

MICROBIAL DIVERSITY AND HEAVY METALS CONCENTRATION OF WOOD SMOKED FISH FROM EDONWHII FISHING SETTLEMENT IN AKWA IBOM STATE, NIGERIA

Abstract

The microbial diversity and heavy metal concentrations of wood smoked fishes (*Clarias gariepinus* and *Pseudotolithus elongatus*) from Edonwhii fishing settlement in Akwa Ibom State, Nigeria were investigated using standard microbiological protocols and analytical procedures. The study revealed variations in the densities of the different microbial groups. The fresh fish samples of *Clarias gariepinus* and *Pseudotolithus elongatus* had Total heterotrophic bacterial count of 7.1×10^5 and 4.1×10^7 cfu/g respectively as compared to the smoke-dried fish which had 4.8×10^2 and 6.7×10^2 cfu/g respectively obtained 16 hours after smoke drying. The study reveals a rich microbial assemblage of the fish samples. *Streptococcus* sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli*, *Vibrio cholerae* and *Bacillus* sp. were the different bacteria isolates encountered in the study while *Absidia*, *Aspergillus niger*, *Candida albicans*, *Candida tropicalis*, *Penicillium italicum*, *Cladosporium* sp., *Apergillus flavus*, *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp., *Candida pseudotropicalis*, *Apergillus fumigatus* and *Mucor* sp were the fungal isolates identified. The metal analysis result showed that Zn was found to be the most abundant metal. However Cd levels in *Clarias gariepinus* (0.23 mg/kg) was relatively higher than the value recorded for *Pseudotolithus elongatus* (0.16 mg/kg) but all were within the FAO/WHO permissible level of (0.5 mg/kg) of Cd in sea food. Similarly, the Pb levels were within the FAO/WHO permissible level of 0.3 mg/kg, indicating that the smoked fish samples were still suitable for human consumption.

Key: microbes, aquaculture, wood smoked fish, heavy metals

1.0 Introduction

Aquaculture development in Nigeria is fast gathering momentum according to findings by Tawari and Abowei (2011). The need for more fish supplies in the market is seen in the increased importation of about 900,000 metric tons annually which is double the local production/catch, estimated at only 450,000 metric tons. Nigeria is Africa's biggest fish consumer according to Ozigbo *et al.* (2013) and Anyanwu (2014). The reason for this high consumption may stem from the fact that fish is adjudged to be nutritionally superior to other sources of animal protein in that they contain most of the essential amino acids particularly lysine, leucine, valine, methionine and tryptophan. Hence fish is regarded as first class protein producer (Ekelemu *et al.*, 2000). Fish is an extremely perishable food item, therefore much interest has also been channeled into its preservation to avoid waste (Pigott, 2015) by adopting various preservation techniques like smoking. Rashed (2001) opined that fishes are also considered as one of the most significant indicators of metal pollution in aquatic environments, asides also hosting microbial populations. It has been proven that fishes absorb dissolved elements and heavy metals from surrounding waters and ingested food. Although these metals and pollutants are found in traced quantities in the water environment, their

ability to bioaccumulate is of interest to researchers as they affect both human and environmental health (Adebayo, 2017). Seymore (1994) found that these pollutants accumulate in various fish tissues in significant amounts, eliciting toxicological effects at critical targets. Preservation of fishes by Smoking is commonest traditional method of fish preservation in Nigeria. This method combines the effect of the destruction of bacteria by compounds (eg phenols) in the smoke and the high temperature cooking of the fish. This results to a longer shelf life, which has been attributed to the drying, antibacterial and antioxidant and cooking effects of smoke (Adebayo, 2017). But this study evaluates the microbial and heavy metal presence in smoked processed fishes in Edonwhii community.

2.0 Materials and Method

Study area

The Edonwhii fishing settlement is located along the coast of the Bight of Bonny at the mouth of Iko River Estuary. The estuary is a major tributary of Imo River. This area is situated in the Niger Delta fringe between Imo River and Qua Iboe River estuaries and lies between latitudes 4°50' and 7°55' East.

Sample collection

Precisely three samples each of two different fish (*Pseudotolithus elongatus* and *Clarias gariepinus*) giving a total of 6 smoked fish samples were subjectively obtained 16 hours after smoked-drying. The smoked fish were conditioned in sterile plastic bags and preserved in ice-packed coolers. Representative fresh samples of selected fishes were also purchased to serve as control. Also collected, were dry samples of red mangrove fire wood used as fuel for the smoking process.

Processing of fish samples

The samples were carefully and aseptically dismembered with sterile surgical blades to obtain intestine and skin. The organs were macerated using a sterile pestle and mortar. One gram (1.0 g) of each organ sample was serially diluted and used for microbiological analysis.

Culture media preparation and sterilization

The media used for the study were: Nutrient Agar (NA), *Staphylococcus* 110 medium, Thiosulphate citrate Bile salt, Eosine Methylene Blue Agar (EMBA) *Salmonella-Shigella* agar (SSA), mineral salt medium, Sabouraud dextrose agar and Dermatophytic medium for the enumeration and isolation of heterotrophic bacteria, *Staphylococcus aureus*, *Vibrio*, faecal coliform (*Escherichia coli*), *Salmonella-Shigella*, hydrocarbon degraders, fungi and dermatophytes species respectively. They were aseptically prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes.

Estimation of Microbial contaminant

The density of heterotrophic and potential pathogens was determined using standard analytical procedures. *Staphylococcus aureus*, *Vibrio*, faecal coliform (*Escherichia coli*), and *Salmonella-Shigella* loads on the samples was determined using the pour plate technique and incubated at 37°C for 24 hours while the densities while the densities of fungi and dermatophytes were determined using spread plate technique and incubated at room temperature for 4 days.

The densities of hydrocarbon utilizing microorganisms were determined using pour plate technique and incubated at (28 ± 2 °C) for 7 days. Discrete colonies that appeared on the

94 culture plates were enumerated with the aid of a Quebec colony counter and recorded as
95 Colony Forming Units (CFU) per gram of fish sample.

96 **Characterization and identification of microbial isolates**

97 The pure colonies obtained from the samples were characterized using standard procedure as
98 described by *Bergey's Manual of Determinative Bacteriology* (1994). The colonies were
99 subjected to Gram's stain and standard biochemical tests. Fungal isolates were identified
100 according to the method Barnett and Hunter (1987).

102 **Determination of Heavy Metals in fresh fish, smoked fish and fire wood Samples**

103 The heavy metals in beach water and sand samples which were analyzed included Cadmium
104 (Cd), Chromium (Cr), Zinc (Zn), Lead (Pb) and Nickel (Ni). The concentrations of heavy
105 metals in the collected samples were determined (after nitric acid digestion) by means of an
106 atomic absorption spectrophotometer (APHA, 2005).

108 **3.0 Results and Discussion**

109 **Microbiological properties of smoked fish skin samples**

110 Figures 1 and 2 show that the fresh fish samples of *Clarias gariepinus* and *Pseudotolithus*
111 *elongatus* had total heterotrophic bacterial count of 7.1×10^5 and 4.1×10^7 cfu/g respectively
112 as compared to the smoke-dried fish which had 4.8×10^2 and 6.7×10^2 cfu/g respectively
113 obtained 16 hours after smoke drying. *Salmonella-Shigella*, *Escherichia coli*, *Vibrio* and
114 hydrocarbon degraders were not detected on the dry fish samples while *Staphylococci* sp
115 were found in all the smoke-dried fish samples, whereas Dermatophytes were found in all the
116 smoked fish samples.

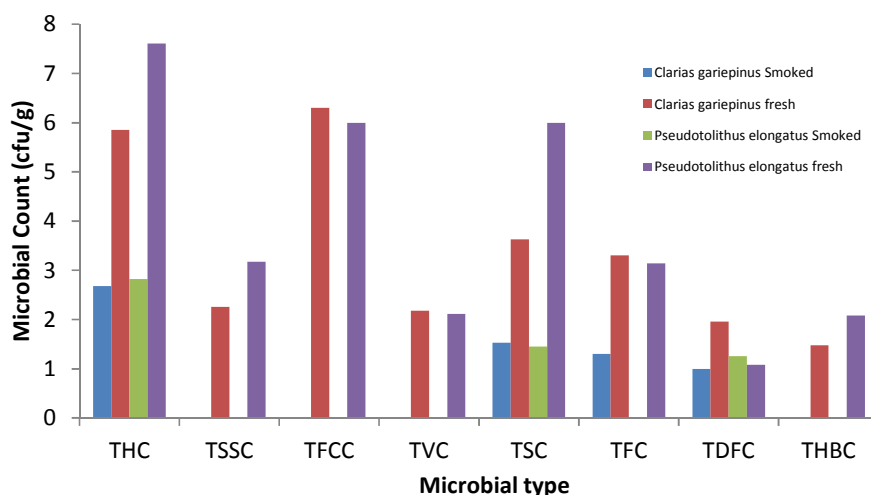


Figure 1: Microbial properties of fish skin samples (Cfu/g)

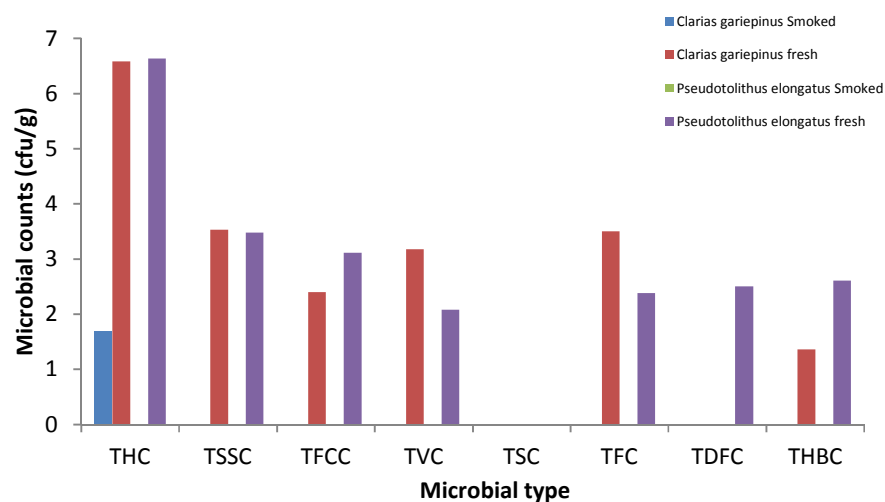


Figure 2: Microbial properties of fish intestinal samples (Cfu/g)

Key:-

Total heterotrophic bacteria count (THC)

Total *Salmonella-Shigella* count (TSSC)

Total *Escherichia coli* count (TFCC)

Total *Vibrio* count (TVC)

Total Dermatophytic fungi count (TDFC)

Total Hydrocarbonoclastic bacteria count (THBC)

Total *Staphylococci* count (TSC)

Total Fungal count (TFC)

Microbial diversity of the fish samples

Streptococcus sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli*, *Vibrio cholerae* and *Bacillus* sp. were the different bacteria isolates encountered in the study while *Absidia*, *Aspergillus niger*, *Candida albicans*, *Candida tropicalis*, *Penicillium italicum*, *Cladosporium* sp., *Apergillus flavus*, *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp., *Candida pseudotropicalis*, *Apergillus fumigatus* and *Mucor* sp. were identified fungal isolates.

Heavy metals load of the fish samples

Heavy metals load of smoked, fresh fishes and the mangrove firewood are presented in Figure 3 and Table 1 respectively. Values recorded for the smoked fish were slightly higher than those of fresh fish samples. Variation in concentrations of metals between fish species was also noticed and Zn had the highest cumulative amount in all the analysed samples.

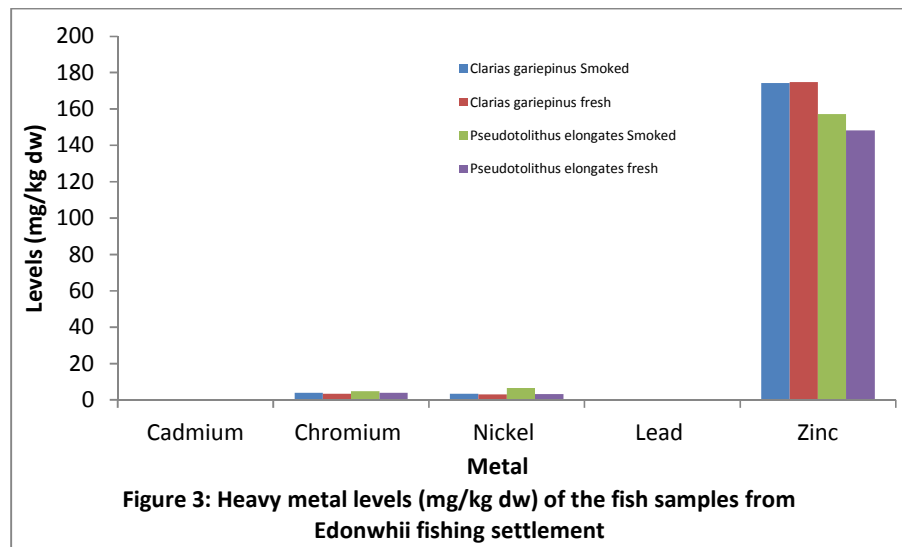


Table 1: Heavy metal levels (mg/kg dw) of mangrove firewood

Heavy metal	Value
Cadmium	0.005
Chromium	2.066
Nickel	4.452
Lead	1.006
Zinc	20.59

Discussion

The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish live and not of the fish species (Shewan, 2000). The results obtained from this study have shown that fresh fish samples are laden with microbial contaminants including pathogenic groups of microorganisms. Fish, because of their soft tissues, aquatic environment, high water content, neutral pH and high amino acids are extremely susceptible to microbial contamination and spoilage (Jeyasejaram *et al.*, 2006). Run-off water may also introduce pathogenic organisms into the aquatic ecosystem from which the fish are harvested. This may result in faecal coliforms contamination resulting with high numbers of coliforms in harvested fish (Oku and Amakoromo, 2013; Okon *et al.*, 2017). The high values in the fresh fish may have been due to contamination from the aquatic habitat, poor or unsanitary handling of harvested fish and invasion of the fish flesh by bacteria due to its high moisture content (Shewan, 2000; Ekpo *et al.*, 2017). This observation also agrees with the findings of Salan *et al.* (2006) who experimented on smoke as a way of preservative method. The elimination of pathogenic bacteria such as *Salmonella* sp and *Shigella* sp from smoked fish intestinal samples as compared to fresh fish intestinal samples is indicative of the preservative function of smoke from the wood. Despite the high heterotrophic bacterial loads of the fish samples, some of which were still within that acceptable range limit of 5.0×10^5 cfu/g (5.7 log cfu/g) for good quality products (ICMSF, 1986). Smoking the fish samples drastically reduced the microbial loads of the fishes sampled.

The bacterial encountered in the smoked fish skin samples included *Streptococcus* sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae* and *Bacillus* sp. The occurrence of *Escherichia coli* and *Staphylococcus aureus* in smoked dried fish skin samples have also been previously reported by Martin (1994). *Staphylococcus aureus* constitute the normal flora of the human skin and mucous membrane, hence their presence in the fish samples might have been through contamination by handling and poor sanitary condition. These organisms prevalence is of public health concern because they have been implicated in food-borne intoxication and infection cases (Umoh and Odoaba, 1999).

Relatively more fungal isolates were encountered on the smoked *Clarias gariepinus* than *Pseudotolithus elongatus* fish samples. The isolates include *Absidia*, *Aspergillus niger*, *Candida albicans*, *Candida tropicalis*, *Penicillium italicum*, *Cladosporium* sp., *Apergillus flavus*, *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp., *Candida pseudotropicalis*, *Apergillus fumigatus* and *Mucor* sp. Some of these fungal isolates especially the dermatophytic species of *Trichophyton* have been reported to be resistant to heat treatment (Essien *et al.*, 2009). Previous studies of Essien *et al.* (2005) have shown strains or species of *Penicillium* sp, *Apergillus flavus* and *Cladosporium* sp isolated from Shark fish have the proteolytic and mycotoxigenic potentials. It is important to state that the majority of the fungal isolates obtained from this study are of veterinary and medical importance. *A. niger* and *A. flavus* are probably the notorious of the common isolates because of their high potentials in producing aflatoxin and ochratoxin respectively.

The results revealed that heavy metals were detected in both smoked and fresh fish samples. However, the results show that slightly higher heavy metals loads were recorded for the smoked fish samples. It shows the mangrove wood fuel had contributed little to heavy metals burden of the smoked fish samples despite being harvested from apparently crude oil contaminated ecosystem.

The metal levels accumulated by the smoked and fresh fish samples is more of an indication of man induced pollution of the water body where the fishes were obtained and less of enrichment through the traditional fish processing method by wood smoking, the former is possible through oil spillage, run off of fertilizer, herbicides, pesticides and discharge of untreated sewage and industrial effluents containing metals into the water bodies, as well as the natural ability of the sediment to act as sink (Taiwo *et al.*, 2014). It has also been reported that mangroves potentially accumulate low metals in contaminated environments (Kathiresan and Bingham 2001). This may be due to the bioavailability in the mangal sediments, exclusion of metals by the mangroves and physiological adaptations that prevent metal accumulation in mangrove plants. It has also been reported that mangrove roots appear to be barriers that prevent metals from reaching the more sensitive part of plants (Tam and Wong 1997). Oxygen exuded by the underground roots forms iron plaques that adhere to the root surfaces and prevent heavy metals from entering the root cells. Where the metals do enter, there are apparent mechanisms to keep them from circulating freely from the plants (Kathiresan and Bingham, 2001). This implies that mangrove wood fuel used in fish smoking may suggestively have contained heavy metals despite being derived from an oil impacted ecosystem. The reason for the comparatively low metals content of the fish samples dried with mangrove wood fuel.

Of the heavy metals examined in the smoked fish samples Zn was found to be the most abundant metal. However cd levels in *Clarias gariepinus* (0.23 mg/kg) was relatively higher than the value recorded for *Pseudotolithus elongatus* (0.16 mg/kg) but all were within the FAO/WHO permissible level of (0.5 mg/kg) of cd in sea food. Similarly, the Pb levels were

within the FAO/WHO permissible level of 0.3 mg/kg, indicating that the smoked fish samples are suitable for human consumption (FAO/WHO, 2000).

Conclusion

The research study has revealed the preservative potency of wood-smoking against microbial contaminants although post treatment contamination may readily occur due to poor or unsanitary handling of smoked products. On the other hand, the levels of Cd, Cr, Pb, Ni and Zn in the smoked and fresh fish samples investigated showed that wood-smoking contributed little or nothing to the concentrations of heavy metals as little variation were recorded for some heavy metals between smoked and fresh fish samples analyzed. The levels of all the metals analyzed were within the FAO/WHO recommended guidelines values implying that the biota may have been exposed to small amount of heavy metals pollution during the smoking process. To check the undesirable addition of chemical contaminants on the smoked fish products, it is important to always be mindful of the type and source of wood used for smoke-drying.

References

1. Bergey, D. H and Holts, J. G. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th ed. Philadelphia: Lipincott Williams and Wilkins Publication.
2. Barnett, H. L. and Hunter, B. B. (1987). *Illustrated Genera of Imperfect Fungi*. New York: Macmillan Publishing Company, pp. 70-80.
3. APHA (2005). Standard Methods for the Examination of Water and Wastewater (20th edition). Washington DC: American Public Health Association.
4. Shewan, J. M. (2000). The Microbiology of Sea Water Fish. In fish as food. Edited by G. Borgstrom, New York: Academic Press, pp 487.
5. Jeyasejaram, G., Ganesan, P., Anandaraj, R. J. and Sukumar, D. (2006). Quantitative and qualitative Studies on the Bacteriological Quality of Indian White Shrimp (*Penaeus indicus*) stored in dry Ice. *Food Microbiology*, 23(6) 526 – 533.
6. Oku, I. and Amakoromo, E. R. (2013). Microflora of Smoked and Fresh Fish in Yenagoa Metropolis, Nigeria. *African Journal of Microbiology Journal Research*, 7(35): 4451-4456
7. Okon, U. C., Umana, S. I., Fatunla, O. K., Abiaobo, N. O. and Essien, J. P. (2017). Bacterial Contaminants and Heavy Metal Accumulating Potentials of Fin-Fishes (*Synodontisobesus* and *Marcuseniuss enegalensis*) from Humic Freshwater. *Journal of Advances in Microbiology*, 6(1): 1-14.
8. Ekpo, U. C., Umana, S. I. Essien, J. P. Bassey, M. P., Uko, M. P. and Abiaobo, N. O. (2017). Virulence Factors and Antibigram of Bacteria Isolated from Fresh Aquatic Produce Sold at Open Air Market Centre in Okepedi, Itu, Akwa Ibom State. *Journal of Applied Life Sciences International*, 14(4): 1-18, 2017
9. Salan, O. E., Juliana, A. G. and Marilia, O. (2006). Use of Smoking to Add Value to *Salmon Trout*. *Brazilian Archives of Biology and Technology*, 49(1), 57-62.
10. International Commission on Microbiological Specification for Food (ICMSF) (1986). Microorganisms in Food: Sampling and Specific Applications. 2nd Edition, Canada: University of Toronto Press.
11. Martin, A. M. (1994). Fish Processing: Biochemical Applications. London: and their Interactions in Foodstuffs. *Food Additives and Contaminants*, 8, 435-440.
12. Umoh, V. J. and Odoba, M. B. (1999). Safety and Quality Evaluation of Street Foods sold in Zaria, Kaduna State, *Nigeria Journal of Food Control*, 10, 9-14.
13. Essien, J. P., Jonah, I., Umoh, A. A., Eduok, S. I., Akpan, E. J. and Umoiyoho, A. (2009). Heat Resistance of Dermatophyte's Conidiophores from Athlete's Kits Stored in Nigerian University Sport; 'scentre. *Actamicrobiologicaetimmunologicahungarica*, 56(1): 71-79.

- 252 14. Essien, J. P., Ekpo, M. A. and Brook, A. A. (2005). Mycotoxigenic and Proteolytic potential of
253 Moulds Associated with Smoked Shark fish (*Chlamydoselachus anguincus*). *Journal of Applied*
254 *Sciences and Environmental management*, 9 (3): 53-57.
- 255 15. Taiwo, I., Henry, A., Imbufe, A. and Adetero, O. (2014). Heavy Metal Accumulation and
256 Biomarkers of Oxidative Stress in the Wild African Tiger Frog (*Hoplobatrachus occipitalis*).
257 *African Journal of Environmental Science and Technology*, 8(1): (6-15).
- 258 16. Tam, N. and Wong, Y. (1997). Accumulation and Distribution of Heavy Metals in a Simulated
259 Mangrove System treated with Sewage. *Hydrobiologia*, 352 (1-3): 67-75.
- 260 17. Kathiresan, K. and Bingham, B. L. (2001). Biology of Mangroves and Mangrove Ecosystem.
261 *Advances in Marine Biology*, 40, 81-251
- 262 18. FAO/WHO (2000). Report of the 32nd Session of the Codex Committee on Food Additives and
263 Contaminant, Appendix XII and XIII. Available online at
264 <http://www.fao.org/docrep/meeting/005/x7137e/x7137e20.htm>. Accessed January 20, 2018
- 265 19. Rashed MN (2001) Monitoring of environmental heavy metals in fish from Nasser Lake.
266 *Environ Int* 27(1): 27-33.
- 267 20. Seymore T (1994) Bioaccumulation of metals in *Barbus marequensis* from the Olifants River,
268 Kruger National Park, and lethal levels of Mn to juvenile *Oreochromis mossambicus*. MSc
269 thesis, Rand Afrikaans University, South Africa.
- 270 21. Adebayo, I. A. (2017). Determination of Heavy Metals in Water, Fish and Sediment from Ureje
271 Water Reservoir. *Oceanography and Fisheries*, 4(1):1-5
- 272 22. Tawari, C. C. and Abowei, J. F. N. (2011). Traditional fish handling and preservation in
273 Nigeria. *Asian Journal of Agricultural Sciences*, 3(6): 437-452.
- 274 23. Ozigbo, E., Anyadike, C., Forolunsho, G., Okechuckwu, R. and Kolawole, P. (2013).
275 Development of an Automatic Fish Feeder” International Institute of Tropical Agriculture
276 Postharvest Unit, Ibadan. *African Journal of Root and Tuber Crop*, 10(1):27-32.
- 277 24. Anyanwu S.O. (2014). Quantitative Analysis of Fish Consumption in Rivers State, Nigeria.
278 *American Journal of Experimental Agriculture*, 4(4): 469-475.
- 279 25. Pigott, G. M. (2015). Fish Processing, A Book by Singh , R. P. *Encyclopaedia Britannia*, Last
280 Updated 1-2-2015, Retrieved 14 January, 2016.
- 281 26. Ekelemu, J. K., Inoru, O. E. and Ojeifo, I. M. (2000). Financial Analysis of Integrated Fish Rice
282 Farming Systems, Proceedings of 5th Annual Conference of Animal Science Association of
283 Nigeria, Port Harcourt, September, 206-208.