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Pesticide concentration in three selected fish species and human health risk in the Lake Tana sub-basin, Ethiopia

Pesticide concentration in three selected fish species and human health risk in the Lake Tana Sub-basin, Ethiopia

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Abstract

Pesticide use has increased in the Lake Tana sub-basin due to increased agricultural activity, potentially endangering nontargeted organisms. To assess its potential impact on fish health and fish-consuming human populations, pesticide concentrations in the fillet and liver tissue of three fish species, namely *Labeobarbus megastoma*, *L. tsanensis,* and *Oreochromis niloticus*, were investigated in Lake Tana. Fish samples were taken from the lake near the rivers of Ribb and Gumara, which flow through agricultural areas where considerable amounts of pesticides have been applied. A total of 96 fish samples were collected. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) revealed the presence of ten pesticides. Pyrimethanil was frequently detected in 96 % of liver and 65 % of fillet samples at a median concentration of 33.9 µg kg^{-1} and 19.7 µg kg^{-1}, respectively. The highest concentration of pyrimethanil was found in *L. megastoma* (1850.0 µg kg^{-1}). *Labeobarbus megastoma* also had the highest concentration of oxamyl (507.0 µg kg^{-1}) and flazasulfuron (60.1 µg kg^{-1}) detected in the liver tissue. The highest concentration of carbaryl (56.5 µg kg^{-1}) was found in the liver tissue of *O. niloticus*. Fish tissue samples from the two study sites contained pyrimethanil, oxamyl, carbaryl, and flazasulfuron. Only pyrimethanil showed a statistically significant difference between the two sites and the species *L. megastoma* and *L. tsanensis*. The amounts of pesticides found in the fish species pose no direct risk to the health of fish consumers human population. However, the results show that the lake ecosystem needs immediate attention and regular monitoring of the rising pesticide usage in the lake watershed.

Keywords: Lake Tana, pesticide, *Labeobarbus*, *Oreochromis*, health risk, toxicity

Introduction

Pesticides have been used in agriculture for a long time to protect crops from weeds, pests, and diseases while increasing crop yields (Lengai et al., 2020; Sabzevari & Hofman, 2022). They have also been used to reduce crop deterioration during storage and extend shelf life, ensuring food security for the world's ever-growing population (Sharma et al., 2019). About 4.2 million tons of pesticides, including herbicides, insecticides, and fungicides, are utilized worldwide annually (FAO, 2021). In developing countries such as Ethiopia, the government has encouraged farmers to use pesticides and fertilizers in smallholder farming (WHO & FAO, 2019). However, because farmers and agricultural workers frequently lack proper personal protection equipment and may not understand the labels with safety instructions, human health and environmental hazards associated with pesticide use are commonly noticed in those nations (Sarkar et al., 2021).

Although pesticides improve crop yield, their extensive use produces residual effects on food products. Moreover, they could develop problems associated with chemical buildup in aquatic organisms, including fish, and negatively impact aquatic life and human health (Amenyogbe et al., 2021). They potentially affect the growth and reproductive efficiency of non-targeted organisms due to their bio-magnification and persistent nature (Deribe et al., 2013). Some pesticides, such as organochlorine pesticides (OCPs), can last for many years in the environment, are deposited in sediments (MacKay & Fraser, 2000; Deribe et al., 2013), and concentrated in the top predators of food chains (Di et al., 2017). As a result, most pesticide-related ecosystem studies focused on those OCPs (Marchand et al., 2010; Sifakis et al., 2017; Yohannes et al., 2017; Worku et al., 2022). Organophosphate pesticides (OPPs) and other synthetic pesticides, which are less persistent than OCPs, are also widely used by farmers worldwide (Manuelmolina-Ruiz et al., 2014). Most of them are water-soluble and taken up by organisms through skin contact, inhalation, or ingestion as food particles. As a result, these pesticides are hazardous to aquatic species, and their residues might affect the phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve terminals in non-targeted animals, causing malfunctioning (Gultekin et al., 2000; Kushwaha et al., 2016). Pesticides can limit the food sources available to fish through their toxicity effect on invertebrate prey, potentially reducing fish growth and survival. At high concentrations in water bodies, pesticides can affect fish by changing their growth rates, progeny survival, and behavior. This influences fish population stability and structure (Holden, 1972). Toxicity effects on fish can also lead to histological and hormonal changes in females, which may reduce the number of oocytes produced (Forsgren et al., 2013).

The fish species of the genus *Labeobarbus* are the most numerous and endemic to Lake Tana. Two of the five *Labeobarbus* species most frequently caught for commercial fishing are *L. tsanensis* and *L. megastoma* (Gebremedhin et al., 2019). *Labeobarbus megastoma* is a piscivores fish that dominantly feeds (up to 67%) on fish, while *L. tsanensis* is feeding dominantly on benthos 43%, 18% mollusks, and 14% detritus/substratum (Sibbing & Nagelkerke, 2001). Similarly, *Oreochromis niloticus*, a highly commercially demanded species in the lake (Nagelkerke et al., 1995; Vijverberg et al., 2013), is an omnivorous fish that feeds on plankton and aquatic plants (Tesfahun & Alebachew, 2023).

The Lake Tana sub-basin, one of Ethiopia's most productive places, is suitable for growing a variety of staple food crops, including Teff (*Eragrostis tef*), Finger millet (*Eluesine coracana*), rice (*Oryza sativa*), maize (*Zea mays*), grass pea (*Lathyrus sativus*) and chickpea (*Cicer arietinum*), as well as cash crops like Khat (*Catha edulis*) and other vegetables (tomato, cabbage, onion, garlic, potato, pepper, etc.) (Abera, 2017). Recently, pesticide consumption has increased due to increased agricultural activity in the area, particularly in irrigation-based dry season cash crops and vegetable cultivations (Agmas & Adugna, 2020; Abera et al., 2022). Farmers spray various pesticides on those crops and vegetables in the field and at their home storage, but they are usually not fully informed or aware of the dangers of the pesticides they use (Agmas & Adugna, 2020). Improper spraying techniques, dropping empty containers into water bodies, careless disposal, or unintentional spills of residual solutions have all been noted as contamination routes in the catchment (Agmas & Adugna, 2020). Additionally, the lake has a large catchment area (approximately 16,750 km^2), and the process of pesticides leaching through soil erosion and surface runoff increases environmental hazards and may have detrimental effects on the lake's biodiversity. For example, due to brain, endocrine, behavioral, and genetic abnormalities, as well as histopathological and hematological changes, fish species may not be able to grow and reproduce normally (Kumari, 2020; Srivastava et al., 2016). Furthermore, pesticide residues may pose a health risk to human communities that consume fish (Agmas & Adugna, 2020; Abera et al., 2022). Despite the increasing use of pesticides in the lake catchment, no research has been done yet to determine the concentrations and the possible harmful impacts on non-target organisms except a recent work by Abera et al. (2022), and to the best of our knowledge, there was no work done on the concentration and effect of pesticide residues, particularly on the economically important and endangered fish species in the lake. Therefore, this study aimed to 1) determine the concentration of pesticides routinely used in the fillet and liver tissue of three commercially important fish species in the study area, 2) assess any potential impact of pesticide residues on fish health and 3) assess the effect of the measured pesticides on the health of fish-consuming communities.

Materials and Methods

Study area

Lake Tana and its tributaries, such as Ribb and Gumara (Fig. 1), provide important ecosystem services to people living in the catchment. The two river watersheds are the most important agricultural areas for most staple crops through smallholder irrigation farming. These water bodies supply drinking water for humans and animals. They are also important for fisheries. *Labeobarbus* species, *Oreochromis niloticus,* and *Clarias garipinus* are commercially important and frequently consumed fish species in the research area. People living near the lake and along the river use rainwater to grow crops and vegetables during the rainy season. While in the dry season, farmers use the lake and river waters through furrow and pump irrigation.

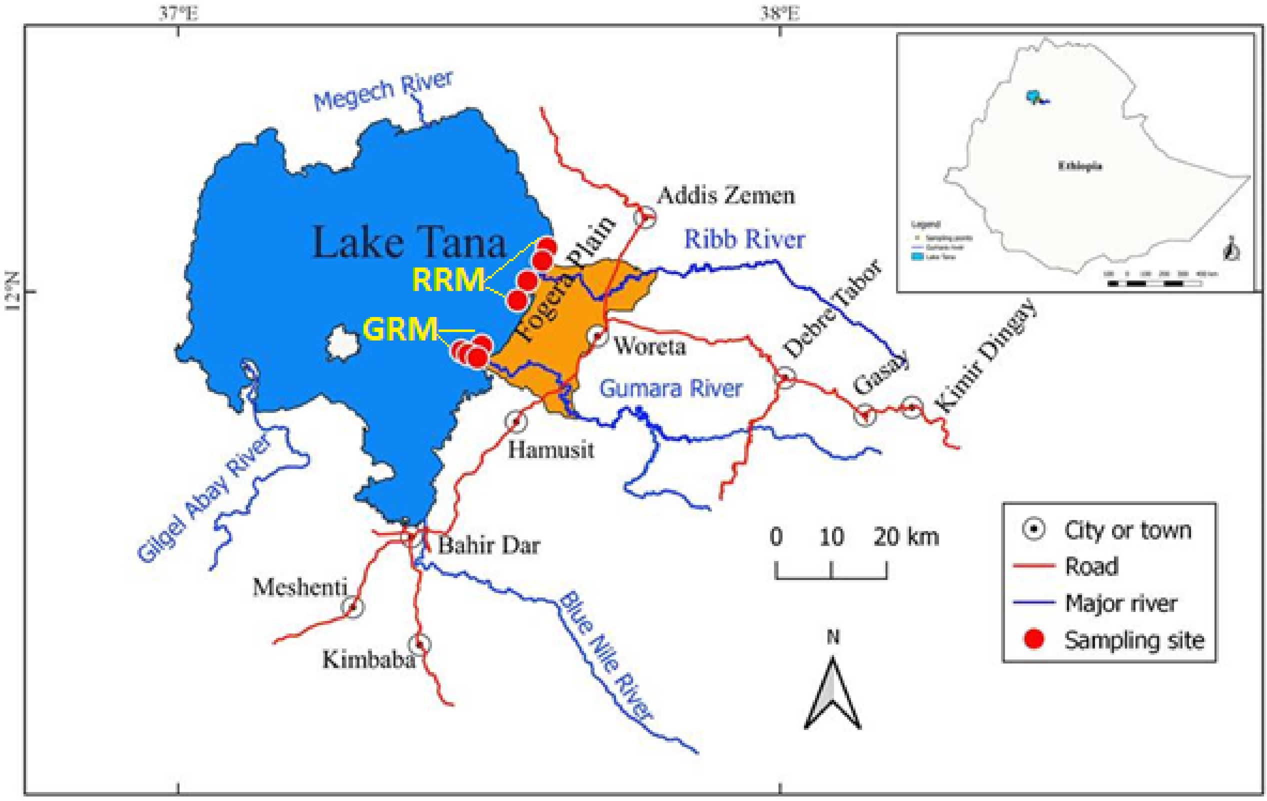

Fig. 1 Map of the study locations in Lake Tana and sampling points (Red circles) at the Ribb River Mouth (RRM) and Gumara River Mouth (GRM) sites.

Fish sampling and preparations

Fish samples were collected from Lake Tana's shore near the mouths of the Gumara and Ribb Rivers. Sampling was executed between September and October 2021 using multifilament gillnets (10, 12, and 14 cm mesh sizes) and catches from fishers at the sampling points. Specimens of three fish species, *Labeobarbus tsanensis*, *L. megastoma,* and *Oreochromis niloticus,* with a weight of more than 200 grams and with a total length of at least 23 cm were identified and selected (Table 1). Tissue samples were collected based on the approach stated by Rosseland et al. (2001). Each fish sample was extracted with stainless steel disposable scalpel blades with a handle, yielding around 50 g (wet weight) of the fillet (white meat taken from the dorsal muscle) and 2 to 5g of the liver. Samples were wrapped in aluminum foil, frozen (-20^{0}C), and transported to Belgium in an ice-filled cooling cabinet. The pesticide analysis was done in the laboratory for phytopharmaceuticals at Ghent University.

Each fillet and the liver sample were homogenized for 10 minutes with an Ultra-Turrax (T 25 ultra Turrax - IKA). The homogenizer was cleaned with acetone after each homogenization to prevent cross-contamination. Based on the analyses' weight requirements, samples were weighed and stored in falcon tubes.

Table 1 Biometric data and feeding mode of the three fish species sampled from Lake Tana between September and October 2021.

Fish species	Feeding mode	Gumara river mouth			Ribb river mouth		
		Total Length (cm)*	Weight (g)*	n	Total Length (cm)*	Weight (g)*	n
L. megastoma	Piscivore	29.7 – 42.5	205 - 615	8	31.7 – 43.5	285 - 700	8
L. tsanensis	Insectivore	30.5 – 39.5	270 - 670	8	30.1 – 35.2	250 - 445	8
O. niloticus	Herbivore	23.2 – 26.4	220 - 295	8	23.5 – 26.5	220 - 290	8

* minimum-maximum values, n: number of samples analyzed.

Pesticide Analysis

Based on data from farmers and district (locally called woreda) agricultural experts on local pesticide use in 2019 and 2020, a list of 109 relevant pesticide products was prepared for the multi-residue analysis before the pesticide study. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with the setting defined in Table S2 (supplementary material) was used to detect and quantify all of the screening pesticides (n = 109) (Table S1 (supplementary material)). The fish tissue samples were extracted using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach. This approach has a small number of steps, is highly reliable, and yields excellent recoveries for a wide range of pesticides from various chemical families (Kim et al., 2019).

In a QuEChERS tube, 10 ml of acetonitrile was added to 10 g of fillet sample and agitated for one minute. Subsequently, a salt solution was added (6 g magnesium sulfate, 0.75 g citrate sesquihydrate, 1.5 g citrate dihydrate, 1.5 sodium chloride), and the tube was vigorously shaken. The sample was then homogenized for 2 minutes in an ultra-turax mixer before being centrifuged for 5 minutes at 3,000 rpm. In a 10 ml flask, 1 ml of the supernatant was diluted in 9 ml ultrapure/miliQ/ water, and 1.5 ml of the solution was then transferred to a vial for LC-MS/MS analysis. The liver samples were extracted by adding 10 ml of acetonitrile to 2 – 5 g of material and then adding water to a total mass of 10 g. The falcon tubes were shaken for 1 minute before adding the salt mixture. The remaining stages were the same as the fillet, except for a clean-up phase. As a result, 7 ml of the supernatant was transferred to an SPE tube, which was agitated for 5 minutes before being centrifuged at 3000 rpm for 5 minutes. In a 10 ml flask, 1 ml of the supernatant was diluted in 9 ml ultrapure (miliQ) water. Finally, 1.5 ml of the sample was subsampled into a vial for pesticide determination.

Method validation

The minimal concentration at which the analyte was identified and established by comparing measured signals from samples with known low amounts of the analyte with those of blank samples. The present method was developed and validated using the ICH Q2(R1) guideline. The pesticide residues in the fish tissue samples were validated and quantified using a calibration curve from samples spiked with the multi-compound stock standard solution. The linear range of the calibration curve was developed using five concentration levels between 0.001 and 0.1 mg l^{-1}. The spike-placebo recovery method was used to determine the validation of the analyte's lowest detectable concentration in a sample (limit of detection or LOD) and the lowest confirmed level with adequate precision and recovery (limit of quantification or LOQ). A blank sample was spiked and analyzed under the same conditions in four replicates. The LOD and LOQ values for the pesticide analysis in LC-MS/MS were set as 0.0003 mg l^{-1} and 0.00086 mg l^{-1}, respectively.

Human health risk assessment

A human health risk assessment was conducted to evaluate if the pesticides found in the lake threatened those who included fish in their diet. The Acute Reference Dose (ARfD) and Acceptable Daily Intake (ADI) were utilized as expected, with no impact levels for acute and chronic pesticide exposure. The Estimated Daily Intake (EDI) for a given pesticide was calculated using the following formula (Mahmood & Malik, 2014; Hamid et al., 2017)

$$\mathrm{EDI} = \frac{C_p \times F_c}{bw} \qquad (1)$$

Cp is the maximum pesticide residue concentration in white fish meat in µg/kg wet weight. Fc is the amount of fish food consumed by a person per day in kg/day. bw is the fish-consuming adult in kg.

The hazard quotient (HQ) for acute and chronic estimations was performed using the following formula (Hamid et al., 2017)

$$HQ_{acute} = \frac{EDI}{ARfD} \qquad (2)$$

$$HQ_{chronic} = \frac{EDI}{ADI} \qquad (3)$$

For the Ethiopian community who live around the water bodies and have high access to fish in their diet, the average daily intake rate was taken as 0.03 kg/day (Yohannes et al., 2014) and assumed the average body weight of 60 kg for an adult Ethiopian for the calculation (Teklu et al., 2015). As liver tissue is not consumed in the area, the pesticide concentration in the fillet was only considered for the risk assessment.

The Hazard Index (HI) was taken as the sum of the chronic or acute hazard quotients separately of all pesticides detected in the fish fillet. Pesticide residues could potentially produce acute/chronic health hazards if the percentage of HI is greater than 100 (Lozowicka, 2015).

Data analysis

The Kruskal-Wallis ANOVA test was used to examine differences in pesticide residue concentrations in the tissue among species, and the Mann-Whitney U test between tissue types using the R package version 4.2.0. The Dunn test was used to evaluate any significant differences between the groups. As a result of an uneven distribution of the pesticide concentration data in the fish tissue, the median values were utilized to represent the pesticide concentrations in the different fish species and tissue types. For these statistical comparisons, a pesticide concentration below the LOQ was taken to be half of the corresponding assigned limiting value. Hence the number of > LOQ values recorded among the pesticides detected was very small (< 60%); the deterministic approach was used to determine the exposure of the fish consumers and the hazard quotients by taking an average pesticide concentration for values that had two or greater than two > LOQ values. For the risk assessment, the LOQ cutoff value, 0.00086 mg/kg, was used for fish fillet samples with none > LOQ value or those detected pesticides with < LOQ values as a maximum pesticide concentration, considering the worst-case scenario. The exposure analysis was performed using Microsoft Excel.

Results

Pesticide concentrations in fish samples

Ten pesticides were found in the fish fillet and liver tissue samples of the three fish species under investigation. The pesticide types insecticides and fungicides were found in equal proportion (40% of each) from the detected pesticides, while herbicides constitute 20%. The level of pesticides quantified ranges from below LOQ to 1850.0 µg kg^{-1} wet weight. The pesticide most commonly found and had the highest concentration was pyrimethanil. The difference between the two research sites was statistically significant ($p < 0.001$) due to the higher median concentration of pyrimethanil (49.7 µg kg^{-1}) at the site near the mouth of the Gumara River (GRM) compared to 2.1 µg kg^{-1} at the site near the mouth of the Ribb River (RRM) (Table 2). Pyrimthanil was detected in 65% of fillet samples with a mean concentration of 96.6 µg kg^{-1} and 96% of liver tissue samples with a mean concentration of 104.0 µg kg^{-1} for the three fish species. A statistically significant difference in pyrimethanil concentration between species ($p = 0.043$) was observed, with the highest concentration in *L. megastoma* and the lowest in *L. tsanensis* (Fig. 2 and Table 3). The mean detected concentration of pyrimethanil was much higher in the liver (258.8 µg kg^{-1}) than in the fillet (55.4 µg kg^{-1}) of *L. megastoma*. However, pyrimethanil was detected in higher concentration in the fillet of *O. niloticus* (mean = 359.8 µg kg^{-1}) and *L. tsanensis* (mean = 99.1 µg kg^{-1}) than in the liver tissue of those species (Table 4). Like pyrimethanil, carbaryl was quantified in the fillet and liver tissue of the three species and found in both studied sites. The highest mean concentration of carbaryl (10.4 µg kg^{-1}) was recorded in *O. niloticus*. However, variations among species were not statistically different.

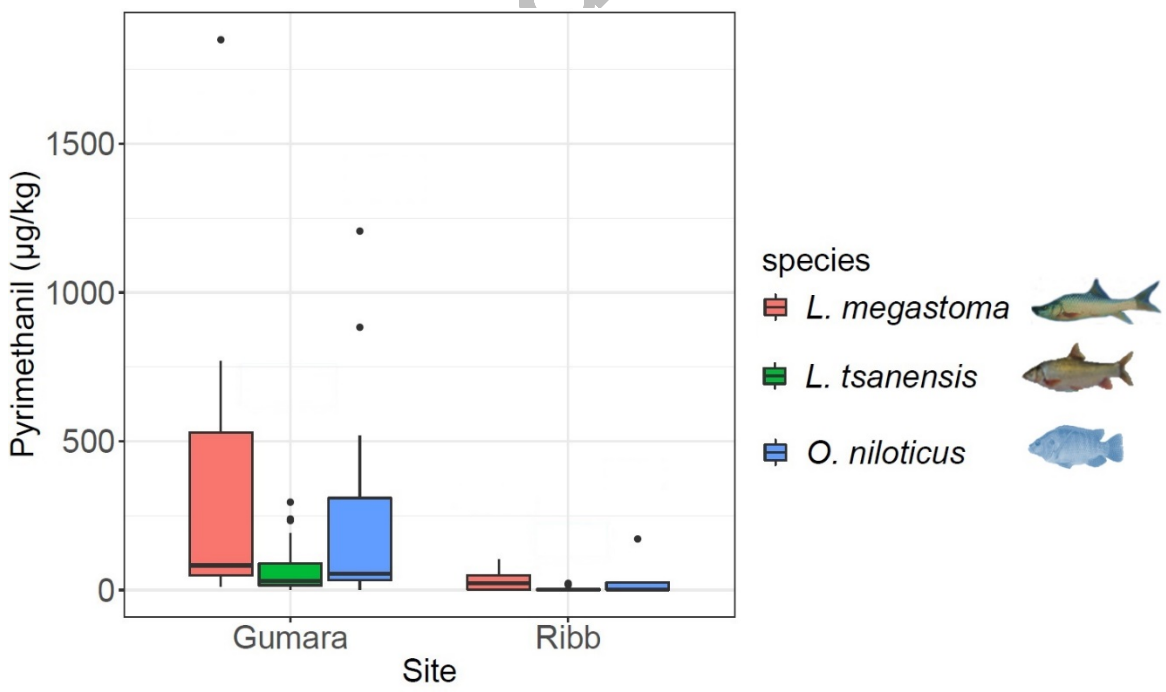

Fig. 2 Boxplot showing pyrimethanil concentration in the tissue of the three studied fish species in the studied sites in Lake Tana.

Table 2 Mean concentrations and range of detected pesticides in Lake Tana fish species sampled between September and October 2021 at the GRM and RRM sampling site.

Pesticide	Pesticide type	GRM Median (µg kg^{-1})	GRM Range (µg kg^{-1})	RRM Median (µg kg^{-1})	RRM Range (µg kg^{-1})
Benalaxyl	Fungicide	2.2*	< LOD – 2.2	< LOD	-
Butachlor	Herbicide	< LOD	-	1.1	< LOD – 1.1
Carbaryl	Insecticide	0.4	0.2 – 56.5	0.4	0.2 – 2.2
Difenoconazole	Fungicide	0.2*	< LOD – 2.2	< LOD	-
Flazasulfuron	Herbicide	2.2	2.2 – 60.1	2.2	< LOD – 2.2
Imidacloprid	Insecticide	< LOD	-	0.9	0.4 – 2.2
Oxamyl	Insecticide	216.5	32.0 – 507.0	24.4	2.2 – 136.9
Pirimiphos-methyl	Insecticide	< LOD	-	0.4*	< LOD – 0.4
Pyrimethanil	Fungicide	49.7	2.2 – 1850.0	2.2	0.4 – 171.7
Tebuconazole	Fungicide	0.2*	< LOD – 0.2	< LOD	-

*quantified only in a single sample; LOD = 0.0003 µg kg^{-1}; LOQ = 0.00086 µg kg^{-1}

Oxamyl and flazasulfuron were detected in both study sites (Table 2). However, oxamyl was found in the tissues of the two *Labeoburbus* species but at a level below LOD in *O. niloticus* (Table 3). Oxamyl was found in *L. megastoma* and *L. tsanensis,* and the highest concentration was detected in the liver tissue of *L. megastoma* (median = 361.7 µg kg^{-1}) (Tables 3 & 4). Though its concentration was small compared to liver tissue, oxamyl was also detected in the fillet of *L. megastoma*, while it was below LOD in *L. tsanensis* and *O. niloticus*. Imidacloprid and flazasulfuron were detected in different quantities in the tissues of the three fish species under investigation at site RRM (Table 5.4), whereas imidacloprid was below LOD at site GRM (Table 3). Imidacloprid was only detected in the liver tissue of *L. tsanensis*; however, it was detected in the fillets of *L. megastoma* and *O. niloticus* at the RRM site (Table 4). However, the concentrations of oxamyl, imidacloprid, and flazasulfuron in neither species nor the tissue types were statistically different.

Table 3. The median pesticide concentration detected in the tissues (fillet + liver) of the three fish species at the two sampling sites in Lake Tana and variations of pesticide types among species is presented in the *p* value.

Sampling site	Pesticide	Pesticide concentrations in fish species (µg kg^{-1}) *L. megastoma*	*L. tsanensis*	*O. niloticus*	*p* value
GRM	Carbaryl	0.2	0.4	4.6	0.041
	Flazasulfuron	2.2	2.2*	2.2	0.446
	Imidacloprid	<LOD	<LOD	<LOD	-
	Oxamyl	361.7	<LOD	< LOD	-
	Pyrimethanil	82.6	30.1	55.1	0.074
RRM	Carbaryl	0.4	-	1.3	0.739
	Flazasulfuron	2.2	2.2	2.2	-
	Imidacloprid	0.4	2.2	0.4	0.223
	Oxamyl	<LOD	24.4	<LOD	-

Pyrimethanil	22.8	2.2	2.2	0.010

* quantified only in a single sample

Benalaxyl, a pesticide that was rarely discovered, was found in the liver tissue of *L. megastoma* at the GRM site (median = 2.2 µg kg^{-1}); nevertheless, difenoconazole was found in the fillet tissue (median = 0.22 µg kg^{-1}) of *L. megastoma*. Similarly, butachlor and pirimiphos-methyl were detected only in RRM. Butachlor was detected in the liver tissue *of L. tsanensis* (median = 1.1 µg kg^{-1}), and pirimiphos-methyl was found in the fillet of *O. niloticus* (median = 0.4 µg kg^{-1}). Tebuconazole was also detected in the fillet of *L. megastoma* in the GRM site (Table 4).

Table 4 Detected pesticide concentration in the tissues of the studied fish species sampled between September and October 2021 at the GRM and RRM sampling site in Lake Tana.

Pesticide	Mean pesticide concentration in µg kg^{-1} wet weight					
	L. megastoma		*L. tsanensis*		*O. niloticus*	
	liver	fillet	liver	fillet	Liver	fillet
Benalaxyl	2.2*	< LOD	< LOD	< LOD	< LOD	< LOD
Butachlor	< LOD	< LOD	1.1	< LOD	< LOD	< LOD
Carbaryl	2.2*	0.2	2.2*	0.4	29.3	3.1
Difenoconazole	< LOD	0.2*	< LOD	< LOD	< LOD	< LOD
Flazasulfuron	2.2	< LOD	2.2	< LOD	2.2	< LOD
Imidacloprid	< LOD	0.2	2.2*	< LOD	< LOD	0.4*
Oxamyl	361.7	32.0*	24.4	< LOD	< LOD	< LOD
Pirimiphos-methyl	< LOD	< LOD	< LOD	< LOD	< LOD	0.4*
Pyrimethanil	15.0	54.1	7.9	35.6	25.4	94.9
Tebuconazole	< LOD	0.2	< LOD	< LOD	< LOD	< LOD

* quantified only in a single sample; LOD = 0.0003 µg kg^{-1}; LOQ = 0.00086 µg kg^{-1}

Human health risk assessment

People eating fish from Lake Tana might be exposed to the seven pesticides detected in the fillet of the three fish species (Table 4). The risk assessment estimation considers all values found in the fillet, including < LOQ values. The highest daily exposure of consumers was recorded for oxamyl (0.016 µg/kg bw/d) (Table 5). The calculated values for HQ acute and HQ chronic were low, with the highest value of 1.60% found for oxamyl. Similarly, the HI values for both HI acute and HI chronic (Table 5) showed a lower value than the threshold value of 100, indicating that adverse non-cancer health effects are not likely to occur or the health risk for the fish meat-consuming community is very low.

Table 5 Adult human exposure to selected quantified (> LOQ) pesticides in the fillet of fish species in the Lake Tana sub-basin. Maximum detected quantity (Cp), Acceptable daily intake (ADI), Acceptable reference dose (ARfD), Estimated Daily Intake (EDI), and the percentage of hazard quotient for acute (HQ acute) and chronic (HQ chronic) cases.

Pesticide	ADI (µg /kg bw/d)	ARfD (µg/kg bw)	Max (µg kg^{-1})	EDI (µg/kg bw/d)	HQ acute (%)	HQ chronic (%)
Carbaryl	7.5	10.0	14.0	0.007	0.07	0.10
Oxamyl	1.0	1.0	32.0	0.016	1.60	1.60
Pyrimethanil	170	1000	1206.6	0.603	0.06	0.35
				HI (%)	**1.73**	**2.06**

Discussion

Detected pesticides and the possible effect on fish species

The development, survival, and reproduction of aquatic animals are known to be impacted when surface waters are contaminated by high pesticide concentrations (Vonesh & Kraus, 2009; Akhter, 2019; Kumari, 2020). It has also been shown that several pesticides in water systems have affected primary and secondary production, including fish species in the food chain (Seeland et al., 2012; Müller et al., 2019). The effect, however, potentially depends on the physicochemical environment and may be ecoregion-specific, long-lasting, or delayed, yet most likely exacerbated by indirect effects resulting from interspecific interactions (Araújo et al., 2012; Müller et al., 2019).

The highest concentration and the frequent occurrence of pyrimethanil in the three fish species in Lake Tana indicate that it is intensively used in the watershed. Pyrimethanil is one of the most widely used fungicides worldwide and has been found in numerous aquatic environments (Lozowicka, 2015; De Cock et al., 2021). It is used mainly on fruits and vegetables and was manufactured to treat resistant fungus strains such as *Botrytis* spp, which affects onions and other vegetables and fruits (Tournas, 2005; Abo-Elyousr et al., 2020). Pyrimethinil is also applied for seed treatment on cereals (EFSA, 2011). It affects algae, zooplankton, and macroinvertebrates which are potential food sources for fish species. For example, for the model organism *Scenedemus acutus* in a 48h chronic test and *Daphnia magna* in a 48-h acute test, the average LC50 was 23.2 mg l^{-1} and 3.6 mg l^{-1}, respectively (Araújo et al., 2012). They also reported that the reproduction of adult *D. magna* was adversely affected when exposed to 0.5 mg l^{-1} of pyrimethanil over many generations under variable temperatures between 20 and 27 °C. Although the pesticide concentration was not directly measured from the water in Lake Tana, the maximum concentration (up to 1.85 mg kg-1) in the fish tissue suggests that the concentration was potentially high. This high concentration of pyrimethanil could affect the normal development of fish organs. According to Bernab et al. (2017), doses up to 50 µgl^{-1} cause a histological alteration in tree frogs (*Hyla intermedia*). The effect of this pesticide also elevated with increasing temperature. For example, Araújo et al. (2012) confirmed that a rise in ambient temperature exacerbated the fungicide's toxicological effects on *D. magna*. Thus, with the relatively high dry season temperature, the effect of pyrimethanil on aquatic invertebrates in Lake Tana might be higher, which could indirectly affect fish species.

Metals and pesticides can be absorbed by aquatic macrophytes and algae (Dosnon-Olette et al., 2010, 2009), and organisms that directly consume those primary sources may have higher concentrations in their tissue. The higher mean concentration of pyrimethanil in the tissue of *O. niloticus* (Table 3) might reflect the assimilation of the fungicide through the food chain. Similarly, the highest concentration in the piscivore species *L. megastoma* (Table 4) could also result from its feeding habit, despite pyrimethanil having less potential in bioaccumulation (Araújo et al., 2015; Lewis et al., 2016).

Flazasulfuron, belonging to the sulfonylurea herbicide family, was detected in 29% of tissue samples from the three fish species with a maximum concentration of 0.06 mg kg^{-1} in *L. megastoma*. This herbicide is known to prevent protein synthesis in plants by blocking branched-chain amino acids protein synthesis (Olette et al., 2008). It has also been shown to impact the chlorophyll pigments and leaf gas exchange (Frankart et al., 2003). While it is not toxic to aquatic animals (US EPA, 2007), it can indirectly have an impact because it might reduce plant and nonvascular aquatic plant productivity (US EPA, 2007). For instance, flazasulfuron reduces the photosynthetic capacity of duckweed (*Lemna minor*) by 16% after seven days of exposure to a 100 µg l^{-1} concentration (Olette et al., 2008).

The other frequently found pesticide in the three sampled fish species and the two study sites in Lake Tana was carbaryl (Tables 2 & 3, Fig. S2 (supplementary material)). This is a carbamate insecticide that has been reported to have an impact on several fish species. For example, Patnaik and Patra (2006) observed its impact on the blood cells in *Clarias batrachus* following a 96-hour acute exposure, with a cell shape modified at 12.6 mg l^{-1} and an LC50 at 15.6 mg l^{-1}. In addition, under a controlled investigation, Boran et al. (2010) found that rainbow trout had an LC50 at 2.5 mg l^{-1} and 1.4 mg l^{-1} over 24 and 96 hours of exposure time, respectively. Matos et al. (2007) also report the effect of carbaryl as it resulted in necrosis of the liver tissue of *O. niloticus* when exposed to a concentration of 0.25 mg l^{-1} for seven days. The pH and temperature of the aquatic environment significantly impact the persistence of carbamate insecticides in natural waters (Aly & El-Dib, 1971). Carbaryl generally has a low water persistence (US EPA, 2003). However, the amount recorded in the fish tissue, up to 56.5 µg kg^{-1} in this study, indicates its excessive pesticide use. The detected amount may not impose risk in the short term. However, prolonged exposure possibly imposes environmental and health impacts, including oxidative stress in the fish species, as observed in several animal groups (Ribera et al., 2001; Matos et al., 2007). In addition, the effect of pesticides is more pronounced on juveniles than on adults of the same species (Lin et al., 2007), so any prolonged and extensive use could affect fish recruitment and population growth.

The other carbamate insecticide that was detected in the two *Labeobarbus* species of Lake Tana was oxamyl (Table 3, Fig. S1 (supplementary material)), which is relatively more toxic than carbaryl for aquatic organisms (Lewis et al., 2016; Alvarez et al., 2022). Oxamyl is applied as an insecticide on field crops, vegetables, fruits, and ornamental plants (Alvarez et al., 2022). It is moderately toxic to fish, and its LC50 after 96 h exposure for species such as bluegill sunfish, goldfish, and rainbow trout were found to be 5.6 mg l^{-1}, 27.5 mg l^{-1}, and 42 mg l^{-1}, respectively (Smith, 1982). Less persistent pesticides such as oxamyl are rarely found in fish tissue. For example, Polat et al. (2018) found a 45 µg kg^{-1} concentration in the fillet of sardine (*Sardine pilchardus*) among nine studied fish species in the Iskenderun Bay of Turkey. However, in this investigation, oxamyl was detected in 7% of the fish tissue sampled from the lake (Table 3), with the highest concentration (507 µg kg^{-1}) in the liver tissue of *L. megastoma*, indicating its excessive use in the catchment. Although additional research on fish sensitivity to pesticides in Lake Tana is needed, the amount of oxamyl in this study showed the necessity for strict regulation and monitoring.

The fungicides, benalaxyl, difenoconazole and tebuconazole were found < LOQ in fish sampled from GRM site. Albeit their concentrations were low, the presence of these pesticides in this site indicates that they have been applied in the Gumara River catchment. Benalaxyl and difenoconazole, however, were not listed in Abera et al. (2022)'s list of pesticides used in the lake watershed, indicating that there are still other pesticides available in the local market that may come through a different marketing route and circulate through an informal supply network as reported in Mengistie et al. (2016). Benalaxyl is a widely used fungicide on tomatoes, onions, and potatoes in the field, while difenoconazole and tebuconazole are applied on cereals and other field crops (Lewis et al., 2016). Although these fungicides had a high log K_{ow} value used (Lewis et al., 2016) compared to other pesticides found in Lake Tana, they were only found in one sample of the fillet of *L. megastoma*. This suggests that they are probably applied infrequently in the catchment or, due to their lipophilic nature (Houbraken et al., 2016; Rasool et al., 2022), adsorbed in organic wastes in sediment. Studies showed that tebuconazole could cause a decrease in hemoglobin and the number of red blood cells in cyprinids when applied with a 2.5 mg l^{-1} concentration for a 96-h exposure (Çilingir Yeltekin et al., 2020). The LC50 of tebuconazole for fish is 4.4 mg l^{-1} (Lewis et al., 2016). Similarly, the broad-spectrum triazole fungicide difenoconazole is extensively used in agriculture (Jiang et al., 2022). It influences immune-related pathways and energy, lipid, and amino acid metabolism in the early stages of zebrafish development (Teng et al., 2018). Although the examined fish species' susceptibility to those pesticides was not assessed, the observed concentration, which was < LOQ, suggests they very likely do not cause any risk to fish at the current application level in the lake catchment.

The other commonly used insecticides, imidacloprid and pirimiphos-methyl, are frequently detected in many aquatic environments (Vignet et al., 2019). Both insecticides are moderately toxic to fish (Lewis et al., 2016). Imidacloprid was detected in the three studied fish species sampled from RRM. According to Mhadhbi and Beiras (2012), when imidacloprid concentrations in the water were above 0.04 mg l^{-1}, it posed pericardial edema and spinal deformities, and it also reduced hatching success when its concentration reached above 0.4 mg l^{-1} in turbot (*Psetta maxima*). In the Ribb and Gumara catchments, crops like rice and other cereals, including maize, are grown and commonly treated with pirimiphos-methyl and imidacloprid. However, the fact that both pirimiphos-methyl and imidacloprid were found in the fish samples taken from the RRM site suggests that farmers in the Ribb River catchment may have used these insecticides in greater quantities than in Gumara.

In general, the number of herbicides, fungicides, and insecticides found in the tissue of the three fish species in Lake Tana indicates how much pesticide use has polluted the lake water. Pesticides used by farmers in the Gumara and Ribb rivers to reduce crop loss and increase the quality of their crops, vegetables, and fruits are indispensable. However, reducing agricultural losses by using too many of these agrochemicals should not come at the expense of aquatic life. Increasing production using intensive agrochemicals could not be sustainable because a rise in pesticide use seriously harms an ecosystem's health and natural resource. The best way to recommend sustainable production and an environmentally friendly approach is using integrated pest management, which weighs natural alternatives to chemical pesticides and only uses them as a last resort (Barzman et al., 2015). Using less toxic and less persistent pesticides is also better for creating more resilient, sustainable food systems. Monitoring the type and quantity of pesticides used and assessing their impact on biodiversity should be part of the management plan of the lake and its catchment, even though the concentrations currently seem to have no adverse toxicological effect on fish species at the current application level.

Pesticides risk to human health

Fish ingest pesticide residues mainly through the epidermis of the gills and ingested food (Braunbeck et al., 1998). When a fish is consumed, the pesticides accumulated in its edible tissue can be transferred to humans. Liver tissue is the detoxification center and stores toxicants in many studied fish species (Braunbeck et al., 1998; Macirella et al., 2022). In this study, however, some pesticides, e.g., pyrimethanil, were found in higher concentrations in the fillet tissue of *L.*

tsanensis and *O. niloticus* (Table 4). Consequently, humans who consume fish meat from the lake are exposed to these pollutants. Two to three different pesticides were found in some fillet samples, and their impact on the health of fish and its consumers is likely greater than that of just a single pesticide due to their combined effect (Kortenkamp, 2007; Hernández et al., 2013). No clear maximum residual limit (MRL) for the three pesticides was available for the risk assessment (Table 5). However, from the metadata for most of the animal products in Great Britain, a maximum value of 50 µg kg^{-1} for carbaryl and pyrimethanil and 10 µg kg^{-1} for oxamyl are used (Lewis et al., 2016). Considering those conservative MRL values, the detected concentration of pyrimethanil in 12 samples and one sample for oxamyl was higher than the limit. The risk assessment, however, might reflect the worst-case scenario because we did not consider the impact of washing, soaking with solutions, cooking, and freezing procedures that could lower the concentration of pesticides in the tissue (Witczak, 2009; Alaboudi et al., 2021; Islam et al., 2022). Overall, the HQ and HI values showed that, at the current application practices, pesticides pose no known harm to human health or consumers of fish collected from the lake. However, the detection of ten pesticides in the fish tissue in this study shows that the use of pesticides along the river catchments needs urgent attention and close monitoring to safeguard the public health of local communities and the population of both culturally endangered and commercially important fish species.

Conclusion

The contamination of ten pesticide residues (2 herbicides, 4 fungicides, and 4 insecticides) was detected in the three commercially important fish species. Variations in pesticide concentrations among fish species and between tissue types were not statistically significant, except for pyrimethanil, which revealed a significant difference between *L. megastoma* and *L. tsanensis*. *L. megastoma* was found to have the highest level of pesticide contamination (eight out of ten) among the three studied fish species, which may be related to its' piscivorous feeding behavior. Pyrimethnil > carbaryl > oxamyl > flazasulfuron were the most frequently occurring pesticides in the studied fish species with > LOQ values. Moreover, most pesticides were found in higher concentrations in the fish's liver than in the fillet. For all of the dominantly detected pesticides, the health risk calculations revealed that the health risk index was below the cutoff value, indicating that, given current pesticide use, there is likely no harm to human health from pesticide exposure in fish. Due to the lack of samples from the water and sediment, the study is limited in its ability to evaluate the effects of combined exposure to toxins and their influence on the lake's biota. Additionally, the small sample size results in an uneven distribution of data about the presence of pesticides in fish tissue. However, although the study was conducted on a limited sample size, only three species and a single developmental stage, the results could serve as a preliminary step for further research, and the information will be used to generate a management strategy for the fish populations in Lake Tana. Further investigations are recommended, including a wide variety of fish species, a sizable sample at various stages of development, and measurements of pesticide concentrations in the water and sediment for a more in-depth ecotoxicological assessment.

Ethics statement

The Ethical Committee of Bahir Dar University, College of Agriculture and Environmental Sciences, approved the study. The care and use of animals followed all applicable international, national, and institutional guidelines.

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Author contribution

Wondie Zelalem: conceptualization, investigation, methodology, formal analysis, writing–original draft; Wassie Anteneh, Minwyelet Mingist: conceptualization, investigation, editing; Alain De Vocht: conceptualization, project administration, methodology, investigation, data curation, editing; Jasmine De Rop, Andrée De Cock, Pieter Spanoghe, Peter L.M Goethals: methodology, data curation, formal analysis, editing; Mulugeta Kibret, Enyew Adgo, Jan Nyssen, Elie Verleyen: investigation, project administration, editing. Felegush Erarto: investigation, editing.

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Data Availability

The corresponding author will provide the data upon reasonable request.

Competing interests

The authors declare no competing interests.

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