Heavy Metals and Microbial Safety of Nile Tilapia (*Oreochromis niloticus*) Fishes Available in Commercial Market of Jimma Town

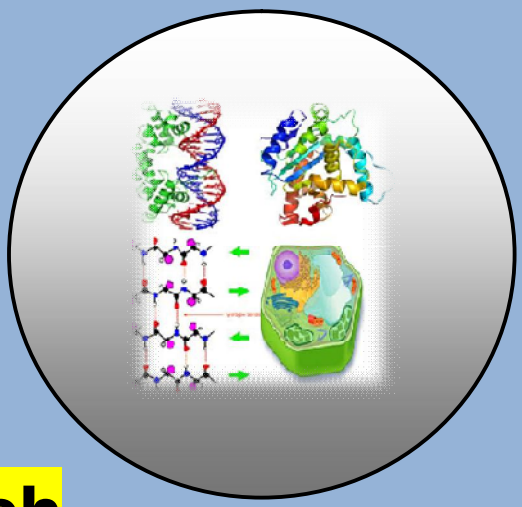
By

Girma Selale Geleta, Adam Biftu, Anbessa Dabassa and Tolessa Lechisa

ISSN 0970-4973 Print
ISSN 2319-3077 Online/Electronic

Global Impact factor of Journal: 0.756
Scientific Journals Impact Factor: 2.597
Index Copernicus International Value
IC Value of Journal 4.21 Poland, Europe

J. Biol. Chem. Research
Volume 32 (1) 2015 Pages No. 36-45



Journal of Biological and Chemical Research

An International Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in Various National and International Scientific Databases of the World

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 1: 36-45, 2015
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Girma Selale Geleta
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 21/08/2014 **Revised: 15/12/2014** **Accepted: 24/12/2014**

Heavy Metals and Microbial Safety of Nile Tilapia (*Oreochromis niloticus*) Fishes Available in Commercial Market of Jimma Town

Girma Selale Geleta, Adam Biftu *Anbessa Dabass and *Tolessa Lechisa
Department of Chemistry, Jimma University, P.O. Box 378, Jimma, Ethiopia
***Department of Biology, Jimma University, P.O. Box 378, Jimma, Ethiopia**

ABSTRACT

In this study the level of heavy metal and microbial safety of Nile Tilapia (Oreochromis Niloticus) fishes available in the commercial market of Jimma town was determined. Thirty five fish meat samples were purchased on different days to make a sample representative from shops in local market of Jimma Town. For heavy metal analysis, samples were digested on a hot plate. The concentrations of heavy metals (Fe, Zn, Pb, Cu, and Co) were investigated in digested fish samples by Atomic absorption spectroscopy. The results of present study indicated that heavy metal concentration (mg/kg, dry weight) recorded as Means± Standared (SD) deviation in fish samples collected were found to be: Iron (16.830±0.005 mg/kg, dry weight), Zinc (3.37±0.0060 mg/kg, dry weight),) Cobalt (2.79±0.0764 mg/kg, dry weight), Lead (0.839864±0.033 mg/kg, dry weight) and Copper (0.198492±0.003 mg/kg, dry weight). Moreover, the fish meat spoilage and poisoning were the results of contamination from different sources. The population of Aerobic mesophilic, anaerobic Spore formers, aerobic Spore formers, Enterobacteriaceae, Coli form groups, Salmonella spp, Staphylococcus spp, Streptococcus spp, Bacillus spp, Enterococcus spp , Yeast and Molds were isolated and identified. The maximum number of the isolates aerobic mesophilic, coliforms, enterobacteriacea, lactic acid bacteria, staphylococci, micrococci, enterococci and yeast and molds were 7.35x107, 2.17x104, 3.24x105,6.24x107,5.34x106, 2.31x103,2.63x105 respectively and anaerobic performer's and salmonella are low in number. Even though, low microbial load with the presence of pathogenic microorganisms and only lead metal concentrations exceeding the limits set by the European commission (EC) 2001 guideline and FAO, the allowable level of lead in fish, is 0.4 and 0.5 mg/kg respectively. This study provides an insight into the potential impact of increased levels of metals and pathogenic microorganisms in the human diets especially estimated of the contaminated of fish tissues.

Key words: Heavy Metal, Pathogen Microorganisms, Safety and Fish Meat.

INTRODUCTION

Heavy metals are defined as a metallic chemical element that has a relatively high density and are toxic or poisonous at low concentrations (Irwandi, J. and Farida, 2009). They are stable and persist in environmental contaminants of aquatic environments and their organisms. They occur in the environment, both as a result of natural processes and as pollutants from human activity (Krishna, Rao, Swaruparani, and Rao, 2014). Some of the metals found in the fish might be essential as they play an important role in the biological system of the fish as well as in human beings, some of them may also be toxic as might cause a serious damage in human health even in trace amounts at a certain limit (Irwandi, J. and Farida, 2009). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs.

Oreochromis Niloticus (Freshwater Fishes of Ethiopia, 2001), Nile Tilapia, local name "Korosso", Family Cichlidae is an inland water fish inhabiting the littoral zone of lakes, mainly up to 5 meters deep. Thus, fishes are major part of the human diet because, it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Boadi, Twumasi, Badu, and Osei, 2011; Irwandi, J. and Farida, 2009; Mukherjee and Bhupander, 2011). Despite their recognized benefits, fish and seafood may represent a risk for human health since they can accumulate contaminants from aquatic environment and magnify them up the food chain (Alshikh and Olfat Mohamed H. Yousef, 2013). The presence of heavy metals leads the marine environmental pollution to be recognized as a serious matter to human health concern. Industrial and agricultural activities were reported to be the leading potential source of the accumulation of pollutants in the aquatic environment, including the (Akif, M., et al, 2002; Gümgüm et al., 1994).

Many studies have been conducted on the contamination of different fish species to determine their heavy metal concentration in different parts of the world(Edem C. et al ,2009 Kamaruzzam, O. et al 2010; Edem et al., 2008). The results of these studies have shown high presence of heavy metals in the organisms studied. Since fishes are highly consumed by human beings and may accumulate large amounts of some metals from the water, it is important to determine the concentration of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption for human health (Irwandi, J. and Farida, 2009) .

Fish is also susceptible to microbial contamination as millions of bacteria are present in the surface slime, gills wastes and in the intestines of live fish. Bacterial growth and invasion of fish are prevented by the body's natural defense system during life but after death, the defense system breaks down and the bacteria multiply and invade aquatic the flesh (Daniel, E.O et al 2013). Microbial action has been known to play a large part in the spoilage of fish (Eyo, 2001). Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odours (Geoff et al., 1991).

In Ethiopia the "Korosso" is the most common and popular fish, found in most lakes and important river systems, and is caught in large quantities. It is a significant contribution to the income and food supply in the rural community, and the second most preferred fish brought to the market.

To our knowledge so far there is no coordinated investigation of the levels of heavy metals in various types of food items including Nile Tilapia (Oreochromis Niloticus) Fishes Commercial Avaiable in Market of Jimma Town. Therefore, in this research an attempt would be made to determine the levels of selected heavy metals in fishes purchased from the local market in Jimma Town, Oromia Regional State.

MATERIAL AND METHODS

Sampling

Fish Samples were collected from a local market of Jimma Town. Thirty five fish, meat sample were collected, transported to the laboratory by an icebox and kept in refrigerator at -4°C prior to the analysis. Whole fish were dissected on a clean bench shortly after thawing with the aid of a stainless steel knife which had been cleaned with acetone and hot distilled water prior to use. The fish samples were finally preserved in the clean dry polyethylene bottle prior to analysis(Kamaruzzaman, M.C. ,et al 2010).

Microbiological examination of fish meat

Twenty five grams (25g) from (randomly samples) of fish meat samples were blended with 225 mL of 0.1% peptone water in a sterile blender jar for 3-5 minutes and decimal dilutions were prepared for testing. Numbers of viable organisms were determined by the plate count method. From the appropriate dilution 0.1 mL was inoculated to the appropriate media for the particular group of organisms to be tested as a Colony forming unit per gram (c.f.u/g). The total aerobic bacterial count was determined according to Shalini, (2010) using plate count agar medium, incubated at 37^{O} C for 48 hours. Anaerobic bacterial count was determined by A.P.H.A, (1992) using cooked meat agar medium with anaerobic jars. Yeasts and molds were counted by spread-plated on pre-solidified surfaces of Potato Dextrose agar supplemented with 0.1g Chloramphenicol and incubated at 25-28 ^{O}C for 5-7 days; according to Dabassa, (2013). Spore former bacteria were determined by heating in a water bath at 80°C for ten minutes and then cooled rapidly in water according to Dabassa, (2013). From appropriate dilution 0.1 ml were spread-plated on pre-dried surface of Plate Count Agar and kept at 35^{O}C for 72 hours. Staphylococcus spp bacterial count was determined to spread plated onto Mannitol Salt Agar by using the spread plate (Acco *et al.,* 2003). Enterobacteriaceae was determined on Violet red bile glucose agar medium after incubated at 37 ^{O} C for 20-24 h, as described by (Spencer *et al.,* 2007). Lactic acid bacterial spp was counted by using the de Mann Rogosa Sharpe (MRS) agar media and incubation for 16- 24 h, at 37^{O} C as described by Pal *et al.,* (2005) . *Salmonella* spp was carried out using (Cheung *et al.,*2007) technique, after enrichment at 37^{O} C for 24 h, in Silent F broth and Rappaport Vassiliadis broth, the cultures were streaked onto Xylose Lysine Deoxycholate Agar . The presumptive Salmonella colonies were picked off and streaked onto Nutrient Agar for purity and incubated at 37 °C for 24 hours. These cultures were further analyzed by standard biochemical test. Staphylococcus spp was enumerated on Baird–parker medium using surface plating technique as recommended by I.A.E.A, (1990), incubated at 37 ^{O} C for 24 h. Enterococci spp was enumerated on Konamycin insulin aside agar medium (Mossel and Tamminge, 1980) positive colonies were confirmed by Microscopic examination for the presence of short chain streptococci.

Micrococci, according to (UK Standards for Microbiology Investigations, 2014). Coliform group was counted using Violet red bile agar medium and incubated at 44 ^{O} C for 24-48 h (Sheth *et al.,* 2005). Clostridum spp used to cooked meat agar medium incubated at 37 ^{O}C for 24 h. in an anaerobic system using gas generation kit as mentioned by Cravene *et al.,* (1979) and Oxoid, (1985).

Experimental Procedure

All glassware used were soaked in detergent solution overnight before being rinsed and soaked in 10% (v/v) HNO3 overnight, followed by rinsing with distilled water. All reagents used were of analytical reagent grade Merck, Germany. Standard stock solutions of lead, copper, Iron, Cobalt, and zinc were prepared by diluting concentrated solutions to obtain solutions of 1000 mgL^{-1}. Nile Tilapia fish's samples were purchased from popular markets in Jimma Town, Ethiopia. A total of thirty five samples were collected. After purchased, the meat was homogenized thoroughly in a food blender with stainless steel cutters. Each sample was then taken and digested promptly as follows: For the determination of selected heavy metals, about 10 ± 0.001 g of homogenized sample were weighed into a 200 mL beaker and 10 mL of concentrated HNO_3 were added. The beaker was covered with a watch glass, and after most of the sample was dissolved by standing overnight, it was then heated on a hot plate with boiling until any vigorous reaction had subsided. The solution was allowed to cool, transferred into a 100 ml volumetric flask and diluted to the mark with distilled water(Kiomars & Amirtaheri, 2011). Corresponding blank samples were digested in the same manner.

Instrumentation

Analysis of metals was carried out using Flame Atomic Absorption Spectrometer (Analytik Jena, ANovAA 300) equipped with air/acetylene flame and hollow cathode lamps of Pb, Co, Cr, Cu and Zn. The wavelength for each of the metals was adjusted according to the description given in the manufacturer's manual while slit width and lamp currents were optimized. The optimized operating conditions are given in (Table 1).

RESULTS AND DISCUSSION

HEAVY METALS AND MICROBIAL LOAD IN FISHES

Meat qualities were deteriorated and had reduced shelf-life as the results of microbial contamination. The microbiology of this study were indicated the most dominant and the detected pathogens (Table 2).

Lead (Pb)

Lead is toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract(Stancheva, M.,2014). Moreover, lead may cause learning disabilities, impaired protein and hemoglobin synthesis and shorten the life span of red blood cells which leads to severe anemia (hypochromic microcytic anemia) in children (Kiomars & Amirtaheri, 2011). In present study the concentration of lead in fish purchased from local market of Jimma town was found to be 0.839864±0.0331 mg/kg. According to European commission (EC) 2001 guideline and FAO, the allowable level of lead in fish, is 0.4 and 0.5 mg/kg respectively (Sivaperumal et. al, 2007).

The maximum concentration of lead which is permitted in prepared foods specifically intended for babies or young children is 200µg/kg, FAO/WHO (Akif, M., et al, 2002) . The concentration of lead in fish commercially available in local market of Jimma town is above permissible level set by, FAO/WHO and European commission (EC) 2001 guideline. Hence, the level of lead from this study could pose health risk since the values were not within the FAO/WHO permissible limit.

Zinc (Zn)

Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities. Zinc is widespread among living organisms, due to its biological significance(Kiomars & Amirtaheri4, 2011) . The maximum zinc level permitted for fish is 50 mg/kg according to Food Codex (Maff et. al, 1993). The recommended daily intakes of zinc are 15 mg for adult males and 12 mg for adult females. In United States environmental protection agency and the European Commission (US-EPA and EC) have not considered any standards or limits for the zinc concentrations (Alimentarius, joint FAO/WHO; 1994, Ashraf et. al, 2006; Mohammad M. et al 2011). The permissible limits for Zn set by FAO/ WHO (1989) is 40 ppm. The average of zinc concentration (3.37±0.0060) was determined in current study is far below permitted level set by FAO/WHO (1998). The concentration of Zinc analyzed in fish is considered safe for human consumption.

Table 1. Optimum analytical operating conditions for FAAS Analysis of metals using air –acetylene flame.

Element	Wavelength(nm)	Current(mA)	Slit width(nm)	Oxidant/Fuel
Chromium	357.9	5	1.2	Air-C_2H_2
Lead	217.0	4	0.8	Air-C_2H_2
Cobalt	240.7	6.0	0.2	Air-C_2H_2
Copper	324.8	3.0	1.2	Air-C_2H_2

In *fish meat s*amples, the minimum counts of Aerobic Mesophilic bacteria (AMB) were over 7.53 x10^6 with the maximum count of 7.02x 10^7 CFU/g. Similarly, the minimum and maximum counts of coliform 1.9x10^3, 2.17 x10 ^{4}**.** The prevalence of enterobacteriacea, coli forms, and *salmonella* spp in the sample indicates the safety problem during processing, and transporting. Yeast and mold have in collected samples has been less than detectable level (Table 2)**.**

The results of present study indicated that heavy metal concentration (mg/kg, dry weight) recorded as Means± Standared (SD) deviation in fish samples collected from Jimma Town, Oromia, Ethiopia were found to be: Iron (16.830±0.005 mg/kg, dry weight), Zinc (3.37±0.0060 mg/kg, dry weight),) , Cobalt (2.79±0.0764 mg/kg, dry weight), Lead (0.839864±0.033 mg/kg, dry weight) and Copper (0.198492±0.003 mg/kg, dry weight). The order of heavy metal concentration was Fe>Zn>Co>Pb>Cu.

Table 2. The microbiological load of the fish meat sold from Jimma town, Oromia Ethiopia, 2014.

Site	Aerobic Mesophilic bacteria	Coli forms	Entero bacteriaceae	Lacticacid bacteria	*Staphylococci*	Enterococci	Anaerobic sporeformer	*Salmonella* spp	Micrococci spp	Yeast and molds
Ajip	$4.75x10^7$	$2.17x10^4$	$3.45x10^4$	$5.93x10^6$	$5.34x10^6$	$2.31x10^3$	$1.5x10^4$	1	$2.83x10^2$	< detectable
Moble	$7.53x10^6$	$3.21x10^3$	$2.58x10^3$	$6.24x10^7$	$4.35x10^5$	$2.45x10^3$	$1.8x10^2$	0	$2.45x10^4$	< detectable
Merkato	$7.02x10^7$	$1.9x10^3$	$3.24xx10^5$	$5.26x10^5$	$5.96x10^5$	$3.02x10^2$	$1.04x10^3$	5	$2.63x10^5$	< detectable

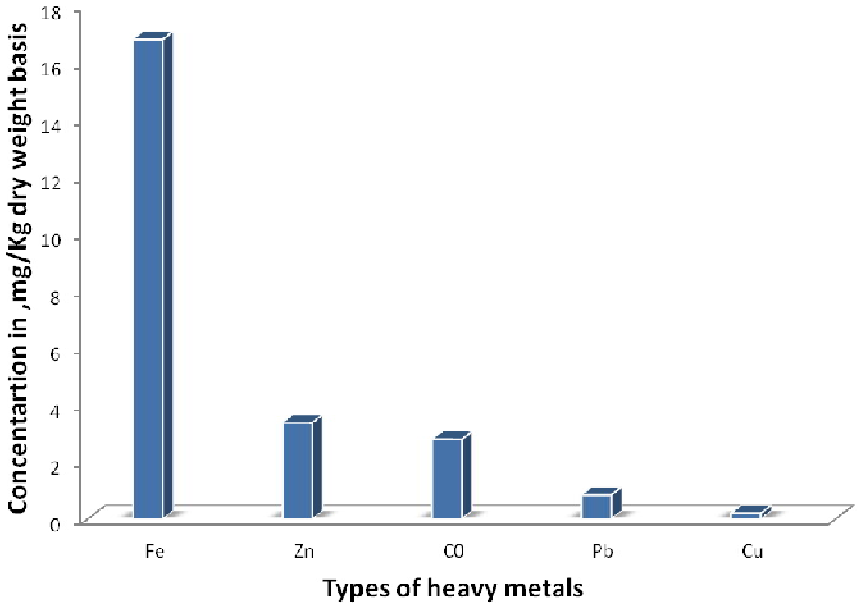


Fig. 1. Different concentrations of heavy metals in fish (mg/kg, dry weight).

Copper (Cu)

Copper, in its many forms is regarded as the third most common minerals in human body. It is a fundamen-tal micronutrient to all forms of life in enzyme activity or random rearrangement of natural protein. Copper is an essential element in the body and is required for different functions.The avarege concentration of copper in present studies was found to be 0.198492±0.0029 mg/ Kg**.** Standard level of 20 mg/kg dw (dry weight) has been reported. However, after new regulation in 2008, no maximum level was specified for Cu in the fish species and sea foods. Also, there is no guideline on acceptable levels of Cu in fish suggested by European Economic Community or FAO/WHO(Fawole, 2013). The concentration of copper analyzed in fish is considered safe for human consumption.

Iron (Fe)

Iron is an essential element in human diet and fish contains relatively high amounts of readily absorbable haem iron which is better absorbed than non-haem iron. Iron forms part of hemoglobin which allows oxygen to be carried from the lungs to the tissues (Daniel, E.O et al 2013). In present study, the concentrations of iron were found to be 16.830±0.005 mg/kg, dry weight. The concentration of Iron analyzed in fish is considered safe for human consumption.

Cobalt (Co)

Cobalt is a component of cyanocobalamin (vitamin B12), constituting nearly 4.5% of its molecular weight. Most animals need the element for the synthesis of the vitamin by intestinal microflora, and such bacteria have also been isolated from the intestinal tract. Cobalt as part of vitamin B 12 is associated with nitrogen assimilation, and synthesis of haemoglobin and muscle protein. In addition, cobalt influences certain enzymes. Cobalt binds to insulin and also reduces plasma glucose levels (Takeshi W. et al, 1997).

Cobalt is beneficial for humans because it is a part of vitamin B12. However exposure to high levels of Co can result in lung and heart defects and dermatitis. Cobalt is used to treat anemia in pregnant women, because it stimulates the production of red blood cells. The levels of cobalt content in analyzed fish were 2.79±0.0764 mg/kg, dry weight. Cobalt concentrations in the literature have been reported in the range of 0.02-0.67 mg/kg for muscles of fish from the fish markets in India, 0.006-0.244 mg/kg for muscles of fish from the coastal waters of the Caspian Sea, 0.04-0.41 mg/kg for muscles and 0.14-0.51 mg/kg for livers of fish from Turkish seas (Mustafa,T. et al,2008). Our results were not generally in agreement with literature.

CONCLUSION

The presence of Coliforms, Enterobacteriacea, *Salmonella spp*. and its count exceeded the maximal acceptable value in all analyzed samples of fish meat. The value of 6 pathogens (*Salmonella* sp.), and coliform bacteria, which are indicators of fecal pollution can be viewed positively. Even if, a low number of pathogen microorganisms, and because of the importance of fish in the human consumption, it is necessary to monitor fish meat for human consumption regularly to ensure continuous food safety.

The counts of Enteronacteriacea exceeded the maximal acceptable value in all analyzed samples of fish meat. The prevalence of 1 to 5 for pathogens (*Salmonella sp*.), and accessibility of coliform bacteria, which are indicators of fecal pollution can be viewed positively. In view of the importance of fish in human diet, it is necessary that biological monitoring of the fish meant for consumption should be carried out regularly to ensure safety.

In the present study the heavy metal levels conform to FAO/WHO and the literature published values in fish except lead. However the estimated lead levels were higher in our samples than WHO/FAO recommended levels and those reported in the literature. This study provides information on selected heavy metal concentrations of Nile Tilapia (Oreochromis Niloticus) fishes available in the commercial market of Jimma town and therefore provides an essential baseline data with which future levels may be compared and evaluated. WHO (1995) reported that heavy metals must be controlled in food sources in order to assure public safety. Excessive concentration of heavy metals in food is associated with the etiology of a number of diseases, especially cardiovascular, renal, neurological and bone diseases.

ACKNOWLEDGEMENTS

We thankfully acknowledge to the Department of Chemistry and Biology, CNS, Jimma University for providing necessary facilities to carry out this research.

REFERENCES

Akif, M., Khan, A. R., Sok, K., Min, K. S., Hussain, Z. and Maal-Abrar, M. 2002. Textile effluents and their contribution towards aquatic pollution in the Kabul River (Pakistan). *Journal of Chemical Society of Pakistan*, *24*(2): 106–111.

Alshikh and Olfat Mohamed H. Yousef 2013. No Title. Histological Changes Induced by Heavy Metals in Fishes at Red Sea- Jazan –*KSA*, *10*(3): 0–5.

A. P. H. A. 1992. Compendium of methods for the microbiological. examination of foods, 3rd edn. Amer. spp (Stph.s), Public. Health Assoc. Washington.

Acco, M., Ferreira, F.S., Henriques, J.A. and Tondo, E. C. 2003. Identification of multiple strains of *Staphyloccocus aureus* colonizing nasal mucosa of food handlers. *Food Microbiol*. 20: 489-493.

Boadi, N. O., Twumasi, S. K., Badu, M., and Osei, I. 2011. Heavy metal contamination in canned fish marketed in Ghana: 877–882. doi:10.5251/ajsir.2011.2.6.877.882

Cravene, E. S., Lillard, H. S. and Mecuri, A. J. 1979. Survival of Clostridium perfringens during preparation of precooked chicken parts. *J. food Tech*. 38:505-508.

Cheung, P. Y., Kwok, K. K. and Kam, K. M. 2007. Application of BAX system, Tecra UniqueTM Salmonella test, and a conventional culture method for the detection of Salmonella in ready-to-eat and raw foods. *J. Appl. Microbiol*., 103: 219–227.

Dabassa, A. 2013. Evaluation of Home Slaughtered Meat Quality used for Human Consumption at Household and Food Seller House in Jimma. *J. Med. Sci.*, 13 (8): 779-784.

Daniel, E.O., Ugwueze, A.U. and Igbegu, H.E. 2013. Microbiological Quality and Some Heavy Metals Analysis of Smoked Fish Sold in Benin City, Edo State, Nigeria *World Journal of Fish and Marine Sciences* 5 (3): 239-243

Edem Christopher, A., Osabor V. and Iniama G. E. R. E. J. 2009. Distribution of Heavy Metals in Bones , Gills , Livers and Muscles of (Tilapia) Oreochromis niloticus from Henshaw Town Beach Market in Calabar Nigeria. *Pakistan Journal of Nutrition*, *8*(8): 1209–1211.

Fawole, F. 2013. Nutritional qualities and trace metals concentration of six fish species from Oba reservoir, Ogbomoso, Nigeria. *African Journal of Food Science*, *7*(8): 246–252. doi:10.5897/AJFS2013.0994

Gümgüm, B., Ünlü, E., Tez, Z. and Gülsün, Z. 1994. Heavy metal pollution in water sediment and fish from Tigris River in Turkey. *Chemosphere*, *29*(1):111–116.

Geoff A., Ivor C. and Susan S. P. 1991. Post-Harvest Losses of Fish in the tropics. Natural *Resources Institute*: 1-5.

Irwandi, J. and Farida, O. 2009. Mineral and heavy metal contents of marine fin fish in Langkawi island, *Malaysia*, *16* :105–112.

I. A. E. A. 1990. Microbiological specification and testing methods for irradiated foods. Technical Report series No. 104, I. A. E. A , m Vienna

Kamaruzzam, B. Y., Ong, M. C., Rina, S. Z. and Joseph, B. 2010. Levels of Some Heavy Metals in Fishes From Pahang River Estuary, Pahang, Malaysia. *Journal of Biological Sciences*. doi:10.3923/jbs.2010.157.161

Kiomars, M. M. M. T. M. D. Amirtaheri 2011. Determination of Pb, Cd, Ni, and Zn Concentrations in Canned Fish in Southern Iran. *Sacha Journal of Environmental Studies*, *1*(1): 94–100.

Krishna, P. V., Rao, K. M., Swaruparani, V. and Rao, D. S. 2014. Heavy Metals Concentration in Fish Mugil cephalus from Machilipatnam Coast and Possible Health Risks to Fish Consumers, *4*(2): 126–135.

Mukherjee, D. P. and Bhupander, K. 2011. Assessment of Arsenic, Cadmium and Mercury Level in Commonly Consumed Coastal Fishes from Bay of Bengal , *2*, 19–31.

Mustafa,T., Aysun, T. and Yalqin, T. 2008 Metal contaminations in five fish species from black, marmara, aegean and mediterranean seas, turkey j. Chil. Chem. Soc. :53

Mossel, D. A. and Tamminge, S.K. 1980. Method for Microbiological Examination of Food (in Deutch). Zeist (1980).

Oxoid, 1985. The oxide manual of culture media ingredients and other laboratory services. Oxide Limited, Hampshire, England St

Pal, V., Jamuna, M. and Jeevaratnam, K. 2005. Isolation and characterization of bacteriocin producing lactic acid bacteria from a South Indian special dosa (appam) *batter. J Cult Collect*. 4:53–60.

Stancheva, M., Makedonski, L. and Peycheva, K. 2014. Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast, *46*(1): 195–203.

Shalini, S. 2010. Study on microbiological aspects of fresh fruit and vegetables (including green leafy vegetables) in and around National Capital Region (NCR). Bhaskaracharya College of Applied Sciences. Dwarka, New Delhi

Sheth, M., Gurudasani, R. and Mudbiidri, R. 2005. Identification of hazards in street foods of Vadodara, India. *Indian J. of Nutrition and Dietetics*.42**:** 266-278

Spencer, K., John, F.T. and Spencer, A. L. 2007. Food Microbiology Protocols, Homana Press, Totowa, New Jersey. (indian reprint).

Takeshi, W., Viswanath, K., and Shuichi, S. 1997. Trace minerals in fish nutrition Aquaculture, 151:185-207.

Corresponding author: Mr. Girma Selale Geleta, Department of Chemistry, Jimma University, P.O. Box 378, Jimma, Ethiopia.
Email: girma245@yahoo.com