Impact of *Lactobacillus rhamnosus* and chitosan on some heavy metals experimentally inoculated in fish fillet

Sara H shaban[1], Abd Elrahman M.Elbagoury[2], Mohammed A.Hassan3 and Marwa A.Saad [4]

[1] Veterinarian at Menoufia Veterinary Directorate, Egypt,

[2, 4] Food Hygiene and Control Department, Faculty of Veterinary Medicine Shebin El-com, Menoufia University, Egypt,

[3] Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University,

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*) an*d imported basa (0.45±0.01mg/kg) ,while the lead mean value of *Oreochromis niloticus* (0.65± 0.0 mg /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)and imported 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 that the 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. The public health significance and some recommendations to produce fish product safe for human consumption were discussed.

KEYWORDS: *Oreochromis niloticus, lates niloticus,* imported Basa, heavy metals

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 come primarily from the industry, agricultural, sewage and ship wastes that pollute [2]. Fish have a greater capacity than the surrounding environment to accumulate heavy metals in their tissues, due to the nature of their feeding systems [3].

Accumulated mercury in Aquatic media is considered highly toxic residues and constitute hazard to human health [4].The organic 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 human bodies. More over lead poisoning symptoms include encephalopathy, anemia, abdominal pain, and colic. Lead is also regarded as one of the immune suppressive 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. Material and Methods

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 analyzed to determine residues using Atomic Absorption Spectrophotometer. Also, the application of biological trials either by using *Lactobacillus rhamnosus* 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, A**u**stralia, model AA240 FS) inhaled all standard, digest and blank solutions before being examined for mercury, lead and cadmium.

3. Effect of *Lactobacillus rhamnosus* on 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 2×10^7 bacteria plus 50 mg/Kg of ionic mercury standard solution. 2×10^7 bacteria plus 20 mg/Kg ionic lead standard solution and 2×10^7 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.

5. 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.

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}

٩٢ Mean values with different superscript letters in the same column are significantly

٩٣ different at ($p<0.05$).

٩٤ Referring to the Egyptian standards for the maximum residual limits as mentioned in

٩٥ table (2) 37.8%, 28.9% and 24.4 of total analyzed samples considered unfit for human

٩٦ consumption due to surpassing MRL presented by Egyptian authority.

٩٧ **Table (2): Incidence of unaccepted samples of fish fillets based on the level of mercury,**

٩٨ **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

٩٩ According to EOS [12] the maximum residual limits of :

١٠٠ Mercury 0.5 mg /kg.

١٠١ 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 (2×10^7 /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 levels while recorded mercury had lower degradation level in experimentally inoculated fish fillet using probiotic.

furthermore obtaind the results in Table (3,4 and 5) showed that, addition of 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, so lead degradation levels were found to be greater than those of cadmium and mercury that recorded lower level.

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		***L.rhamnosus***		**Chitosan**	
Storage time	**Control (mg/Kg)**	**Treated group (mg/Kg)**	***L.rhamnosus* Reduction %**	**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

6. Discussion :

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 *Oreochromis niloticus ,lates niloticus and* imported Basa .

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) in the examined sample of *Oreochromisniloticus, lates niloticus,* imported Basa, respectively.

Nearly similar results were obtained by Fatma [15] (0.037+_0.01 mg /kg in *Oreochromis niloticus***),** Hamada [16] (0.73±0.09 mg /kg in *Oreochromis niloticus***) ,**Helmy [17] (1.9±0.12mg/kgin *Oreochromis niloticus)***,** 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±0.01 mg/kg in *Lates niloticus* and *Oreochromis niloticus* respectively . According to [12] who stated that the allowable limit of mercury should not exceed that 0,5mg/kg.In Table (2) recorded (50%, 36.7% and 26.7%)□of investigated fish fillets of *Oreochromis niloticus, lates niloticus* and imported Basa were not accepted for human consumption where they exceed the permissible limits .

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].

Considering table (1) it is obvious that the mean values of lead were (0.65±0.01, 0.41±0.01and0.2±0.01mg/kg) in *Oreochromi sniloticus, lates niloticus and,* imported Basa , respectively. The recorded results were lower tthan that obtained by Abd ElAziz [22] (6.90±4mg/kg in imported filleted fish) **,**While nearly similar to Hamada[16] (0.34±0.05 mg

/kg in Oreochromis niloticus) ,Helmy[17] (0.53±0.06mg/kg) in *Oreochromis niloticus*, 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/kg in *Lates niloticus and Oreochromis niloticus,* respectively.

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 .

. 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 hand adult 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 levels of lead can cause severe issues such as malfunctioning of the nerves, joints ,muscles, memory loss, heart problems, bone abnormalities, and kidney illnesses [23].

Considering table(1)acquired results of Cadmium mean value (0.36±0.01,0.22±0.01 and 0.10±0.01mg/kg) in *Oreochromis niloticus, lates niloticus and* imported basa respectively, When comparing the cadmium concentration readings whith that reported by Abd ElAziz [22] (2.44±1.5mg/kg) was higher in imported fish fillets , Hamada [15] (0.10±0.01mg/kg in *Oreochromis niloticus* **),** Helmy [17] (0.19±0.01mg/kgin *Oreochromis niloticus***),** Shoker [18] (0.17±0.01mg/kg in *Oreochromis niloticus)***,** Samir [19**]** (0.37±0.04mg/kg *in Oreochromis niloticus*) and Morsy [20] (0.13±0.02 and 0.07±0.01mg/kg)in *Lates niloticus and Oreochromis niloticus* respectively were 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.

. 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]**.

Significant differences ($P \geq 0.05$) 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.

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.

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, 4 and 5), demonstrated a significant decline in the levels of lead, cadmium and mercury respectively.

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, 26.7,15.9 and 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 22.7 mg/kg) in treated groups with reduction percentage (00%,21%, 49.6% and 54.6%) , respectively.

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 3.3 mg/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.

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 4.0 mg/kg) in control groups with reduction percentage (00%,32.0%,51.0%and 60.0%), respectively.

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].

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.

7. CONCLUSION

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.

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

8. REFERENCES.

1. Özden, Ö., Erkan, N. and Kaplan, M: Toxic metals and omega-3 fatty acids of *blue fin Tuna* from aquaculture: (2018) Health risk and benefits. Expo. Hlth, 110.

2. Ayenimo, J. G.; Yusuf, A.M. and Adekunle, A.S: Heavy metal exposure from personal care products. (2010) Bull. Environ. Contam. Toxicol. 84 (1): 8-14

3. Olaifa, F., Olaifa, A., Adelaja, A. and Owolabi, A.: Heavy metal contamination of *Clarias gariepinus* from a lake and fish farm in Ibadan, (2004) Nigeria. Afric. J. Biomed. Res., 7: 23-27.

4. Clarkson, T.W: The three modern faces of Mercury. (2002) Environmental Health. Prespect, 110(1): 1-32

٢٤٢ 5. Alina, M.; Azrina, A.; Mohd Yunus, A. S.; Mohd Zakiuddin, S.; Mohd Izuan Effendi, H.and
٢٤٣ Muhammad Rizal, R : Heavy metals (mercury, arsenic, cadmium, plumbum) in
٢٤٤ selected marine fish and shellfish along the Straits of Malacca (2012). Int.Food Res.
٢٤٥ J., 19 (1):135-140.

٢٤٦ 6. Dorea, J.G.: Mercury and lead breast- feeding. (2004) British Journal Nutrition. 92 (1): 21.

٢٤٧ 7. Chisolm, J: Management of increased lead absorption and lead poisoning. (1973) England
٢٤٨ Journal Medicine, 289: 10161017.

٢٤٩ 8. Achparaki, M.,Thessalonikeos, E., Tsoukali, H., Mastrogianni, O., Zaggelidou, E.,
٢٥٠ Chatzinikolaou, F., Vasilliades, N., Raikos. N: Heavy metals toxicity. (2012) Aristotle
٢٥١ University Med. J. 39 (1): 20-31.

٢٥٢ 9. Staniskiene, B.; Matusevicius, P.; Budreckiene, P. and Skibniewska, K.A.: Distribution of
٢٥٣ heavy metals in tissues of freshwater fish in Lithuania. (2006) Polish J. Environ
٢٥٤ Studies, 15(4): 585-591.

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

٢٥٧ 11.Halttunen T., Collado, M., El-Nezami, H., Meriluoto, J., Salminen, S. : Combining strains
٢٥٨ of lactic acid bacteria and heavy metal removal efficiency from aqueous solution
٢٥٩ (2008). Letters Appl. Microbiol. 46:160–165.

٢٦٠ 12. EOSQ" Egyptian Organization for Standardization and Quality Control. :. Maximum
٢٦١ residue limits for heavy metals in food. (2010) Ministry of industry No. 7136/2010.
٢٦٢ Egyptian standards, Ministry of industry, Egypt.

٢٦٣ 13. Heidarieh, M., Maragheh, M.G., Shamami, M.A., Behgar, M. and Ziaei, F.: Evaluate of
٢٦٤ heavy metal concentration in shrimp (*Penaeus semisulcatus*) and crab (*Portunus pelagicus*)
٢٦٥ with INAA method. (2013) Springer plus, 2: 72-76.

٢٦٦ 14. Fatma .H.M. Alia, Nasser S. Abdel-Attya :Heavy metal residues in local and imported fish
٢٦٧ in Egyp Food Hygiene Dept., (2016)Faculty of Veterinary Medicine, Beni-Suef
٢٦٨ University Benha Veterinary Medical Journal.

٢٦٩ 15. Hamada .E .O, Zakaria H. Elbayoumi, Reda A. Khader، , Abdel. Rahman M.
٢٧٠ Elbagory:Assessment of Heavy Metal Concentration in Fish Meat of Wild and
٢٧١ Farmed Nile Tilapia (Oreochromis Niloticus), (2018) Egypt Alaxandria Journal of
٢٧٢ Veterinary science 57(1):30.37

٢٧٣ 16. Helmy .N .A , Mohamed A. Hassan, Faten S. Hassanien and Ahmed A. Maarouf:
٢٧٤ Detection of Heavy Metals Residues in Fish and Shellfish(2018)BENHA
٢٧٥ VETERINARY MEDICAL JOURNAL, VOL. 34, NO. 2: 255-264, JUNE 2016.

٢٧٦ 17. shoker L. A.: Heavy metals residues contaminating Nile and marine fishes Thiese (2019)
٢٧٧ Degree of Doctor of Philosophy in Veterinary Science Faculty Veterinary Medicine,
٢٧٨ Benha University Medicine.

٢٧٩ 18. Samir. O, Edris. A .M, Heik .G:Degradation effect of Lactobacillus rhamnosus on some
٢٨٠ heavy metals experimentally inoculated in fish fillet(2021)model Benha

٢٨١ Veterinary Medical Journal 41 (1), 132-136, 2021

٢٨٢ 19. Morsy.N. S: Risk of toxic residues among some freshwater. And marine water fish
٢٨٣ species (2023)Thies master degree in Food Control Department, Faculty of Veterinary
٢٨٤ Medicine, Zagazig University, Zagazig, Egyp assessment.

٢٨٥ 20. Kathleen, F. L., David, C.E., Kimberly, A.W., Susannah, L.K., Noelle, E.S:Integrating
٢٨٦ Mercury Science and Policy in the Marine Context: (2012 Challenges

٢٨٧ 21. ABD EL-AZIZ .D and Z.M. ZAKY: ASSESSMENT OF HEAVY METAL IN LOCAL
٢٨٨ AND IMPORTED FILLETED FISH :(2010) IN ASSIUT CITY, EGYPT Dept. of
٢٨٩ Forensic Med. and Toxicology, Fac. of Vet. Med., Assiut Univ.
٢٩٠ 22. Environmental Working Group “EWG”: Lead compounds. Retrieved November 5,
٢٩١ (2010), from http// www.ewg: org/chemindex/ term/ 455.
٢٩٢ 23. Hassan, M.A. and Salem, A.M.: Risk assessment of some heavy metals contaminating
٢٩٣ fish and fish products. (2003) Benha Vet. Med. J., 14 (2):67-75.
٢٩٤ 24. Agency of Toxic Substances and Disease Registry "ATSDR": Toxicological profile for
٢٩٥ Lead. (2008) Retrieved November, http://www.atsdr.cdc.gov/ toxprofiles.Aspid =96& tid=22.
٢٩٦ 25. Mirlean N., Larned, Nikora S.T., V., Kulters V.T. et al: Mercury in lakes and lake
٢٩٧ fishes on a condensation-industry gradient in Brazil.(2005)Chemosphere, 60(2); 226-236.