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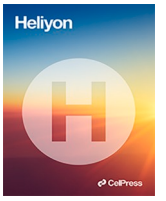

Research article

Proximate composition, microbial quality and heavy metal concentration of fresh Nile tilapia fillet in Lake Tana, Ethiopia

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ABSTRACT

Nowadays, consumption of fish is becoming a public health concern due to quality and safety issues. This study was designed to assess the proximate composition, microbial quality, and heavy metal accumulation in the Nile tilapia fillet at three selected landing sites in Lake Tana. Fifteen samples were collected and analyzed. The mean moisture, ash, fat, protein, salt, and water activity were 81.76%, 0.98%, 1.88%, 14.04%, 0.14%, and 0.9869, respectively. The proximate contents varied slightly among sites but were not significantly different ($p > 0.05$). The mean aerobic mesophilic bacteria, *Staphylococcus aureus*, total coliform, and fecal coliform counts were 6.30 log CFU/g, 2.91 log CFU/g, 1.51 log MPN/g, and 0.89 log MPN/g, respectively. Such high microbial loads and the high counts of fecal coliforms are indicative of poor handling practices and unsanitary processing that might lead to foodborne illnesses and economic losses. In addition, the mean concentration of heavy metals in the tissue samples decreased in the order of chromium (0.165 mg/kg) > arsenic (0.085 mg/kg) > lead (0.054 mg/kg) > cadmium (0.010 mg/kg). Except for chromium, the concentrations of the assessed metals were below the maximum permissible limits. Long-term chromium exposure, especially in its hexavalent form, can pose significant health risks like respiratory issues, gastrointestinal distress, and even cancer. Therefore, identifying contamination sources, employing proper waste management strategies, continuous monitoring of heavy metal levels, and proper fish handling practices are highly recommended to address the health implications of microbial contamination and elevated chromium concentrations.

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1. Introduction

Fish have a crucial role in nutrition and global food security, as they represent a valuable source of high-quality animal proteins vital for physical and cognitive development [1]. Nile tilapia (*Oreochromis niloticus*) is the most commercially important fish species in tropical freshwater [2], contributing more than 50% of the annual catch in Ethiopia [3] and more than 65% of the total catch in Lake Tana [4,5]. However, the nutritional benefit of fish is challenged due to a lack of adequate infrastructure and services for ensuring fish quality, such as hygienic landing sites, electric power supply, potable water, roads, ice, refrigerated transport, and appropriate processing and storage facilities in developing countries [6], and particularly in Lake Tana [7–11]. Such limitations in fish production might lead to quality deterioration, contamination, or other safety risks [6,12].

Fish are highly susceptible to contamination with biological and chemical hazards, leading to consumers' health risks [13]. Inadequate hygiene practices may be associated with an increased risk of exposure to pathogens and microbial spoilage [14]. Contamination of fish from external sources during processing and storage may add pathogenic bacteria like *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., and *Escherichia coli* [15]. Aerobic mesophilic bacteria (AMB) are a general indicator of handling practice, while *Escherichia coli* indicates contamination with pathogens originating from the gastrointestinal tract of humans [16]. Inadequate handling, processing, storage, and preservation practices of fish were reported in Ethiopia [9,17–19], and also in Lake Tana [11].

In addition to microbial quality and safety, chemical hazards due to urbanization, industrialization, and intensive agricultural activities, large amounts of metals are discharged into aquatic ecosystems, risking aquatic organisms [20,21]. In the discharged wastes, elements with a relatively high density are heavy metals, which are toxic at low concentrations [22]. Especially, the bioaccumulation and biomagnification of heavy metals have a higher effect on human beings and other living things [23]. Some heavy metals like mercury, chromium, cadmium, lead, and arsenic are categorized as potentially harmful elements [24,25] and are metals of public health concern that result in a risk to humans through the food chain [26,27]. Fish are highly exposed to these metals in the food chain interaction with phytoplankton and zooplankton [28,29]. As a result, fish can act as bioindicators for monitoring aquatic pollution and the implications for human consumption [30]. For instance, excess Hg affects the early stages of development in children [31], Pb damages the nervous system [32], and Cr causes pulmonary disorders and organ damage [33,34]. In Ethiopia, different water bodies are affected by heavy metals due to anthropogenic activities with poor waste management practices [35–39]. For example, tributary rivers that flow towards Lake Tana, like the Megech River, have been contaminated with heavy metals [40]. Kindie et al. [41] also recommend the need for continuous monitoring of the level of heavy metal pollution in Lake Tana. Information on the chemical and biological quality of fresh *O. niloticus* fillet (the major primarily processed product) in Lake Tana is limited. Geremew et al. [42], Kindie et al. [41], and Mitiku et al. [43] have made valuable contributions by investigating specific aspects of these issues in Lake Tana. However, Lake Tana is the largest lake in Ethiopia, and their research is limited in scope, focusing on the southern (Gulf) of Lake Tana with a restricted set of parameters. This leaves gaps on the overall proximate, microbial, and heavy metal issues, and this study was designed to fill the gaps by assessing the proximate composition, microbial quality, and heavy metal accumulation in the muscle tissue of *O. niloticus* at selected landing sites in Lake Tana. These might provide valuable information for regulatory bodies to monitor food quality and for consumers to cook the product properly before consumption.

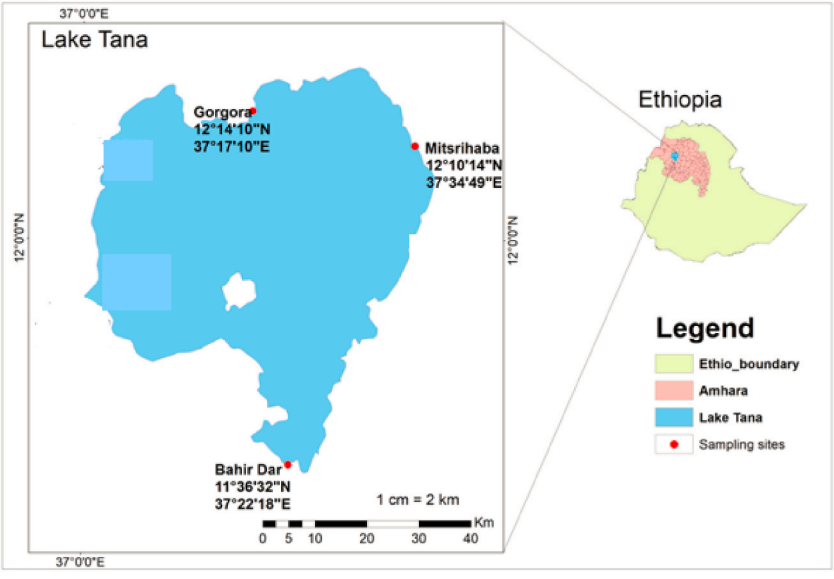

Fig. 1. Map of the study area and sampling sites. Source: own map.

2. Materials and methods

2.1. Description of the study area

This study was conducted in Lake Tana, the largest freshwater body in Ethiopia (Fig. 1). It is situated at 12°N, 37°15′E, 1830 altitude, and covers an area of 3050 km^2 [44]. The lake is shallow, with an average depth of 8 m [45]. The area experiences an annual rainfall of up to 2000 mm [46]. The main tributary rivers are Gilgel Abay, Rib, Gumara, and Megech, accounting for 93% of the inflow to the lake [47]. The catchment is considered one of the agricultural growth corridors by the government of Ethiopia [48]. Lake Tana, its tributary rivers, and the associated wetlands provide fish and drinking water for people living around the lake [44]. A total of 27 fish species have been identified in Lake Tana [49,50], and most of them are endemic and commercially important (*Oreochromis niloticus*, *Labeobarbus* species, and *Clarias gariepinus*) [49,51]. However, fish processing is mostly practiced traditionally under non-hygienic conditions on bare ground for gutting, filleting, or drying with little to no regard for safety [52].

2.2. Study design and sample collection

The study design was cross-sectional, and it was conducted from January to May 2023. The sampling procedures for the microbial and heavy metal analyses were determined based on the International Commission for Microbiological Specifications for Food (ICMSF) [53] and the United States Environmental Protection Agency (USEPA) [54], respectively. In Lake Tana, freshly caught fish are traditionally filleted into market-ready products at the landing sites by fishers and processors. Out of these products, an appropriate size of fresh Nile tilapia fillet was collected for proximate, microbial, and heavy metal analysis. A total of 15 samples (5 samples at each site) were collected at Bahir Dar, Mitsrihaba, and Gorgora landing sites (Fig. 1). We selected the three sampling sites because they are the major landing and processing sites, representing the Lake Tana fisheries. The samples were collected during similar seasons and times of the day (the morning between 8 and 10 a.m.) across the different landing sites to avoid the effect of diurnal and seasonal variations. Clean and sterilized sample collection tools were used to collect an equal number of samples at each site with a similar sampling protocol. The collected samples were handled in a similar manner during transportation to Bahir Dar University (with an ice box). All aseptic procedures were followed to avoid cross-contamination during sample collection and preparation.

2.3. Proximate analysis

The proximate composition was determined following the specific instructions outlined in the Association of Official Analytical Chemists (AOAC) manual. An appropriate weight of sample was used for assessing the proximate composition (moisture, fat, protein, and ash) and salt content.

2.3.1. Moisture content

The moisture content was determined using the methods outlined in AOAC [55]. The moisture content was calculated as:

$$Moisture\ (\%) = \frac{W1 - W2}{W1} \times 100 \qquad \text{Equation 1}$$

Where, W1 = weight (g) of sample before drying; W2 = weight (g) of sample after drying.

2.3.2. Ash content

Following the specific instructions outlined in the AOAC [55] manual, the ash content was calculated as:

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100 \qquad \text{Equation 2}$$

2.3.3. Crude protein

The crude protein was determined using the Kjeldahl method, as outlined by AOAC [55]. The total nitrogen and crude protein content in the sample were estimated using the appropriate formula.

$$\%N = \frac{(S\text{-}B)*M*14.01}{Weight\ of\ sample} \times 100 \qquad \text{Equation 3}$$

$$Crude\ protein\ (\%) = \%N * 6.25 \qquad \text{Equation 4}$$

Where, %N and %P are nitrogen and protein content in percent, respectively; S is the volume of HCI in sample titration; B is the volume of HCI consumed in blank titration; M is the normality of HC1; 14.01 is the molecular weight of nitrogen; 6.25 is a correction factor.

2.3.4. Fat content

The fat content was determined using the Soxhlet method according to the guidelines provided by the AOAC [55]. The fat content was calculated as:

$$Crude\ fat\ (\%) = \frac{Weight\ of\ fat}{Weight\ of\ sample} \text{ x } 100 \quad \text{Equation 5}$$

2.3.5. Salt content

The salt content was determined using Mohr's titration method and calculated with following equation.

$$NaCl\ (\%) = \frac{Titer\ value \times Normality\ of\ AgNO_3 \times 58.4 \times 100}{Weight\ of\ \text{the}\ sample \text{ x } 1000} \quad \text{Equation 6}$$

2.3.6. Water activity

The water activity of the fish sample was measured using a water activity meter (AQUALAB 4 TE, USA, SN: S40003072) at a range of 24–27°C.

2.4. Microbial analysis

For microbial analysis, 25 g of the fish fillet were weighted under aseptic conditions inside the biosafety hood and mixed with 225 ml of sterilized peptone and a normal saline solution (0.1% peptone and 0.85% NaCl) in a sterilized plastic bag. The mixture was homogenized for 2 min by using a stomacher*400 circulator (England, SN: 42110). Then, from the homogenized sample, tenfold serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} were prepared with sterile peptone and normal saline solution using a sterile pipette. From each serially diluted sample, 1 ml was taken and pour-plated on sterile 15–20 ml media for enumeration of microbial load [56]. The Petri dish was then incubated at the appropriate temperature and time. Then, colonies were counted using a colony counter (UK, SN: R000100372) and expressed as the mean colony-forming units per gram of sample.

2.4.1. Aerobic mesophilic count

For the aerobic mesophilic bacteria count, inoculated plate count agar (HiMedia, India) was incubated at 37°C for a duration of 24–48 h. Plates with 30–300 colony-forming units were counted to determine the total load of aerobic mesophilic bacteria [56]. The recorded result was expressed as the mean colony forming units per gram using the following formula [56]:

$$N = \frac{\sum C}{[(1 \text{ x } n1) + (0.1 \text{ x } n2)]d} \quad \text{Equation 7}$$

Where, N = number of colonies per gram; $\sum$C = sum of counted colonies; n1 = number of plates counted in the first dilution; d = dilution of the first count obtained; n2 = number of plates counted in the second dilution.

2.4.2. Staphylococcus aureus count

Plates with inoculated mannitol salt agar (HiMedia, India) were incubated at 37°C for a period of 24–48 h. Colonies from plates with 20–200 colonies were counted. Representative colonies exhibiting a golden yellow color were selected, and then the catalase and coagulase tests were conducted for confirmation [57].

2.4.3. Total coliform and fecal coliform counts

The most probable number (MPN) technique was used to enumerate both total and fecal coliforms [58]. A presumptive, confirmed, and complete test was performed using Lactose Broth (HiMedia, India), 2% Brilliant Green Bile Broth (HiMedia, India), and EC broth (HiMedia, India), respectively. For the presumptive test, 1 ml of sample was taken from 10^{-1}, 10^{-2}, and 10^{-3} diluted homogenate and inoculated into three tubes containing 10 ml of lactose broth and an inverted Durham tube. Following an incubation period of 24–48 h, the number of tubes exhibiting positive gas production was recorded. To confirm the total coliforms, a loopful of broth from each presumptive positive lactose broth tube was gently transferred to tubes containing 10 ml of brilliant green lactose bile 2% broth and an inverted Durham tube. The tubes were then incubated at 37°C for a maximum of 48 h [58]. For fecal coliform confirmation, a loopful of broth from each presumptive positive lactose broth tube was gently transferred to tubes containing 10 ml of sterilized EC broth and an inverted Durham tube, which were subsequently incubated at 44.5°C for 24–48 h. Positive tubes identified during the confirmation tests were recorded, and the results were reported using the MPN table [58]. In the completion stage of the testing process, a loopful of broth from each EC broth tube showing positive results was streaked onto eosin methylene blue agar (HiMedia, India) and incubated at 37°C for 24 h [58].

2.4.4. Detection of Salmonella and Shigella spp.

The homogenized sample was incubated at 37°C for 24 h to enrich the sample with buffered peptone water (HiMedia, India). Then, an inoculum from the enriched culture was streaked on Salmonella and Shigella agar (HiMedia, India) and incubated at 37°C for 24 h. The detection of suspected *Salmonella* and *Shigella* colonies was based on their distinctive appearance on SS agar. The suspected colonies were also subjected to further biochemical tests [59,60].

2.5. Heavy metal analysis

The digestion tubes were washed using detergent, followed by rinsing with distilled water and overnight soaking in nitric acid

(HNO_3). Subsequently, a homogenized 1 g of powdered muscle tissue was weighed and transferred to a digestion tube. A digestion tube was then filled with a 1:3 ratio of concentrated analytical reagent aqua regia (HNO_3: HCl) and subjected to the digestion process using a digestion unit (Italy, DK 20). The resulting digested sample was diluted with 30 ml of distilled water and transferred to a 100 ml volumetric flask through the use of filter paper. Simultaneously, a blank without any sample was prepared in the same manner. Then heavy metal analysis for Cr, Cd, Pb, and As was performed using inductively coupled plasma optical emission spectroscopy (USA, OptimaTM 8000).

2.6. Risk assessment on human health

The human health risk of Nile tilapia (*O. niloticus*) consumption with heavy metals (Cr, Cd, Pb, and As) was assessed by calculating the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and the cancer risk (CR) values.

2.6.1. Estimated daily intake (EDI)

The daily exposure to metals for adult fish consumers was calculated using the following formula Culha et al. [61].

$$EDI = \frac{HC\ x\ IR}{BW} \quad \text{Equation 8}$$

Where, HC is the mean heavy metal concentration in fish muscle (mg/kg); IR is the ingestion rate, which is the average daily consumption of fish; and BW is the average adult body weight.

For this calculation, we have used the following information: In Ethiopia, fish consumption per capita remains among the lowest in the world and is estimated at 2 kg per year [62], which is 5.48 g/day/person, but it may be higher than this around fish production areas including Lake Tana. The average body weights of adults were 64.65 kg (67.0 kg for males and 62.3 kg for females) [63]. The fish from Lake Tana are commercially important and can be sold in different parts of the country (Ethiopia).

2.6.2. Target hazard quotient (THQ)

The target hazard quotient (THQ) is the measure of non-carcinogenic risk due to contaminant exposure, and it was calculated using the following equation [64].

$$THQ = \frac{EF\ x\ ED\ X\ EDI}{RfD_o\ x\ AT_{non\text{-}cancer}}\ \text{x}\ 10^{-3} \quad \text{Equation 9}$$

Where, EF is the exposure frequency (365 days/year); ED is the exposure duration (in Ethiopia, the average life expectancy is 67.8 years [65]); EDI is the estimated daily intake; RfD_o is the reference oral dose; $AT_{non\text{-}cancer}$ is the average exposure time (365 days/year x ED); 10^{-3} is the unit conversion factor. RfD_o is the safe reference oral dosage of heavy metals, and it is 0.003 mg kg^{-1} day^{-1} for Cr, 0.001 for Cd, 0.0015 for Pb, and 0.0003 for As [130].

2.6.3. Hazard index (HI)

The hazard index (HI) is the sum of the THQ, and it was calculated using the following equation [64].

$$HI = \sum_{i=1}^{n} (THQ_i) \quad \text{Equation 10}$$

2.6.4. Cancer risk (CR)

The cancer risk (CR) of exposure was calculated using the following equation [66].

$$CR = \frac{EF\ x\ ED\ x\ EDI\ x\ CSF}{AT_{cancer}}\ \text{x}\ 10^{-3} \quad \text{Equation 11}$$

Where, CSF is the cancer slope factor and the CSF values of Cr, Cd, Pb, and As are 0.5, 6.3, 0.0085, and 1.5, respectively [64,67].

Table 1
Standard concentration, regression equation, correlation coefficient (R^2), detection limit (DL) and quantitation limit (QL) for chromium, cadmium, lead and arsenic using ICP-OES method.

Metals	Standard concentrations (mg/L)	Regression equation	R^2	DL	QL
Cr	0.05, 0.5, 1, 2, 5, 10 and 20	y = 102532× - 6255.5	0.9992	0.0037	0.0113
Cd	0.05, 0.5, 1, 2, 5, 10 and 20	y = 766833× - 6498	0.9996	0.0003	0.0009
Pb	0.05, 0.5, 1, 2, 5, 10 and 20	y = 53509× - 3427.5	0.9989	0.0009	0.0029
As	0.05, 0.5, 1, 2, 5, 10 and 20	y = 11251× - 1049.8	0.9993	0.0048	0.0146

Note: ICP-OES = Inductively Coupled Plasma Optical Emission Spectrometry.

2.7. Analytical method validation

To ensure the acceptance of the analytical method used (inductively coupled plasma optical emission spectrometry), the linearity of the analytical response was determined by plotting calibration curves of the intensity values versus the concentration values, and then the correlation coefficient (R^2) of the calibration curve was determined according to the validation of analytical procedures guideline [68]. Calibration was done using seven standard concentrations (0.05, 0.5, 1, 2, 5, 10, and 20 mg/L). The correlation coefficient (R^2) was close to 1 ($\geq$0.997), ranging from 0.9989 to 0.9996 (Table 1), which indicates good linearity of the method over the specified concentrations. Based on these data, it was concluded that the method has fulfilled the linearity acceptance criterion. The detection limit and quantitation limit were also calculated using standard formulas [68].

$$DL = \frac{3.3\sigma}{S} \quad \text{Equation 12}$$

$$QL = \frac{10\sigma}{S} \quad \text{Equation 13}$$

Where, σ is the standard deviation of y-intercepts of the regression lines and S is the slope of the calibration curve. The slope (S) was estimated from the calibration curve, and the standard deviation (σ) was calculated from the analytical response of the blank according to the validation of analytical procedures guideline [68].

2.8. Data analysis

The data was analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, Version 26.0). Descriptive statistics, such as frequency, means, graphs, and tables, were used to visualize the collected data. A one-way ANOVA was used to compare the mean difference in proximate composition, microbial counts, and heavy metal levels among sites. Tukey's multiple comparison tests were used to find the difference between sites. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of fresh *O. niloticus* fillet varied slightly among sites but was not significantly different ($p > 0.05$) (Table 2).

3.1.1. Moisture content

The mean moisture content of *O. niloticus* fillet was 81.76 ± 0.92%, and it was within the acceptable range of 66%–81% [69]. The value was comparable with that of Geremew et al. [42] in the same lake (Ethiopia), Olopade et al. [70] in Nigeria, and Suwannatrai et al. [71] in Thailand, but higher than Desta et al. [72] and Shahrier et al. [73] reports. However, a higher amount of moisture (89.5%) was also reported in Sri Lanka by Premarathna et al. [74]. The variation might be associated with the size and maturity of the fish examined and other factors like the water body they live in [75,76]. The higher moisture content in this study suggests the

Table 2
Proximate composition (%) of fresh *Oreochromis niloticus* fillet samples collected from different landing sites around Lake Tana (2023).

Parameters	Landing sites	Mean ± SD	Minimum	Maximum
Moisture content	Gorgora	81.04 ± 1.19[a]	80.00	82.35
	Bahir Dar	81.85 ± 0.19[a]	81.63	82.00
	Mitsrihaba	82.39 ± 0.76[a]	81.91	83.27
	Gorgora	0.87 ± 0.23[a]	0.63	1.10
Ash content	Bahir Dar	1.07 ± 0.34[a]	0.68	1.27
	Mitsrihaba	0.99 ± 0.36[a]	0.60	1.32
	Gorgora	1.62 ± 0.08[a]	1.53	1.67
Fat content	Bahir Dar	1.66 ± 0.57[a]	1.28	2.33
	Mitsrihaba	2.35 ± 0.54[a]	2.02	2.99
	Gorgora	14.62 ± 0.93[a]	13.60	15.44
Crude protein	Bahir Dar	14.38 ± 1.40[a]	13.20	15.94
	Mitsrihaba	13.13 ± 0.74[a]	12.28	13.61
	Gorgora	0.9870 ± 0.001[a]	0.9861	0.9883
Water activity	Bahir Dar	0.9872 ± 0.002[a]	0.9870	0.9874
	Mitsrihaba	0.9866 ± 0.018[a]	0.9851	0.9887
	Gorgora	0.13 ± 0.60[a]	0.07	0.19
Salt content	Bahir Dar	0.18 ± 0.00[a]	0.18	0.18
	Mitsrihaba	0.12 ± 0.02[a]	0.12	0.13

Lowercase superscript (a) indicates no statistical difference among the mean values of the sites in the same column for each parameter using a one-way ANOVA ($p > 0.05$).

susceptibility of this product to microbial growth and contamination during the filleting process. Furthermore, moisture has an inverse relationship with fat, protein, and relative energy, where the higher the moisture content, the lower the fat, protein, and relative energy of the product [77].

3.1.2. Ash content

The mean ash content was 0.98 ± 0.28%, and it was comparable with that of Ghassem et al. [78] and Suwannatrai et al. [71] reports but lower than that of Olopade et al. [70], Mazumder et al. [79], Desta et al. [72], Geremew et al. [42], Shahrier et al. [73], and Jim et al. [80]. Premarathna et al. [74] reported a lower value of ash content compared to our finding. The variation might be associated with differences in geographical location, species, and season of sampling [81]. However, the value was within the acceptable range of 0.6 %–1.5% [78], suggesting *O. niloticus* fillet as a good source of minerals for consumers.

3.1.3. Fat content

The mean fat content was 1.88 ± 0.53%, ranging from a minimum value of 1.28% to a maximum value of 2.99%. The fat content in this study was higher than that of Olopade et al. [70], who reported 0.53% in Nigeria, and Geremew et al. [42], who reported 0.6% in the same lake (Ethiopia), but lower than that of Desta et al. [72], Shahrier et al. [73], Suwannatrai et al. [71], and Jim et al. [80], who reported 3.98%, 2.92%, and 2.44%, respectively. These variations might be due to differences in locations, maturity, water temperature, season, and food availability at the site [82,83]. According to Ackman's [84] categories of fish based on their fat content, *O. niloticus* can be grouped as a lean fish whose fat content is <2%.

3.1.4. Crude protein

The mean crude protein content was 14.04 ± 1.15%, ranging from a minimum value of 12.28% to a maximum value of 15.94%. The finding revealed that *O. niloticus* is a good source of protein compared to its fat and mineral contents. The value is comparable with that of Olopade et al. [70], Suwannatrai et al. [71], and Jim et al. [80], but lower than that of Geremew et al. [42] and Shahrier et al. [73] reports. According to Ryu et al. [85], the protein content in fish muscle generally ranges from 15% to 25%. The variation might be associated with age, size, season, feeding, and habitat [86].

3.1.5. Salt content and water activity

The mean salt content was 0.14 ± 0.04%, ranging from a minimum value of 0.07% to a maximum value of 0.09%. This suggests that the salt content of fresh *O. niloticus* fillet is low, and it might not be a concern for fish consumers with special health conditions like hypertension. However, a lower salt content and a higher water activity (0.9869 ± 0.0011) in the fillet might provide a suitable environment for microbial growth and contamination.

3.2. Microbial quality and safety analysis

3.2.1. Aerobic mesophilic bacteria count

The average aerobic mesophilic bacteria count in fresh *O. niloticus* fillet samples was 6.30 ± 0.16 log CFU/g. An ANOVA test revealed that there was no significant statistical difference in aerobic mesophilic bacteria count among the landing sites around Lake Tana ($p > 0.05$) (Fig. 2). This indicates a general lack of sanitation practices in fish handling and processing around Lake Tana. Such poor handling can allow naturally occurring bacteria (inherent to the fish) to multiply and might also introduce additional microbial contaminants to the product. The microbial load recorded in this study was above the international standard limit set for fresh fish [53] (Table 3), implying a lack of proper handling and a risk of illness if consumed undercooked. This might be due to the traditional fish processing and handling practices in Ethiopia [9,11,17,19]. The high microbial load in this study might result in a shorter shelf-life of the fillet and susceptibility to spoilage and contamination. Therefore, proper handling, hygiene, and preservation practices are needed. The load was higher than that of Monteiro et al. [87], Rodrigues et al. [88], Saadia [89], Onjong et al. [90], and Wendwesen et al. [91] reports. However, it was lower than that of the Admasu et al. [92] report, while it was comparable with the load reported by Abelti [93], Eltholth et al. [94], and Sarkar et al. [95]. The variation might be associated with differences in available facilities,

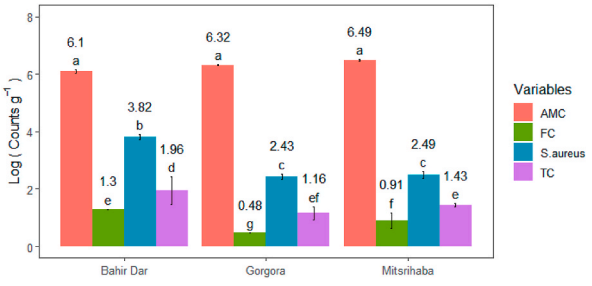

Fig. 2. The microbial counts per gram of fresh *Oreochromis niloticus* fillet samples at three landing sites in Lake Tana (2023). Lowercase letters (a–g) indicate the statistical difference of microbial counts among the sites, ANOVA ($p < 0.05$, $n = 15$). Abbreviations: AMC = Aerobic Mesophilic Count (cfu/g), FC = Fecal Coliforms (MPN/g), TC = Total Coliforms (MPN/g).

Table 3
The mean microbial counts of fresh *Oreochromis niloticus* fillet sample (n = 15) in Lake Tana (2023) and the acceptable limits set by the International Commission for Microbiological Specifications for Food (ICMSF) [53].

Loads	Mean ± SD (current study)	Acceptable limits [53]
Aerobic mesophilic counts (CFU/g)	6.30 ± 0.16	<5.5
Staphylococcus aureus (CFU/g)	2.91 ± 0.67	<3
Total coliform counts (MPN/g)	1.51 ± 0.44	<2
Fecal coliform counts (MPN/g)	0.89 ± 0.37	<1.04

environmental conditions, storage practices, and processing operations [96,97]. In our study, all assessed sites need to practice proper fish handling, establish a cold chain system, and educate fish handlers to enhance fish quality and consumers' confidence.

3.2.2. *Staphylococcus aureus* count

The average load of *S. aureus* was 2.91 ± 0.67 log CFU/g, and it was below the limit set by the ICMSF [53]. A significantly higher count was recorded at the Bahir Dar site compared to the Gorgora and Mitsrihaba sites (Fig. 2). The site variation might be associated with different factors like geographical locations, water quality, harvest and post-harvest handling, and processing practices. The presence of *S. aureus* in the sample indicates cross-contamination of the fillet product due to poor fish handling practices among fish handlers. *S. aureus* is not considered as part of the normal fora of fish [15], and its presence in Nile tilapia fillets likely reflects cross-contamination due to poor fish handling practices among fish handlers. Therefore, avoiding fish handling with bare hands, cleaning surfaces of processing materials, and regular monitoring of food safety practices might decrease cross-contamination of the fish product in the value chain. The load in this study was comparable with that of Admasu et al. [92] and Onjong et al. [98], but lower than Rondón-Espinoza et al. [99] and Pohoroo and Ranghoo-Sanmukhiya [100]. Further research on identifying the source of *S. aureus* contamination is needed for developing effective management strategies that can enhance food safety and minimize the health risks associated with *S. aureus* in fish. This is because the consumption of raw fish in some parts of Ethiopia is common, as reported by Bedane et al. [101].

3.2.3. Total and fecal coliform counts

The mean total and fecal coliform counts were 1.51 ± 0.44 and 0.89 ± 0.37 log MPN/g, respectively. There was a significant mean difference in total and fecal coliform counts among sites ($p < 0.05$), with the highest count recorded at the Bahir Dar landing site (Fig. 2). The variation might be associated with differences in human and urbanization exposure of the landing sites. For example, the high urban pressure from Bahir Dar City leads to direct waste discharge into the lakeshore, which results in fecal contamination of the lake water [102,103]. The count value in this study was comparable to that of the Admasu et al. [92] and Quaiyum et al. [104] reports, but lower than that of Onjong et al. [90] and Eltholth et al. [94]. Fecal contamination of fish was also reported by Lerma-Fierro et al. [105], Sarkar et al. [95], Mitiku et al. [43], and Rondón-Espinoza et al. [99]. This contamination can lead to serious health issues in humans, including gastrointestinal infections and other diseases. Therefore, regular monitoring of the lake water and fish products for fecal indicators is essential to minimize risks to public health. In addition, governments at national and local offices should strengthen policies and regulations related to water quality and fish safety.

3.2.4. *Salmonella and Shigella spp.*

The samples tested positive for both *Salmonella* and *Shigella* spp., indicating significant health risks to consumers that might lead to outbreaks of foodborne illness unless the fish is cooked properly. As mentioned by Fernandes et al. [106], *Salmonella* and *Shigella* can be introduced into fish products through contact with contaminated water or due to inadequate handling and processing practices. Similarly, Mitiku et al. [43] also detected *Salmonella* and *Shigella* spp. in the same lake, even though the occurrence was higher in the present study, where all of the samples were contaminated. Admasu et al. [92] and Wendwesen et al. [91] also reported the presence of *Salmonella* spp. in Hawassa and Arba Minch, Ethiopia. The prevalence of *Salmonella* and *Shigella* contamination in Nile tilapia was also reported in Kenya [90]. The finding from this study revealed the need for educating fish handlers about proper hygiene practices like regular handwashing and cleaning processing materials to avoid cross-contamination. In addition, efficient cooking of the fillet before consumption is highly recommended.

Table 4
The mean concentration of selected heavy metals (mg/kg, dry weight) in the muscle tissue of *Oreochromis niloticus* in Lake Tana (2023) and the acceptable limits set by different international standards.

Heavy metals	Current study (Mean ± SD)	Acceptable limits (mg/kg)				
		FAO [107]	WHO [108]	FAO/WHO [109]	EC [110]	USEPA [111]
Cr	0.165 ± 0.037	0.15–1.0	0.15	–	–	8
Cd	0.010 ± 0.009	0.05	0.5	0.5	0.05	–
Pb	0.054 ± 0.019	0.2	0.5	0.5	0.3	–
As	0.085 ± 0.003	1	1	1	–	–

Note: WHO = World Health Organization; USEPA = United States Environmental Protection Agency; FAO = Food and Agricultural Organization; EC = European Commission.

3.3. Heavy metal analysis

The mean length and weight of *O. niloticus* were 22.21 ± 1.70 cm and 247.13 ± 37.68 g, respectively, and statistically did not differ among sites ($p > 0.05$). The mean concentrations of selected heavy metals in the tissue samples decreased in the order of Cr > As > Pb > Cd (Table 4).

3.3.1. Chromium

The mean concentration of Cr was 0.165 ± 0.037 mg/kg dry weight, and it was above the acceptable limit set by WHO [108] but within the limits set by FAO [107] and USEPA [111] (Table 4). The mean value of Cr in this study was lower than that of Kindie et al. [41] in the same lake (Ethiopia), Tessema et al. [112] in Lake Koka (Ethiopia), and Hossain et al. [113] in the lower Meghna River (Bangladesh), but higher than the value reported by Samuel et al. [114] in Lake Hawassa (Table 5). The variation might be related to differences in the geographical location of sites, different human exposures to the lakes, and the methods or instruments used for analysis. As shown in Fig. 3, there is a statistically significant difference in the concentration of heavy metals among the sites ($p < 0.05$). The variation among sites might be associated with differences in anthropogenic activities and agricultural and urbanization influences [116]. Specifically, the concentration was higher at the Mitsrihaba site than at the Bahir Dar site, which has increased urbanization influence on the lake. The reason might be associated with the natural conditions and the agricultural activities in and around the Mitsrihaba site.

The source of chromium might be from anthropogenic activity or naturally from the environment. Anthropogenic sources include solid waste disposal, industrial, and agricultural activities [117]. We believe that the potential chromium source in Lake Tana might be the combination of untreated municipal wastes and agricultural activities. This is because Lake Tana has a large catchment area that includes areas with intensive agricultural activities [118,119], and large cities like Bahir Dar and Gondar with small and large-scale industries working on metal, chemical, and wood processing. As a result, runoff from agricultural land and discharge of untreated industrial and municipal waste into this lake might introduce heavy metals, including chromium, which later entered and accumulated in the fish through the food chain. For example, about 50% of Cd and Cr inputs in the soil of agricultural land were from mineral fertilizers [120]. Cr is a priority heavy metal, particularly in agricultural regions, due to its common presence [121]. In addition, natural leaching of chromium from rocks and topsoil might enter the lake and contribute as a source.

Our result revealed the bioaccumulation of Cr in muscle tissues of *O. niloticus*, suggesting a risk to fish consumers that might cause different health-related problems, including organ damage [33,34]. Especially hexavalent chromium (Cr (VI)) long-term exposure in higher concentrations can lead to carcinogenicity (risk of lung, bladder, and stomach cancer), and it is classified as a class A carcinogen [122]. In addition to the carcinogenic effect, it can also cause DNA damage and genomic instability [123]. To address this issue, measures must be taken to prevent the contamination of water bodies through the proper utilization of agricultural inputs like pesticides and fertilizers and also through the proper disposal of waste. Furthermore, the continuous monitoring and regulation of chromium levels in the aquatic environment and fish is necessary to minimize its health risks.

3.3.2. Cadmium

The mean concentration of Cd was 0.010 ± 0.009 mg/kg dry weight, and there was no significant mean difference among sites ($p < 0.05$) (Fig. 3). The concentration of Cd in this study was below the acceptable limits set by FAO [107], WHO [108], FAO/WHO [109], and EC [110] (Table 4). The value was also lower than that of the Tessema et al. [112], Hasanein et al. [124], Morshdy et al. [125], Mendoza et al. [126], and Oyeleke et al. [127] reports. This might indicate less exposure of Lake Tana fishing sites and fish to mining activities and Cd containing fertilizers, which are anthropogenic sources of Cd [128]. However, the value recorded in this study was higher than that of the Ishak et al. [129] report. Contamination of edible fish species with cadmium was also reported by Ustaoglu and Yuksel [130]. Chronic exposure of cadmium through fish consumption might increase the risk of kidney, lung, and bone damage even at low concentrations. Therefore, continuous monitoring of cadmium levels in various fish species and strengthening regulations regarding industrial waste discharges into the lake are needed to minimize the health risk to fish consumers.

3.3.3. Lead

The mean concentration of Pb was 0.054 ± 0.019 mg/kg dry weight, and it was below the acceptable limit set by most international standards. The mean value in this study was lower than that of Tessema et al. [112] in Ethiopia, Hasanein et al. [124], and Morshdy

Table 5
Comparison of selected heavy metals concentration in the muscle tissue of *Oreochromis niloticus* in different Ethiopian water bodies (mg/kg).

Ethiopian water bodies	Heavy metals				
	Cr	Cd	Pb	As	References
Lake Tana	0.165	0.010	0.054	0.085	Current study (2023)
Lake Koka	15.76	0.34	2.68	NA	Tessema et al. [112]
Lake Hawasa	0.14	NA	0.01	0.31	Samuel et al. [114]
Gilgel Gibe Dam	0.80	<0.01	<0.2	NA	Bayissa et al. [115]
Lake Ziway	0.93	<0.01	<0.2	NA	Bayissa et al. [115]
Lake Langano	0.96	<0.01	<0.2	NA	Bayissa et al. [115]

Abbreviation: NA = not assessed.

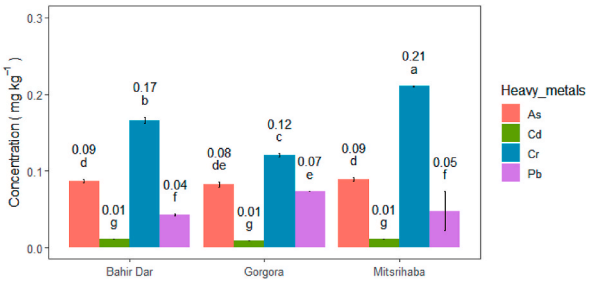

Fig. 3. The concentration of selected heavy metals in fresh *Oreochromis niloticus* fillet at three landing sites in Lake Tana (2023). Lowercase letters (a–g) indicate the statistical difference in heavy metal concentration among the sites, ANOVA ($p < 0.05$, $n = 15$). Abbreviations: Cr = Chromium, Cd = Cadmium, Pb = Lead, and As = Arsenic.

et al. [125] in Egypt. However, Mendoza et al. [126] did not detect lead in their report, while Ishak et al. [129] and Samuel et al. [114] reported a lower value compared to our findings. Considerable levels of lead in edible fish species were also reported in Turkiye [130–133]. There was a significant difference in the mean values of Pb between Gorgora and the rest two landing sites ($p < 0.05$), but the Tukey HSD post hoc test for multiple comparisons found that the mean value of Pb was not significantly different between Bahir Dar and Mitsrihaba landing sites ($p > 0.05$) (Fig. 3). Even though Pb is an urban pollutant in the chemical industry [134,135], the result of this study revealed an insignificant mean difference in Pb concentration between urban-influenced areas (Bahir Dar site) and agricultural-influenced areas (Mitsrihaba site). However, the value was higher at the Gorgora site, which might be associated with the nearby Megech River mouth, where it is receiving much more pollutants from Gondar town. Long-term lead exposure, even at a low level, can affect the kidneys and cognitive functions, especially in children and pregnant women [136]. Therefore, efforts should be made to minimize the release of lead contaminants into the ecosystem through regulatory measures and public education.

3.3.4. Arsenic

The mean concentration of As was 0.085 ± 0.003 mg/kg dry weight, and it was below the acceptable limit set by FAO [107], WHO [108], and FAO/WHO [109] (Table 4). There was no significant As mean difference among sites ($p > 0.05$) (Fig. 3). In comparison, Mendoza et al. [126] did not detect arsenic in their report, while Hasanein et al. [124] and Samuel et al. [114] reported higher values compared to our finding. Since chronic arsenic exposure increases the risk of cardiovascular diseases, neurological effects, and developmental delays in children [137,138], regular assessment of arsenic levels in fish and strengthening regulations on industrial activities and agricultural inputs are needed.

3.4. Human health risk assessment of heavy metals

3.4.1. Estimated daily intake (EDI)

For adults, the mean estimated daily intake for Cr, Cd, Pb, and As was 0.0117, 0.0007, 0.0038, and 0.0061 mg/kg of body weight per day, respectively (Table 6). The EDI values were below the tolerated daily intake (TDI) set by the joint FAO/WHO [139], which is 0.003 mg kg^{-1} day^{-1} for Cr, 0.001 for Cd, 0.0015 for Pb, and 0.003 for As. The values lower than TDI guidelines suggest a lower possible health effect of the metals on consumers, even though it depends on the quantity of fish consumed daily and the average body weight of individuals. This is in line with Tesema et al. [112] report in Lake Koka (Ethiopia) and Ustaoglu and Yuksel [130] in Black Sea Lagoon lakes (Turkiye).

Table 6
Estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and cancer risk (CR) values of selected heavy metals from the consumption of *Oreochromis niloticus* muscle tissue in Lake Tana.

Risk assessment		Landing sites		
	Heavy metals	Gorgora	Bahir Dar	Mitsrihaba
EDI	Cr	0.0085	0.0118	0.0149
	Cd	0.0006	0.0008	0.0008
	Pd	0.0052	0.0030	0.0034
	As	0.0058	0.0062	0.0063
THQ	Cr	0.0028	0.0039	0.0050
	Cd	0.0006	0.0008	0.0008
	Pd	0.0035	0.0020	0.0023
	As	0.0194	0.0205	0.0210
CR	Cr	4.26×10^{-6}	5.89×10^{-6}	7.46×10^{-6}
	Cd	4.02×10^{-6}	4.91×10^{-6}	4.91×10^{-6}
	Pd	4.41×10^{-8}	2.59×10^{-8}	2.88×10^{-8}
	As	8.71×10^{-6}	9.25×10^{-6}	9.44×10^{-6}
HI		0.0263	0.0273	0.0290

3.4.2. Target hazard quotient (THQ) and hazard index (HI)

The mean THQ value for Cr, Cd, Pb, and As was 0.0039, 0.0007, 0.0026, and 0.0203, respectively. The hazard index (HI) for the assessed heavy metals was <1, as shown in Table 6. The finding revealed that the THQ and HI values were below the acceptable limit set by USEPA [140], which indicates no potential non-carcinogenic human health risks from ingestion of these metals through *O. niloticus* from Lake Tana. Similarly, Samuel et al. [114] in Lake Hawassa (Ethiopia) and Ustaoglu and Yuksel [130] in black sea Lagoon lakes (Turkiye) reported THQ and HI values below 1 for Cr, Pb, and As in fish.

3.4.3. Cancer risk (CR)

The mean CR value for Cr, Cd, Pb, and As was 5.87×10^{-6}, 4.61×10^{-6}, 3.29×10^{-8}, and 9.13×10^{-6}, respectively. According to USEPA [140], a cancer risk value lower than 10^{-6} is negligible, above 10^{-4} is unacceptable, and a value between 10^{-4} and 10^{-6} is acceptable. That means the carcinogenic risk values of Cr, Cd, and As in our finding were acceptable, and for Pb it was negligible. The finding revealed that there is no human carcinogenic risk from consumption of *O. niloticus*, even though monitoring of the heavy metals level needs to be continued.

4. Conclusion

In conclusion, the findings from this study provide an overview of the nutritional content of the fresh *O. niloticus* fillet, which is essential for the growth and development of the human body. However, our result revealed microbial and heavy metal contamination of the fillet, suggesting a risk to fish consumers that might cause foodborne illness, organ damage, cancer, and cognitive effects. Long-term exposure to heavy metals, even at low concentrations, might pose significant health risks like kidney, lung, and bone damage. However, the human health risk assessment value of heavy metals suggests a lower possible health effect of the metals on consumers, even though it depends on the quantity of fillet consumed daily and the average body weight of individuals. To address contamination issues and the associated human health risk, tracing the source of contamination, strengthening regulations on waste disposal, continuous monitoring of contamination levels, establishing a cold chain system and sanitation protocols, and educating fish handlers in the value chain are highly recommended.

CRediT authorship contribution statement

Solomon Birie: Writing – original draft, Investigation, Conceptualization. **Minwyelet Mingist:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Mulugeta Kibret:** Writing – review & editing, Visualization, Conceptualization. **Tadlo Yitayew Atlog:** Writing – review & editing, Methodology, Investigation. **Hirut Geremew:** Writing – review & editing, Investigation. **Banchiamlak Getnet:** Writing – review & editing, Investigation. **Dagnew Mequanent:** Writing – review & editing, Investigation.

Data availability statement

The authors declare that data are available from the corresponding author upon request.

Ethical approval statement

The authors have collected fish tissue samples from the fish caught by fishers, and we certify that this study followed all the applicable guidelines for the care and use of fish.

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Declaration of competing interest

The authors declare no conflict of interest.

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