http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (18): 895-899, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.895.899

Heavy Metal (Lead, Cadmium) and Antibiotic (Tetracycline and Chloramphenicol) Residues in Fresh and Frozen Fish Types (*Clarias gariepinus*, *Oreochromis niloticus*) in Ibadan, Oyo State, Nigeria

Adetunji Victoria Olusola, Popoola Amirah Folashade and Odetokun Ismail Ayoade
Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: This study was carried out to assess the level of heavy metals (lead (Pb) and cadmium (Cd)) and antibiotics (tetracycline and chloramphenicol) residues in frozen and fresh fish types obtained from Eleyele river, Officer's mess, Alfa farm and a major frozen meat outlet in Ibadan, Oyo State Nigeria. The Atomic Absorption Spectrophotometer (AAS) and High Performance Liquid Chromatography (HPLC) were used to analyze the heavy metals and antibiotics residue levels in fresh and frozen fish, respectively. The results showed mean concentrations of antibiotics was higher ($p<0.05$) in fresh than in frozen fish samples while there were no significant differences in the mean concentrations of heavy metal residue. The differences of mean residue levels in both antibiotics and heavy metals tested in the cranial and caudal parts of the fish samples were not significant ($p<0.05$). However, there was a significant difference between species (tilapia and catfish) and sources. The highest heavy metals and tetraxycline residues were observed in Alfa's farm (Pb: 0.039±0.004 ppm; Cd: 0.020±0.006 ppm; tetraxycline: 2.185±0.412). Chloramphenicol was highest in Officers mess (0.837±0.165 ppm). The heavy metals (Pb and Cd) concentrations determined were below the maximum permissible limits set by both local and international safety agencies. Tetraxcycline exceeded international limits of 0.2 ppm while Chloramphenicol which has a zero tolerance level was also detected from all sources. This study accentuates the need for control of heavy metals and antibiotics in fish sold for human consumption in Ibadan, Nigeria. The need to ban chloramphenicol in treatment of fish and other animals is emphasised.

Key words: Fish, heavy metals, antibiotics, permissible limits

INTRODUCTION

An investigation of heavy metals in fish is an important aspect of environment pollution control. Environmental pollution has long been recognized as a biological hazard to wildlife as well as to mankind. Fish contaminants can reach man through the food chain (De Lacerda *et al*., 1987). Industrial pollutants, pesticides and some heavy metals are known to exhibit biomagnifications as they move up the food chain. Discharges of metal effluents into rivers may cause deleterious effects to the health (Tavares and Carvalho, 1992).

Fish are of socio-economic importance globally since they contribute to livelihood of people. Aquaculture or capture fishes provide employment and an income opportunity for people and are responsible for at least 70% of the national feed needs in Nigeria (Aboaba, 1998). Non-commercial aquaculture plays an important role in rural livelihood (Ahmed and Lorica, 2002) and fish families in general are better nourished than non-fish farming families. In particular tilapia culture in Nigeria contributes to food security, poverty alleviation, employment, trade and income generation (Omotoso and Fagbenro, 2005).

The African catfish (*Clarias gariepinus*) is highly appreciated as good aquaculture specie because of its resistance to disease, ability to tolerate a wide range of environmental parameters including high stocking densities under culture conditions and relative fast growth rate (Goos and Richter, 1996). Disease is one of the most serious limitations in fish production as the condition so far reported in Nigeria have been mainly of pathogenic origin. Another setback is nutritional deficiencies due to management practices (Jegede and Fagbenro, 2007).

Heavy metals and antibiotics residues are well known environmental pollutants that can result in serious health hazards to humans, their effects may not manifest immediately but show up after several years. The indiscriminate use of antibiotics therapy in fish may lead to adverse effects such as antibiotics resistance in humans. This may inadvertently lead to an increased incidence of non-healing wounds, secondary bacterial infections and death (Chinabut *et al*., 2011).

Corresponding Author: Adetunji Victoria Olusola, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria Tel: +234 (0)704 097 9193

Lead (Pb) and Cadmium (Cd) are among the main toxic metals that accumulate in food chains and have a cumulative effect (Cunningham and Saigo, 1997). Lead is the number one environmental poison amongst the toxic heavy metals all over the world, causing serious health hazards to humans, especially to young children. Over exposure to Pb causes damage to foetal nervous system, increasing the risk of premature birth or low birth weight. Other effects associated consumption of foods containing high level Pb contents include: inhibition of biosynthesis of haem, severe vomiting, intestinal cramps, circulatory disorder, madness and death (Cunningham and Saigo, 1997). Chronic exposure to cadmium is associated with heart disease, anaemia, skeletal weakening, depressed immune system response and kidney and liver diseases.

Antibiotic have been used extensively in human, veterinary medicine, agriculture and aquaculture business and it has steadily increased especially in the developing countries (Kumar *et al*., 2009). Chloramphenicol has never been approved for use in food-producing animals in the developed world. It causes aplastic anemia in humans. Adverse reactions are not dose dependent and a safe level of exposure has not been determined (Koonse, 2007). It is worthy of note that chloramphenicol is still being used in the treatment of bacteria diseases of fish in Nigeria. Chloramphenicol when detected, even though at a low concentration, may be toxic or carcinogenic for humans (Chinabut *et al*., 2011).

The tetracycline group includes tetracycline, chlortetracycline (CTC), oxytetracycline (OTC), methacycline, demeclocycline, doxycycline and minocycline. The entire group are derivatives of the polycyclic compound naphthacenecarboxamide.

Oxytetracycline is a broad-spectrum antibiotic used to treat a variety of infections and is also used as a growth promoter in animals (Moller, 2011). Resistance in human intestinal coliforms and allergies have been (Moller, 2011). Currently, antimicrobial resistance is a growing public health threat and has been designated by the WHO as an emerging public health problem.

This study therefore, aims at assessing the level of heavy metal residues (Pb and Cd) and antibiotic residues (Tetracycline and Chloramphenicol) in frozen and fresh fish types (*Clarias gariepinus*, *Oreochromis niloticus*) in Ibadan, Oyo state, Nigeria.

MATERIALS AND METHODS

Study location: Ibadan is located on latitude 7°26'N 3°54'E in southwestern Nigeria. River Eleyele is located in north eastern part of Ibadan. This river provides water to the city of Ibadan and fishing activities are also carried out on the river. Oficers mess, Alfa farms and major meat outlet are major sources of fish public consumption.

Samples: Sixty fish samples including fresh fish samples (*Clarias gariepinus*, *Oreochromis niloticus*) were randomly obtained from River eleyele, officers mess and Alfa's farm in Ibadan city over a 2 week period. Frozen fish samples were obtained from a major meat outlet in Ibadan. Fifteen samples each were obtained from each location. A total of 120 replicate samples (60 each from caudal and cranial tissue) were screened for antibiotic and heavymetal residues.

Preparation of the fish samples: The samples were placed on ice and transported immediately to the Food Hygiene Laboratory, of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for analysis. Thirty gram of fish muscle was cut aseptically and placed in sterile plastic containers and stored at -20°C until analysed. Samples were taken from the cranial and caudal portions of the fish samples in 2 replicates.

Determination of heavy metals: This was carried out using the Atomic Absorption Spectrophotometer (AAS Perkin Elmer AAnalyst, USA) according to Iwegbue *et al*. (2008).

Extraction/digestion process: The 0.5 g of the samples was weighed into a set of digestion tubes. Te milliliter of perchloric+nitric acid was digested into the sample vessels. The digestion tubes were then mounted on the digestion block at a temperature of 120°C for 2 h. At the end of the digestion, the samples were allowed to cool to room temperature. The samples were mixed with ultra-pure water to make a volume of 50 mL. The samples were shaken for 10 min. The shaken sample solutions were then centrifuged at the rate of 4500 rpm for 5 min. The supernatants were then decanted into a set of test tubes. The combined stock standard (Pb and Cd 1000 ppm each) was prepared from reference standards and stored in the refrigerator at 7-10°C until use. The certified sensitivity check of standard solutions for each element was used in optimizing the efficiency of the AAS. The working standards were first determined to create the standard curve; this was followed by the measurement of the unknown analytes. The atomic absorption spectrophotometer was adjusted to specific wavelength corresponding to each of the metals to be measured. Measurements were done using the hollow cathode lamps at the wavelength of 283.3 and 228.8 for Pb and Cd, respectively.

Determination of chloramphenicol

Extraction: Twenty five grams of the sample was weighed into a set of volumetric flasks (50 mL). Five milliliter of ultra-pure water and 2.5 mL of methanoic acid were dispensed and 1.5 mL of HCl and 5 mL of methanol were added. The mixture was shaken for 45 min and centrifuged at 1000 rpm for 25 min. The supernatant was then collected into plastic vinyls for the determination on High Performance Liquid Chromatography (HPLC) (Wang *et al*., 2008).

Determination of tetracycline

extraction: 2.5 g of the blended samples in solution was weighed into a set of centrifuge tubes. 1.8 mL of SN HCl was dispensed and mixed properly. Twenty milliliter of a mixture of phosphate buffer solutions+sodium hydroxide+sodium metabisulphite solution in the ratio of 2:1:4, respectively into the samples and covered properly, shaken for 15 min and then centrifuged for 20 min at 4500 rpm. The supernatant was collected into plastic vinyls for the determination on HPLC (Wang *et al*., 2008).

Statistical analysis: Mean values of residues of antibiotics and heavy metals among sample location of the fish were compared using one-way analysis of variance (ANOVA) while mean values between the two species, parts of the fish sampled and the storage type was compared using the T-test. p-values less than 0.05 were considered significant. All analyses were carried out using the SPSS, version 17.

RESULTS

The mean residue levels of both heavy metals and antibiotics analyzed varied across the sources sampled (Table 1). This variation is significantly different ($p<0.05$) for each residue tested across the sources where the fish were sampled from. Cadmium levels are in the range of 0.012 to 0.02 mg kg^{-1}. The lowest Cd level was from the major frozen meat outlet and officer's mess sampled while the highest was from Alfa's farm. Lead levels ranged from 0.026±0.005 to 0.039±0.004 mg kg^{-1}. Officer's mess and Alfa's farm had the lowest and highest concentrations respectively. Tetracycline levels varied from 1.845±0.394-2.185±0.412 mg kg^{-1}, with the frozen meat outlet having the lowest and Alfa's farm with the highest residue level. A low chloramphenicol level (0.720±0.201 mg kg^{-1}) was recorded in the major meat outlet while the highest chloramphenicol residue levels (0.837±0.165 mg kg^{-1}) were recorded from Officers mess.

Based on fish species, the mean concentrations of both heavy metals and antibiotics residues were higher in Tilapia fish (*Oreochromis niloticus*) than for catfish (*Clarias gariepinus*) (Table 2). This higher residues levels in Tilapia were significant at $p<0.05$.

Table 1: Mean concentrations (mg kg^{-1} ppm^{-1}) of heavy metals and antibiotic residues in fish from different sources

Source	Cadmium	Lead	Tetracycline	Chloramphenicol
Officers mess	0.012±0.006^{a}	0.026±0.005^{a}	1.952±0.512^{a}	0.837±0.165^{a}
Alfa's farm	0.020±0.006^{b}	0.039±0.004^{b}	2.185±0.412^{b}	0.835±0.379^{b}
Meat Outlet	0.012±0.003^{c}	0.030±0.006^{c}	1.845±0.394^{c}	0.720±0.201^{c}
Eleyele river	0.019±0.004^{d}	0.031±0.005^{d}	nd	nd

Within columns, means with different superscript letters are statistically different ($p<0.05$), nd: Not determined

Table 2: Mean concentrations (mg kg^{-1} ppm^{-1}) of heavy metals and antibiotic residues in fish

Type	Cadmium	Lead	Tetracycline	Chloramphenicol
Species				
Tilapia	0.019±0.004^{a}	0.031±0.005^{a}	3.077±0.538^{a}	1.108±0.300^{a}
Catfish	0.014±0.006^{b}	0.029±0.007^{b}	1.9820±0.486^{b}	0.822±0.223^{b}
Part				
Cranial	0.022±0.008^{a}	0.041±0.005^{a}	2.031±0.218^{a}	0.992±0.203^{a}
Caudal	0.018±0.002^{a}	0.037±0.004^{a}	2.338±0.519^{a}	0.679±0.464^{a}
Storage				
Frozen	0.014±0.003^{a}	0.030±0.006^{a}	1.845±0.394^{a}	0.720±0.201^{a}
Fresh	0.016±0.006^{a}	0.030±0.007^{a}	2.539±0.139^{b}	0.972±0.282^{b}

Means with different superscript letters are statistically significantly different ($p<0.05$) within columns

Generally, higher residue concentrations 0.022±0.008, 0.041±0.005 and 0.992±0.203 mg kg^{-1} of Cd, Pb and chloramphenicol, respectively were deposited in the cranial part of the fish (Table 2) though not significant at $p<0.05$. However, higher tetracycline contents (2.338±0.519 mg kg^{-1}) were found in the caudal part (Table 2).

Concentration of tetracycline and chloramphenicol in fresh fish (2.539±0.139 and 0.972±0.282 mg kg^{-1}) was higher ($p<0.05$), respectively than in the frozen fish (1.845±0.394 and 0.720±0.201 mg kg^{-1}). Significant differences at $p<0.05$ were not observed in the heavy metal residue concentrations analyzed in both the frozen and fresh fish samples.

DISCUSSION

This study revealed that lead, cadmium, tetracycline and chloramphenicol residues detected in fish sampled from the various sampling locations showed significant variations. The heavy metals (Pb and Cd) concentrations determined were below the maximum permissible limits set by both local and international safety agencies. EC (2005) set the maximum tolerable limits in fish muscle to be 0.3 and 0.2 ppm, respectively for Pb while EC (2005) reported a maximum limit of 0.05 ppm for Cd. The residue concentrations obtained in this study is comparable to that determined by Sireli *et al*. (2006) where the Cd range found was 0.003-0.036 ppm and Pb was 0.001-0.791 ppm in Mackerel, *Salmo salar* and *Oncorhynhus mykiss* marketed in Ankara, Turkey. The various levels in Pb and Cd residue concentrations indicates that fish could serve as potential source of Pb and Cd contamination.

Tetracycline residue levels determined in the fish samples were markedly higher than the maximum permissible limit set by the Export Inspection Council of India (EIC, 2002) which stipulated a limit of 0.1 ppm. This is a clear indication of the misuse of antibiotic in aquaculture in Nigeria. The release of large quantities of antibiotics into the environment due to animal production (including aquaculture) and human use has produced the phenomena of microbial resistance, although different bacteria can acquire resistance to antibiotics and the development of antibiotic resistance by pathogenic bacteria is considered to be one of the most serious risks to human health at the global level (WHO, 2011).

Chloramphenicol was detected in all the sample locations. Chloramphenicol remained a banned antibiotic in the treatment of bacteria diseases of fish but the results obtained showed that this drug is evidently still in use in the country. The highest chloramphenicol residue levels of 1.108±0.300 ppm obtained in Tilapia in this study is much lower than 1.51 μg kg^{-1} (0.00151 ppm) noted in the branchia of carp fish by Lu *et al*. (2009). The Pb residue levels in both species of fish studied were higher than the Cd levels determined comparable to the results obtained Eneji *et al*. (2011) but contrary to the residue values recorded by Raphael *et al*. (2011) in both Tilapia and catfish. Also, Pb and Cd accumulation vary with fish species. Sireli *et al*. (2006) equally found varying Pb and Cd levels in three different fish species. The significant higher concentrations determined in the Tilapia than catfish are similar to the findings of Raphael *et al*. (2011). Pb residues were higher in Tilapia than in catfish in this study, similar to reports by Eneji *et al*. (2011). It is possible that the Tilapia has a better ability to concentrate metals in their muscles. Accumulation of bioactive metals like Pb and Cd is actively controlled by fish through different metabolic processes and the level of accumulation usually depends on the ambient concentration (Raphael *et al*., 2011).

Long-term storage of fish probably has no effect on the residue concentrations of both metals tested owing to the insignificant differences observed in the fresh and frozen fish sampled in this study. Accordingly, most tested heavy metals by Ganbi (2010) including Pb and Cd in some Hammour fish exhibited a high stability in freezing and in frozen storage for up to six months. On the other hand, significant low levels in both antibiotics analysed was observed in the frozen fish samples; the reason for this is not clear, however, the antibiotics residues may have been denatured due to the long term storage of the fish samples at very low temperatures. The fact that the fish consumes its food and also breaths from the cranial region might be responsible for the higher residue levels noticed in the cranial part of its body though this was not significant. There is a high possibility of some residue being trapped in the gills. Entry of metals Raphael *et al*. (2011) as well as antibiotics occurs either through the gill membrane or through ingestion in fish.

CONCLUSION

It could be inferred from the results of this study that despite the presence of heavy metals residues tested below the maximum residue limit set by both local and international food safety agencies, the possibility for bioaccumulation overtime is still a public health concern. It is recommended that antibiotics be used with maximum caution on fish farms while strict law enforcing the ban of chloramphenicol (and other similar harmful drugs already banned among the international community) should be enacted. Regulation of veterinary medicine administration to food animals should be sufficiently strict so that potentially toxic antibiotics residues are unlikely to be found in commercially produced animal products. Furthermore, an alternative to the use of antibiotics as growth promoters in food animals have been suggested; these include the addition of digestive enzymes to animal feed to help break down certain feed components, the addition of probiotic microbes to animal feed and the introduction of more effective infection controls, such as improved biosecurity measures.

REFERENCES

Aboaba, J.O., 1998. Vet needs in aquaculture in Ogun state for fish inspection services. Proceedings of the Seminar on Aquaculture in Ogun State: Management and Veterinary Needs, November 11, 1997, Ogun State, Nigeria.

Ahmed, M. and M.H. Lorica, 2002. Improving developing country food security through aquaculture development-lessons from Asia. Food Policy, 27: 125-134.

Chinabut, S., T. Somsiri, K.L. Bartie, G. Huys and D.T.H. Oanh *et al*., 2011. The use of antimicrobials in Asian aquaculture: Aims of the EU Asia resist project. http://www.medinfo.dist.unige.it/Asiaresist/Publications/WAS%20Abstract.pdf

Cunningham, W.P. and B.W. Saigo, 1997. Environmental Science: A Global Concern. Wm. C. Brown Publisher, New York, USA., Pages: 389.

De Lacerda, L.D., W.C. Pfeiffer and M. Fiszman, 1987. Heavy metal distribution, availability and fate in Sepetiba Bay, S.E. Brazil. Sci. Total Environ., 65: 163-173.

EC, 2005. Commission regulation (EC) No. 78/2005 of 19 January 2005 amending regulation (EC) No. 466/2001 as regards heavy metals. Official J. Eur. Union, L16: 43-45.

EIC, 2002. Orders and notification. Order No. 722, Export Inspection Council of India. http://eicindia.gov.in/eic/qc&i/enotfn-mrl-522.htm

Eneji, I.S., R.S. Ato and P.A. Annune, 2011. Bioaccumulation of heavy metals in fish (*Tilapia zilli* and *Clarias gariepinus*) organs from River Benue, North-Central Nigeria. Pak. J. Anal. Environ. Chem., 12: 25-31.

Ganbi, H.H.A., 2010. Heavy metal pollution level in marine hammour fish and the effect of popular cooking methods and freezing processes on these pollutants. World J. Dairy Food Sci., 5: 119-126.

Goos, H.J. and C.J. Richter, 1996. Internal and External Factors Controlling Reproduction in African Catfish (*Clarias gariepinus*). In: The Biology and Culture of Catfish, Aquatic Living Resources, Legendre, M. and J.P. Proteau (Eds.). Vol. 9. Academic Publisher, UK., pp: 46-58.

Iwegbue, C.M.A., G.E. Nwaje and E.H. Iyoha, 2008. Heavy metal residues of chicken meat and gizzard and turkey meat consumed in southern Nigeria. Bulgar. J. Veterin. Med., 11: 275-280.

Jegede, T. and O.A. Fagbenro, 2007. Tilapia husbandary vs. midwifery: Use of antifertility plants. Proceedings of the 3rd Annual Conference of School of Agriculture and Agricultural Technology, April 16-19, 2007, Federal University of Technology Akure, Nigeria, pp: 118-122.

Koonse, B., 2007. JIFSAN good aquacultural practices manual section 10: Food laws and regulations. Joint Institute for Food Safety and Applied Nutrition (JIFSAN) Good Aquacultural Practices Program, University of Maryland, pp: 1-6.

Kumar, P.A., J. Patterson and P. Karpagam, 2009. Multiple antibiotic resistance profiles of *Vibrio cholera* non-01 and non-0139. Jap. J. Infect. Dis., 62: 230-232.

Lu, X.W., Z. Dang and C. Yang, 2009. Preliminary investigation of chloramphenicol in fish, water and sediment from freshwater aquaculture pond. Int. J. Environ. Sci. Technol., 6: 597-604.

Moller, J.K., 2011. Fighting drug-resistant hospital bacteria-less antibiotics and more infection control. Ugeskr Laeger, 173: 2264-2266.

Omotoso, F.O. and O.A. Fagbenro, 2005. The role of aquaculture in poverty alleviation in Nigeria. World Aquacult., 36: 6-8.

Raphael, E.C., O.C. Augustina and E.O. Frank, 2011. Trace metals distribution in fish tissues, bottom sediments and water from okumeshi river in delta state, Nigeria. Environ. Res. J., 5: 6-10.

Sireli, U.T., M. Goncuoglu, Y. Yildirim, A. Gucukoglu and O. Cakmak, 2006. Assessment of heavy metals (cadmium and lead) in vacuum packaged smoked fish species (Mackerel, *Salmo salar* and *Oncorhynhus mykiss*) marketed in Ankara (Turkey). Eur. J. Fish. Aquat. Sci., 23: 353-356.

Tavares, T.M. and F.M. Carvalho, 1992. Avaliacao da exposicao de populaces humanas a metais pesados no ambiente exemplo do Reconcavo Baiano [Assessment of exposure of human populations to heavy metals in the environment: The Reconcavo Baiano examples]. Quimica Nova, 15: 147-154.

WHO, 2011. Antimicrobial resistance. Fact Sheet No. 194. http://www.who.int/mediacentre/factsheets/fs194/en/index.html

Wang, L., H. Yang, C. Zhang, Y. Mo and X. Lu, 2008. Determination of oxytetracycline, tetracycline and chloramphenicol antibiotics in animal feeds using subcritical water extraction and high performance liquid chromatography. Anal. Chim. Acta, 619: 54-58.