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Relationship between lactobacillus species and heavy metal concentration in fish fillet

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ABSTRACT

This study was applied to investigate levels of lead, cadmium, and mercury in commercially sold fish species (*Oreochromis niloticus* and *Lates niloticus*) and the effect of the addition of lactobacillus species on metal levels in fish fillet. A total of 120 fish fillet samples, represented by *Oreochromis niloticus* (O. niloticus) and *Lates niloticus* (*L. niloticus*) (60 samples of each), were obtained from different markets in Qalubiya Governorate, Egypt. The obtained results indicated that O. niloticus exhibits higher levels of heavy metal contamination, specifically lead, cadmium, and mercury, compared to *L. niloticus*. The average levels of lead, cadmium, and mercury (mg/kg) were 1.07 ± 0.01, 0.49 ± 0.01, and 1.53 ± 0.02 in O. niloticus compared with those of 0.65 ± 0.01, 0.32 ± 0.01, and 0.98 ± 0.01 in *L. niloticus*, respectively. A higher percentage of O. niloticus samples were deemed unfit for consumption due to excessive heavy metal levels compared to *L. niloticus* ones, according to Egyptian standards. The experimental inoculation of a mixture of Lactobacillus rhamnosus (L. rhamnosus) and (L. plantarum) probiotic strains into fish fillets resulted in a higher reduction (83%) in lead levels after 72 hours than reductions seen with L. plantarum (67%) and L. rhamnosus (75%), respectively. So, we recommended the potential application of probiotics to ensure the safety of seafood consumption.

1. INTRODUCTION

Seafoods are a healthy food in today's diet because they contain essential elements that are vital to our body. Seafood can also be taken on a regular basis to reduce the danger of heart disease, enhance the human brain and improve the immune system; thus, it should be included in the group of recommended diet foods (Özden et al., 2018). A balanced and varied diet that includes seafood can provide important nutrients, but consumers should be aware of potential heavy metal contamination and moderate their intake of certain fish species due to the ability of heavy metals like mercury, lead, cadmium, and arsenic to accumulate in their muscle tissue (Li et al., 2017). While low levels of these heavy metals are not a major concern, excessive accumulation can pose potential health hazards for consumers (Rahman et al., 2012; Djedjibegovic et al., 2020).

The deposition of such elements in the human body leads to severe impacts, like the accumulation of lead in human tissue, which hampers the immune system and circulation and reduces hormonal and enzymatic activity (Chen et al., 2015). Moreover, long-term accumulation of mercury in humans, even in low concentrations, causes neurological disorders and increases the chances of developing cancer (Chałabis-Mazurek et al., 2021). Moreover, the consumption of fish contaminated with Cd can lead to seafood poisoning, affecting renal and skeletal health due to its persistence in biological systems (Azar and Vajargah, 2023).

Contamination by heavy metals can arise from various origins, including waste from agricultural activities, by-products of industrial processes, emissions from burning fossil fuels, extractive mining operations, and effluents from wastewater treatment facilities, all of which can impact the natural environment (Gheorghe et al., 2017). Fish can absorb and concentrate heavy metals from the water and sediments they live in (Authman et al., 2015). The long-term toxicity of certain heavy metals, such as mercury and lead, impacts the neurological system, especially in young and pregnant mothers. Therefore, it is important to monitor heavy metal levels in commercially sold fish and seafood to ensure they do not exceed safety limits and pose a health risk to consumers (Djedjibegovic et al., 2020).

Several studies have shown a promising result in the capability of different strains of *lactobacilli* to reduce the level of heavy metal in examined samples. The reduction is attributed to the bacteria binding to metal ions through adsorption or ion exchange (Elsaid et al., 2023). In other in vitro studies, *L. plantarum* strains showed the ability to alleviate toxicity from heavy metals like chromium, cadmium, and lead. These strains can promote the elimination of heavy metals through feces, recover the altered gut microbiota, and make the metals less soluble and absorbable (Abdel-Megeed, 2020; George et al., 2021). So, the existing study aimed to analyze the examined fish for estimation of their heavy metals' concentrations (lead, cadmium, and mercury) to determine their validity for human consumption. And the application of *Lactobacillus* cultures (*L. plantarum* and *L. rhamnosus*) as biological trials to control such serious residues in fish fillets.

2. MATERIALS AND METHODS

1.1.Sample collection

A total of 120 random fish fillet samples, consisting of equal numbers of *O. niloticus* and *L. niloticus*, were systematically collected from various fish markets in Benha City, Qalubiya governorate, Egypt. Each sample was meticulously preserved in a separate insulated ice container to ensure freshness and was promptly transported to the laboratory for analysis. To standardize the study parameters, the average mass of individual fish samples was kept at approximately 100 grams.

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2.2. Determination of heavy metals

2.2.1. Cleaning procedures (Lars, 2003).

Glassware and vessels underwent a cleaning process using deionized water, followed by a 24-hour immersion in a warm, diluted solution of 10% nitric acid (HNO3). After multiple deionized water rinses, the items were left to dry. Subsequently, all containers were rigorously cleansed with deionized water and then left to air-dry in an incubator.

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

In a precise manner, each sample, weighing one gram, was pulverized and then subjected to a digestion process using 10 ml of a solution composed of 60 ml of 65% nitric acid and 40 ml of 70% perchloric acid. This mixture was placed in a screw-capped tube, which was then vigorously shaken and subsequently heated for four hours in a water bath maintained at 110 °C to ensure the samples were thoroughly digested. Following this, the contents of each tube were diluted with deionized water until the volume reached 25 ml. The resulting solution was then filtered through Whatman filter paper. The filtered liquid was then stored at ambient temperature for subsequent analysis to determine the levels of mercury, lead, and cadmium present.

The examined sample, control, and reference standards were extracted by an atomic absorption spectrophotometer and assessed for the levels of specific elements within these solutions.

2.3. Experimental part (effect of both L. plantarum and L. rhamnosus) in concentration of lead experimentally inoculated into fish fillets.

The probiotic strains used in this study, "*L. plantarum and L. rhamnosus," were* obtained from the Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan. Lead standard solution (1000 mg/L) in HNO3 (2%, w/w) was obtained from Fluka (Sigma-Aldrich) and used to prepare the diluted solutions required for binding studies.

2.3.1. Preparation of bacterial suspension

A strain of Lactobacillus species was propagated in Brain Heart Infusion (BHI) Broth (provided by Fluka, Sigma-Aldrich Chemie GmbH) and incubated for 24 hours at a temperature of 37 °C to develop an overnight culture. Subsequently, one milliliter of this bacterial culture was serially diluted in sterile 0.1% peptone water (w/v) (sourced from Merck, Darmstadt, Germany). Following this, the enumeration of the *Lactobacillus* species strain was performed on BHI Agar (Fluka, Sigma-Aldrich Chemie GmbH) utilizing the plate count technique, with a culture broth volume equivalent to roughly 1×10^{6} CFU. Then the culture broth underwent centrifugation at 500 rpm for 15 minutes, and the bacterial pellets were washed twice with deionized water (Halttunen et al., 2007).

2.3.2. Binding assay.

The bacterial pellet of both strains (10^{6} CFU/ml) and 10 mg/kg ionic lead solution were mixed with fish fillet samples as follows: the 1st group control group was mixed with lead solution only (G1), the 2nd group (G2) (*L. plantarum* and lead solution), the 3rd group (G3) (*L. rhamnosus and lead solution), and the 4th group (G4) (both strains and* lead solution) (Halttunen et al., 2008). Samples were acidified with ultrapure HNO3 and examined at zero-, 24-, 48-, and 72-hour time points for measuring each free metal by flame atomic absorption spectrophotometer. The scheme was replicated five times.

2.3.4. Determination of lead: as before.

2.4. Statistical analysis:

The results were statistically analyzed using the Analysis of Variance (ANOVA) test, as outlined by Feldman et al. (2003).

Reduction rate (%) = $B-A\ /A\ x100$

B = the mean value of the next heavy metal level.

A is the mean value of the previous heavy metal level.

3. RESULTS

Table 1 presents data indicating that *O. niloticus* has higher levels of heavy metal contamination, specifically lead, cadmium, and mercury, compared to *L. niloticus*. Lead concentrations in *L. niloticus* extended from 0.13 to 1.09 mg/kg, with an average of 0.65 ± 0.01, whereas 0.18 to 1.52 with an average of 1.07 ± 0.01 mg/kg in *O. niloticus*, in cadmium, were 0.01 to 0.57 in an average of 0.32 ± 0.01 in *L. niloticus*, 0.03 to 0.85 in an average of 0.49 ± 0.01 mg/kg in *O. niloticus*, and also, 0.39 to 2.74 in an average of 1.53 ± 0.02 in *O. niloticus*, compared to 0.27 to 1.81 mg/kg at a range of 0.98 ± 0.01 B mg/kg of mercury in *L. niloticus*.

Table (1) Mean values of different heavy metals levels "mg/ Kg" in the examined fish fillets (n=60).

Heavy metal	*latus niloticus*		*Oreochromis niloticus*	
Lead residue	Mean ± S.E: 0.65 ± 0.01 ^{B}	Min: 0.13	Mean ± S.E: 1.07 ± 0.01 ^{A}	Min: 0.18
		Man:1.09		Man: 1.52
Cadmium residue	Mean ± S.E: 0.32 ± 0.01 ^{B}	Min: 0.01	Mean ± S.E: 0.49 ± 0.01 ^{A}	Min: 0.03
		Man:0.57		Man: 0.85
Mercury residues	Mean ± S.E: 0.98 ± 0.01 ^{B}	Min: 0.27	Mean ± S.E: 1.53 ± 0.02 ^{A}	Min: 0.39
		Man:1.81		Man: 2.74

-Means with different superscripts in the same row are significantly different ($P<0.05$).

The results exhibited in Table 2 indicate the detection of heavy metals in fish samples. Specifically, 35, 31, and 39 samples of *L. niloticus* tested positive for lead, cadmium, and mercury, respectively. Similarly, 41, 36, and 46 samples of *O. niloticus* were found to contain these metals. Results were compared to the Egyptian standards for permissible heavy metal levels; 22 (36.7%), 24 (40%), and 28 (46.7%) samples of *L. niloticus* were unfit for lead, cadmium, and mercury contents, respectively. In *O. niloticus*, the percentage of unacceptable samples were higher as 29 (48.3%), 32 (53.3%), and 33 (55%) samples exceeded the safe limits for lead, cadmium, and mercury, respectively.

Table (2) Edibility of the examined fish samples based in their heavy metal contents according to "EOS" (2010).

Type of heavy metal	Edibility	*latus niloticus*		*Oreochromis niloticus*		MRL (mg/Kg)*
		No.	%	No.	%	
Lead	Positive samples	35	58.3	41	68.3	0.3
	fit samples	13	21.7	12	20	
	Unfit samples	22	36.7	29	48.3	
cadmium	Positive samples	31	51.6	36	60	0.05
	fit samples	7	11.6	4	6.6	
	Unfit samples	24	40	32	53.3	
Mercury	Positive samples	39	65	46	76.6	0.5
	fit samples	11	18.3	13	21.7	
	Unfit samples	28	46.7	33	55	

Maximum residual limit of * Egyptian Organization for Standardization "EOS" (2010).

Results reported in Table 3 illustrate the reduction effect of the addition of *lactobacillus* strains to the fish fillets inoculated with lead (10 mg/kg), in which the level in the control group remained for 72 hours. of the experiment while the combination treatment of *L. rhamnosus* and L. plantarum in (G3) resulted in the highest reduction of 83%, compared to 67% for *L. plantarum* (G1) and 75% for *L. rhamnosus* (G2), separately. This suggests a synergistic effect when using the two probiotic strains together.

Table (3) Influence of *Lactobacillus* culture (1x10^{6}) on the levels of lead experimentally inoculated to fish fillets (10 mg/Kg).

Treatment Storage time	Control (mg/Kg)	*L. plantarum* Treated group (mg/Kg)	R%	*L. rhamnosus* Treated group (mg/Kg)	R%	*L. rhamnosus + L.plantarum* Treated group (mg/Kg)	R%
Zero time	10	10	----	10	----	10	--
24 hours	10	6.1	39	5.3	47	4.9	*51*
48 hours	10	4.8	52	3.6	64	3.1	*69*
72 hours	*10*	*3.3*	*67*	*2.5*	*75*	*1.7*	*83*

4. DISCUSSION

Seafood, particularly fish, is a notable source of metal exposure for many individuals. Eating food with a toxic metal content that surpasses established safety thresholds can be harmful to health and is banned from trade by various local and international regulations (Djedjibegovic et al., 2020). High levels of metal exposure can have severe adverse effects on both fish and humans; for example, in fish, lead accumulation in various tissues can lead to structural damage and functional disorders (Jezierska and Witeska, 2001). In humans, it is known to be carcinogenic, with studies indicating that mercury and lead may contribute to developmental abnormalities in children (Gibb), and prolonged consumption of cadmium has been linked to kidney, prostate, and ovarian cancers (Eisa and Awad, 2020). Therefore, the current research aims to assess the levels of various significant heavy metals found in commercially sold fish, such as *O. niloticus* and *L. niloticus*. Lead is recognized as a highly toxic heavy metal that tends to accumulate predominantly in the liver, spleen, kidneys, and gills, as noted by Cretì et al. (2010). When fish, such as *O. niloticus*, are exposed to high levels of lead, it can lead to significant functional disruptions. This was evidenced by Tanekhy et al. (2015), who observed a drop in hemoglobin levels, red blood cell count, and hematocrit values in these fish. According to Table 1, the average amount of lead residue found in the *O. niloticus* that was studied was 1.07 ± 0.01 A mg/kg. This is higher than what El-Said (2016), Helmy et al. (2018), and Samir et al. (2021) found, which were 0.24, 0.53, and 1.05 ± 0.11 mg/kg for *O. niloticus*. While the mean lead level at *L. niloticus* was 0.65 ± 0.01 ^{B} mg/kg, this was higher than the mean value of lead at *L. niloticus* reported by Eisa and Awad (2020) during two different seasons in Lake Nubia from 2016 to 2017.

Cadmium is particularly harmful to aquatic life, posing a severe threat due to its tendency to accumulate rapidly in organisms. It accumulates mostly in the liver, kidneys, and gills, with the least accumulation occurring in the skin. The buildup of cadmium in the environment is a significant issue due to its prolonged elimination process. The gills serve as the primary organ for eliminating cadmium efficiently (Handy, 1992). Cadmium is a highly toxic heavy metal that poses a very serious health threat to humans. The main hazards of cadmium toxicity include kidney damage, skeletal effects, cancer, respiratory effects, cardiovascular effects, reproductive effects, and neurological effects (Zuhra et al., 2024). Table 1 shows that the average amount of cadmium in *O. niloticus* was 0.49 ± 0.01 mg/kg. This is higher than what Sohsah (2009) (0.26 mg/kg), El-Said (2016) (0.051 mg/kg), Helmy et al. (2018) (0.19 mg/kg), and Samir et al. (2021) (0.37 ± 0.04 mg/kg) found. As for *L. niloticus*, its mean cadmium level was 0.32 ± 0.01 mg/kg. This was higher than the mean cadmium level found by Eisa and Awad (2020) for the same species during two seasons.

Mercury is recognized as a highly toxic heavy metal and is listed as the third-most hazardous substance in the environment, following lead and arsenic. This ranking is provided by the United States Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) (Pack et al., 2014). Mercury pollution originates from both natural and human-made sources. Naturally, it can be released into the environment through forest fires and volcanic activities. Human activities contribute significantly to mercury emissions, with the use of fungicides, the disposal of electronic equipment and batteries, and the application of certain paints being primary sources. Additionally, the combustion of fossil fuels and mining operations are substantial contributors to the release of mercury into our environment (Boening, 2000). The mean value of mercury residue in the examined *O. niloticus* (Table 1) was about 1.53 ± 0.02 mg/kg which is higher than that stated by El-Nahas (2015), El-Said (2016), and Helmy et al. (2018), who registered levels of mercury in the same fish at 0.46, 0.037, and 1.29 mg/kg, respectively. And lower than that detected by Samir et al. (2021), who counted the level of mercury to be 1.91 ± 0.27 mg/kg in the same examined fish. In addition, the mean value of mercury in the examined *L. niloticus* was 0.98 ± 0.01 mg/kg, which is higher than that reported by Eisa and Awad (2020) during the two seasons in Lake Nubia from 2016 to 2017.

The data presented in Table 2 assesses the suitability of the tested fish against the maximum allowable limits of the Egyptian Standards (EOS, 2010). These figures are almost consistent with the edibility levels reported by Samir et al. (2021) for the same species, highlighting concerns over bioaccumulation. Moreover, results showed that *Lactobacillus* can reduce the effects of heavy metal toxicity. This is done by changing the population and activity of microbes within the gastrointestinal tract through a process known as “gut remediation." Simply put, this type of bacterium has the ability to neutralize toxins within the gut via control of the microbes inhabiting it (Zhai et al., 2016; Wang et al., 2020 b). The current study indicates that using a combined treatment of *L. rhamnosus* and *L. plantarum* on lead-contaminated fish fillets (10 mg/kg) is more effective in reducing the heavy metal concentration than when these strains are applied individually, as shown in Table 3. The effectiveness of this treatment is attributed to these strains' capacity to enhance the removal of heavy metals via feces, which helps in rebalancing the gut microbiota that is otherwise disturbed by such toxic elements. Specifically, *L. plantarum* has been recognized for its detoxification actions against various heavy metals. This is in agreement with the results of Breton et al. (2013) and Yu et al. (2016), who found that *L. plantarum* CCFM8610 can reduce cadmium toxicity. Similarly, Milatovic et al. (2017) observed its effectiveness in mitigating lead toxicity. Furthermore, Breton et al. (2013) also reported that *L. plantarum* CCFM8610 can counteract the adverse effects on fish suffering from cadmium poisoning. In addition, adding *L. rhamnosus* GR-1 to yogurt has been found to decrease the levels of heavy metals in both pregnant women and children (Wang et al., 2020 a). Also, the study by Jama et al. (2012) generally indicates that introducing *L. rhamnosus* to a rat model can notably decrease the occurrence of genotoxicity and hepatotoxicity, which are typically induced by cadmium exposure. This suggests that *L. rhamnosus* may play a beneficial role in mitigating the harmful effects of cadmium on the liver and genetic material. This highlights the potential of using specific microbial strains as a therapeutic strategy to alleviate the negative impacts of excessive heavy metal presence in the body.

Their actions appear to be a result of their potential mechanisms for enhancing the excretion of heavy metals, including lead, through various pathways. It produces antimicrobial agents such as organic acids and bacteriocins, which can help protect the gut lining and facilitate the detoxification process by promoting beneficial gut flora that competes with harmful bacteria and enhances the overall metabolic function of the intestines (Hao et al., 2024). Additionally, *L. rhamnosus* has been associated with the modulation of the gut microbiome, which can influence the absorption and bioavailability of heavy metals. By improving intestinal barrier function and increasing the production of mucins, *L. rhamnosus* can reduce the uptake of toxins like lead into the bloodstream (Wang et al., 2022). Both strains also exhibit antioxidant properties, which can mitigate oxidative stress caused by heavy metal exposure, further supporting their role in detoxification processes.

5. CONCLUSIONS

The findings from this research highlight the significant health dangers associated with consuming fish contaminated with excessive levels of heavy metals like lead, cadmium, and mercury. Also, the results showed that using both L. rhamnosus and L. plantarum together is better at lowering the amount of lead in fish fillets than using each one separately.

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