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Heavy and Essential Trace Metals Analysis in Frozen and Fresh Tilapia Fish Consumed in Nairobi County

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Abstract: Fish is a very popular source of protein among Nairobi County residents, however; pollution of fish habitat with heavy metals has led to accumulation of these metals in the different parts of fish including the edible parts. This study aimed to analyze Tilapia fish for metals and further extended to compare their concentrations with the permissible limits by international standards bodies like the Food and Agriculture Organization (FAO) and World Health Organization (WHO). The study also involved trace/essential metals analysis. Samples were collected from two points Muthurwa market (for fresh Tilapia fish being prepared for hotel consumption) and Navias supermarket (frozen tilapia fish from Lake Victoria). Analysis for heavy metals was done using the Atomic Absorption Spectrometer (AAS) instrument (spectra AA-6300) and concentrations were determined as follows. For fresh Tilapia fish concentrations in mg/kg were; Pb (0.21± 0.08), Ni (0.45±0.10), Cu (0.45±0.10), Fe (0.68±0.47), Mn (0.12± 0.01), Mg (2.54± 0.10) and Zn (0.43± 0.04). For frozen fish, results were; Pb (0.22±0.13), Ni (0.32±0.05), Cu (0.04±0.02), Fe (0.92±0.10), Mn (0.11±0.05), Mg (1.58±0.16) and Zn (0.26± 0.01). Cadmium levels were found to be below the detection limits of the instrument. Fresh tilapia fish recorded high concentrations in almost all the metals when compared to frozen tilapia fish except for the Pb and Fe which were more in frozen than fresh fish, probably due to the different habitat characteristics from which the samples were obtained. Generally fresh tilapia fish contained higher levels of the metals with magnesium recording the highest concentration of the metals analyzed. All the results fell within the permissible limits of most references made with the World Health Organization (WHO) and Food and Agriculture Organization (FAO) 1989. The study further recommended that future studies should be done in other parts of Nairobi County that the research did not extend to ascertain the safety of the fish consumed by Nairobi County residents. The study further recommended that future studies should be done in other parts of Nairobi County that the research did not extend to ascertain the safety of the tilapia fish consumed by Nairobi County residents.

Keywords: Fish, Heavy Metals, Trace Metals, Identify, Quantify, Compare, Concentrations, International Standard Bodies, World Health Organization (WHO), Food and Agriculture Organization (FAO), Digesting, Aqua Regia Reagent, Atomic Absorption Spectrometer, Permissible Limits.

I.0: Introduction

Fish are aquatic, craniate, gill-bearing animals that don't have limbs with digits. Fish are mostly cold-blooded making their body temperature vary as ambient temperature though some like white sharks and tuna can hold a higher core temperature. Fish can communicate with each other most often in the context of feeding, aggression, or courtship [1]. Fish have been utilized by humans as food throughout history. In Kenya, the fish-eating culture is believed to have started around L. Victoria by the original inhabitants of that region, over a long period, this culture has spread to most parts of the country. Heavy metals are considered metallic elements of relatively high density and are usually poisonous at even lower concentrations. They are very toxic even at the slightest exposure. Some of the effects derived include; kidney and bone damage, developmental disorders, abnormal, neural-behavior, increased blood pressure, and potential lung cancer among others. Essential metals include Fe, Cu, Zn, and Mn while non-essential metals include Hg, Pb, Ni, and Cd. Fish have the potential to form the link for transferring toxic heavy metals from water to humans [2]. Essential or Trace elements are elements not present in significant amounts but are needed in only minute amounts in a particular sample or environment. In analytical chemistry, it is considered one whose concentration is below 100 parts per million(mg/kg). They include copper, Iron, Zinc, Cobalt, iodine, Molybdenum, and Selenium among others. They are very important for cell functions at biological, chemical, and molecular levels. Most of them act as catalysts in the enzyme system, others like Iron act as constituents of hemoglobin and myoglobin playing a very important role in oxygen transportation in blood. Trace elements can be very harmful when consumed above recommended limits.

2.0: Problem Statement

Tilapia fish has been a major protein source in Nairobi County for decades. As a result, very, many people have found it irresistible given the delicacy it offers and how pocket-friendly it is compared to other sources of protein. As demands for land increase due to population growth, urbanization, and agricultural expansion, forests and wetlands have been cleared leading to environmental degradation and increased sediment deposition in rivers and lakes [3]. This means that these deposited sediments may act as an additional source of heavy metals in the fish habitats (water bodies). Tilapia fish get their food from their habitat so

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there are high chance the heavy metals too interact with the fish directly through ingestion or indirectly. This necessitates a study to determine the level of these heavy metals in fish.

Keeping tilapia fish in a cool environment will slow down the rate of fish getting spoilt. Cool fish keep longer than uncooled fish, although both will spoil in a matter of hours [4]. Many fish dealers have exploited this idea of freezing fish as a means to make them last longer as they wait for their customers. The study also focuses on the effect of freezing on the concentration of heavy and trace essential metals. This study also assesses the concentration of heavy metals and trace metals in fish tissues of a sample of tilapia fish consumed in Nairobi County. The results were then compared to the allowed limit from the European Commission (EC), Food and Agriculture Organization (FAO), and World Health Organization (WHO) to detect whether the heavy metal contamination in tilapia fish consumed in Nairobi County exceeds the safe consumption permissible limits.

3.0: Main Objective

The main objective of this study was to analyze heavy and trace essential metals in frozen and fresh fish consumed in Nairobi County.

3.1: Specific Objectives

The specific objective of this study was:

(i) To identify heavy metals and trace metals in frozen and fresh tilapia fish consumed in Nairobi County

(ii) To quantify heavy and trace metals in frozen and fresh tilapia fish consumed in Nairobi County

(iii) To assess if there is any significant difference in in concentration of heavy metals and trace metals between frozen and fresh tilapia fish consumed in Nairobi County.

(iv) To compare the quantity of heavy and trace metals determined with permissible limits by international bodies such as WHO and FAO

4.0: Materials and Methods

4.1 Study area

Nairobi is the largest and the capital city of Kenya. The city and its surrounding area are the ones that constitute Nairobi County. The county has a total of seventeen constituencies and eighty-five wards. The study covered 2 of the constituencies; Starehe constituency and Kamukunji constituency. Figure 4.1 shows a map of Nairobi County showing all the 17 constituencies.

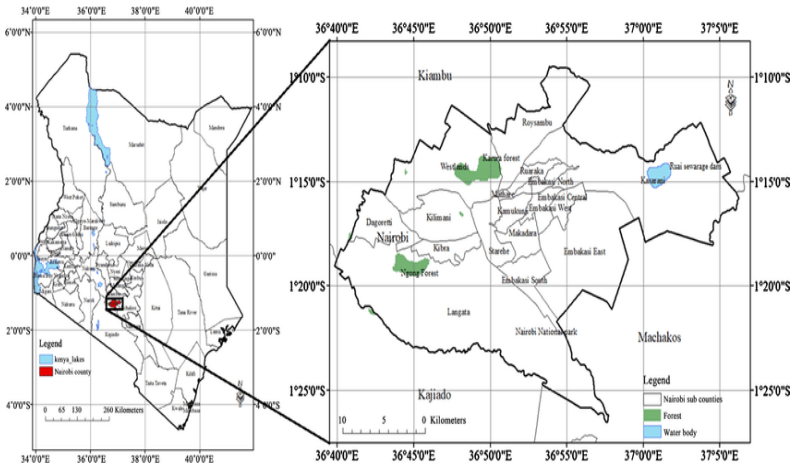

Fig 4.1 Map of Nairobi County

Muthurwa market is located in Kamukunji constituency and that is where fresh tilapia fish was sampled whereas; the frozen tilapia fish was sampled from Starehe constituency where Naivas supermarket Hazina trade center is situated.

4.2 Project Design

The study involved the following steps to realize its objectives; Sample collection, sample and standard preparation, and then finally sample analysis.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

4.3 Sample Collection

Sampling was done randomly. Most residents consume fresh fish obtained from sources around for example Sagana River or nearby ponds and preserved fish (frozen) from our supermarkets. Therefore; fresh fish was obtained from Muthurwa market (from a fishmonger supplying to different hotels within the market) and frozen fish was obtained from Naivas supermarket, Hazina Trade Center Nairobi, first floor, Monrovia Street. Figures 4.2 and 4.3 show fresh fish from Muthurwa and frozen tilapia from Lake Victoria obtained from Naivas supermarket, Hazina trade center, first floor, Monrovia Street, Nairobi.

Fig 4.2: Frozen Tilapia Fish from Lake Victoria Obtained from Naivas Supermarket

Fig 4.3: Fresh Tilapia Fish from Muthurwa Market

4.4 Sample Preparation

Tilapia fish flesh was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. Fish flesh was cut and oven-dried at 110°C to a constant weight [5]. A wet digestion method was used based on the Analytical Methods for Atomic Absorption Spectrometry. Before use, all glassware was previously soaked in diluted nitric acid for 24 h and then rinsed with distilled water. The dry fish sample was ground to a fine powder to increase the surface area to volume ratio for easy digestion. 1g of each of the fish flesh samples was weighed in triplicate and put into a 250 ml conical flask with 10ml aqua regia reagents (mixture of HCL: $HN0_3$ in the ratio 3:1).

When the fish tissue stopped reacting with aqua regia reagent (mixture of HCL: $HN0_3$ in the ratio 3:1), the beaker was then placed on a hot plate and heated till the sample became clear, 10 ml of distilled water was then added and returned to the hot plate to be heated to reduce the volume to 5ml. The content of the beaker was left to cool and then filtered before being transferred into a 100 ml volumetric flask and diluted to the mark with distilled water. All the steps were performed in the fume hood. The above procedure followed the guidelines from the Analytical Methods for Atomic Absorption Spectroscopy [6].

4.5 Sample Analysis.

All the samples were analyzed in the laboratory. This section discusses the apparatus used, the reagents used, and how calibration was done.

4.5.1 Apparatus Used

The apparatus that was used in this study included 250ml beakers, 100ml volumetric flask, 100 and 10ml measuring cylinders, analytical balance (Fischer Scientific A-160Analytical weighing balance), hot plate with heat control knob, and stirrer knob, conical flasks, wash bottle, pipette, Gallenkamp Oven model OV-160 for drying samples and glassware, Atomic Absorption Spectrometer AAS instrument. (spectra AA-6300)

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

4.5.2 Reagents Used

The reagents that were used were: Nitric (v) acid, HCL, and distilled water, The digested samples were placed in sample bottles

previously washed and rinsed with nitric acid to prevent ions from being absorbed on walls of the bottle, ready for analysis in the AAS. Aqua regia reagent was prepared by adding 10m; of nitric acid to 30ml nitric acid in a storage bottle.

4.5.3 Calibration

Calibration was done by introducing water as blank to adjust the AAS instrument reading. Stock solutions were diluted in series to produce different standard solutions of heavy metals (Pb, Cu, Fe, Mg, Mn, Ni, Zn, Cd). After calibration, each of the samples was then injected into the AAS instrument and constant readings were recorded. The apparatus was washed using distilled water which acted as a blank to ensure accurate reading.

4.6 Atomic Absorption Spectroscopy

Atomic absorption Spectrometer measures the amount of light that resonates and the wavelength that is absorbed as it passes through the cluster of vaporized atoms. The number of vaporized atoms has a direct relationship with the amount of light absorbed, therefore; by measuring the amount of light absorbed in terms of absorbance, the analyte can be quantified. The use of light sources and careful selection of wavelength allow the specific quantitative determination of individual elements in the presence of others. Beer- Lambert's law is applied. This law defines the logarithmic dependence between the transmission, (T), of light through a substance and the product of the absorption coefficient (α) of the substance of a specific wavelength (ℓ). This can be expressed as a simple relationship;

$A = \alpha \ell = £\ell c$

where A is absorbance and c is the concentration of the absorbing species. 23 Thus, if the path length and the molar absorptivity (£) are known and the absorbance is measured, the concentration of the substance can be deduced.

The technique uses a flame to atomize the sample, there are other atomizers such as a graphite furnace or plasmas, for instance inductively coupled plasmas. The liquid sample is transformed into atomic gas in three steps; de-solvation, vaporization, and atomization. Hollow cathode lamps are used as radiation sources in atomic absorption spectroscopy. Inside the lamp, filled with argon or neon gas, is a cylindrical metal cathode containing the metal for excitation, and an anode. When a high voltage is applied across the anode and cathode, gas particles are ionized. As voltage is increased, gaseous ions acquire enough energy to eject metal atoms from the cathode. Some of these atoms are in excited states and emit light with the frequency characteristic of the metal. Atomic absorption spectroscopy can also be performed by lasers, for instance; diode lasers are obsessed by their good properties for laser absorption spectrometry.

The technique is then either referred to as diode laser atomic absorption spectrometry or since wavelength modulation most often is employed, wavelength modulation absorption spectrometry. Therefore; the general procedure involved, the sample (in liquid form for this case) got aspirated into a flame, the liquid evaporated and the solid left behind was atomized. The exited atoms vaporized and emitted light with the same frequencies as that absorbed by the analyte in the flame used. The detector then measured the amount of light that had passed through the flame. This signal was amplified and recorded as a read-out. Based on the cost, sensitivity, and availability of the method, atomic absorption spectrometry was preferred as the technique of choice in this study. Figure 4.4 showsthe spectra AA-6300 AAS instrument used to analyze the samples.

Fig 4.4: samples being analyzed in AAS machine

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

5.0: Results And Discussion

The current study involved the analysis of heavy and trace essential metals in frozen and fresh tilapia fish consumed in Nairobi County. The results obtained from the study were presented in this using tables and figures. The concentration levels of the essential elements are compared against the levels stipulated by the WHO as depicted in Table 5.1.

Table 5.1 Levels of metals analyzed in frozen and fresh tilapia fish samples analyzed in (mg/kg)

Metal	Concentration(mg/kg)		Permissible limits (mg/kg)	Organization
	Frozen fish	Fresh fish		
Mg	1.57±0.16	2.54± 0.10	64.29	WHO
Mn	0.11±0.01	0.12±0.01	0.2-0.4	WHO
Zn	0.26± 0.01	0.43±0.04	70	WHO
Fe	0.68 ± 0.47	0.92±0.10	56	WHO
Cd	BDL	BDL	0.49	WHO
Cu	0.05±0.02	0.04± 0.02	35	WHO
Ni	0.32±0.05	0.45± 0.10	11	WHO
Pb	0.22± 0.13	0.21± 0.08	0.025- 1.75	WHO

The study investigated the concentrations of magnesium, manganese, zinc, iron, cadmium, copper, nickel, and lead in both fresh and frozen fish samples, comparing the results with recommended limits to assess the safety of consumption. The concentration of magnesium in fresh fish was found to be 2.54±0.10mg/kg, and in frozen fish, it was 1.57±0.16mg/kg, both within recommended limits. Manganese levels in both fresh and frozen fish were 0.12±0.01mg/kg and 0.11±0.01mg/kg, respectively, meeting permissible limits. Zinc concentrations in fresh and frozen fish were 0.43±0.04mg/kg and 0.26±0.01mg/kg, respectively, falling within recommended limits. Iron levels in fresh and frozen fish were within the recommended limits of 0.68±0.47mg/kg and 0.92±0.10mg/kg. Cadmium was below the detection limit in both types of fish, making them safe for consumption. Copper concentrations in fresh and frozen fish were 0.05±0.02mg/kg and 0.04±0.02mg/kg, within recommended limits. Nickel and lead concentrations were also within acceptable levels. Although lead concentrations were slightly higher than previous recommendations, they still fell within an acceptable range, confirming the safety of the analyzed fish samples.

The results in Table 5.1 were further represented in figures as bar graphs comparing; levels of metals analyzed in fresh fish, level of metals analyzed in frozen fish, and level of metal analyzed in both frozen and fresh fish samples analyzed.

5.1 Comparisons

5.1.1 Comparison of levels of metals analyzed in Fresh Fish

Levels of metals analyzed in fresh fish were further compared in a bar graph in Figure 5.1.1

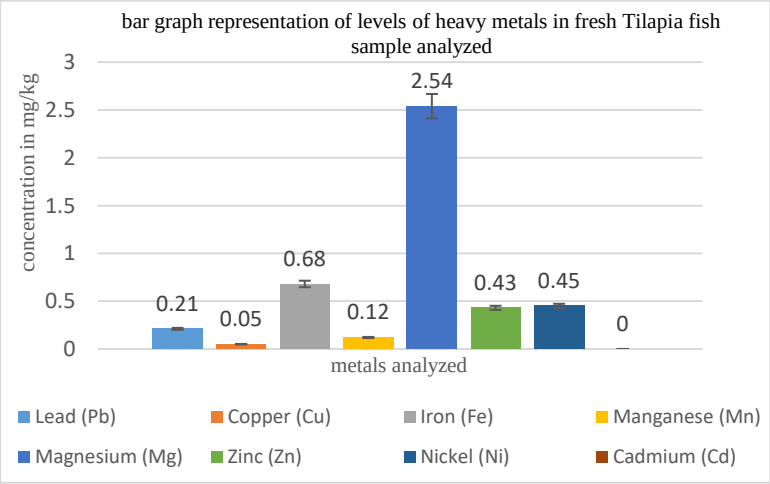

Fig 5.1.1: Bar graph comparing levels of metals analyzed in fresh tilapia fish

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

From figure 5.1.1 magnesium recorded the highest concentration of the metals analyzed while copper recorded the lowest concentration levels. Cadmium was given a zero figure because the instrument was not able to quantify it. Zinc and Nickel were at almost the same level.

5.1.2 Comparison of Levels of Metal Analyzed in Frozen Fish

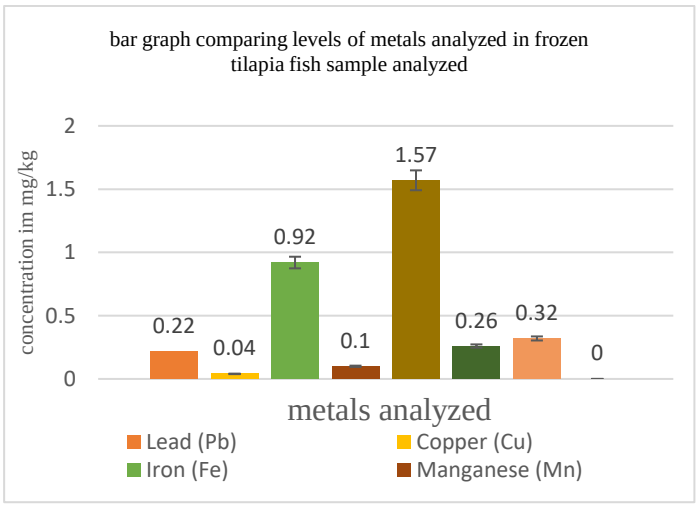

Fig 5.2.2: Bar graph comparing levels of metals analyzed in frozen fish

From Figure 5.1.2 it was reported that magnesium recorded the highest concentration of the metals analyzed while copper recorded the lowest concentration levels. Cadmium was given a zero figure because the instrument was not able to quantify it

5.1.3 Comparison of levels of metal analyzed in frozen and fresh tilapia fish samples analyzed

Levels of metals analyzed in fresh and frozen tilapia fish were further compared in a bar graph in Figure 5.1.3

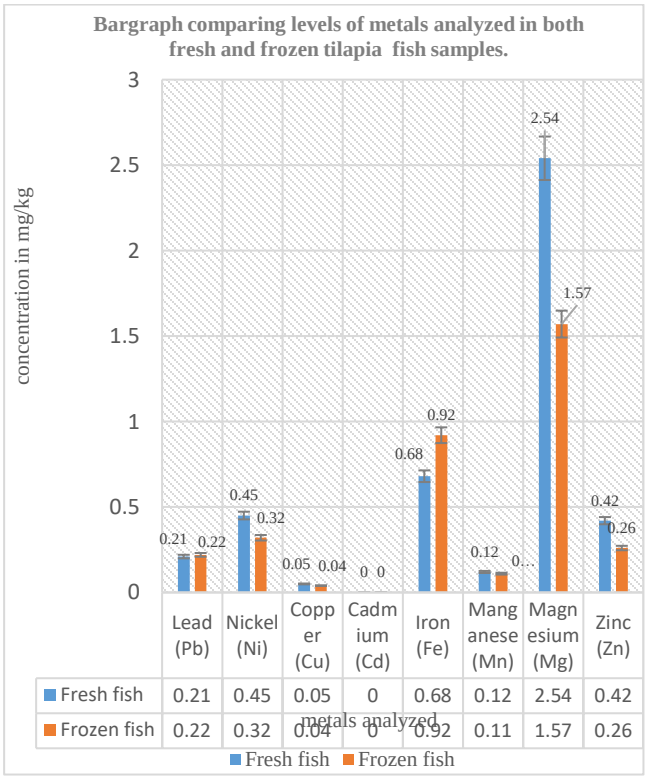

Fig 5.1.3: Bar graph comparing levels of metals analyzed in fresh and frozen tilapia fish samples analyzed

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

From Figure 5.1.3, it was reported that; frozen fish had relatively lower concentrations of nickel, copper, manganese, magnesium, and zinc when compared to fresh fish. Frozen fish only recorded slightly higher concentrations of lead and iron. Magnesium had the tallest bar meaning it had the highest concentration compared to all other metals analyzed. Cadmium was assumed a zero concentration not necessarily zero but it was just because the concentration was below the minimum the instrument could quantify. Copper bars were also very small meaning they had the lowest concentration of the metals identified and quantified by the instrument.

5.2 F-test

The F-test is a means of analyzing variations to assess the extent of variation of the samples analyzed if the variation is significant or insignificant. It utilizes means squares and degrees of freedom both within samples (replicate measurements) and between samples (different samples). This study further subjected the results to analysis of the variation observed between samples (fresh and frozen tilapia fish samples) during analysis. F-statistics are the ratio of two variances that are approximately the same value when the null hypothesis is true which yields F- F-statistics near 1. F-tests sum the predictive power of all independent variables it is unlikely that all of the coefficients equal zero. However, it is possible that each variable isn't predictive enough on its own to be statistically significant.

F-values were calculated by dividing mean squares by degrees of freedom. This was termed as $F_{calculated}$ and was compared with tabulated values F values as shown in table 5.3. The null hypothesis developed was that there is no significant difference between the mean concentration of the metal analyzed between the frozen and the fresh fish studies.

Metal	$F_{calculated}$	$F_{tabulated}$	Difference
Fe	3.20	18.51	Not significant
Mn	30.17	18.51	Significant
Mg	32.54	18.51	Significant
Zn	47.57	18.51	Significant
Pb	0.01	18.51	Not significant
Cu	6.84	18.51	Not significant
Ni	2.32	18.51	Not significant

Table 5.3 F-test

F is the ratio of two mean square values, if the null hypothesis is true, it is expected that F should have a value close to one. A large F ratio means the variation among group means is more than what is expected by chance. The difference was termed significant if F was $_{calculated} > F_{tabulated}$ otherwise, it was termed not significant. From Table 4.3, it was observed that variations in the mean concentration of iron, lead, copper, and nickel were not significant in fresh and frozen fish samples. This is to say that other factors such as freezing did not affect the variation of the mean concentrations observed. It was also observed that the variation in mean concentrations of manganese, magnesium, and zinc differed significantly between fresh and frozen fish samples analyzed; this is to say that other factors such as freezing had effects on the variation of the means observed.

6.0: Conclusion and Recommendations

6.1: Conclusion

The study aimed at analyzing heavy and trace essential metals. A total of 3 heavy metals were analyzed; cadmium, lead, and copper. Five trace essential metals were analyzed; iron, nickel, magnesium, manganese, and zinc, since they were of interest for investigation. Although the numbers of heavy and trace metals analyzed were not equal, the study was able to make significant comparisons of their concentrations in fresh and frozen fish samples. In comparison; magnesium recorded the highest concentration in both fresh and frozen fish among all the heavy and trace essential metals analyzed, and lead recorded the highest concentration among the heavy metals analyzed. Cadmium was found to be below the detection limit. Fresh fish recorded high concentrations in almost all the metals when compared to frozen fish except for the Pb and Fe which were more in frozen than fresh fish, probably due to the different habitat characteristics from which the samples were obtained. Generally fresh fish contained higher levels of the metals. While the samples were not from the same river sources, they were from different habitats which were Muthurwa and Kamkunji constituencies. The results were compared to WHO and FAO standards, and the concentrations were found to fall within the permissible limits. While certain metals were not analyzed due to instrument readiness, the study still provides valuable insights into the concentration levels of essential metals in fish. The mean concentration of iron, lead, copper, and nickel was not significant in fresh and frozen fish samples; whereas, the mean concentrations of manganese, magnesium, and zinc differed significantly between fresh and frozen fish samples analyzed. Mercury, selenium, chromium, and iodine were not analyzed because, at the moment of analysis, their lamps were not ready for use in the atomic absorption spectrometer instrument.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XIII, Issue III, March 2024

6.2 Recommendations

The study only covered two of the constituencies in Nairobi County; therefore, further studies on the same should be extended to other parts of the county to ensure the safety of the fish consumed by Nairobi County residents. Since only two species of fish were analyzed, another study that would involve more fish species would be a nice addition. The study further recommended further work to be done on other metals that were not covered by this work but are of great threat like mercury or of benefit like iodine to increase further understanding of the quality of fish that circulates among Nairobi County residents.

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