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ASSESSMENT OF SEASONAL VARIATION OF HEAVY METALS IN THREE DIFFERENT FISH SPECIES FROM SOUTHEAST COAST REGION OF CUDDALORE

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ABSTRACT

Fish has been consider as a one of the most important source of nutrients. These nutrients can be affected by manmade pollution and leads to some toxic effects. The main purpose of this study to assess the seasonal variation of proximate composition, level of heavy metals such as mercury, arsenic and lead in seawater and different fish species. The muscle tissues of three commonly demanded fish species [*Lutjanus sanguineus*, *Loligo duvauceli* and *Leiognathus splendens*] and sea water samples was collected from southeast coast region of Cuddalore at three different seasons [winter, summer and Monsoon]. The level of heavy metals in different fish species was determined by atomic absorption spectrophotometer. The result of the current study revealed that all the metals were present below the permitted level in sea water and fish sample at all three seasons. They are present in the following order in all the seasons Hg<As<Pb in the sea water.

Keywords: Fish, heavy metals, sea water, AAS.

INTRODUCTION

A wide variety of animal species can be found in the sea. But from the past decade, day by day fish consumption is increased by large population in the world, due to the presence of rich source of protein, essential fatty acids and micronutrients. The analysis of proximate composition of fish such as moisture content, crude fat crude protein and ash contents are essential factors to evaluate the nutrient content of fish. Because they can vary widely, not only from fish to fish of the same species, but also within an individual fish, meticulousness is impossible [1, 2]. The use of regular consumption of fish can offer many possibilities like reduction of cancer risk factors [3, 4], dementia, Alzheimer`s diseases [5] and prevent the cardiovascular diseases [6]. Fish meat is very safe and healthier to consume compared to other animal meat. However, heavy metals pollution is one of the major threats in the aquatic environment [7]. There are increasing concerns regarding the environmental pollution like industrial wastes, geochemical structure, agricultural activities and mining of metals are deposit slowly in the surrounding water and sediments and cannot be degraded [8]. As a result heavy metals can enter the fish through different routes such as inhalation and ingestion. They may then be trans located to blood causing adverse biological reactions in several organs [9]. Significantly, fish are frequently used as an indicator of heavy metal pollution in the aquatic ecosystem because they live in high trophic levels and are vital food source [10] [11]. The bioaccumulation of metals in fish varies from species to species, depending on age, developmental status, sexual condition, feeding, time of the year and other physiological factors [12]. A few metals are found to be essential due to their beneficial role in the physiological activity of the human beings. Iron, copper, zinc and manganese are essential metals but mercury, lead, cadmium and arsenic are considered as the toxic metals, because of their undesirable effects [13]. These toxic metals are found naturally in the earth. Due to human activities like paints, fertilizers, lead – acid batteries and aging water supply infrastructure metals concentrations become more and affect the human beings through consumption of aquatic organisms. The toxicity of various heavy metals affect the metabolical activity of the various marine organisms [14, 15]. The study was designed to provide information on seasonal variations of proximate composition, concentration of different heavy metals in sea water and different fish species available at the southeast coast region of Cuddalore.

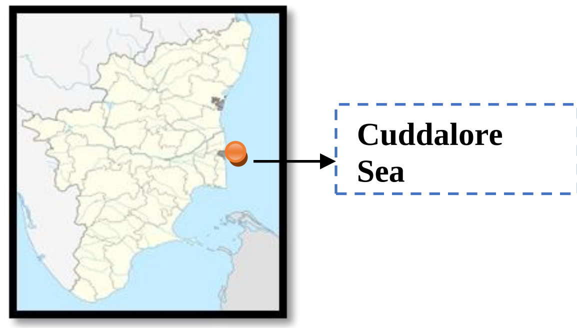
MATERIALS & METHODS

Three marine food fishes Red snapper (*Lutjanus sanguineus*), Squid (*Loligo duvauceli*) and Pony (*Leiognathus splendens*) were collected from the south east coast region in Cuddalore, during the winter (February), summer (April), and monsoon (June) seasons. Total fish lengths, & weight were measured and significant differences were found among the different species. After collection, the samples were kept in different plastic bags and transported in an insulated ice box to the laboratory and were beheaded, gutted, washed and filleted and stored for the analysis. Then, the samples were stored in the icebox and transferred to the laboratory.

Each species of the fish sample was oven-dried in an electric oven at between 70 – 80°C until the samples had constant weight. From each composite sample 2g were measured and taken as analytical sample. The proximate composition was analyzed chemically according to the method of analysis described by the Association of Official Analytical Chemist (AOAC, 1975)[16] while the percentage trace elemental concentration were determined using (AAS) Atomic Absorption Spectrophotometer.

STUDY AREA

Cuddalore is located at 11^{0}45\`N 79^{0}45E/ 11.75^{0}N 79.75^{0}E. It has an average elevation of 6m (20 Ft.). The Pennayar River runs north of the town, while Gadilam River runs across it. Cuddalore is situated at a distance of 200 km (120 mi) from the state capital Chennai and 18 km from puducherry, the neighboring union territory. Cuddalore Sea is polluted by companies manufacturing pesticides and intermediates, pharmaceuticals, chemicals, plastics, dyes and textiles. And also some major companies such as EID parry, Arkema peroxide, clariant and Bayer operate outside the SIPCOT limits are located but in the vicinity of the estate.



Map showing the study area.

Determination of heavy metals in sea water

Adjust the pH of sea water sample to 4.0 ± 0.1 by careful, drop wise addition of HNO_3. Divide one liter of the sample into four equal aliquots (250 ml each) and transfer them into a separatory funnel and add 2.5 ml of the APDC solution and 10 ml of MIBK (5 ml of this will dissolve in the sample). Shake for 5 mts, venting the separatory funnel carefully and regularly during this period. After shaking, allow the sample to stand for 10 mts while then phases separate. Drain off the aqueous phase and collect the organic layer containing the metals chelates. Add a further 5 ml of MIBK to the sample and re-extract it for further 5 mts and allow the phases to separate, drain off the aqueous layer and retain it for standard extraction if necessary. Combine the organic layer extracted. To the small bottle containing the organic extract add 1 ml of 50% (v/v) nitric acid. Seal the bottle and shake vigorously for one minute to decompose the dithiocarbamate. Transfer the contents of the bottle to a separatory funnel and rinse the sample bottle with two 1 ml aliquots of deionized water. Allow the phase to separate, collect the aqueous layer and dilute accurately to 5 ml. Discard the organic layer. The pre-concentrated metals in the aqueous layer can then directly be aspirated into an AAS or ICP and quantified against known standards.

Determination of heavy metals in fish samples

Add 20 ml of concentrated HNO_3 to a known quantity of dried samples in the beaker, and leave the mixture for 24 hours. Then the sample has to be evaporated to dryness on a hot plate at
120^{0} C, until the residue turns from yellow to white. After drying add 20 ml of a 4:1 mixture of nitric and perchloric acids. Evaporate the mixture to near

dryness. If the supernatant solution is either clear or slightly yellow fume the sample to dryness. In case if the supernatant liquid is orange colour, add a further aliquot of nitric and perchloric acid and fume the sample to dryness. Care must be taken while adding perchloric acid as indiscriminate use can lead to explosions. Allow the dry residue to cool and add 10 ml of 10% nitric acid. Transfer the contents to a 20 ml polytop vial and allow this mixture to stand for 2 hours for the residue to settle. The supernatant liquid can also be filtered if necessary, by using cotton. The filtered sample in the vial can directly be aspirated for metals analysis into an AAS or ICP and quantified against known standards.

RESULTS AND DISCUSSION

Heavy metal level in sea water:

Heavy metal pollution in the marine environment is determined by measuring its concentration in water and living organisms. Annual mean concentration of heavy metals in sea water varied spatially from species to species and seasons to seasons.

Seasonal variation of the mean concentrations of heavy metals are presented in the (Figure: 1) Hg, As and Pb are more or less equal at three seasons. The level of mercury is found to be in the same level both in the winter and summer season, the level is slightly increased in the monsoon season. The results were compared with Federal ministry of environment water standards for aquatic life (FWA), USEPA and World Health Organization standard for permissible limit of heavy metals in water. The concentration of Hg was found to be within the normal value of USEPA value and WHO limits. But compared to the FME limit which was found to be high.

Arsenic acts to coagulate protein, forms complexes with coenzymes and inhibits the production of adenosine triphosphate (ATP) during respiration (INECAR, 2000). It is possibly carcinogenic in compounds of all its oxidation states and high-level exposure can cause death. The level of arsenic was found to be high in the summer season compared to other two seasons. With refer to USEPA and WHO value the concentration of Arsenic is found to be present below the limited value. But the level was increased according to the FME limit in all the three seasons. The value was ranged from 0.005 mg/L to 0.01 mg/L.

Lead is the most significant toxin of the heavy metals, and the inorganic forms are absorbed through ingestion by food and water, and inhalation. The results of the present study revealed that the concentration of lead was found present in the higher level in all the seasons compared to the FME limit. The value was found to be remain similar both in winter and the monsoon seasons. But the values increased in the monsoon season above the normal value of FME. According to USEPA and WHO limit the metal is present below the detected limit.

The present study revealed that the levels of heavy metals were present in the sea water is found in the following increasing order:

In Winter Season: Hg<As<Pb.

In summer season: Hg<As<Pb.

In monsoon seasons: Hg<As<Pb.

Among the three metals monitored Pb was found to be most abundant one when compared to other metals in these three seasons, and the level of mercury was found to be present in lower concentration. High concentration of Pb (0.01-0.03mg /L) observed in the water may be associated to deposit of air born particulate matter as well as spill from fishing boat used as mean of transportation.

Proximate composition:

Fish has the potential to be considered as a balanced food and can therefore be expected to give relief from malnutrition. The proximate composition of three different species were shown in (figure: 2, 3, 4 &5).Results of the present study revealed that the different fish species having different nutrient content at different seasons. The composition of fish however, varies with diet, feed rate, genetic strain and age.

Proteins, lipids and moisture contents were the major constituents, which had been considered as a major tools in evaluating the nutritional value of the fishes studied. The nutritional elements showed variable values in all the fishes analyzed.

Changes in moisture content

The inverse link had appear between the moisture content and lipid content of the fish. The percentage of moisture content found in fish species indicates the virtual content of energy, proteins and lipids. Figure 2: displays seasonal variation of moisture content in different fish species (values expressed in %). The average moisture content of fish *L.saguineus* species varies from 71.09% to 74.50%. *L.duvauceli* shows the values from 82.01% to 85.15% and *L.splendens* species displays from 74.1 to 80.1. There is a slight variation was found among the three fishes in three different seasons. *L.duvaceli* fish shows the higher moisture content between 82.01 % to 85.15 in all the seasons, compared to other two species. This result revealed that the *L.duvauceli* may have low fat content because of the high moisture content. The similar result was found in the nemipterus japancius which had an average moisture content of 74.72%. Majority of the fish species (61%) contained moisture contents between 75 and 80%.

Changes in Ash content

The ash content of the species is an indication of the mineral concentration in the organisms. Ash content of fish species shows the slight variations in the three different seasons. . Figure 3: displays seasonal variation of ash content at different fish species (values expressed in %). Among the three fishes *L.saguineus* contains the higher ash content compared to the remaining two fish species. *L. duvauceli* had the lowest ash content among the three fish species in all the seasons. This results gave an indication, that the fish samples are the good sources of minerals such as zinc, manganese, calcium, copper and iron. Asuquo *et al*., [18] stated that marine species have high ash content compared to fresh water species, because they live in high salinity environment. Compared with three fishes the red snapper (*Lutjanus sanguineus)* has high ash content compared with the rest of the two fishes.

Protein

Protein is the major growth promoting factor in feed. Biochemical components such as protein and fat are essential for body growth and maintenance. Results of the present study (Figure 4) revealed that the protein content of the *L.saguineus* was high in monsoon season (19.09%) followed by the winter season (18.19%) and it was found that the level was decreased to (14.8%) in the summer season. The fish *L.duvauceli* shows the high protein content in the summer season (17.02%) but it shows same values both in winter season and monsoon season (15.18%). *L.splendens* species shows the high protein content at the monsoon season (13.61%) and winter season (12.9%) compared to the summer season (10.52%).

Fat

Fats are highly efficient as source of energy and they contain more than twice the energy of proteins. Variations in lipid content at different seasons are shown in figure 5. The average lipid content of *L.saguineus* is 8.12%, *L.duvauceli* is 6.24% and L.splendens is 4.03%.

Variations in the lipid content in the summer season was found to be 7.23%,in *L.saguineus* whereas in *L.duvauceli* is 6.01% and in *L.splendens* is 3.49% and in monsoon season it was found to be 9.23% in *L.saguineus,* whereas in *L.duvauceli* is 7.51% and in *L.splendens* is 3.76%. From the results of the present study it was found that the *L.saguineus* had the higher lipid content in monsoon season and *L.splendens* had lower lipid in summer season.

Protein, crude fat and moisture contents were the major constituents, which had been considered in evaluating the nutritional value of the fishes studied. The nutritional elements showed variable values in all the fishes analyzed. The total protein and fat content was found to be high in L.saguineus. This makes the fishes important living resources of dietary protein and fat. High crude fat fishes had less water and more protein, similar results were observed in our study also.

Metals content of three different fish species at three different seasons

Mercury

Mercury (Hg) is one of the most toxic elements among the premeditated heavy metals and exposure to high level of this element could permanently damage the brain, kidneys and developing foetus (WHO/FAO, 2004).Figure: 6 shows the variation in the level of Hg at different season in different fish species. The value of Hg was increased in *L.sanguineus* at the monsoon season (0.002mg/kg wet weight of sample) compared with the other two seasons. While other three fish samples the level of Hg was found to be below the detected limit (BDL) in all the three seasons. According to Bulgarian food codex and European community, the maximum mercury level permitted for sea fish is 1mg/kg.In the present study the level was found to below the permitted level in all the three seasons.

Arsenic

General population exposure to arsenic is mainly intake via the food and drinking water. Only a few percent of the total arsenic in fish is present in organic form, which is the only form about which a PTWL has been developed by JECFA (WHO/FAO, 2004) [18].

Arsenic found in the highest concentration of the edible tissue of the species studied. Inorganic As is the most toxic and considered as a Group A human carcinogenic and can effects mainly lungs, kidneys and skin (ATSDR, 2003)[19]. In the present study, the concentration of Arsenic in fish samples of three species during the three seasons (winter, summer and monsoon) was detected.

Figure: 7 shows variations in the level of arsenic at three different seasons. The average value found in winter season in *L.sanguineus* 0.001 mg/kg, whereas in *L.duvaceli* is 0.002 mg/kg and in *L. splendens* is 0.001 mg/L. The higher average value of Arsenic was found in *L.duvaceli* (0.002 mg/kg), whereas *L. splendens* and *L. sanguineus* contains 0.001 mg/kg which showed that there was a decrease in the concentrations of As.

In the summer season the average values of As found in *L. sanguineus* is 0.004 mg/kg, whereas in *L.duvaceli* is 0.002 mg/kg and in *L.splendens* is 0.002 mg/L. The study revealed that *L. sanguineus* shows the higher value of As *(*0.004 mg/kg) whereas *L.splendens* and

L.duvaceli showed similar values of0.002 mg/kg.
In the monsoon season the average value of As in *L. sanguineus* is 0.003 mg/kg, whereas in *L.duvaceli* is 0.002mg/kg and in *L. splendens* is 0.001 mg/L. The Arsenic concentration in *L.sanguineus* was found to be high whereas the fish *L.splendens* shows lower concentration. The WHO guideline for maximum permissible limit of Arsenic in fish is 0.026mg/kg. The Arsenic detected was found to be below the permissible limit in all the three fish samples at all the three seasons.

Lead

Lead is toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract. Some of the known symptoms of Pb poisoning are headache, irritability, abnormal pain and various symptoms related to nervous system.

The joint FAO/WHO (2004) Expert committee on Food Additives establishes a provisional tolerable weekly intake (PTWI) for lead as 0.3 mg. kg^{-1} body weight. European community (No 1881/2006) and Bulgarian food codex (No 31/ 2006) set maximum permitted level for Pb in fish of 0.4 $mg.kg^{-1}$.

In the present study, the concentrations of lead in fish samples of these species during the three seasons (winter, summer and monsoon) were detected and the results are discussed below. Figure: 8 shows variations in the level of lead at three different seasons. In the winter seasons the average values of lead (Pb) found in *L.sanguineus* is 0.671mg/kg, whereas in *L.duvaceli* is 0.263mg/kg and in *L.splendens* is 0.124 mg/L. Among the three Species The *L.sanguineus* exhibit higher value of Pb (0.671 mg/kg) which indicates that there was an increase in level of lead in the species of *L.sanguineus* compared to the normal range, which was very toxic to human consumption as well as other living beings in food chain .But the other two fish species exhibit the concentrations of Pb within the normal level.

In summer season the average values of lead for *L. sanguineus* is 0.236mg/kg, whereas in *L.duvaceli* is 0.474mg/kg and in *L.splendens* is 0.205 mg/L which are graphically represents in Figure 8. The highest average value of Pb was found in *L.duvaceli* was 0.474mg/kg which showed that there was increase in the concentration of lead in this fish compared two other two seasons.

In monsoon season the level of lead in *L. sanguineus* is 0.471mg/kg, whereas in *L.duvaceli* is 0.311mg/kg and in *L.splendens* is 0.163 mg/kg. The level of Pb was found to be high in *L.sanguineus* 0.471mg/kg in the monsoon. But the level was found to be decreased compared to the winter season and gradually decreased in the summer season also. The WHO guidelines for maximum permissible limit of Lead in fish are 2 mg/kg. The Lead detected was below the permissible limit in three fish species at all the three seasons. So the results of the present study confirms that the fish sample collected from this coastal area can be safe for the consumption, because the detected heavy metals were present in below the detected level.

CONCLUSION

In the present study all the species shows high value of moisture content compared to fat which is found to be low. The inorganic (ash) and organic (protein) compound also found to present in the appreciable amount in these three fish species at all these three seasons. The result of the present study shows the all the metals were present below the permitted level in the all the three samples at three different seasons.
This observation concluded that the fish sample collected from the Cuddalore area can be safe to consume in terms of these selected heavy metals concentrations, but some strict action should be taken in order to avoid the further pollution of the some of the heavy metals .In conclusion the routine analysis of proximate composition fish and trace element is an important one in determining the nutrient composition of fishes and better understanding of human nutrition.

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PROXIMATE COMPOSITION

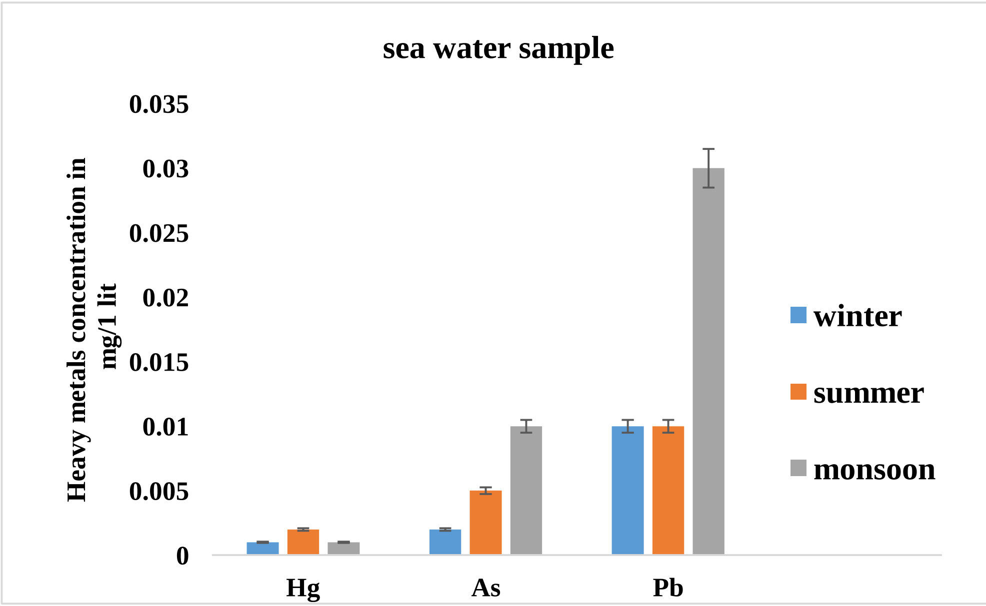

Figure 1:variations in the level of heavy metals in the sea water at three different seasons (values expressed in mg / 1 lit)

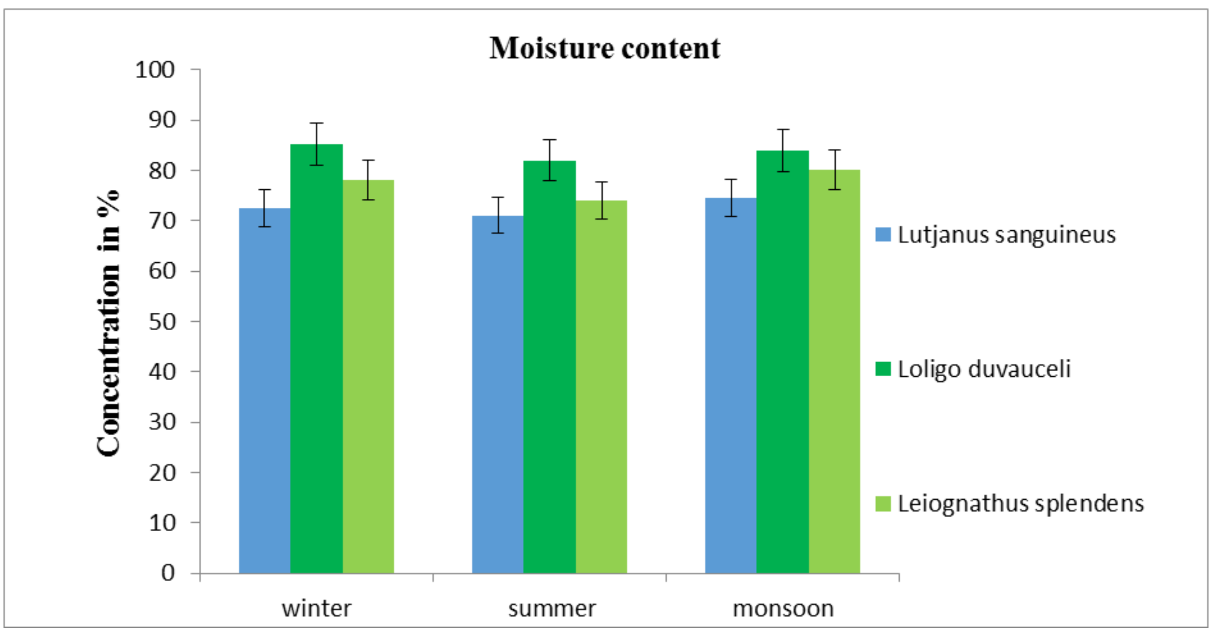

Figure 2: seasonal variation of moisture content at different fish species (values expressed in %)

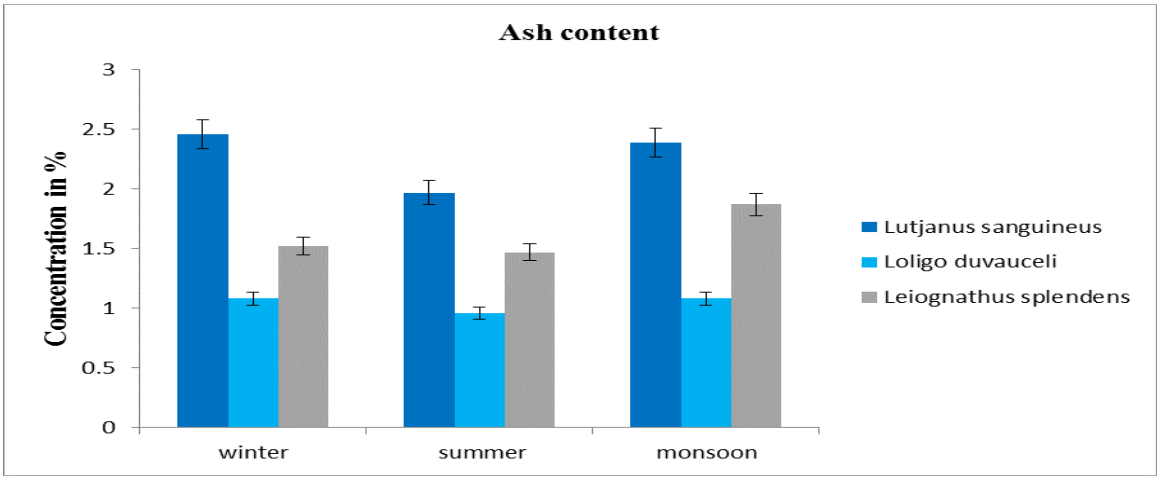

Figure 3: seasonal variation of ash content at different fish species (values expressed in %)

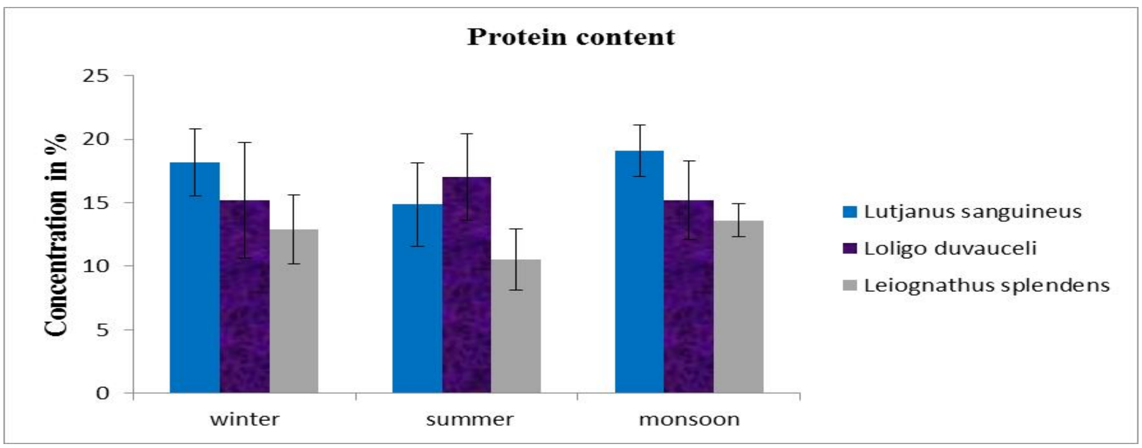

Figure 4: seasonal variation of protein content at three different sea fishes (values expressed in %)

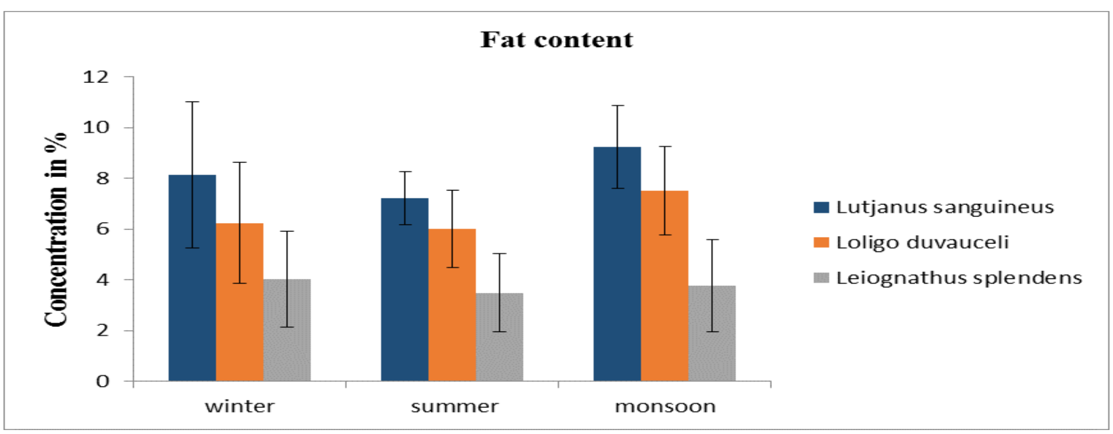

Figure 5: seasonal variation of fat content at different fish species (values expressed in %)

METALS CONCENTRATION OF THREE DIFFERENT FISH SPECIES AT THREE DIFFERENT SEASONS

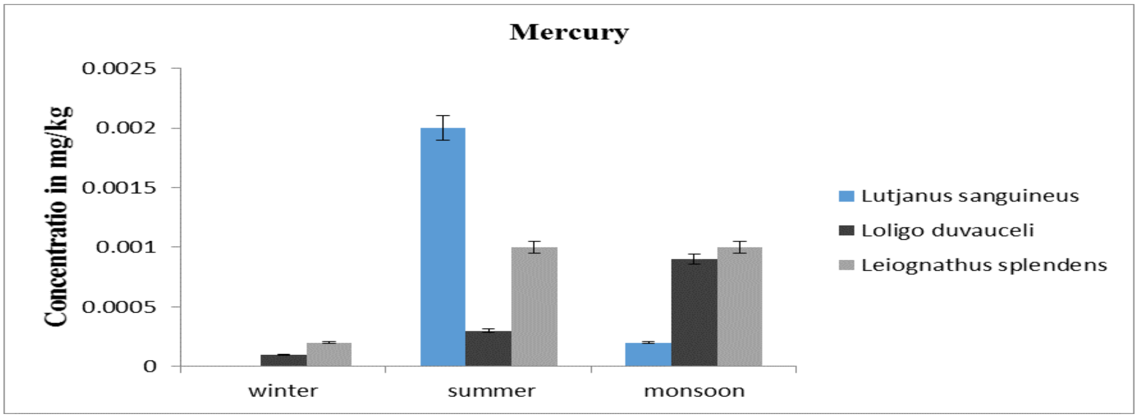

Figure 6: seasonal variation of mercury at different fish species (values expressed in mg/kg)

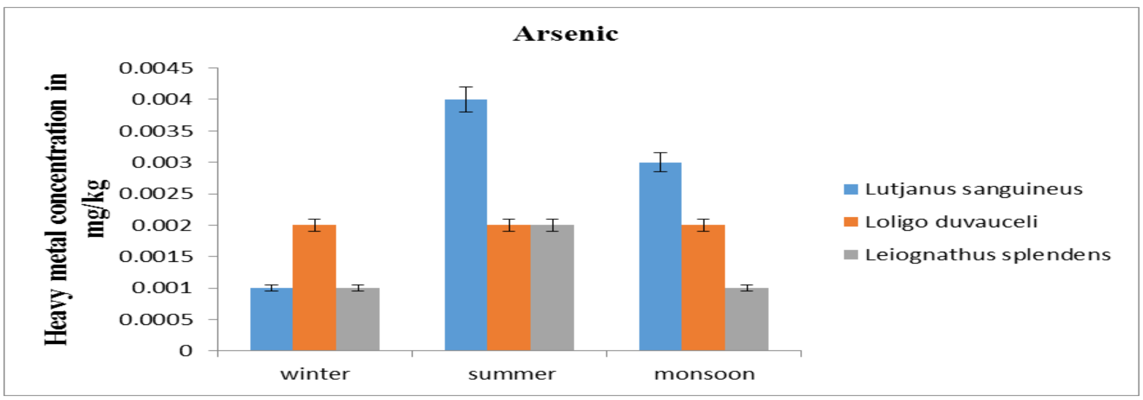

Figure 7: seasonal variation of arsenic at different fish species (values expressed in mg/kg)

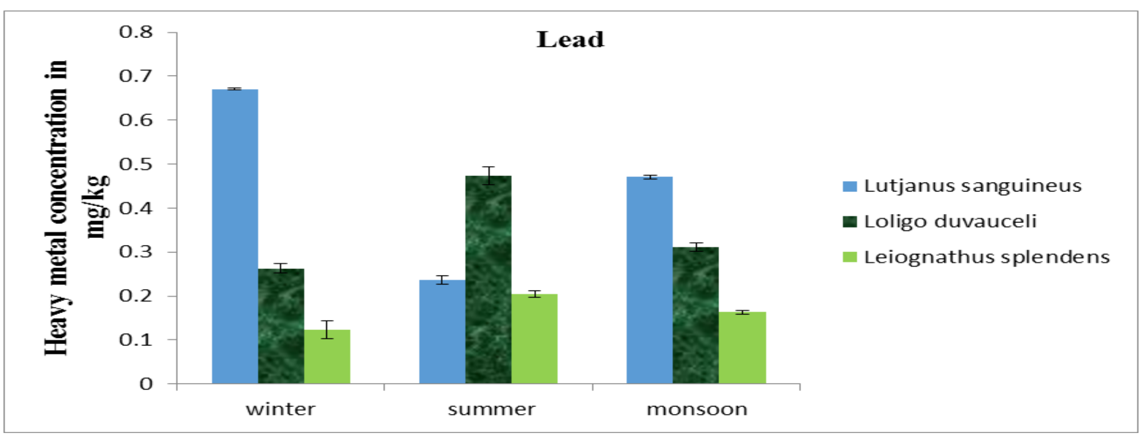

Figure 8: seasonal variations of lead level at different fish species (value expressed in mg/kg)

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