chemie GmbH).

3.2. Binding assay[11]

Fish fillets weighting 1 kg were hung with the bacterial strain. A final concentration of 2×107 bacteria plus 50 mg/Kg of ionic mercury standard solution. 2×107 bacteria plus 20 mg/Kg ionic lead standard solution and 2×107 bacteria and 10 mg/Kg ionic cadmium standard solution was achieved by adjusting the mixture according to [11] with a little adjustment. 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.

4. Effect of chitosan on heavy metals inoculated into fish fillets.

Chitosan was obtained from Fluka Chemie GmbH, Sigma-Aldrich Chemie GmbH, Switzerland.Accurately, after adjusting the pH to about 7 (neutral), 0.5 gram of the produced chitosan products was added to 50 ml of distilled water solution. To ascertain the effect of 1% chitosan on heavy metals, fish fillets that were tested and contaminated with standards were subjected to zero, twelve, twenty-four, and thirty-six hours of observation.**Table (1):** This is a table. Tables should be placed in the main text near to the first time they are cited.

3. RESULTS

The results recorded in Table (1) among the investigated fish samples*Oreochromis niloticus* samples indicated the highest contamination with mercury, lead, and cadmium levels (mg/kg), followed by *lates niloticus* finally imported Basa,respectively.

Mean values with different superscript letters in the same column are significantly different at ($p<0.05$).

Referring to the Egyptian standards for the maximum residual limits as mentioned in table (2)37.8%, 28.9% and 24.4 of totalanalyzed samples considered unfit for human consumption due to surpassing MRL presented by Egyptian authority.

According to EOS [12]the maximum residual limitsof :

Mercury 0.5 mg /kg.

Lead 0.1mg/kg.

Cadmium 0.05 mg/kg.

Using the results that have been showed in Table (3, 4 and 5), addition of L. rhamnosus culture (2×107 /g) showed a quick, encouraging decline in the impact of mercury, lead and cadmium levels as they were reduced by 72.8, 83.0 and 79% within 36 hours of the incubation, additionally, lead degradation levels were found to be greater than those of cadmium levelswhile recorded mercury had lower degradation level in experimentally inoculated fish fillet using probiotic.

furthermoreobtaind the results in Table (3,4 and 5) showed that, additionof chitosan revealed a quick, encouraging decline in the impact of mercury, lead and cadmium levels as they were reduced by 54.6,67.0and 60.0%within 36 hours of the incubation,solead degradation levels were found to be greater than those of cadmium and mercury that recorded lower level.

Table (1): Mean values of heavy metals levels (mg/kg) in the analyzed fish fillets samples (n=30).

Fish fillets	Mercury	Lead	Cadmium
Oreochromis niloticus	1.02 ± 0.01^{A}	0.65 ± 0.01^{A}	0.36 ± 0.01^{A}
Lates niloticus	0.67 ± 0.01^{B}	0.41 ± 0.01^{B}	0.22 ± 0.01^{B}
Imported Basa	0.45 ± 0.01^{C}	0.24 ± 0.01^{C}	0.10 ± 0.01^{C}

Table (2): Incidence of unaccepted samples of fish fillets based on the level of mercury, lead and cadmium (n=30)

Fish fillets MRL (mg/Kg)*	Unfit samples (mercury)		Un fit samples (lead)		Un fit samples (cadmium)	
	No.	%	No	%	No	%
Oreochromis niloticus	15	50	11	36.7	10	33.3
Oreochromis lates	11	36.7	9	30	7	23.3
Imported Basa	8	26.7	6	20	5	16.7
Total(90)	34	37.8	26	28.9	22	24.4

Table (3) Effect of *L. rhamnosus* ($2x10^7$/g) and chitosan on the concentration of mercury experimentally injected into fish fillets (50 mg/Kg).

Treatment / Storage time	Control (mg/Kg)	*L.rhamnosus* Treated group (mg/Kg)	*L.rhamnosus* Reduction %	Chitosan Treated group (mg/kg)	Chitosan Reduction %
Zero time	50	50	-------	50	....

12 hours	50	26.7	46.6	39.5	21.0
24 hours	50	15.9	68.2	25.2	49.6
36 hours	50	13.6	72.8	22.7	54.6

Table (4) Effect of *L. rhamnosus* ($2x10^7$/g) and chitosan on the concentration of lead experimentally injected into fish fillets (20 mg/Kg).

Treatment **Storage time**	**Control (mg/Kg)**	***L.rhamnosus*** **Treated group (mg/Kg)**	**L.*rhamnosus*** **Reduction %**	**Chitosan Treated group (mg/kg)**	**Chitosan Reduction%**
Zero time	20	20	-------	20	....
12 hours	20	8.4	58.0	12.9	35.5
24 hours	20	5.1	74.5	8.5	57.5
36 hours	20	3.3	83.0	6.6	67.0

Table (5) Effect of *L. rhamnosus* ($2x10^7$/g) and chitosan on the concentration of cadmium experimentally injected into fish fillets (10mg/Kg).

Treatment **Storage time**	**Control (mg/Kg)**	***L.rhamnosus*** **Treated group (mg/Kg)**	**L.*rhamnosus*** **Reduction %**	**Chitosan Treated Group (mg/kg)**	**Chitosan Reduction%**
Zero time	10	10	-------	10	....
12 hours	10	5.2	48.0	6.8	32.0

24 hours	10	2.9	71.0	4.9	51.0
36 hours	10	2.1	79.0	4.0	60.0

4. DISCUSSION

5. Heavy metals are hazardous contaminants in food and environment. These are non-biodegradable with long biological half-lives[14], the present study was carried out to survey and investigate the concentrations (mg/kg) of mercury, lead and cadmium in some commercially present fish in the Egyptian markets represented *Oreochromisniloticus ,lates niloticus and* imported Basa .

6. Considering table (1) it is obvious that the mean values of mercury were (1.02±0.01,0.67±0.01and0.45±0.01mg/kg)inthe examined sample of *Oreochromisniloticus, lates niloticus,* importedBasa, respectively.

7. Nearlysimilar results were obtained by Fatma [15] (0.037+_0.01 mg /kgin *Oreochromis niloticus*)**,** Hamada[16] (0.73±0.09mg /kg in *Oreochromis niloticus*) **,**Helmy[17] (1.9±0.12mg/kgin*Oreochromisniloticus)***,** Shoker[18](0.89±0.01 mg/kg*Oreochromis niloticu*), Samir [19](1.91±0.27mg/kg*in Oreochromis niloticus* and Morsy[20](0.42±0.02 and 0.07±0.01mg/kg in *Lates niloticus*and *Oreochromis niloticus* respectively . Accordingto[12]who statedthat the allowable limitof mercury should not exceed that 0,5mg/kg.In Table (2) recorded (50%, 36.7% and 26.7%)[8]of investigated fish fillets of *Oreochromis niloticus, lates niloticus* and imported Basa were not accepted for human consumption where they exceed the permissible limits .

8. The neurotoxic consequences of mercury toxicity are especially noticeable in developing organisms, such as fetuses, newborns and young children, who are typically more susceptible to these effects than adults [21].

9. Considering table (1) it is obvious that the mean values of leadwere(0.65±0.01, 0.41±0.01and0.2±0.01mg/kg) in *Oreochromisniloticus, lates niloticusand,* importedBasa ,respectively.The recorded results were lower tthanthat obtained by Abd ElAziz [22] (6.90±4mg/kg in imported filleted fish) **,**While nearly similar toHamada[16] (0.34±0.05 mg /kgin Oreochromis niloticus) **,**Helmy[17] (0.53±0.06mg/kg) in *Oreochromisniloticus***,** Shoker [18](0.49±0.01mg/kg) in *Oreochromis niloticus***,** Samir [19](1.05±0.11mg /kg *in Oreochromis niloticus)and* Morsy[20] (0.31±0.04 and 0.09±0.02mg/kgin *Lates niloticusand Oreochromis niloticus,*respectively.

10. Established a 0.10 mg /kg maximum allowable level for lead in fish meat [13]In light of this allowable limits table(2) showed that(36.7%,30% and 20%) of investigated fish fillets of *Oreochromis niloticus, lates niloticus* and imported Basa were un accepted for human consumption .

11. . Chronic poisoning from high lead doses can cause hematological, renal, GIT, and neuromuscular diseases. The two main symptoms of lead poisoning in kids are aberrant behavior and learning disabilities. On the other handadult male reproductive dysfunction, weakness, headaches, abdominal pain, kidney damage, memory loss, and tremors in the extremities are the main signs of lead poisoning. Exposure to higher than allowed levelsof lead can cause severe issues such as malfunctioning of the nerves, joints ,muscles, memory loss, heart problems, bone abnormalities, and kidney illnesses[23].

12. Consideringtable(1)acquired results ofCadmiummean value (0.36±0.01,0.22±0.01 and 0.10±0.01mg/kg)in*Oreochromis niloticus, lates niloticusand* importedbasa respectively, When comparing the cadmium concentration readings whith that reported byAbd ElAziz [22]

(2.44±1.5mg/kg) was higher inimported fish fillets , Hamada[15](0.10±0.01mg/kgin*Oreochromisniloticus*)**,**Helmy[17](0.19±0.01mg/kgin*Oreochromisniloticus*)**,**Shoker [18](0.17±0.01mg/kgin *Oreochromisniloticus)***,** Samir[19**]**(0.37±0.04mg/kg*in Oreochromisniloticus*)andMorsy[20](0.13±0.02and0.07±0.01mg/kg)in *Lates niloticus and Oreochromis niloticus* respectivelywere nearly similar results. Established a 0.050 mg /kg maximum allowable level for Cadmium in fish fillets [12] In light of this allowable limit (33.3%, 23.3%and 16.7%) of investigated fish fillets of *Oreochromis niloticus, lates niloticus* and imported Basa were unaccepted for human consumption.

13. . Cadmium as a heavy metal can build up in the body and cause astroglial toxicity and brain damage [24].Acute cadmium exposure causes vomiting, gastritis, and diarrhea while chronic cadmium toxicity causes bone deformities and renal damage. Furthermore, cadmium inhalation has been linked to lung cancer cases **[25]**.

14. Significantdifferences ($P \geq 0.05$) were found through statistical analysis of the recorded results, where *Oreochromis niloticus and lates niloticus* were the most common highly contaminated samples in the fish fillet under examination. These findings may be attributed to either the bioaccumulation process or the highly contaminated surrounding environment, depending on how old the collected samples.

15. The observed discrepancies between the recorded outcomes and the remaining records might be ascribed to changes in the sample collection locations, fish age, which plays a crucial role in the bioaccumulation process and the kinds of fish under examination.

16. An investigation into the degradation of mercury, lead, and cadmium levels in fish fillet models under the influence of *L. rhamnosus* and chitosan were carried out through an experimental study. The findings, which are listed in Table (3, 4and 5), demonstrated a significant decline in the levels of lead, cadmiumand mercury respectively.

17. The results recorded in Table(3) showed that the influence of *L.rhamnosus* culture (2×10^7/g) and the impact of chitosan on fish fillets on the levels of mercury experimentally inoculated into fillets of fish (50mg/kg) at zero time, 12 h, 24 h and 36 h as the level of mercury decreased from(50, 26.7,15.9and 13.6 mg/kg) in treated groups to with reduction percentage (00%,46.6% ,68.2% and 72.8%) , respectively ,while the impact of chitosan on fish fillets' levels of mercury inoculation (50mg/kg) at zero time, 12 h, 24 h and 36 h , the level of mercury decreased to (50,39.5 ,25.2 and 22.7mg/kg) in treated groups with reduction percentage (00%,21%, 49.6% and 54.6%) , respectively.

18. The results recorded Table(4) showed that the influence of *L.rhamnosus* culture (2×10^7/g) and the impact of chitosan on fish fillets on the levels of lead experimentally immunized into fillets of fish (20mg/kg) at zero time, 12 h, 24 h and 36 h as the level of lead decreased to (20,8.4,5.1 and 3.3mg/kg) in treated groups with reduction percentage (00%,58.0%,74.5%and 83.0%) ,respectively ,while the impact of chitosan on fish fillets' levels of lead (20mg/kg) at zero time, 12 h, 24 h and 36 h the level of lead decreased about (20,12.9,8.5 and 6.6mg/kg) in treated groups with reduction percentage (00%,35.5%,57.5%,67.0%), respectively.

19. The results recorded in Table(5) showed that the influence of *L.rhamnosus* culture (2×10^7/g) and the impact of chitosan on fish fillets on the levels of cadmium experimentally injected into fillets of fish (10mg/kg) at zero time, 12 h, 24 h and 36 h the level of Cadmium decreased to (10,5.2,2.9 and 2.1mg/kg) in treated groups with reduction percentage (00%,48%.0,71.0% and79.0%), respectively .While the impact of chitosan on fish fillets' levels of cadmium inoculation (10mg/kg) at zero time, 12 h, 24 h and 36 h the level of Cadmium decreased to (10,6.8,4.9 and 4.0mg/kg) in control groups with reduction percentage (00%,32.0%,51.0%and 60.0%), respectively.

20. *Lactobacillus* have the capacity to associate with the heavy metals intra or extracellular by biosorptiona passive process that binds metal ions to the *Lactobacillus*cell wall without metabolic activity, *Lactobacillus* interaction effect with heavy metals is decreasing. This prevents any detrimental interactions in the host cell [25].

21. **CONCLUSIONS**

22. This study can concluded that the frozen fish fillets were highly contaminated with mercury, lead and cadmium above the safe permissible limits constituting ,public health hazard.

23. Further the using of *Lactobacillusrhamnosus* and chitosan were highly effective on the reduction and control of such serious pollutants of heavy metals.

24. ACKNOWLEDGEMENTS

25. The current investigation demonstrated that there are significant differences in the amounts of lead, cadmium and mercury in the fish samples that were analyzed. Furthermore, the tested samples had high concentrations of hazardous metals, which have a negative impact on human health, and were thus highly contaminated. To put it another way, consuming these tainted fish over time may lead to public health risk through causing a gradual and permanent build-up of these hazardous substances in the body. Possible harm of these metals may cause, people to consume a variety of fish in addition to lesser amounts of fish that are known to be contaminated with heavy metals in order to prevent consuming harmful levels of these metals.

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