DETECTION OF PARASITES AND LEAD CONTAMINATION IN THE FOUR FAMILIES OF FISH CAUGHT FROM THE ARABIAN SEA BELT OF INDIAN OCEAN

Adil Shehzad and *Maqsood Ali Ansari

Department of Genetics, University of Karachi, Karachi-75270, Pakistan.
maansari@uok.edu.pk; *shehzadadil@yahoo.com*

ABSTRACT

The main aim of this study is to get the idea of the degree of contamination in the coastal fishing area of Pakistan and to highlight the importance of solving the problem of environmental pollution. For this purpose fifteen fish species belonging to four different families i.e. *Clupeidae, Scombridae, Coryphaenidae* and *Engraulidae* were collected from Karachi Fish Harbor and presence of parasites and lead was detected in them through microscopy and atomic absorption spectrophotometry. Presence of parasites in gills and GIT although is a common phenomenon but we have found these in the muscles of almost all the families studied, an indication that the fish is not suitable for the consumption. The presence of lead, a heavy metal, in the fish is a strong indicator that the environment is contaminated with heavy metals which usually is from the effluents of industries. Although the analyzed fish samples showed the lead values to be under the permissible limits established by Food safety authority of Ireland (FSAI), but is giving the indications that there is a need of continuously monitoring the levels of heavy metals in the aquatic environment.

Key-words: Fish parasites, lead contamination, Arabian sea, Indian Ocean.

INTRODUCTION

Seafood, specially fish and fish products are one of the major sources of food consumed by humans as it is rich in protein (Kristinsson and Rasco, 2000). Fish inclusion in regular diet is highly recommended by nutritionists (Kris-Etherton *et al*., 2003; Calder, 2004) and it is recommend that fish should be eaten at least thrice a week (Olsen, 2002).

Contamination in fish is broadly categorized into Biological (e.g. parasites) and Non- Biological (e.g. Heavy Metals) contamination (de Magalhães *et al*., 2001; Olaifa *et al*., 2004). Parasites are present in all kind of cultured and wild fish, mainly in the gills and gastro intersectional tract but the presence of these in the fish muscles generally renders fish not fit for consumption (Du Preez *et al*., 2003). Parasites are small dependent organisms that can be unicellular e.g. *Trypanoplasma* species, *Amoeba* species and *Mesomycetozoea* group or multicellular e.g. nematodes, cestodes, isopods and helminthes (Chubb, 1982). These parasites directly affect the fish health and severity of infection depends upon the site and number of parasites per fish. In an aquatic system, schools or brood stocks affected with low number of parasites may or may not show the signs of illness however, reproductive capacity in different fish species is directly affected with parasitic contamination (Yanong, 2002).

It is reported that food borne parasitic infections are an important public health issue as improper techniques of food handling and preservation accelerate the parasite based food borne infections (Van *et al*., 2010). According to World Health Organization, more than 18 million people were infected with fish parasitic infections at different regions globally (WHO, 1995). Eating of raw or improperly cooked fish may cause parasitic infections, gastric infections, serious gastrointestinal pain, sudden stomach pain, nausea, vomiting, ulcers, food poisoning, and intestinal lesions sometimes even may cause perforation in the intestine (Park *et al*., 2009; Kliks, 1983 and 1986). *Capillaria philippinensis* (nematode) is a causative death agent of a Filipino patient who died 40 years ago (Khalil *et al*., 2014).

Metals accumulation in biological system is the major hazard in sea food (Kristinsson and Rasco, 2000; Kris-Etherton *et al*., 2003). Due to the high level of embedding and bioaccumulation properties, heavy metals supposed to be more dangerous as compared to any other pollutants (Öztürk *et al*., 2009; Bozkurt *at el*., 2014)**.** Metals like lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), Barium (Ba) and arsenic (As) are severe hazards to human health even if present in the body in small amounts. Although maximum limits for these toxic elements have been established by different food and environmental control agencies (Food Safety Authority of Ireland (FSAI.), 2009; Blanco *et al*., 2008; Nauen, 1983; E. C., 2006; FDA., 2008), yet most of them are referred as toxic heavy metals as they have no role in biological system (Canli and Atli, 2003).

Fish took suspended metal particles in water by ingestion and through respiration, thus these particles adsorb and accumulate in the tissues (Mansour and Sidky, 2002). Several studies have reported that diet is the main route of

*corresponding author: Dr. Maqsood Ali Ansari, e-mail:maansari@uok.edu.pk

exposure of heavy metals in humans (Ahmed *et al*., 2016; Zhuang *et al*., 2009). It is also reported that consumption of fish with heavy metals accumulated in its body is serving as a major source of exposure of heavy metals in humans (Castro-González and Méndez-Armenta, 2008). Lead poisoning may have severe effects on brain and intellectual development in children, long-term exposure cause damage to the kidneys (Tuzen, 2009; WHO 1995), in addition to effects on the nervous, reproductive and immune systems in both children and adults (Fang *et al*., 2014; Goldhaber, 2003).

The aim of the conducted study is to assess the quality of the fish meant for consumption locally and for export. This was done by looking for the presence of fish parasites and the presence of heavy metal lead (Pb) in fish muscles. This will also indicate the environmental conditions surrounding the fish that is caught for consumption and export.

Materials and Methods

Collection and Identification of Fish

Around 950 fish were collected from the coast of Karachi or purchased directly from the fishermen. They were sorted and around ten members randomly of each of fifteen species belonging to four families of fish namely *Clupeidae, Scombridae, Coryphaenidae and Engraulidae* were separated. All hundred and fifty fish were identified on the basis of morphology as per the field guide specie identification (Strauss and Bond, 1990; Psomadakis *et al*., 2015) in collaboration with Biological section of Marine Fisheries Department, Karachi, Pakistan. Preferably we collect the samples of same size.

Isolation and identification of Parasites in fish

Analysis of the fish was done immediately once taken to the lab and checking of the gills, gill slits and gill lids was done for the presence of any parasite. Identification of the parasites in fish was done as by Bamidele (2007) and Yanong (2002). Briefly, fish were dissected longitudinally into two halves and placed on board under the high light beam to look for targeted regions in the muscles (dark spots), near to GIT and gills. The GIT was cut longitudinally into two halves and stomach and intestinal regions were analyzed and macroscopic parasites were identified by the naked eyes and preserved in formalin whereas the regions of fish suspected for the presence of the microscopic parasites were used for preparing permanent slides and were observed under the microscope at different magnifications i.e. 4X, 10X and 40X.

Preservation of macroscopic parasites in formalin

Large parasites including isopod and *ascaris* and *anisakis* species (nematodes) are commonly found in the gills and GIT, they are large enough to be easily visible without microscope, these animals are simply picked with the help of forceps and placed in glass bottles, washed with distilled water and then air dried, after drying they were placed permanently in 40 % formalin solution.

Preparing permanent slides of parasites

Initial washing of parasitic targeted tissue was performed with normal saline (0.85% NaCl), whereas distilled water was used in case of isopod and *ascaris and anisakis* species. Dehydration of tissue was done by placing them serially in the increasing concentrations of ethanol (low, 10% to high 90% and then absolute). This was followed by placing in the mixture of formalin and glycerin (04% formalin and 01% glycerin) to shine the specimen. Canada-balsam was then applied followed by air drying. Completely dried slides were visualized under the microscope at different magnifications 4X, 10X and 40X and photographed.

Detection of lead in Fish

Twenty five fish muscle samples out of one hundred and fifty were randomly selected belonging to different families and species. Estimation of lead concentration was done by Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer) as per manufacturer's instruction. Samples were prepared as per Yildirim *et al*., (2009).

RESULTS

Collection and Identification of Fish

The collected fish were identified as *Sardinella albella*, *Sardinella sindensis, Sardinella longiceps, Dussumieria acuta*, *Hilsa kelee*, *Anodontostoma chacunda*, *Nematalosa nasus*, *Escualosa thoracata*, *Coryphaena hippurus, Thryssa mystax, Rastrelliger kanagurta*, *Katsuwonus pelamis*, *Thunnus albacares*, *Auxis thazard* and *Euthynnus affinis.* The collected samples were immediately transported on ice to the laboratory

Almost all the 150 fish samples examined showed the presence of various parasitic species in different regions of the body. Samples belonging to *Clupeidae, Coryphaenidae and Engraulidae* families showed mostly the presence of microscopic parasites in muscular regions near GIT and gills (Fig.**1-12**). Three different types of microscopic parasites recovered and identified were *Pentastomes* specie (Fig. **1**), *Capillaria* specie (Fig. **2**), and *Acanthocephala* specie (Fig**. 3**), few relatively large macroscopic organisms such as typical nematodes of family Anisakidae were also recovered. Members of the family *Scombridae* showed large /macroscopic parasites in their gills and GIT and very few microscopic parasites in muscles. Cestodes from the coelom whereas isopods and nematodes (*Anisakis*) were recovered from the gills and GIT, respectively.

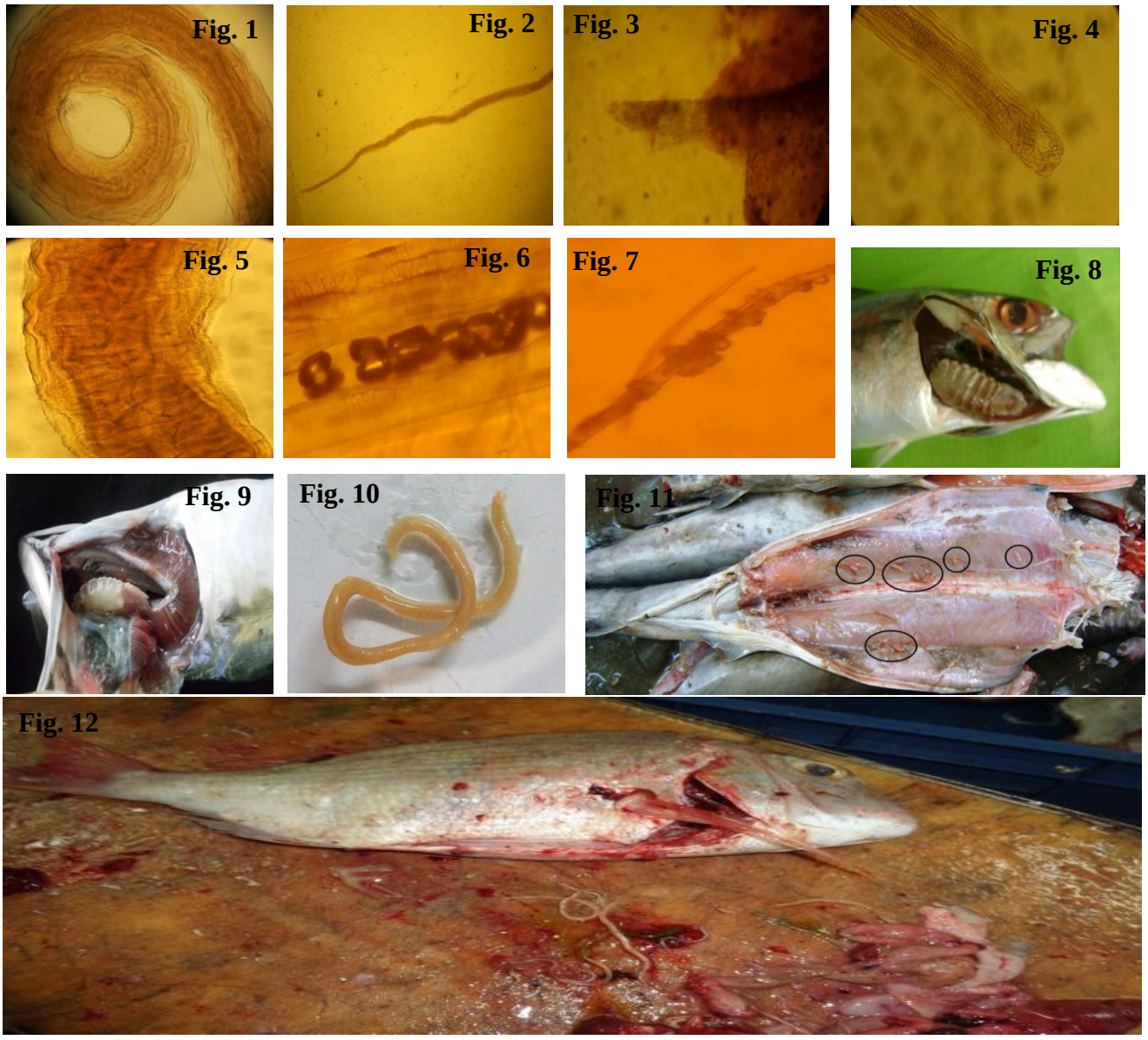

Fig.1-12. Parasites in fishes.
Key: Fig. 1. Typical loop of *Pentastome* in muscles, Fig. 2. *Capillaria* (nematode) in fish muscles. Fig. 3. Acanthocephalan (hook worm), Fig. 4. Buccal cavity and neck region of worm, Fig. 5. Two distinct body layers, Fig. 6. Eggs in parasite body, Fig. 7. Parasite near blood vessel, Fig. 8. Isopod in gill (Indian Mackerel family *Scombridae*), Fig. 9. Invasion of isopod near Buccal cavity, Fig. 10. *Anisakis* specie recovered from GIT, Fig. 11. Cestodes attached in coelom, Fig. 12. Large size Nematodes recovered from fish intestine at Biology Lab. Marine Fisheries Department.

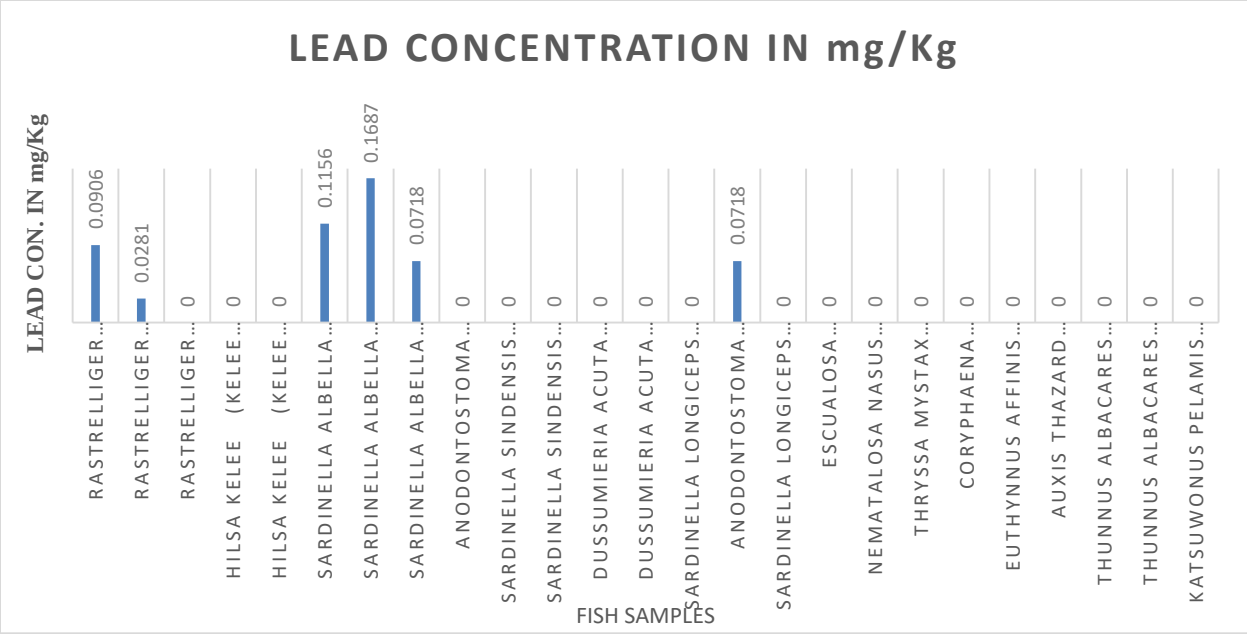

Fig.13. Lead concentration in different fish species.

Lead in Fish

Out of twenty five fish samples analyzed for the presence of lead in the muscles, six samples in belonging to three species *Sardinella albella*, *Anodontostoma chacunda* and *Rastrelliger kanagurta* showed the presence of lead with the range of concentration between 0.1687 to 0.0281mg/Kg. (Fig. **13**). The remaining nineteen fish samples showed to have no lead as was below the detection Limit (BDL).

DISCUSSION

Fish is the major source of proteins with repairing and protective values for human body (Kristinsson and Rasco, 2000), marine fish is high in heart friendly fatty acids like omega 3 and other basic components and vitamins (Burr *et al*., 1989; Elvevoll and Osterud, 2003). Fish export is serving an important role in the economy of many countries (Ababouch, 2006). Maintenance of fish quality from C to C (catch to consumption) is one of the major issues, for this purpose different studies and plans were made from time to time by different agencies.

Infections to fish not only can cause economic loss but also pose threat to the consumer. Up to 50 species of fish-borne intestinal parasites has been reported belongs to different groups including nematodes, cestodes, flukes and others widespread in South-east Asia (China, Korea and Thiland) (Khalil *et al*., 2014; Yu and Xu, 2005; Chai *et al*., 2005; Waikagul and Radomyos, 2005). Percentages of these groups are not same in all different regions, may be it depends upon the location, host specificity, salinity, contamination or pollution, habitat and fish migration.

In our collected fish samples significant amount of contamination with different microscopic and macroscopic parasites were found. Different parasitic species were recovered from muscles, gills and GIT regions in different fish samples. Samples belonging to family *Clupeidae, Coryphaenidae and Engraulidae* showed relatively more microscopic parasites as compared to the macroscopic organisms. In fish, nematode species are found higher in number as compared to any other group of parasites (Onyedineke *et al*., 2010) Members of *Capillaria* group (nematode) were recovered from some of the fish species in the muscles near GIT. It is known that intestinal capillariasis, mostly caused by eating the raw fish, is the most common disease that appeared in many parts of the world including the Philippines, Iran, Egypt and Taiwan (Cross, 1992). Adult round worms of *Anisakis* family were also recovered from GIT of different fish species. According to the study in New Zealand by Grabda and Slósarczyk (1981), some nematode species including Anisakis and Capillaria that usually occurs in fish muscles are serving as the main hazardous agent for humans. Fish parasites (nematodes) belonging to the *Anisakidae* and *Capillariidae* families are considered as the main causative agents of different GIT syndromes in humans (Eiras *et al.*, 2016), *Capillaria philippinensis* was the causative agent of endemic diarrhea in Philippines and Thailand populations (Cross and Belizario, 2007) and sporadic cases have also been reported from Korea and Japan territory (Eiras *et al.*, 2016). According to Eiras *et al.* (2016) five cases of nematodiasis have been reported in Brazil with Anisakis and

gnathostoma species. The major risk factor in all fish associated parasitic infections is the consumption of raw or under cooked fish, physical inspections and removing of parasites highly recommended to control these hazards (Newell *et al*., 2010; FDA, 2001). According to FDA the most effective way for removing or killing of parasites are freezing and heating (FDA, 2001).

Another important hazard to human health is the heavy metal poisoning, especially of lead. Lead poisoning is considered as the most common environmental problem worldwide with loss of hearing ability, blood disorders (anemia and anisopoikilocytosis of RBCs), nephritis, premature births, miscarriages etc as its common signs (Yildirim *et al*., 2009). In our study six samples out of twenty five (24%) belonging to three species *Sardinella albella*, *Anodontostoma chacunda* and *Rastrelliger kanagurta* showed the presence of lead with the concentration range of 0.1687 mg/Kg to 0.0281mg/Kg. Highest lead concentration recovered in our study was 0.1687mg/Kg found in *Sardinella albella* (Fig. **13**). These values were found to be below the Europe Union established maximum limit for lead in fish muscles i.e. 0.3 mg/Kg (Food Safety Authority of Ireland (FSAI, 2009) and Blanco *et al*. (2008). Values of lead concentration in fish caught in other countries were relatively high. For instance in Turkey, average concentrations of lead found in the three species of fish namely *S. sarda*, *E. encrasicholus* and *S. scombrus* were 1.27 mg/Kg, 2.15 mg/Kg and 1.9 mg/Kg respectively (Yildirim *et al*., 2009). In same territory lead concentration in different parts of fish samples including muscles, gills, GIT, air sac, liver and heart also been studied and all results found to be normal < 0.3mg/Kg as WHO recommended (Öztürk *et al*., 2009).

The present study on the fish quality suggests that fish of our coastal areas does contain various parasitic nematodes that can be a significant threat to humans if consumed in raw or improperly cooked form. Although the fish were not found to contain significant amount of lead but still there should be proper and continuous monitoring of the presence of lead and other heavy metals in the fish samples.

Conclusion

Conducted study confirms the presence of parasites in the Arabian Sea belt of Indian Ocean, Pakistan. These parasites ranged from microscopic organisms to macroscopic parasites; however as per FDA (2001) proper cooking or freezing of fish destroys these types of parasites. In some fish samples heavy metal lead was found but the concentration was found to be lower than the permissible limit. Majority of the fish samples did not showed any traceable amount of lead in them.

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