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Climate-smart aquaculture in Tanzania: Assessment of transitional and heavy metals concentrations in a commonly used local feed ingredient for Tilapia farming

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ABSTRACT

A study conducted from January through May 2023 to assess the concentrations of heavy and transitional metals in a commonly used local feed ingredient in a farmed Nile tilapia diet (Oreochromis niloticus) in Tanzania. Eleven fish feed ingredients such as, sunflower seed cake (SFSC), wheat pollard (WP), maize bran (MB), fish meal (FM), freshwater shrimp (FWS), cattle blood meal (CBM), bone meal (BM), soya bean meal (SBM), and rice bran (RB), brewers' spent grain (BSG) and Taro leaves (Colocasia esculenta; TL) were randomly sampled from feed manufacturers, animal feeds' centers and other animal feeds suppliers in Arusha and Dar es Salaam region for inclusion in this study. Heavy metals and transition metals in feed ingredients were analyzed using the Energy-Dispersive X-rays Fluorescence (XRF) (Xla Pro-Spectrometer/German) at the Tanzania Atomic Energy Commission (TAEC) laboratory. The results showed that most of the fish-feed ingredients used in this study comply with the maximum allowable concentrations in Nile tilapia diets-according to the Tanzania bureau of standards and the European commission. However, the results showed that, the concentrations of reported metals (As, Pb, Cd, Hg, Co, Cu, Mo, Mn, Ni, Ag, V, Cr, Fe and Zn) varied significantly ($p < 0.05$) in most of the analyzed local feed ingredients collected in Tanzania. The study has paved the way for other researchers to further assess more feed ingredients used not only on heavy metals but other potential contaminants in feeds to ensure sustainable fish farming in Tanzania.

Keywords: Concentration, Feed ingredients, Heavy and transitional metals, Minerals, Nile tilapia, Toxic metals.

1. INTRODUCTION

Aquaculture is the world's fastest food producing sub-sector and the most diverse activity, contributing to about half of world fisheries and aquaculture production in 2018 [1]. It provides about 50% of total human animal protein consumption in most Asian countries, 30% of total animal protein consumption in Tanzania, and 17% of world human protein consumption [2-3]. The aquaculture operations are continuously increasing worldwide to meet global market demand for fish and fishery products, driven by the increasing human population and over-exploitation of wild capture fisheries [4]. The continuous expansion of this venture was due to the intensification of the private sector, advances and innovations in modern technology and farming methods in terms of farm inputs (quality fish seeds and feeds), culture systems, culture methods and high-quality fish feed production [5]. Recently, the aquaculture sub-sector has been rapidly expanding and become one of the most commercialized sub-sectors of Tanzanian agriculture following post-government efforts to attain the blue economy targets as well as the 30 global agendas for sustainable development.

The most cultured fish species in Tanzania and across the East Africa region is Nile tilapia *Oreochromis niloticus*, followed by the African catfish *Clarias gariepinus* [3, 6-8]. The cultured fish species are normally nourished by the feed they consume [9]. High quality fish feed usually results in successful aquaculture production [10-11]. However, feed ingredients used in commercial fish feeds (local-made and imported) production have been associated with toxic metal contamination which may in turn cause a potential toxicity in fish and human beings as well as in other aquatic biota [12-13]. The most commonly toxic metals in both local feed ingredients and commercial animal feeds include lead (Pb), cadmium (Cd), copper (Cu), chromium (Cr), arsenic (As), mercury (Hg), manganese (Mn), silver (Ag). These metals are toxic or poisonous even at a low concentration for fish when present in ecosystems [14-15]. The metals are easily accumulated in fish body parts such as body surface, digestive tract, gills, liver and muscles [15]. Fresh water fish (Nile tilapia) are more prone to heavy metal toxicity because of their ability to bio-accumulate, hence they are easily exposed and vulnerable [16]. Information on both transition and heavy metals reported in local feed ingredients is missing in Tanzania and across the East Africa region, and it is urgently needed.

The adverse health effects associated with heavy metal intake through fish feed include cellular and nuclear deformities, genetic damage, reproductive complications particularly reduced GSI, fecundity, hatching rate, fertilization success, abnormal embryonic and larval development of fish, increased mortality rate and deformed shape [13, 17]. Embryonic and larvae development stages of fish are more vulnerable to heavy metal toxicity compared to juvenile and adult fish and are usually used as bio-indicators to determine the toxicity of metals [18]. Taslima, K et.al, reported that fish development stages such as blastula, gastrula, segmentation and hatching respond differently to the intoxication depending on the type of metals, mode of action, exposure period and concentration of heavy metals [19]. For instance, the study by silva, J.P et.al, on Nile tilapia (*Oreochromis niloticus*) has reported high values for markers of genetic damage with the highest concentration of metals, particularly Iron, Zinc, Manganese, Cadmium and Chromium [20]. Another study by Çiftçi, N et.al, [21] on the effects of copper (Cu) and zinc (Zn) on hepatosomatic index (HIS), Gonadosomatic index (GSI) and condition factor (CF) discovered that Cu and Zn caused HIS and CF to decrease. Also, the study further reported that despite the increase in GSI as the Cu concentration increases, with longer exposure time GSI tends to decrease and negatively affect fish reproduction.

The effects of toxic metals are not only a threat to inhabitants of the aquatic ecosystem, but also to human health. When humans consume fish, metals tend to accumulate in various tissues of the human body and, when they exceed safety levels, cause various kinds of diseases [22]. Metals like cadmium, mercury, lead and arsenic are carcinogenic and mutagenic and tend to compromise human health [23-24]. For instance, specific effects on human health associated with exposure to Cd are kidney damage, especially the proximal tubular cells which are the main site of accumulation; Cd also causes bone demineralization as a result of renal dysfunction [25]. Lead toxicity tends to occur very slowly but it may cause abdominal pain, constipation, headache, memory loss, brain damage, reduced fertility and kidney damage in human beings. In pregnant women, even at a very low concentration, lead can cross the placenta barrier and affect the unborn child's nervous system, behavior, intelligence and cause death [26]. Arsenic is very toxic in inorganic form, and its effects include; skin cancer and hyperpigmentation, bone marrow depression, bladder damage and lung cancer. Other possible effects of arsenic toxicity include neurotoxicity, diabetes, cardiovascular diseases, papillary and cortical necrosis and hyperkeratosis which lead to the soles of the feet [27]. On the other hand, mercury is the most dangerous toxic metal among all due to its ability to affect the distribution and retention of other metals in the body [28]. The human body can excrete amounts of mercury through urine, sweating and feces but not to a safer amount. When ingested, mercury tends to accumulate in the brain, kidney and placenta in women [29]. In the body, mercury outcompete copper and iron in the active sites of enzymes involved in energy production and causes mitochondrial dysfunction and oxidative damage. It can also accumulate in the thyroid and increase the risk of autoimmune disorders [30]

In Tanzania, most of our local feed ingredients are multipurpose and used in the formulation of different animal feeds like pig feeds, poultry pellets, cattle feed and fish pellets. When taking up the feed, animals may also take some concentrations of heavy metals, transitional metals and end up metabolizing, excreting, storing or bio-accumulating them in the body [31]. Therefore, it is important for the feed ingredients to comply with the maximum limits of heavy and other toxic metals established by various regulatory authorities in the world and the present study aims to assess the concentration of transitional and toxic heavy metals in the commonly used feed ingredients for Nile tilapia (*Oreochromis niloticus*) diets obtained from northern part of Tanzania and ensure that fish feeds are not contaminated with toxic metals or do not exceed their maximum permissible limits. So far, no study has been carried out to show the concentration of transition and heavy metals in feed ingredients used in Tanzania.

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2. MATERIALS AND METHODS

2.1 Study area

The present study was conducted during the period of January to May 2023 at Tanzania Atomic Energy Commission (TAEC) laboratory (latitude 3°23′ S and longitude 36°47′ E) in Arusha region. The climate condition in Arusha is sub-tropical, with mean annual temperature of 20.5°C and mean annual rainfall of 1125 mm.

2.2 Samples collection of local feeds ingredients

A total of nine (9) different local feed ingredients were collected randomly from Animal feed centers located in different geographical locations in both es Salaam, and Arusha regions. The samples were collected according to FAO / WHO (2004) general guidelines for sampling with a little modification [32]. The collected samples include sunflower seed cake (SFSC), wheat pollard (WP), maize bran (MB), fish meal (FM), fresh water shrimp (FWS), cattle blood Meal (CBM), bone Meal (BM), soya bean meal (SBM), and rice bran (RB). Brewery Spent' grain (BSG) was purchased from Tanzania Breweries Limited (TBL), and Taro leaves (*Colocasia esculenta*) (TL) were collected along the river bank of Nduruma river in Arusha near The Nelson Mandela African Institution of Science and Technology. The collected samples were then placed in a clean polyethylene bags, transported, sun-dried and processed before transported to the Analytical Chemistry Laboratory of the Tanzania Atomic Energy Commission for analysis.

2.3 Preparation of taro leaves sample

For sample preparation, Taro leaves powder was prepared according to the method suggested by Alcantara et al. (2013) with some little modification [33]. Briefly, the taro leaves were harvested, sorted into water, and washed /cleaned before chopped into small pieces (strips) at the laboratory. The strips were then soaked into tap water for 24 hours and dried at room temperature for 14 days prior for analysis. Prior to mineral analysis, samples were milled in a blender (JYL-D020 Powerful Multifunctional Blender Food Processor, Joyoung, China) and sieved by hand to pass through a sieve with 1.0 mm circular openings.

2.4 Determination of toxic heavy metals and transitional metals concentrations in feed ingredients

Toxic heavy metals such as; mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd), and transitional metals such as; copper (Cu), zinc (Zn), chromium (Cr), vanadium (V) manganese (Mn), iron (Fe), cobalt (Co), molybdenum (Mo) and silver (Ag) were analyzed in triplicate according to the method described previous by Croffie, M.E et.al., [34]. The feed samples were grounded using mortar and pestle into powder and sieved in a 60-micron sieve (shanghai sieve/China) to obtain the fine powder of the samples. About 4g of each feed sample was measured and mixed with 0.9 g of a Cereox-BM-0002-1 Binding Additives (Fluxana GmbH Co. KG, Bedburg-Hau, Germany) in a bowl for homogenization by using a pulverizer (Fritsch GmbH, Ider-Oberstein, German) at the speed of 180 rev /min for 10 min. The homogenized sample mixture was then compressed to form a pellet using a hydraulic press machine (Vaneox Fluxana PP25, Bedburg-Hau, Germany) at 15 psi (pounds per square inch) for 1 min. The Energy-dispersive X-ray Fluorescence (EDXRF) (Xla pro-Spectro xepos Spectrometer/Germany) was used to quantify the concentrations of transitional and heavy metals in powdered feed ingredients' samples.

2.5 Statistical analysis

Statistical analysis was performed using R Commander Plug-in for University Level Applied Statistics (RcmdrPlugin.NMBU) Version 1.8.14. Data were subjected to One way Analysis of variance (ANOVA) to compare the mean values. The values obtained (mean ± standard error means) were compared by Post hoc pairwise test using Tukey's Honest Significant Difference (Tukey's HSD) and the differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Toxic heavy metals

The concentrations of toxic heavy metals (As, Pb, Hg and Cd) found in a commonly used local feed ingredients were presented in Table 1 and Fig. 1. The results were expressed as mg/kg. ND: Not detected. The highest concentration of As was reported in the BM feed sample (8.07 mg/kg) with the lowest concentration of 0.34 mg/kg in SFSC, whereas

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the concentration of As was below the detection limits in MB, RB and SBM samples. The highest concentration of Pb was reported in CBM (50.96 mg/kg) and the lowest concentration in SFSC (0.49 mg/kg) with undetected concentrations of Pb in two feed samples such as RB and SBM. Moreover, the highest value 0.59 mg / kg of Hg was reported in both BSG and SFSC and the lowest concentration of Pb was reported in WP sample (0.48 mg/kg), however, the concentrations of Hg were below the detection thresholds in BM, CBM, FM, FWS, MB, RB and SBM. Nonetheless, the highest concentration of Cd was reported in BSG and TL samples with a recorded values of 2.28 mg/kg and 2.23 mg/kg respectively, and the lowest concentration was reported in both SFSC (2.07 mg/kg) and WP (2.09 mg/kg) samples, while Cd was not detected also in ingredients such as BM, CBM, FM, FWS, MB, RB and SBM. The results of the present study indicated that the concentration of As and Pb were varied significantly ($P < 0.05$) in amongst the ingredients analyzed, refer Table 1. However, there was no significant difference ($P > 0.05$) in concentrations of Hg and Cd observed between local feed ingredients analyzed, refer to table 1.

Table 1. Toxic heavy metals analyzed in local feed ingredients collected in Arusha region (mg/kg)

Feed Ingredient	As	Pb	Hg	Cd
BM	8.07^{a}	2.84^{a}	ND	ND
CBM	1.85^{b}	50.96^{b}	ND	ND
FM	2.50^{b}	2.50^{a}	ND	ND
FWS	3.20^{b}	2.44^{a}	ND	ND
MB	ND	2.00^{a}	ND	ND
RB	ND	ND	ND	ND
TL	0.80^{b}	10.03^{c}	0.54	2.23
BSG	0.45^{b}	3.23^{a}	0.59	2.28
SBM	ND	ND	ND	ND
SFSC	0.34^{b}	0.49^{a}	0.59	2.07
WP	0.44^{b}	9.07^{c}	0.48	2.09
SEM	0.90	1.13	0.31	0.06
P-value	0.001	0.000	0.971	0.076

[1] Mean value (n=3); Means with a different superscript letter within a column are significantly different $p < 0.05$

[2] BM: Bone meal, CBM: Cattle blood meal, FM: Fish meal, FWS: Freshwater shrimp, MB: Maize bran, RB: Rice bran, TL: Taro leaves, BSG: Brewers' Spent grain, SBM: Soy bean meal, SFSC: Sunflower seed cake, WP: Wheat pollard,

[3] The results were expressed as mg/kg. ND: Not detected, [4]SEM standard error means, P-values at 95% confidence level

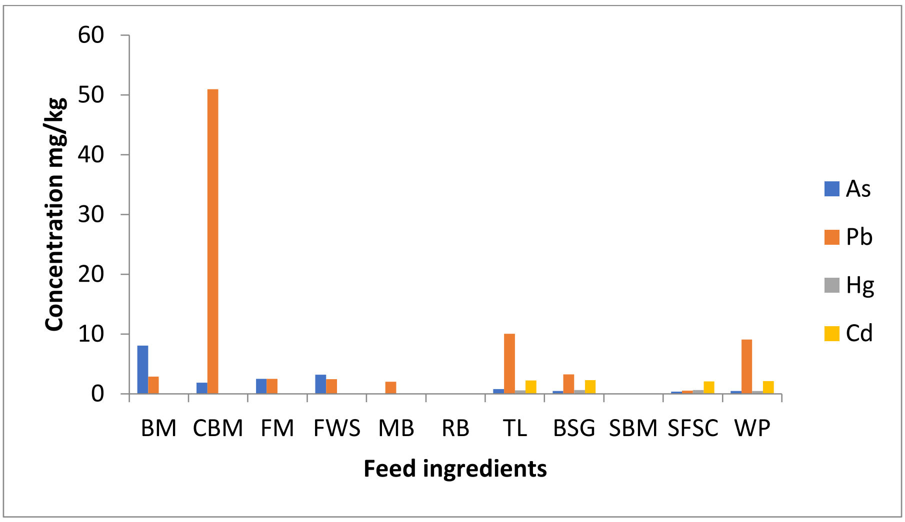

Figure 1: Concentration of heavy metals (As, Pb, Hg and Cd) in mg/kg of commonly used local feed ingredients collected from Arusha region, Tanzania

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Presence of heavy metals in various animal, agricultural, terrestrial and aquatic plant leaves, industrial by-products as well as aquaculture environmental are of global issues. According to the U.S Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease Registry (ATSDR) in Atlanta, Georgia has documented the "Priority list of hazardous substances for 2001". Among the top twenty hazardous substances; arsenic, lead and mercury were at the 1^{st}, 2^{nd}, 3^{rd} positions respectively, while Cadmium was at the 7^{th} position (Huseen and Mohammed, 2019). In this regard, these heavy metals are considered to be toxics to fish, humans, animals and environment.

In the present study, the results of the analysis reported that the concentrations of these heavy metals were below the maximum permitted limits as proposed by the Tanzania Bureau of Standards with the exception of Pb in CBM. The analyzed results indicated that the ingredients CBM reported the highest concentration of lead (50.96 mg/kg), while its lowest concentration was detected in SFSC (0.491 mg/kg). However, agricultural by-products such as RB and SBM reported a very minimal concentration of heavy metals. The highest concentration of lead in CBM may be due to the fact that cattle may obtain lead from different on-farm sources such as lead batteries, especially burnt ones, painted surfaces, paint tins, drinking sump oil, automotive grease and oil filters, eating linoleum, caulking, putty or hungry stock seeking feed around hazardous areas such as the farm dump or around sheds [35]. On the other hand, BM reported the highest concentration of arsenic (8.065 mg/kg) among the ingredients with the lowest concentration in SFSC (0.340 mg/kg), while the mercury and cadmium were not detected in most of the feed ingredients, even in those detected ingredients their values were within the maximum permissible limits in Nile tilapia feeds. The Tanzania Bureau of Standard (TBS) has specified the maximum permissible concentrations of heavy metals in various types of feed ingredients used in the country like maize, maize bran, rice bran, wheat bran, bone meal, blood meal, fish meal etc., for instance, the specified maximum limits (mg/kg) for Hg, Pb, As and Cd are 8, 15, 10 and 10, respectively [36]. Therefore, based on these TBS standards, with the exception of CBM, the concentrations of heavy metals detected in all local feed ingredients analyzed in this study comply with the maximum permissible concentration limits. However, The European Commission also recommended the maximum acceptable limits (mg/kg) for Pb, Cd and Cr in animal feed to be 5, 2, 5 respectively (EU Commission, 2013). Considering the European commission, the BM sample contains As concentration beyond the maximum permissible limits but all other ingredients comply with the commission standard. Other ingredients such as WP, TL, BSG, FM, CBM and BM contain Pb concentrations above the maximum permissible limits. The higher concentration of Pb in FM may be attributed to the fact that the aquatic ecosystem is highly contaminated with heavy metals from various sources that may bio-accumulate and enter into the fish body [26, 37]. Moreover, the concentration of Cd content in all analyzed feed ingredients was within the standards established by the European commission [38].

3.2 Essential transitional metals

The concentrations of transitional metals such as Co, Cu, Fe, Mn, Mo Zn and Ni in commonly used Nile tilapia feed ingredients were presented in Table 2 and Fig. 2. The results were expressed as mg/kg. ND: Not detected. The highest concentration of Co was reported in CBM (18.56 mg/kg) with the lowest concentrations in WP (0.26 mg/kg) and SFSC (0.38 mg/kg),while undetectable concentrations of Co were reported in FM, BM, MB, RB, and SBM. Similarly, the high value of Cu concentration was reported in FWS (58.56 mg/kg), and 55.62 mg/kg for CBM while the lowest value was reported in FM (4.50 mg/kg), Cu was not detected only in BM sample. For Fe, the highest concentration was reported in FM (805.50 mg/kg) and CBM (688.00 mg/kg), while the lowest concentration was detected in WP (27.95 mg/kg), SSC (64.50 mg/kg) and TL (67.56 mg/kg). Fe was detected in all sampled ingredients and only differed in its concentrations. The concentration of Mn was high in RB (163.00 mg/kg) followed by CBM (137.00 mg/kg), while the lowest concentration was reported in MB (13.73 mg/kg) and FM (18.00 mg/kg). Mo was not detected in BM, CBM, FM, FWS, MB, RB and SBM and only detected in BSG, SSC, TL and WP with the highest concentration of 18.98 mg/kg in TL and the lowest concentration in SSC (14.51 mg/kg). Moreover, the highest concentration of Zn was reported in FM (135.50 mg/kg) and SBG (116.75 mg/kg), while the lowest concentration was reported in RB (40.50 mg/kg) and CBM 40.50 mg/kg). The results of the present study indicated that the concentration of Co, Cu, Fe, Mn, Mo, Zn and Ni varied significantly ($P < 0.05$) in most of the local feed ingredients analyzed, refer to table 2 below. However, there were no significant differences in Mo concentration observed between analyzed local feed ingredients, refer to Table 2.

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Table 2. The concentration of essential transitional metals in feed ingredients (mg/kg)

Ingredient	Co	Cu	Fe	Mn	Mo	Zn	Ni
BM	ND	ND	360.50[a]	19.91[a]	ND	75.00[a]	ND
CBM	18.56[a]	58.56[a]	688.00[b]	137.00[b]	ND	40.50[b]	1.99[a]
FM	ND	4.26[b]	805.50[c]	18.00[a]	ND	135.50[c]	3.40 [a]
FWS	4.12[b]	55.62[a]	248.00[d]	62.45c	ND	44.00[b]	ND
MB	ND	4.50[b]	116.00[e]	13.73[a]	ND	52.50[b]	1.50 [a]
RB	ND	5.57[b]	108.50[d]	163.00[d]	ND	40.50[b]	1.62 [a]
BSG	0.40[c]	15.46[c]	87.08[f]	43.58[e]	15.25	116.75[d]	1.54 [a]
SBM	ND	12.76[c]	268.50[g]	35.15[e]	ND	42.11[b]	3.17 [a]
SFSC	0.38[c]	32.88[cd]	64.50[h]	45.52[e]	14.51	109.49[d]	15.64[b]
TL	1.52[bc]	15.46[c]	67.56[h]	42.40[e]	18.98	94.06[de]	5.40 [ac]
WP	0.26[c]	8.06[b]	27.95[g]	81.59[f]	14.62	74.39[a]	0.90 [a]
SEM	0.90	1.16	4.50	2.77	1.28	2.86	0.91
P-value	0.000	0.000	0.000	0.000	0.068	0.000	0.000

[1] Mean value (n=3) ± SD; Means with a different superscript letter within a column are significantly different $p < 0.05$
[2] BM: Bone meal, CBM: Cattle blood meal, FM: Fish meal, FWS: Freshwater shrimp, MB: Maize bran, RB: Rice bran, TL: Taro leaves, BSG: brewers Spent' grain, SBM: Soy bean meal, SSC: Sunflower seedcake, WP: Wheat pollard,
[3] The results were expressed as mg/kg. ND: Not detected [4]SEM standard error means, P-values at 95% confidence level

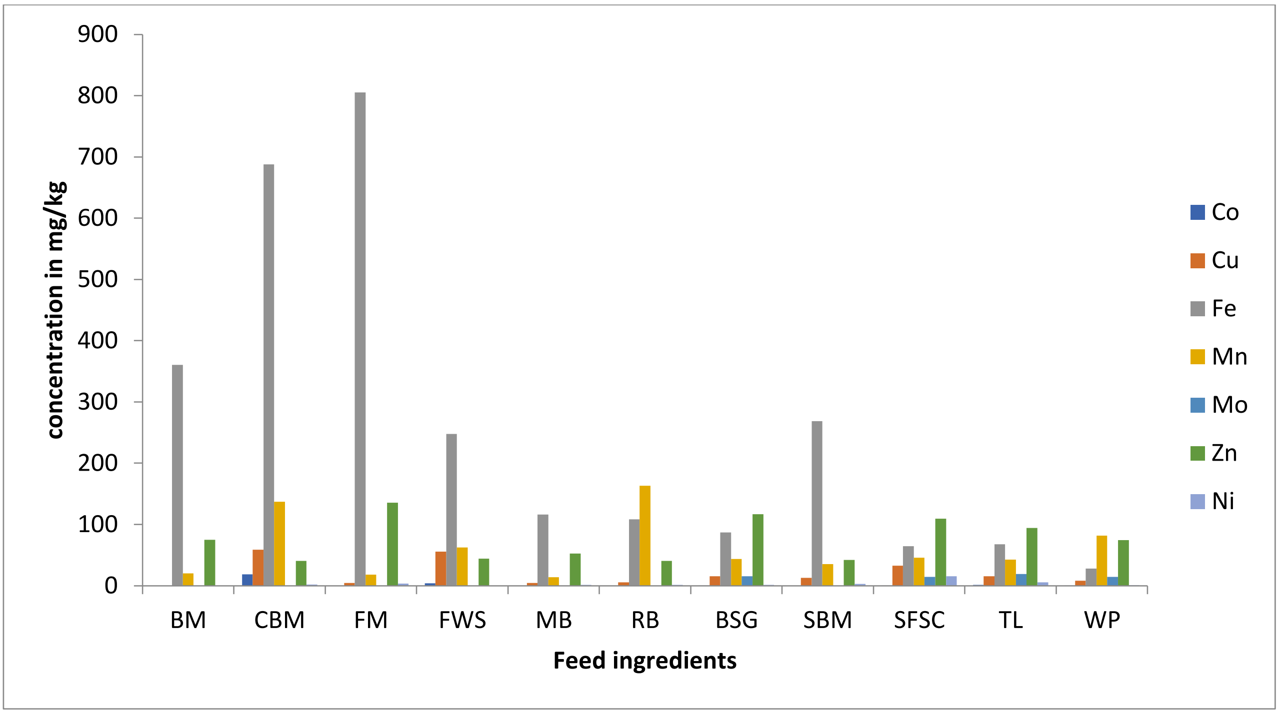

Figure 2: Concentrations of essential transitional metals (Co, Cu, Fe, Mn, Mo, Zn and Ni) in mg/kg of commonly used local feed ingredients in collected from Arusha region, Tanzania

These metals have interesting roles in aquatic toxicology because they are both nutrients and toxic [39]. As nutrients are required in the fish diet at lower concentrations and their deficiency may result in reduced growth, anemia, exudative diathesis, muscular dystrophy and high mortality rate [22]. When their concentrations exceed the maximum permissible limits, they become toxic to cells, tissues, systems and biochemical reactions [37].

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Table 3: Transition element (Mineral) requirements (mg /kg) of different tilapia species

Species	**Co**	**Cu**	**Fe**	**Mn**	**Mo**	**Zn**
Blue tilapia	0.05-1[6]	3 - 5[7]	200[1]		[8]Trace	20[2]
Nile tilapia	5-10[1]	1.5 – 6.0 [1]	24.7-200[1]	13-15[4]	[8]Trace	30[2]
Red tilapia	0.05-1[6]	26.81[5]	23.6-209[1]	12[4]	[8]Trace	12[4]
Hybrid tilapia	0.05-1[6]	4[3]	260-290[1]	7[3]	[8]Trace	45-80[2]
Tilapia Mossambicus	0.05-1[6]	3 - 5[7]	85[2]		[8]Trace	30[2]

([1]NRC [40]; [2]Prabhu et.al. [41]; [3]Lall and Kaushik [42]; [4]Hangsapreurke et.al. [43]; [5]Rafiee et.al. [44]; [6]Makwinja and Geremew [45]; ; [7]Zhang and Nakajima, [8]NRC [46])

Transitional metals are d-block elements which are characterized by having multiple oxidation states, high melting and boiling point, high density and colored compounds. These metals play an important critical role in both fish and other animals including human being. The roles played by this element of this group were described briefly as follows:

For Cobalt (co), Co is one of the essential elements in fish nutrition. It is a component of cyanobalamin (vitamin B12), which prevents pernicious anemia, coupled with production of vitamin B by gut microbiota [47]. Dietary Co has beneficial effects on growth performance and an increased number of white blood cells in Nile tilapia, carps and rainbow trout. Tonye and Sikoki; Nasri, Heydarnejad and Nematollahi showed that, Nile tilapia has shown better growth performance, survival rate, feed conversion efficiency and carcass composition with Cobalt chloride incorporated into the diet [47-48]. The Co dietary requirement in the Nile tilapia diet is ranged from 5 -10 mg Kg-1 of the dry diet. Intake of Co above the maximum permitted limits was found to be toxic to Nile tilapia. It may result in hemorrhages in the fish intestinal tract, reduced number of white blood cells and reduction in intestinal synthesis of vitamin B [49]. In the present study, the results showed that the concentration of Co analyzed amongst the feed ingredients assessed, FWS, SBG, TL, SSC and WP were within the maximum permissible limits (5 – 10 mg/kg) with the exception of CBM containing the Co concentration above the maximum permitted limit (18.555±2.19). Co was undetected in BM, FM, MB, RB and SBM local feed ingredients' samples.

For Copper (Cu), Cu is another essential trace element in the fish diet and acts as a co-factor in ascorbic acid oxidase, co-factor in tyrosinase and dopamine hydroxylase, cytochrome and a component of heme in cephalopods which play roles in cellular metabolism and other biochemical reactions [50-31]. The Cu requirement in tilapia feed ranged from 1.5 to 6 mg/kg [52]. Among the local feed ingredients, FWS and CBM reported the highest concentration of Cu (55.62±2.28) mg/kg and (58.56±2.21) mg/kg, respectively, while the lowest concentrations were reported in ingredients such as FM and MB with (4.255±0.36) mg/kg and (4.49±0.69) mg/kg, respectively. Cu was not detected in BM. The study by Shiau and Ning reported a high concentration of hemoglobin in hybrid Nile tilapia fed with a diet containing 2 mg Cu/kg and 3 mg Cu per kg [50]. The study further showed that high Cu concentration (above 20 mg/kg) significantly reduced tilapia growth and feed utilization efficiency. Nile tilapia fed on a Cu enriched diet (2000 mg/kg) to satiation for 42-days exhibited a significant high growth and enzymatic action reduction.

For iron (Fe), Fe plays an important role in oxidation-reduction reactions and the electron transport chain of the respiration system. It is an essential component of hemoglobin in skeletal muscles and myoglobin in skeletal and heart muscles. Fish takes Fe through the gills and intestinal mucosa [53]. The study done by El-serafy, S et.al, revealed that, Nile tilapia fed on a Fe free diet had significantly reduced body weight, specific growth rate, survival rate and higher feed conversion rate, whereas Fe supplementation to the diet resulted in improved growth performance and feed utilization [54]. Feeds of animal origin such as FM, CBM, BM and meat meal contain high Fe content ranging from 400 to 900 mg/kg while feeds of plant origin contain a minimum amount of Fe ranging from 100 to 200 mg/kg but its availability depends on its form [49]. The results of this study also show that ingredients of animal origin such as FM, CBM, BM and FWS contain 805.5±6.36, 688.00±1.41, 360.50±6.36, and 248.00±11.31 (mg/kg) respectively. Of the

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original plant's ingredients, SBM contains the highest amount of Fe (268.50±2.12 mg/kg), while the lowest concentration was reported in WP, 27.95±0.07 mg/kg.

For Manganese (Mn), Mn is most important in fish and widely distributed in fish tissues. It acts as a co-factor for enzymes, kinase, peptidase, arginase, succinic decarboxylase and DNAse [55-56]. It is highly available in mitochondria when compared to its availability in the cytoplasm. Manganese deficit in young Nile tilapia results in loss of equilibrium, reduction in feed intake, stunting growth, cataract of eyes and sometimes death [49]. The dietary Mn requirement in the Nile tilapia fish diet ranges between 20 -50 mg/kg of dry matter. Among the ingredients, CBM, FWS, RB and WP contain Mn concentrations above the maximum permissible limit (20 – 50 mg/kg) i.e., 137.00±4.24, 62.45±2.05, 163.00±5.66 and 81.591±1.72 mg/kg respectively. The high concentration of manganese in rice bran was in accord with the study by Manzoor, A et.al shows that RB is a good source of Mn [57].

For Molybdenum (Mo), Mo is an essential micronutrient required for normal growth and development in all organisms [58]. There are about fifty major enzymes, including nitrate reductases, sulphite oxidases, xanthine dehydrogenase and oxidases, and aldehyde oxidases which use Mo-cofactor to perform various metabolic functions [60]. In Nile tilapia, Mo acts as a co-factor of xanthine, oxidase, hydrogenase and reductase enzymes [60]. Mo was not detected in BM, SMB, CBM, FM, FWS, MB and RB but detected in SBG, SSC, TL and WP. It is required in a very trace amount and therefore, even in detected feed ingredients' samples, concentrations were all within the EAC fish feed standards.

For Zinc (Zn), Zn is essential in fish nutrition for the structure and function of the insulin hormone but also as a co-factor for carbonic anhydrase enzyme. Zn deficiency in Nile tilapia (*Oreochromis niloticus*) may result in symptoms like growth retardation, increased fish mortality, cataracts [61]. The maximum permitted level of Zn in tilapia feed is between 30 -100 mg/kg according to the EAC fish feed standards [60]. But the requirements of Zn in a fish diet tend to vary depending on the fish development stages. For instance, according to the EAC fish feed standard starter feed, finisher feed and brooder feed are recommended to contain 100 mg/kg of Zn, while grower feed should at least contain 50 mg/kg of Zn. Therefore, with the exception of SFSC (109.49±1.31 mg/kg), spent brewers' grain (116.75 mg/kg) and fish meal (135.5±2.12 mg/kg), all other feed ingredients analyzed contain Zn within the permitted concentration. The high Zn concentration in FM may be due to the fact that seafood usually tends to contain high amounts of Zn when compared to plant ingredients [62]. Zn usually works antagonistically with Cu hence increasing one in the fish diet tends to reduce the other.

3.3 Toxic transitional metals

The concentrations of toxic transitional metals; Ag, V Cr and Ni in commonly used local feed ingredients were presented in Table 4 and Fig. 3. The results were expressed as mg/kg. ND: Not detected. The highest concentration of Ag was reported in the TL (38.48 mg/kg) sample, while the WP sample had the lowest concentration (15.01 mg/kg). However, the highest concentration of V in feed ingredients' samples was reported in the TL sample (22.40 mg/kg), whereas the lowest concentration of V was reported in the WP sample (2.45 mg/kg). For Cr, the highest concentration of Cr was reported in the TL sample (37.69 mg/kg), while the lowest detected concentration of Cr in CBM (8.59 mg/kg). In addition, Cr was not detected in BM, FM, FWS, MB, RB and SBM ingredients' samples. Furthermore, the highest concentration of Ni in analyzed feed ingredients' samples was reported in the SFSC sample (15.64 mg/kg) with the lowest concentrations in MB and BSG samples such as 1.50 mg/kg and 1.54 mg/kg respectively, and undetected concentration in two ingredients' samples such as BM and FWS. Therefore, the results of the present study showed that, there was a statistical significant difference ($P < 0.05$) in concentrations of toxic transitional metals (Ag, V, Cr and Ni) in all local feed ingredients sampled and analyzed, refer Table 4.

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Table 4. Toxic transitional metals assessed in the local feed ingredients (mg /kg)

Ingredient	Ag	V	Cr	Ni
BM	ND	6.95 [a]	ND	ND
CBM	ND	ND	8.59 [a]	1.99 [a]
FM	ND	11.71 [a]	ND	3.40 [a]
FWS	ND	10.28 [a]	ND	ND
MB	ND	ND	ND	1.50 [a]
RB	ND	2.61[a]	ND	1.62 [a]
BSG	19.56 [a]	6.40 [a]	19.30 [b]	1.54 [a]
SBM	ND	3.32 [a]	ND	3.17 [a]
SFSC	26.01 [b]	4.00 [a]	27.52 [c]	15.64 [b]
TL	38.48 [c]	22.40 [ab]	37.69 [d]	5.41 [ac]
WP	15.01 [a]	2.45[a]	14.52 [ab]	0.90 [a]
SEM	1.52	2.77	1.82	0.91
P-value	0.000	0.001	0.000	0.000

[1]Mean value (n=3) ± SD; Means with a different superscript letter within a column are significantly different p< 0.05.
[2]BM: Bone meal, CBM: Cattle blood meal, FM: Fish meal, FWS: Freshwater shrimp, MB: Maize bran, RB: Rice bran, TL: Taro leaves, SBG: Spent brewers' grain, SBM: Soy bean meal, SSC: Sunflower seedcake, WP: Wheat pollard,
[3]The results were expressed as mg/kg. ND: Not detected [4]SEM standard error means, P-values at 95% confidence level

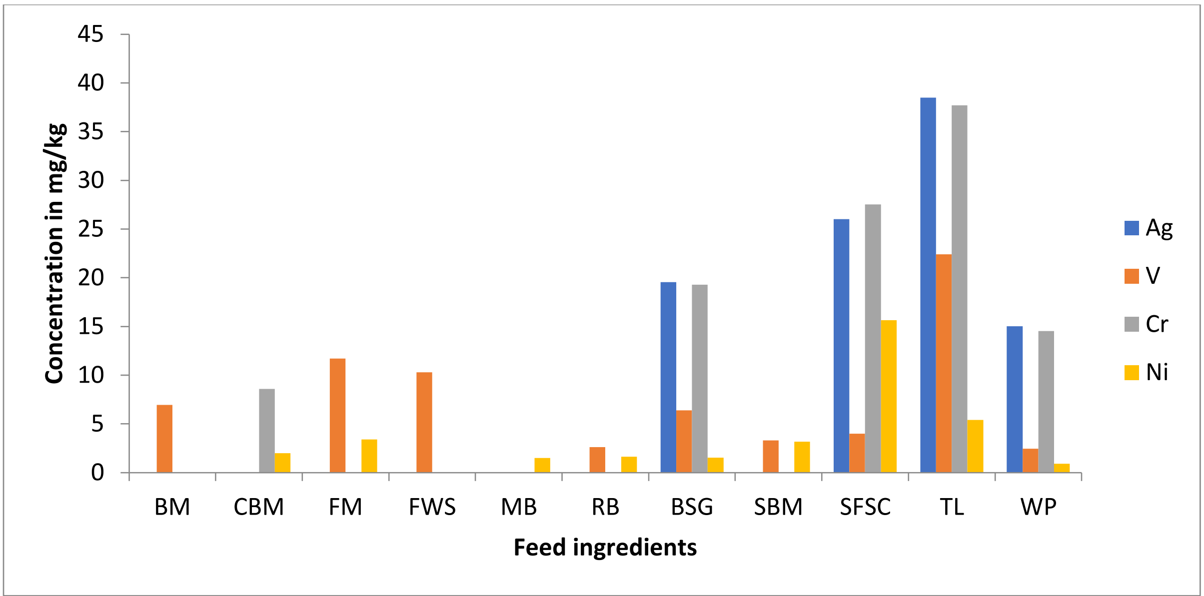

Figure 3: Concentrations of toxic transitional metals (Ag, V, Cr, and Ni) in mg/kg of the commonly used local feed ingredients collected from Arusha region, Tanzania.

For silver (Ag), Ag is one of the most toxic elements to aquatic organisms in the form of silver nitrate and silver chloride [63]. Ag is not a dietary requirement in fish. In freshwater fish like Nile tilapia, Ag tends to damage the gills and even cause death but does not bio-accumulate in the body as it is excreted via bile and urine [64]. The acute toxicity of Ag changes depending on the hardness of water and it tends to increase as hardness decreases. Depending on hardness, the lethal dosage of Ag concentration for freshwater fish is between 4 - 280 mg/kg [65]. Plants absorb the Ag element even though it has no biological value and can be passed to fish through plant feed materials when they are included in the diet. Ag was not detected in most of the ingredients' samples analyzed and was only found in

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ingredients of plant origin such as spent brewers' grain, taro leaves, wheat pollard and sunflower seedcake. The findings were consistent with study done by Courtois, P et.al, found that plants (feed of plant origin) are more susceptible to Ag contamination than ingredients of animal origin which later pass to fish, animals and human beings and cause major health problems [66].

For Vanadium (V), V was discovered in 1971 as a trace mineral essential for normal growth and has been found to regulate the activity of various enzymes [67]. Nothing is known about V concentration in the Nile tilapia fish diet. But the study done by Anderson, P.D et.al in lethal tests on rainbow trout showed that a vanadium concentration of 11.5 mg/kg caused death throughout the experimental period (14 days) [68]. Taking into consideration this lethal dose, TL, and FM contain V concentrations above the maximum permissible levels. The highest concentration of V in taro leaves, 22.40 mg/kg, may be due to the fact that taro leaves were harvested along the river bank where farming activities took place. According to previous studies documented, phosphate containing fertilizers are contaminated with high amounts of V that may cause vanadium leaching into soil, water and vegetables [69]. In FM the concentration of V is too high (11.72 mg/kg), second to taro leaves as a consequence of anthropogenic activities such as industrial activities that release appreciable amounts of V to increase natural background concentrations [70].

For Chromium (Cr) concentration in the feeds/foods used in Nile tilapia, nutrition has been classified among the most crucial trace elements, being involved in glucose metabolism and collagen formation [71]. Studies on the optimum Cr level to be included in the Nile tilapia diet have reported varying results. For instance, the study done by Ng and Romano recommended a 1.3 - 2.04 mg/kg Cr concentration in tilapia nutrition [72]. The study by Makwinja and Genemew on the other hand, reported that the supplementation of the Nile tilapia diet with Cr, 1.2 mg/kg, led to a significant decrease in serum cholesterol, total protein, and albumin concentration [73]. According to Shaukat and Javed the estimated lethal concentration of Cr in Nile tilapia is 129.77 mg/kg [74]. In this study, Cr was undetected in most of the feed ingredients but all were below the lethal dosage, making the ingredients safe for inclusion in the Nile tilapia diet here in Tanzania.

For Nickel (Ni), Ni is widely used in industries and it is the dominant chemical pollutant in natural water bodies [65, 75]. It is usually released into the environment through both anthropogenic and natural sources, and it is found in soil, water, air and biosphere [65, 76]. Ni is not an essential component of fish feed and may not be present in a sufficient amount in fish feed as well as in aquatic ecology, although its presence has both positive and negative impacts in both fresh water organisms and human beings [77]. According to previous studies done elsewhere revealed that no exposure to the Tilapia environment leads to behavioral changes such as rapid swimming accompanied by increased rate of opercular opening [76, 78]. It was further reported that fish mortality may occur as a result of absorption and bio-accumulation of Ni in the fish body. For example, the lethal value was reported to be 51.39 mg/kg in *Oreochromis mossambicus,* 40 mg/kg in *Labeo rohita,* 19.3 mg/kg in Rain bow trout *Oncorhynchus mykiss,* 33.7 mg/kg in Three-spined stickleback (Gasterosteus aculeatus), 48.1 mg/kg in Perch (*Perca fluviatilis*), 48.7 mg/kg in Roach (*Rutilus rutilus*) and 61.2 mg/kg in Dace (*Leuciscus leusciscus*). Therefore, we concluded that the lethal toxicity of Ni varied significantly depending on species, size and age of the fish [79-80]. However, in low concentration, Ni is an important trace metal required in the production of red blood cells [81]. In the present study, the concentration of Ni in all feed ingredients' samples was below the lethal acute value, making them safe for using in fish feed formulation, refer to table 4.

4. CONCLUSION

The analyses performed in this study reported concentrations of toxic heavy metals and transitional metals in commonly used local feed ingredients' samples used for tilapia diet formulation by fish farmers in Tanzania. These data will provide a guideline on carrying out pre-assessment of many more ingredients to be included in farmed fish feed formulations to ensure optimal growth performance, welfare of the farmed fish species, other aquatic species as well as human beings. The results have reported that few ingredients' samples have exceeded the maximum permissible limits of toxic metals and, without monitoring, can interfere with early life and normal physiological processes in fish including enzymatic and genetic effects, immune response, susceptibility to diseases and death. Also, this paper will call the attention of researchers and practitioners to the importance of conducting pre-assessment of

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ingredients, not only metals, but also on other potential contaminants like pesticides and insecticide residuals, to ensure the smooth and sustainable development of the Tanzania blue economy.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests

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