Assessment of Some Heavy Metal Contents in Fresh and Salted (Feseakh) Mullet Fish Collected from El-Burullus Lake, Egypt

Yosef TA and Ghada M Gomaa

Department of Forensic Medicine and Toxicology,
Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
E-mail: tarektoxicology@yahoo.com

Abstract: Fe, Zn, Cu, Pb and Cd concentrations were determined in muscle, gills and liver of fresh and salted (either by analytical or industrial salt) mullet fish caught from El-Burullus Lake. Heavy metal concentrations varied significantly depending on the type of the tissue and salt. Generally, the lowest concentrations of the studied metals were found in fresh fish, while the highest concentrations were recorded in salted fish by industrial salt which were higher than salted fish by analytical one. Fish organs of fresh and salted fish by analytical salt contain metals followed the order: liver>gills>muscle, while in salted fish by industrial one showed: gills>muscle>liver. The metal levels were ranked as follow: Fe>Zn>Cu>Pb>Cd. Fe and Cd concentrations exceeded the upper limit of standards while, Zn one recorded much below level in all deliberate fish organs. Cu content was slightly higher than the permissible limit in muscle and gills of salted fish by industrial salt. Concerning Pb level, it was almost double the concentration of the permissible limit in the liver of all groups and muscle of salted fish by industrial salt, while it were triple the concentration of the permissible limit in gills of that group.
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Introduction

Fish, apart of being a good source of digestible protein vitamins, minerals and polyunsaturated fatty acids, are also an important source of essential heavy metals **(Irwandi and Farida, 2009),** in the future, seafood will even be a more important source of food protein than they are today and the safety for human consumption of products from aquaculture is of public health interest **(WHO, 1999)**.

Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from the water **(Mansour and Sidky, 2002)**. Unlike organic contaminants that loose toxicity by the time with biodegradation, heavy metals cannot be degraded; their concentration can be increased by bioaccumulation **(Aksoy, 2008)**. Metal bioaccumulation is largely attributed to differences in uptake and depuration period for various metals in different fish species **(Tawari-Fufeyin and Ekaye, 2007)**.

Multiple factors including season, physical and chemical properties of water can play a significant role in metal accumulation in different fish tissues **(Kargin, 1996)**. Industrial development; fertilizers; livestock manure; air pollution; increases in pesticide usage and mining have led to increasing levels of heavy metals in aquatic environments **(Cooper, 1993 and Guerrero & Kesten 1993).** Metal residues problems in the fish tissues are serious, as reflect by the high metal concentrations recorded in the water and sediments **(Wong et al., 2001)**. The gills are directly in contact with water. Therefore, the concentration of metals in gills reflects their concentration in water where the fish lives, whereas the concentrations in liver represent storage of metals in the water **(Romeo et al., 1999)**.

Assessment of heavy metals residues in fish tissues have been a major environmental focus of many researches in different countries: Egypt **(Saeed and Shaker, 2008)**; Finland **(Voigt, 2007)**; India **(Abid et al., 2009)**; Jordan **(Al-Weher, 2008)**; Malaysia **(Irwandi and Farida, 2009)**; Nigeria **(Olowu et al., 2010)**; Pakistan **(Rauf et al., 2009);** Saudi Arabia **(Mohammed, 2009)** and Turkey **(Öztürk et al., 2009).**

Salting is one of the earliest and most widespread preservation technique practiced by man **(Wheaton and Lawson, 1985)**, it is used as commonly traditional method in some Mediterranean areas and tropical countries because of simplicity of the process, low production cost and its ability to be combined with other methods in order to satisfy consumer's habit and requirements **(Berhimpon et al., 1991)**. It is well known that fish salting is a process which aims to reach the saline equilibrium between fish muscle and the surrounding salt solution in a specific time **(Zugarramurdi and Lupin, 1980).** Feseakh is a traditional Egyptian fish dish consisting of fermented salted and dried mullet, of the Mugil family, a saltwater fish that lives in both the Mediterranean and the Red Seas.

This study was conducted to determine the concentration of cadmium, lead, copper, zinc and iron in fresh and salted mullet fish (Feseakh) either by analytical or industrial salt caught from El- Burullus Lake, Egypt. Heavy metal contents of the two types of salts were also studied to give a better picture on the effect of salting process in fish metal contents.

Material and Methods

Study area

El- Burullus Lake, Egypt is the second largest lake in the Nile Delta, situated in the far north of the Delta and mediates between the two branches, Damietta and Rosetta. The average area of the lake is 370 km^2 with an average tall and width about 70 km^2 and 17 km^2 respectively, **(GAFRD 2007)**. The lake related to the Mediterranean through the strait slot Burullus while related to the Nile through Brembel channel in addition to many irrigation canals (The canal 3- the main Gharbeya canal [Kotchner]- Terah Sea- Batalah Sea- Canal 7- Canal Nashart- Canal 9- Canal Al-Moheet), from which it receives mainly agriculture drainage water (3.2 X 109 m3 y-1). El-Burullus Lake is considered as one of the major site of commercial and recreational fisheries in Egypt. (Fig.1)

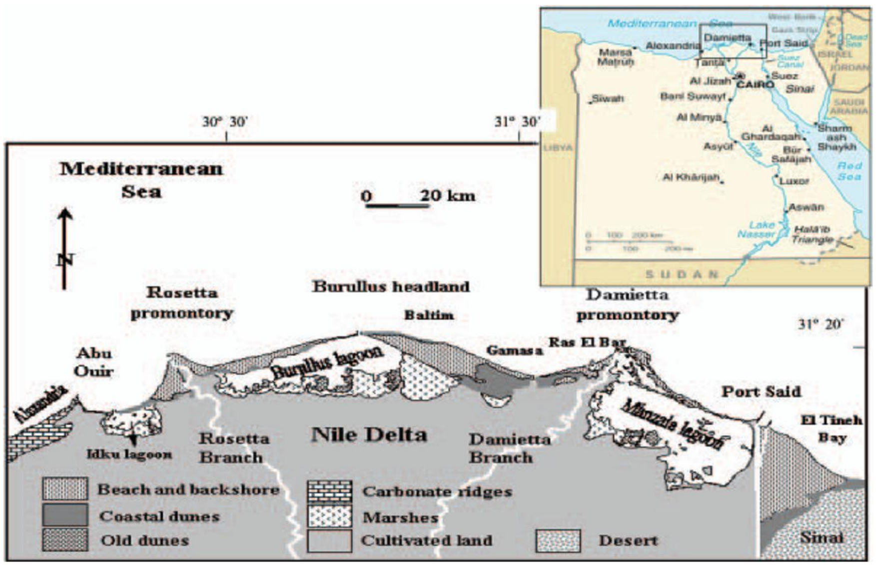

Figure 1: The Nile delta coast of Egypt and the study area (Burullus region).

Sampling

A total of 90 mullet fish (Mugil family) were collected from commercial catch in El- Burullus Lake, Egypt. Cleaning with distilled water to remove adhering dirt was done. Fish were randomly divided into three groups 30 fish each. The first group was considered as control while the second and third groups were salted according to Kench method **(Codex, 2005)** by using two types of salts namely, analytical salt (fine salt, extra pure 99.5%) produced by Laboratory Rasayan (S.D. Fine-Chem Ltd.) Company, India and industrial salt (coarse salt) produced by El-Nasr Company, Egypt. All samples were placed in polyethylene bags, identified and kept frozen at -20°C till the analysis was conducted.

Sample treatment

Fish and salt samples were digested according to **AOAC (1996)**. All frozen fish samples were allowed to thaw at room temperature, washed with distilled water and placed on filter paper to remove the excess liquid. Their liver, gills and muscle tissues were dissected separately and minced using a domestic blender, then approximately 1.0g was placed in a 150mL beaker and 10mL concentrated nitric acid was added. After a short soaking period, 5mL of 60% perchloric acid was added and the mixture was slowly heated on a hot plate until the conclusion of growth (approximately 2h). The mixture was then heated until the appearance of dense white fumes that indicate the nitric acid had evaporated and perchloric acid had reached its boiling point. The mixture was cooled; 10mL of 25% hydrochloric acid was added then, the solution was transferred to a 100mL volumetric flask that was subsequently brought to volume with deionized water. Blank solution was prepared for the background correction. Atomic absorption spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK) instrument was used to determine Fe, Zn, Cu, Pb and Cd concentrations which were expressed as µg/g. in the Toxicology Unit of Central Laboratory, Kafrelsheikh University, Egypt.

Statistical analysis

Statistical analysis of data was conducted using "Statistics for Veterinary and Animal science **(Petric and Watson 1999).**

Results and Discussion

The heavy metal contents in three major body divisions of fresh and salted (either by analytical or industrial salt) mullet fish caught from El- Burullus Lake, Egypt, in addition to the two types of salts are presented in Tables 1, 2, 3 and 4 respectively. Metal contents in fresh fish indicate the level of water metal pollution from which it is caught while, in salted one it may give an idea about the extent of salt impurity rather than the level of water metal pollution.

The present results exposed that, the lowest concentrations of Cd, Pb, Cu, Zn and Fe were found in fresh fish, followed by salted fish by analytical salt while the highest concentrations were recorded in salted fish by industrial salt.

In the three examined fish organs, the metal found mostly abundant in the fish was Fe which ranged between 52.524-133.842 µg/g in fresh fish and between 58.527-173.333 µg/g in salted fish by analytical salt while between 177.773- 189.380 µg/g in salted fish by industrial salt. For Zn element, the studied fish samples had various concentrations, ranged from 22.674 to 34.194 µg/g, in fresh fish and from 29.472 to 38.482 µg/g, in salted fish by analytical salt while from 39.860 to 48.227 µg/g, in case of salted fish by industrial salt. However, Cu contents of fish organs varied from 3.226-8.852; 7.672-8.961and 8.645-13.342 µg/g in fresh and salted fish either by analytical or industrial salt respectively. Concerning Pb element, its concentrations were ranged from 0.322 to 0.974 µg/g, in fresh fish and from 0.448 to 1.226 µg/g, in salted fish by analytical salt while from 1.230 to 1.864 µg/g, in case of salted fish by industrial one. Cd contents varied from 0.093 to 0.246 µg/g, in fresh fish and from 0.164 to 0.273 µg/g, in salted fish by analytical salt while from 0.281 to 0.712 µg/g, in case of salted fish by industrial one. (Tables 1-3)

Heavy metal contents in analytical and industrial salt used in fish salting were 0.009, 0.174; 0.000, 0.224; 2.510, 7.512; 3.240, 34.221 and 11.227, 60.123 for Cd, Pb, Cu, Zn and Fe respectively. (Table 4)

Different levels of metals were reported in different species or within the same species. The differences were explained due to the fact that the concentration of metals depends on species, sex biological cycle and on the portion of the fish analyzed. Moreover, ecological factors such as season, place of development, nutrient availability, temperature and salinity of the water may also cause the inconsistency of metal concentration in fish organs **(Tuzen, 2003)**. Our results confirmed the differences of heavy metals accumulation in different tissues.

Table 1. Concentrations of Cd, Pb, Cu, Zn and Fe in µg/gm wet wt in three organs of fresh mullet fish collected from El- Burullus Lake, Egypt

Organ / Metal	Muscle			Gills			Liver		
	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.
Cadmium	0.016	0.157	0.093±0.023	0.042	0.365	0.180 ±0.037	0.133	0.445	0.246 ±0.102
Lead	0.082	0.745	0.322 ±0.097	0.114	1.001	0.512 ±0.054	0.351	1.665	0.974 ±0.123
Copper	1.522	6.480	3.226 ±0.281	2.156	8.650	5.864 ±0.229	3.170	9.452	8.852 ±0.329
Zinc	7.552	39.753	22.674 ±0.551	9.773	55.665	29.144 ±0.952	13.860	69.654	34.194 ±1.125
Iron	31.815	90.673	52.524 ±0.859	67.273	193.822	118.842 ±1.813	78.27	213.824	133.842 ±2.132

Table 2. Concentrations of Cd, Pb, Cu, Zn and Fe in µg/gm wet wt in three organs of salted mullet fish (by analytical salt) collected from El- Burullus Lake, Egypt

Organ / Metal	Muscle			Gills			Liver		
	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.
Cadmium	0.028	0.277	0.164±0.031	0.043	0.394	0.183 ±0.045	0.172	0.536	0.273 ±0.335
Lead	0.091	0.963	0.448 ±0.081	0.123	1.122	0.564 ±0.072	0.361	1.971	1.226 ±0.162
Copper	2.579	9.112	7.672 ±0.413	2.965	9.149	7.712 ±0.358	3.835	9.794	8.961 ±0.435
Zinc	13.935	48.121	29.472 ±0.841	15.641	59.442	31.796 ±1.052	19.364	67.381	38.482 ±1.538
Iron	32.76	98.331	58.527 ±1.213	69.631	202.394	128.931 ±1.774	77.523	281.480	173.333 ±2.532

Table 3. Concentrations of Cd, Pb, Cu, Zn and Fe in µg/gm wet wt in three organs of salted mullet fish (by industrial salt) collected from El- Burullus Lake, Egypt

Organ / Metal	Muscle			Gills			Liver		
	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.
Cadmium	0.055	0.612	0.321±0.034	0.171	1.233	0.712±0.125	0.153	0.790	0.281 ±0.411
Lead	0.279	2.341	1.142 ±0.154	0.322	2.598	1.864±0.166	0.311	1.957	1.230 ±0.562
Copper	5.754	21.832	12.536 ±0.510	5.981	23.877	13.342 ±0.467	3.735	9.216	8.645 ±0.532
Zinc	19.836	61.734	41.442 ±0.833	22.532	76.747	48.227 ±1.416	14.560	65.122	39.860 ±1.346
Iron	34.377	295.250	177.879 ±1.441	75.458	237.120	189.380 ±1.962	82.523	215.217	177.773 ±2.478

Table 4. Concentrations of Cd, Pb, Cu, Zn and Fe in µg/gm in analytical and industrial salts

Salt / Metal	Analytical salt			Industrial salt		
	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E.
Cadmium	0.005	0.015	0.009±0.001	0.087	0.573	0.174±0.009
Lead	0.000	0.000	0.000± 0.000	0.112	1.523	0.224±0.014
Copper	1.334	6.832	2.510±0.142	3.981	15.836	7.512 ±0.355
Zinc	2.500	8.146	3.240±0.521	12.532	66.747	34.221 ±0.347
Iron	7.157	18.454	11.227±0.632	35.572	114.538	60.123 ±1.464

Fish organs of fresh and salted fish (by analytical salt) contain metals followed the command: liver>gill>muscle, while salted fish (by industrial salt) showed: gill>muscle>liver. The metal levels were ranked as follow: Fe>Zn>Cu>Pb>Cd. (Table 1-3)

The current results display that the contents of heavy metals in the liver and gills of fresh and salted fish by analytical salt are much higher than that in muscles. **Jobling (1995)** attributed the high accumulation of heavy metals in liver and gills tissues to the metallothionein proteins which are synthesized in these tissues when fishes are exposed to heavy metals to detoxify them. These proteins are thought to play an important role in protecting tissues from damage by heavy metal toxicants. Moreover, fish liver plays a primary role in the metabolism and excretion of xenobiotic compounds **(Rocha and Monteiro, 1999)**, besides to higher metal accumulation tendency of the liver, followed by gills and muscle **(Adhikari et al., 2006).** Gills are the site directly exposed to the ambient conditions and also are known for their excretory function even for some metals **(Matthiessen and Brafield, 1977)**. Alongside, gills were the initial site for the accumulation of waterborne metals, where they bind to the external gill cytosolic compounds via convalent bonds **(Wepener et al., 2001). Karadede & Ünlü, (2000) and Khan et al., (1989)** mentioned that muscle is not an active tissue in accumulating heavy metals. Similar observations were reported by many studies carried out with various fish species **(Alam et al. 2002, Guerrin et al. 1990 and Saeed and Sakr, 2008).**

Our results stated that the contents of heavy metals in muscle and gills of salted fish by industrial salt are much higher than that in liver. This result may be owing to the direct contact of muscle and gills with industrial salt during ripening process. Salinas have been identified as possible sinks for heavy metals **(Nixon, 1980)**, which can be absorbed by simple diffusion through gill pores and skin **(Sindayigaya et al 1994)** in addition to the suggestion of binding between heavy metals, which have high affinity for thiol groups, with proteins and peptides in skeletal muscle **(Mohammed, 2009).**

Moisture content may be play an important role in the increasing of metal contents in salted fish, as it decreased by 10-15% after ripening which may be attributed to the loss in the water holding capacity of fish protein as a result of proteolysis **(Foda et al., 1986)**, consequently ash content increased by 10-15% as a result of loss of moisture content and the diffusion of salt in the fish during salting and ripening process **(Awad, 1999 and Hernandez-Herroro, 1997)**.

Contamination levels of heavy metal in fish are normally compared to the permissible limits recommended by Food and Agriculture Organization and World Health Organization **(FAO/WHO, 1984 and 1992)**. However, Ministry of Industry Egypt also has set standards called "Egyptian Organization for Standardization and Quality Control **(E.O.S.Q.C, 1993)**. Unlike for iron, there is no limit revealed in FAO/ WHO or E.O.S.Q.C so, we were compared its levels to that reported by **Food Stuff (1972*)***. Tables 5; 6 and 7 shows the frequency distributions of heavy metals in different fish organs compared to the permissible limits set by FAO/WHO; E.O.S.Q.C and Food Stuff.

Results from Tables 1-6 indicate that all organs of fish studied were found to contain Fe element much above the recommended limits set by Food Stuff, which is 30 ppm. Pertaining to Zn element, its content was much below the recommended limits set by FAO/WHO which is 150 ppm. The concentration of Cu element was found lower than the permissible limit set by FAO/WHO in all organs of fresh and salted fish by analytical salt in addition to liver of salted fish by industrial salt, while it was slightly higher in muscle and gills of salted fish by industrial salt. The level of Pb was slightly lower compared to the permissible limit set by FAO/WHO in muscle of fresh and salted fish by analytical salt, whereas it was slightly higher in gills of the same groups. Pb level was almost double the concentration of the limit set by FAO/WHO in liver of all groups and muscle of salted fish by industrial salt, while it was triple the concentration of the limit set by FAO/WHO in gills of that group. Generally, the contents of Pb were found higher than the limit set by E.O.S.Q.C in all examined organs of all groups. However, Cd level was generally higher than the permissible limit in all examined organs compared to the permissible limit set by FAO/WHO and E.O.S.Q.C.

Table (5): Frequency distribution of Cd, Pb, Cu, Zn and Fe residues in muscle samples of fresh and salted (by analytical or industrial salt) mullet fish collected from El- Burullus Lake, Egypt (n = 30).

Heavy Metals	Permissible limit (ppm)	Less than permissible limit %			More than permissible limit %		
		Row fish	Salted fish by analytical salt	Salted fish by industrial salt	Row fish	Salted fish by analytical salt	Salted fish by industrial salt
Cadmium	0.05 (1)	40.00	20	0.0	60.00	80	100
	0.1 (2)	83.33	66.67	26.67	16.67	33.33	73.33
Lead	0.5 (1)	76.67	10	6.67	23.33	90	93.33
	0.1 (2)	6.67	0.0	0.0	93.33	100	100
Copper	10 (3)	100	100	53.33	0.0	0.0	46.67
Zinc	150 (3)	100	100	100	0.0	0.0	0.0
Iron	30 (4)	0.0	0.0	0.0	100	100	100

NB: (1): FAO/WHO (1992); (2): EOSQC (1993); (3): FAO/WHO (1984) and (4): Food Stuff (1972).

Table (6): Frequency distribution of Cd, Pb, Cu, Zn and Fe residues in gills samples of fresh and salted (by analytical or industrial salt) mullet fish collected from El- Burullus Lake, Egypt (n = 30).

Heavy Metals	Permissible limit (ppm)	Less than permissible limit %			More than permissible limit %		
		Row fish	Salted fish by analytical salt	Salted fish by industrial salt	Row fish	Salted fish by analytical salt	Salted fish by industrial salt
Cadmium	0.05 (1)	6.67	6.67	0.0	93.33	93.33	100
	0.1 (2)	66.67	66.67	0.0	33.33	33.33	100
Lead	0.5 (1)	76.76	60	6.67	23.33	40	93.33
	0.1 (2)	0.0	0.0	0.0	100	100	100
Copper	10 (3)	100	100	23.33	0.0	0.0	76.76
Zinc	150 (3)	100	100	100	0.0	0.0	0.0
Iron	30 (4)	0.0	0.0	0.0	100	100	100

NB: (1): FAO/WHO (1992); (2): EOSQC (1993); (3): FAO/WHO (1984) and (4): Food Stuff (1972).

Table (7): Frequency distribution of Cd, Pb, Cu, Zn and Fe residues in liver samples of fresh and salted (by analytical or industrial salt) mullet fish collected from El- Burullus Lake, Egypt (n = 30).

Heavy Metals	Permissible limit (ppm)	Less than permissible limit %			More than permissible limit %		
		Row fish	Salted fish by analytical salt	Salted fish by industrial salt	Row fish	Salted fish by analytical salt	Salted fish by industrial salt
Cadmium	0.05 (1)	0.0	0.0	0.0	100	100	100
	0.1 (2)	0.0	0.0	0.0	100	100	100
Lead	0.5 (1)	10	6.67	16.67	90	93.33	83.33
	0.1 (2)	0.0	0.0	0.0	100	100	100
Copper	10 (3)	100	100	100	0.0	0.0	0.0
Zinc	150 (3)	100	100	100	0.0	0.0	0.0
Iron	30 (4)	0.0	0.0	0.0	100	100	100

NB: (1): FAO/WHO (1992); (2): EOSQC (1993); (3): FAO/WHO (1984) and (4): Food Stuff (1972).

Comparing metal accumulations in fresh fish organs in this study with other studies from different sampling areas in Egypt, it is obvious from table (8) that **El-Moselhy (1999)** recorded lower metal concentrations in fish organs from Lake Manzala than those in the present study. Higher values of metals were recorded in fish organs collected from Shanawan Drainage Canal **(Khallaf et. al., 1998)**; Manzala Lake (**Saeed and Shaker, 2008)** and Edku Lake (**Saeed and**

Shaker, 2008)** except Cu levels in muscle and gills which were lower than our results.

Comparison of metal levels in muscle of salted fish from different studies in Egypt vs. present study showed, higher metal concentrations than those in the current study **(EL-Tahan et al., 1999)**. Nearly similar values were recorded by **Zaki (1998).** While, lower levels were mentioned by **Nassar et al., (1996)**.

Table 8. Comparison of heavy metals concentrations in various organs of fresh fish with previous studies in other localities in Egypt.

Organ	Locality	Fe	Zn	Cu	Mn	Cd	Pb	Refer.
Muscle	Shanawn Canal	530.9	55.4	5.1	20.9	5.3	48.7	(1)
	L. Manzala	4.32	5.96	0.51	-	0.03	0.13	(2)*
	L. Edku	75.19	27.60	2.80	1.98	0.19	0.59	(3)
	L. Manzala	256.66	212.44	48.84	22.98	10.36	10.1	(3)
	L. Manzala	18.46	18.46	1.09	-	0.08	0.52	(2)*
Gills	L. Edku	515.23	87.46	4.24	26.25	1.96	3.41	(3)
	L. Manzala	2056.82	1006.88	242.12	30.32	32.22	56.12	(3)
	Shanawn Canal	1489.3	107.5	205.5	39.6	12.6	91.9	(1)
Liver	L. Edku	720.48	112.15	154.43	13.89	2.16	2.88	(3)
	L. Manzala	2256.42	1226.34	277.82	33.55	39.12	42.22	(3)

(1): Khallaf et al., (1998); (2): El-Moselhy (1999); (3): Saeed and Shaker (2008); *wet weight.

Conclusions

Fe, Cd and Pb levels in most studied samples were above the international permissible limits, while Cu and Zn residues were at a relatively low safety levels. Therefore this fish species caught from El-Burullus Lake may pose health hazards for consumers. Salted fish by industrial salt had greater concentrations of most studied metals than those of fresh and salted fish by analytical one. These results were probably credited to the usage of impure salts during salting process; going by this result it could be inferred that impurity of industrial salt is more than

analytical one. Catching of fish from uncontaminated water, controlling the industrial and agriculture effluents into lakes and surface water; also using salts of food grade quality which meets the purity requirements of standards for iodized **(Republic Act, 1996)** in salting process are our recommendation to produce a hygienic salted fish for human consumption.

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