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A Comparative Analysis of Potential Toxic and Essential Elements in *in-situ* Derived and Commercial Fish Feeds Applied in Cage Aquaculture Fish, Lake Victoria Basin, Kenya

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Abstract

The uses of feeds are critical to success of aquaculture farming on Lake Victoria Basin. The composition of the feeds can either be derived from in situ plants or animals e.g. the Caridina niloticus or Rastrineobola argentea within the basin or externally sourced from outside the catchment. The net effect from the input of those feeds from outside could increase eutrophication in the lake while feeds derived from fauna and flora from the lake may not. A potential to exploit the in-situ products compromising C. niloticus and R. argentea is immense and this may offer an environmentally sustainable feeds sources for aquaculture. The nutritional values of the in-situ based feeds and the formulated feeds from local flora and fauna within the ecosystem of the lake are compared. Samples from both the formulated and in-situ based fish feeds were collected from aquaculture farms or from local sources within the basin. The samples were then freeze-dried and ground to fine local powder and dissolved using acid digest process. The resulting sample-acid solution was then analyzed using an Agilent 7500cx inductively-coupled plasma mass spectrometer (ICP-MS). In essential element, the C.niloticus samples had higher values of 6.87 ± 1.49, 32.97 ± 6.62, Fe 942.70 ± 246.01, 137.30 ± 34.13, 0.68 ± 0.13 and 105.48 ± 16.04 mg/kg for Boron (B),Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo) and Silicon (Si) respectively compared to R.argentea. While in lower values of 1.03 ± 0.10 and Zn 181.16 ± 18.76 mg/kg were observed in the Selenium (Se) and Zinc (Zn) respectively in C. niloticus compared to R. argentea samples. In Potentially Toxic Elements (PTEs), in-situ based feeds specifically C. niloticus had significantly high PTEs of 680.76 ± 203.34, 0.99 ± 0.17, 98.56 ± 23.69 and 9.69 ± 3.21 mg/kg in Aluminium (Al), Arsenic (As), Barium (Ba) and Chromium (Cr) respectively compared to formulated feed pellets. Our study therefore; indicates that, the in-situ based feeds are potential competitors with the formulated fish feeds as they are the most promising source of animal based protein and also contain essential micronutrients and macronutrients that add nutritive value to the fish. The local cage farmers are therefore encouraged to use more often the insitu-based feeds together with the formulated feeds to add nutrients to the caged fish for human consumption.

Keywords: Fish, Eutrophication, PTEs, Malnutrition

INTRODUCTION

Good animal nutrition is critical for the cost-effective production of healthy, high-quality fish meal products (Bohsale et al., 2010). Nutrition is crucial in fish aquaculture farming since feed accounts for almost half of the variable production cost of about 50 percent (Mousavi et al., 2021). *Oreochromis niloticus*, the Nile tilapia is currently the most available and economically valuable fish in Lake Victoria Basin (Yongo et al., 2018). Nile tilapia is a typical omnivorous warm water fish species, with global production exceeding 2500 thousand tonnes per year (FAO, 2006). Fish and shrimp meals, on the other hand, are becoming increasingly rare and expensive due to a global drop in fisheries products. As a result, nutritionists and feed suppliers have been exploring for non-traditional sources of dietary animal protein to replace traditional sources and lower feed prices (Munguti et al., 2009). Because the Nile tilapia species consumes a wide range of foods, it's important to check *Caridina nilotica's* (the lake shrimps) potential as a protein ingredient in fish feed. We used the lake shrimp to substitute Formulated Meal (FM) in order to advance aquaculture growth by utilizing locally underutilized feed sources (Mugo-Bundi and Okoth-Oyoo, 2015). Omena and lake shrimp diet are two fish protein sources extensively utilized in Kenya. Omena, on the other hand, is consumed by humans whereas lake shrimp, a waste of the Omena fishery in Lake Victoria, is becoming highly minimal and more costly due to the lake's constant closures (Munguti et al., 2006). As an outcome, substituting lake shrimp with the less expensive and readily accessible animal protein feedstuffs is likely to lower the cost of Nile tilapia diets (Kubiriza et al., 2018).

With the development of new, balanced commercial diets that support optimal fish growth and health, fish nutrition has evolved substantially in recent years (Golden et al., 2016). The expansion of aquaculture business is aided by creation of novel species-specific food formulations in order to meet rising demand for economical, safe, high-quality fish and seafood (Craig et al., 2017). Regardless of the culture system in which they are kept, fish and other aquatic creatures rely on an adequate supply of nutrients, both in terms of quantity and quality, for growth, health, and reproduction. The nutrients and energy requirements of the species under cultivation are fulfilled, and the system's production goals are reached, with an adequate supply of inputs (feeds, fertilizers, etc.). Malnutrition is one of the world's most serious issues (Hasan et al., 2001). Generally, fish is known to be rich in protein diet that is becoming increasingly popular among individuals who feed on it. Fish, which are high in nutrients, can help a lot in the fight against malnutrition (Farzad, et al., 2019). Fish are a good supply of Omega 3, a polyunsaturated fatty acid, as well as a very reliable source of high-quality protein (PUFA). Essential fatty acids can help prevent heart disease and are necessary for brain development. Furthermore, fish are high in highly accessible micronutrients including minerals and vitamins, which are essential for human health, growth, and development, as well as illness prevention (Golden and Allison, 2016). Essential metal elements in fish flesh that are vital for human biological functions and are integral in metabolism of proteins (Oyoo-Okoth et al., 2010). However, the importance of fish as a source of micronutrients in the diet is underappreciated and understudied (Beveridge et al., 2013).

Minerals are involved in a variety of critical biological processes in both humans and animals since they are part of numerous enzymes. The micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), Cobalt (C) and zinc (Zn)) as well as the macronutrients such as phosphorus (P), calcium (Ca), potassium (K), sulfur (S) and magnesium (Mg), are the most interesting elements in this perspective (Nieder et al., 2018). For example, selenium is beneficial to human health in a variety of ways, including immunological function, thyroid hormone metabolism, cardiovascular health, neurodegeneration prevention, anti-cancer, and antagonism to methylmercury and other heavy metal toxicity (Alissa et al., 2003). The health of fish depends on selenium, and a lack of it might damage the animal's immunity and increase mortality. Previous research has shown that Nile tilapia fish may require a greater Se element in their food, and it will be more absorbed and biologically active if administered in organic form (Wang et al., 2006).

Despite the fish's nutritional value to humans, there are concerns that widespread environmental contamination caused by man-made activities in and around the Lake Victoria basin it could have negative consequences due to toxic element bioaccumulation in both wild and cage-cultured fish. Mercury (Hg), one probable pollutant, has been the most extensively researched hazardous element (Hightower et al., 2003), from Minamata phenomenon in Japan to the consequences of gold mining in the Amazonian rivers (Forsberg et al., 1988). Other potentially toxic elements for example; lead (Pb), arsenic (As) and cadmium (Cd) have also been detected in fish and feed samples from different parts of the LVB in Kenya which continuously receives increased toxic metals including copper, lead, cadmium and chromium (Oyoo-Okoth et al., 2010) from the various activities in its catchment.

In Kenya, the in-situ based fish feeds i.e. *Rastrineobola argentea* (omena) and *Caridina niloticus* (the lake shrimps) are the most commonly used as animal protein sources. Omena as locally termed in Kenya, is a native fish to the Lake Victoria region and a diminutive endemic silver cyprinid that is approximately 5 cm long. It is a significant economic and commercial product that drifts in massive shoals and is exploited for human consumption as well as animal feed production (FAO, 2017). Lake shrimp is a by-product of the Omena fishery in the lake which is exclusively used as animal feed and it's supply as a feed is impeded by the lake's fisheries being closed on a regular basis (Kirima et al., 2019). There are few research addressing the hazardous and important components in Kenyan feeds and fish (Huntington and Hasan, 2009). As a result, one of the goal of this study is to investigate dietary intake of vital micronutrients and macronutrients, and hazardous components in fish and fish feeds from various aquaculture cages and sites within the Lake Victoria by Kenyans. This research looked at the quality of various feeds, such as element content (both essential and potentially toxic components) and the element content of Nile tilapia fish, and also the effects on the fish's quality in terms of human health (Kundu and Aura 2017).

MATERIALS AND METHODS

Study Area

The field work study was carried out in the Winam Gulf of the Lake Victoria Basin, Kenya. The Lake is home to one of the world's greatest commercial freshwater fisheries and is a vital source of nutrition for millions of people (Simonit and Perrings, 2005). The Lake's watershed sustains a fast rising population of almost 40 million people, whose activities have a significant impact on it (Yongo et al., 2005). As a result, the importance of the Lakes from a socio-economic viewpoint cannot be overstated. It provides a source of money through fishing and fisheries employment, as well as goods and services like as food to the riparian settlement (Bokea and Ikiara, 2000). Exotic Nile perch (*Lates niloticus*), native cyprinid fish (*Rastrineobola argentea*), and non-native introduced Nile tilapia (*Oreochromis niloticus*) are the most important commercial fish species (Njiru et al., 2005; Njiru et al., 2007). One of the three productive fish species is the cyprinid fish omena (*Rastrineobola argentea*), which is the main source of protein for some Lakeside communities (Wanink et al., 1999).

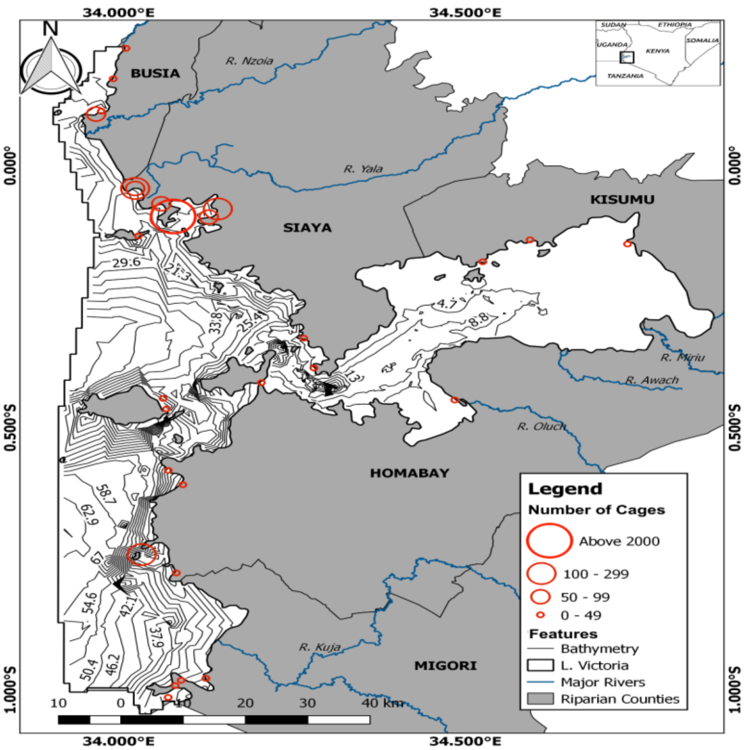

Figure 12: Map of the Kenyan Lake Victoria Basin, indicating number of aquaculture cages and their locations (KMFRI).

Study Design

A completely randomized sampling design was employed due to the wide spread of beaches at the lake. At each sampling points, random samples of potential fish feeds i.e. formulated feeds and the *in-situ* based feeds (omena and lake shrimps) samples were collected from the twenty stations of the Winam Gulf of the Lake Victoria Basin (LVB) (mean values ± standard error). The sampling points was guided by the presence of aquaculture farming activities. Samples were collected from all accessible aquaculture farming sampling points.

Fish Feeds Samples Collection

The species sampled included *R. argentea*, *C. niloticus* and formulated feeds (pellets).

R. argentea, *C. niloticus* and Formulated Feeds (Pellets) Collection

The feed samples, i.e. *R. argentea* and *C. nilotica* were collected from local vendors whereas the formulated feed pellets were collected from local cage-fish farmers at Lake Victoria, Kenya. The number of samples collected for each feed were from at least three vendors per site and local fishermen/cage owners on two sampling occasions. This was done between May and November 2019 from twenty (20) sites. The sampling sites were defined using a Global Positioning System (GPS) and using a mobile application Maps. Me®. After feed collection, they were locally sundried. The feed samples were well stored in a clean plastic bag and placed in the cooler box for transportation to the University of Eldoret, School of Environmental Studies (SES) Biotechnology Laboratory Center, Eldoret, Kenya, for freeze drying prior to transportation for analysis at the British Geological Survey, UK.

Sample Preparation and Storage of Fish Feeds Prior to Digestion

The commercial feeds, as well as *R. argentea* and *C. niloticus* meat, were sampled individually and homogenized samples (50g each) were stored at -20°C until further examination. Each sample was freeze dried at -60°C using a freeze drier (Harvest right) until a homogeneous weight was attained before proximate analysis was done. Using a grinder, the samples were ground into powder (MX-151SG1, Panasonic, China). The samples were then sealed and held at -20 °C until the formation in close proximity was determined.

Acid Digest Process for Feeds and Multi-Element Analysis

In-situ based fish feed samples of *R. argentea* and *C. nilotica* (0.25 g) were digested using a mixed acid solution of HNO3: 10mL, HNO3: 5mL, H2O2: 1Ml in an open vessel on a programmable hot block as described previously by Joy et al. (2015) and Watts et al. (2013, 2017) and left to cool for 10 minutes, 20 minutes, and 30 minutes, respectively.

As detailed by Joy et al. (2015) and Watts et al. (2015), multi-elemental studies were performed using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7500cx) (2013, 2017). Using a DMA-80 direct mercury analyzer (Milestone Inc.) and 0.07–0.010 g of freeze-dried fish, total mercury (Hg) in fish feed was determined. Feed samples were weighed into individually pre-cleaned nickel weighing boats (heated to 550°C for 5 minutes), randomized in batches of 10, thermally degraded (using an O_2 rich furnace) at 650°C, and quantifiably analyzed by atomic absorption.

Statistical Analyses

The element concentrations in the fish meals were statistically examined using SPSS version 21 and a one-way analysis of variance (ANOVA). For differentiation between significantly different means, *post-hoc* analysis was utilized. Following an outlier analysis, linear regression analysis was used to examine the correlations between nutritional element concentration in fish feeds at different sites. The data were presented in box plots, table of frequencies and bar graphs. The 5% significance threshold (significance probability level of $p<0.5$) were subjected to parametric analyses and computations.

RESULTS AND DISCUSSION

Micronutrients Concentrations in the Different Cage Aquaculture Feeds

The different feeds comprising the formulated pellets and *in-situ* derived feed consisting of *R. argentea* and *C. nilotica* were compared for concentrations of the micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si) and zinc (Zn). The different feeds contained comparable mineral content for *R. argentea*, *C. niloticus* and formulated feeds (pellets) (**Fig. 2**).

Higher concentrations of the elements B (6.87 ± 1.49 mg/kg), Cu (32.97 ± 6.62 mg/kg), Fe (942.70 ± 246.01 mg/kg), Mn (137.30 ± 34.13), Mo (0.68 ± 0.13 mg/kg) and Si (105.48 ± 16.04 mg/kg) were observed in shrimp compared to omena where B (1.89 ± 0.72 mg/kg), Cu (5.59 ± 0.50 mg/kg), Fe (203.11 ± 10.30 mg/kg), Mn (52.59 ± 4.70 mg/kg), Mo (0.19 ± 0.01 mg/kg) and Si (35.78 ± 2.01 mg/g) were in lower concentrations. While in lower levels of Se (1.03 ± 0.10) and Zn (181.16 ± 18.76) were observed in the shrimp compared to omena samples Se (1.44 ± 0.06 mg/kg) and Zn (286.33 ± 19.05) (**Fig. 2**).

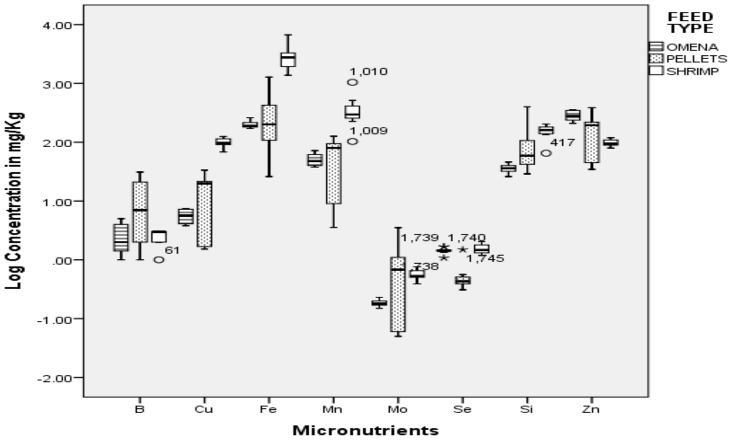

Figure 2: Box Plots showing micronutrients concentration in *in-situ* based feeds and formulated feeds (mg/kg; wet weight).

Macronutrients Concentrations in the Different Cage Aquaculture Feeds

The different feeds comprising the commercially formulated pellets and *in-situ* derived feed consisting of omena and shrimp were compared for concentrations of the macronutrients Ca (calcium), Potassium (K), Magnesium (Mg), Phosphorus (P) and Sulphur (S). The different feeds contained comparable mineral content in omena, shrimp and formulated feeds (**Fig. 3**.).

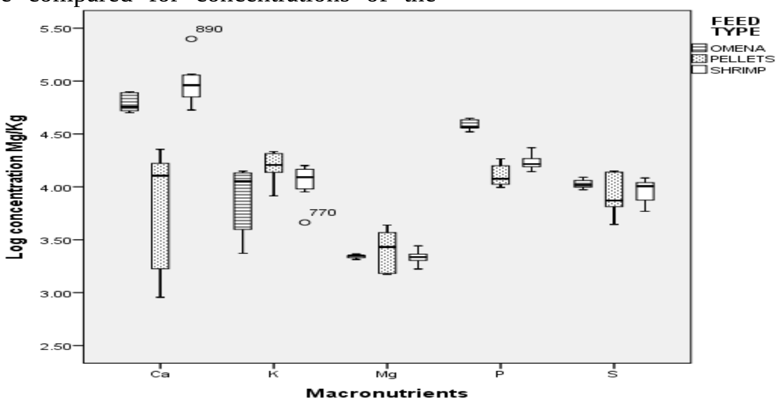

Figure 3: Box Plots showing macronutrients concentration in *in-situ* based feeds and formulated feeds (mg/kg; wet weight).

Higher levels of key minerals of K and Mg and lower concentrations of P, S and Ca were observed in the formulated mixed pellets than in the *in-situ* feeds i.e. shrimp and omena. The *in-situ* based feeds contain comparable mineral content to the formulated feeds (pellets) (**Fig. 3**). For instance, the mean concentrations of higher level of key minerals of K (16075.15 ± 1299.19 mg/kg). and Mg (2684.38 ± 309.91 mg/kg) were observed in the formulated feed pellet sample. The mean concentrations of key minerals of P (13028.85 ± 845.76 mg/kg), S (9520.15 ± 1092.80 mg/kg) and Ca (11248.92 ± 2318.40 mg/kg) were observed in the omena samples (**Table. 1)**.

Table 1: Mean (± SE) concentrations of essential nutrients (micronutrient and macronutrient) in *in-situ* based feeds and formulated feeds from Winam Gulf, Lake Victoria (mg/kg; wet weight). SE=Standard Error of mean, Means with different letters are significantly different from each other

Nutrient	**Element**	**Omena**	**Lake Shrimp**	**Formulated feeds**
Micronutrient	B	1.89 ± 0.72	6.87 ± 1.49	11.62 ± 2.95
	Cu	5.59 ± 0.50	32.97 ± 6.62	15.48 ± 3.35
	Fe	203.11 ± 10.30	942.70 ± 246.01	340.92 ± 97.73
	Mn	52.59 ± 4.70	137.30 ± 34.13	61.21 ± 12.97
	Mo	0.19 ± 0.01	0.68 ± 0.13	0.90 ± 0.29
	Ni	0.90 ± 0.19	5.48 ± 1.41	3.16 ± 0.99
	Se	1.44 ± 0.06	1.03 ± 0.10	0.52 ± 0.08
	Si	35.78 ± 2.01	105.49 ± 16.04	112.38 ± 34.17
	Zn	286.33 ± 19.05	181.16 ± 18.76	151.89 ± 30.76
Macronutrient	Ca	63276.11 ± 4181.89	47307.03 ± 8575.06	11248.92 ± 2318.40
	Co	0.13 ± 0.02	0.68 ± 0.12	0.61 ± 0.17
	K	8964.56 ± 1755.42	12930.33 ± 906.52	16075.15 ± 1299.19
	Mg	2202.33 ± 29.02	2573.64 ± 150.27	2684.38 ± 309.91
	Na	5200.33 ± 1062.27	2421 ± 94.46	3857.15 ± 315.01
	P	38716.56 ± 1489.35	21159.70 ± 2008.51	13028.85 ± 845.76
	S	10777.22 ± 357.41	9536.06 ± 537.23	9520.15 ± 1092.80

Potential Toxic Elements' Concentrations in the Different Cage Aquaculture Feeds
The different feeds comprising the commercially formulated pellets (Pellets) and *in-situ* derived feed consisting of *R. argentea* (Omena) and *C. nilotica* (Shrimp) were compared for concentrations of the Potential Toxic Elements (PTEs), Aluminum (Al), Barium (Ba), Mercury (Hg), Lead (Pb), Cadmium (Cd), Silver (Ag), Tin (Sn), Arsenic (As), Chromium (Cr) and Lithium (Li).

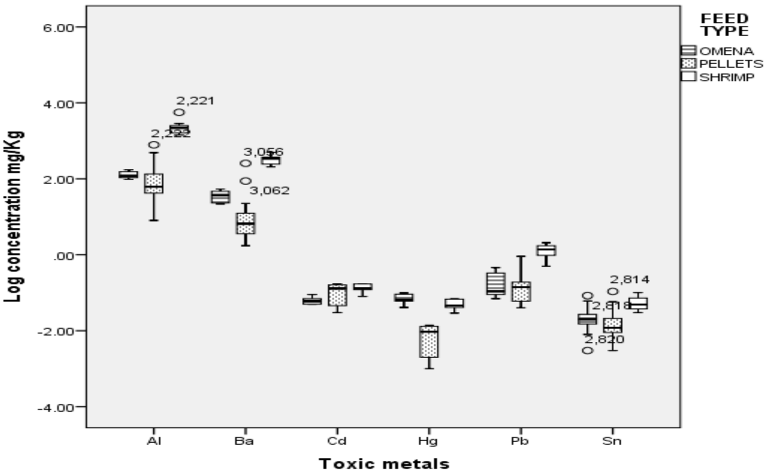

Figure 4: Box Plots showing potential toxic elements (PTEs) concentration in mg/kg for *in-situ* based feeds and formulated feed pellets (mg/kg; wet weight).

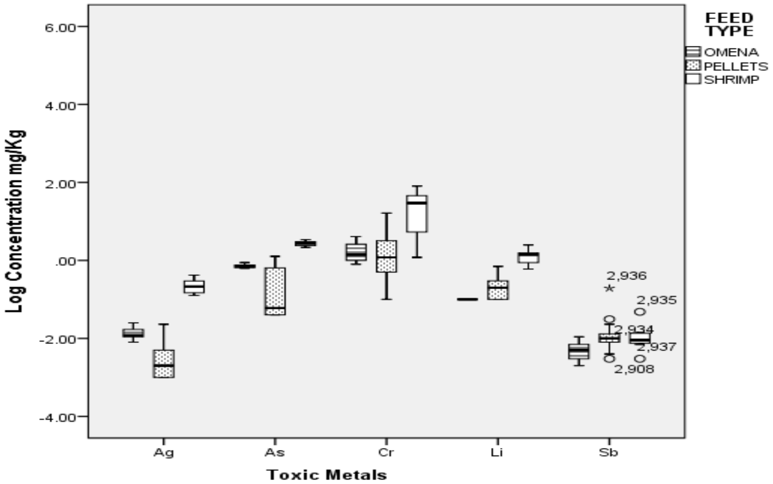

Figure 5: Box Plots showing potential toxic elements (PTEs) concentration in *in-situ* based feeds and formulated feed pellets (mg/kg; wet weight).

C. niloticus (the lake shrimp) is highly abundant in the Lake and is a key component of *O.niloticus*, tilapias diet. However, elevated levels of PTEs of Hg, Al, As, Ba, Cd, Cr, Li, Pb and Sn were observed in shrimp tissue (**Fig. 4 & 5**).

For instance, the mean concentrations of PTEs; Ag (0.06 ± 0.02 mg/kg), Al (680.76 ± 0.02 mg/kg), As (0.99 ± 0.17 mg/kg), Ba (98.56 ± 23.69 mg/kg), Cd (0.09 ± 0.01 mg/kg), Cr (9.69 ± 3.21 mg/kg), Li (0.45 ± 0.10 mg/kg), Pb (0.45 ± 0.10 mg/kg) and Sn (0.06 ± 0.02 mg/kg) were observed in the Lake shrimp samples (**Table 2).**

Table 2: Mean (± SE) Concentrations of Potentially Toxic Elements (PTEs) in *in-situ* Based Feeds and Formulated Feeds from Winam Gulf, Lake Victoria (mg/kg; wet weight). SE=Standard Error of mean, means with different letters are significantly different from each other

Potentially Toxic elements	**Omena**	**Lake Shrimp**	**Formulated feeds**
Ag	0.01 ± 0.002	0.06 ± 0.02	0.00 ± 0.002
Al	126.44 ± 8.94	680.76 ± 203.34	173.85 ± 65.40
As	0.73 ± 0.03	0.99 ± 0.17	0.35 ± 0.11
Ba	37.61 ± 4.27	98.56 ± 23.69	33.01 ± 19.62
Cd	0.06 ± 0.005	0.09 ± 0.01	0.07 ± 0.02
Cr	1.92 ± 0.42	9.69 ± 3.21	2.71 ± 1.19
Hg	0.03 ± 0.01	0.01 ± 0.00	0.04 ± 0.01
Li	0.03 ± 0.02	0.45 ± 0.10	0.21 ± 0.06
Pb	0.20 ± 0.05	0.45 ± 0.10	0.21 ± 0.07
Sb	0.01 ± 0.001	0.01 ± 0.01	0.03 ± 0.01
Sn	0.03 ± 0.01	0.06 ± 0.02	0.02 ± 0.01

DISCUSSION

This study aimed at comparing the concentration of PTES and essential elements in *in-situ* derived fish feed components with those in commercial fish feeds applied in cage aquaculture fish, LVB, Kenya.

The formulated feed pellets were more variable in PTEs levels than the in situ feeds (*R.argentea* and *C.niloticus*). For example, the mean concentrations of As in pellets were observed to be lower compared to As in Omena and shrimps respectively. Similarly, Hg in pellets were lower compared to the Hg concentrations in Omena and shrimps respectively . This could be due to trophic feeding and also mixing processes in pellet manufacture. The different feeds contain comparable PTEs for Omena, shrimp and pellet .

Regarding the micronutrients and macronutrient essential elements in this findings, the formulated fish pellets had higher B, K and Mg compared to the *in-situ* fish feeds. Boron is a trace mineral micronutrient that plays a variety of key roles in metabolism, making it essential for the well-being of flora, fauna, and people. The gastrointestinal system, skin, fins, and gills of fish and crustacea are all good sources of potassium (Mehaffey et al., 2017). Magnesium in fish meal is scarce, and some commercial feeds may fall short of fish Mg needs (Van der Velden et al.,1992). Poor development in fish, starvation, lethargy, muscular flaccidity, seizures, vertebral curvature, high mortality, and low magnesium concentrations in the whole body, blood serum, and bone are all indicators of dietary magnesium shortage (Janz et al., 2010). Therefore, aquaculture cage fish fed in formulated fish pellets containing these micronutrients may indicate fish has good sources of B, K and Mg. As a result, our findings clearly revealed that Mg concentrations in fish meals were significantly higher in formulated feeds than in *in-situ* feeds.

Regarding Potentially Toxic Elements, *in situ* feeds i.e. Omena and shrimps in this findings, had higher concentration of Cr, Al, Ba, Li and Pb as compared to formulated fish pellet feeds. Cr element is known to be carcinogenic while Ba, Li and Pb is non-carcinogenic. The number of studies on the environmental monitoring and impact of Li in aquatic systems is limited, probably due to the lack of regulation of this element (Immenhauser et al., 2016). Lead (Pb) element is a common metals in the ecosystem and biological systems, despite having no recognized physiological effects in humans. It may be found in all phases of the anaerobic environment and biochemical pathways. Pb has also been linked to neurotoxicity, nephrotoxicity, hepatotoxicity, and a variety of other human health problems (Abarshi et al., 2017).

CONCLUSION AND RECOMMENDATIONS

Aquaculture as practiced currently in the lake offers nutritionally fit fish products that have a potential to address protein and hidden hunger. A potential to exploit the *in-situ* products comprosing *C. niloticus* and *R. argentea* is immense and this may offer an environmentally sustainable feed sources for aquaculture. Based on the feed ingredient availability and mineral concentration level, the *in-situ* based feeds (i.e. *R. argentea* and *C. niloticus*) are potential competitors with the formulated fish feeds as they are the most promising source of animal based protein and also contain essential micronutrients and macronutrients that add nutritive value to the fish. The local cage farmers are therefore encouraged to use more often the *in situ*-based feeds together with the formulated feeds to add nutrients to the caged fish for human consumption,

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