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Multi residue analysis of persistent organochlorine pesticides in fish from Baro River, Gambella, Southwest Ethiopia

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ABSTRACT

In this study, the concentrations of organochlorine pesticides in thirty (30) randomly collected *Tilapia Zilli* fish samples from the Baro River, Ethiopia were determined. The pesticide residues were extracted by soxhlet extraction technique using a mixture of n-hexane and acetone. The extracts were cleaned with silica gel and analyzed using a GC-MS. Concentrations of organochlorine pesticides were calculated from linear calibration curves of nine mixtures of standards with a concentration range of 5-200 μg/kg in the fish sample and had correlation coefficients of 0.999 for all concentrations. The ranges of the limit of detections (LODs) and limit of quantifications (LOQs) were 0.008–0.017 μg/kg and 0.025–0.052 μg/kg respectively. Recovery was determined from spiked samples at concentrations of 5 μg/kg, 60 μg/kg, and 200 μg/kg. Recoveries of 90.87–102.6% were obtained and the method precision calculated from relative standard deviation (RSD) was found to be 1.00–8.62%. Of the thirteen studied organochlorine pesticides five of them were detected in collected fish samples with concentrations of 67.08 μg/kg, 15.35 μg/kg, 16.6 μg/kg, 3.87 μg/kg, and 17.1 μg/kg for Endosulfan II, Endrin, Endosulfan I, *4,4'*-DDD, and Chlordane, respectively. The occurrence of organochlorine pesticide residues in fish samples shows that these compounds have been used in the study areas. The levels of chlordane and Endosulfan II detected in fish samples were above the maximum residual limits (MRLs) set by the Codex Alimentarius Commission. Therefore public awareness of pesticide use and management needs to be worked on by concerned bodies.

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1. Introduction

Pesticides are a group of chemicals used for the destruction of insects, weeds, fungi, bacteria, etc. They are generally called insecticides, fungicides, bactericides, herbicides, or rodenticides. Most pesticides can destroy a wide variety of pests or weeds, but some are developed against specific pathogens. Most of these chemicals are designed in such a way as to disturb the physiological activities of the target organism, leading to dysfunction and reduced vitality. The worldwide production and application of pesticides, especially organochlorine in agriculture can have adverse environmental pollution and human health risk (Kim et al., 2017; Wondimu and Geletu, 2023). The characteristics of pesticides, such as high lipophilicity, bioaccumulation, long half-life, and the potential for long-range transport, have increased the chance of contaminating the air, water, and soil, even after many years of application.

Organochlorine pesticides are synthetic pesticides widely used all over the world. They belong to the group of chlorinated derivative hydrocarbons, which have vast applications in chemical industries and agriculture. These compounds are known for their toxicity, slow degradation, and bioaccumulation. Even though many of the compounds, which belong to organochlorine pesticides, were banned in developed countries, the use of these agents has been rising. Though pesticides have been developed with the concept of target organism toxicity, often non-target species are affected badly by their applications (Ambaye, 2016).

Chlorinated organic pesticides are very stable in both fresh and saltwater and resistant to photodegradation (El-Mekkawi et al., 2009). They will disappear from the water with secondary mechanisms such as absorption of sediment, biological breakdown by microflora and fauna, and absorption by fish through gills, skin, and feeding. They are poorly hydrolyzed and slowly biodegrade in the environment. Therefore, these compounds are persistent in food chains and are readily accumulated in animal tissues. Fish absorb these compounds directly from water or by ingesting contaminated food (Rani et al., 2021). In particular, organochlorine pesticides are highly stable under different environmental conditions and have chronic adverse effects on wildlife and human beings (Yadav and Devi, 2017).

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Ethiopia has a rich diversity of Ichthyo-fauna in its lakes, rivers, and reservoirs. The fish fauna of Ethiopia contains a mixture of Nilo-Sudan, Highland East African, and endemic forms. There are 175 fish species in Ethiopia and 38 of them are endemic and found only in Ethiopia (Kebede et al., 2017; Eschmeyer et al., 2020). The Number of fish species recorded from the seven drainage basins of Ethiopian has Baro (87), Abay (36), Rift Valley Lakes (32), Wabe-Shebele (26), Omo (26), Awash (15), and Tekeze (10) (Mengesha, 2015). The highest fish species diversity in Ethiopia has been recorded from the Baro basin (87) (Mitiku and Mitiku, 2022), followed by the Blue Nile, Wabishebele, and Omo-Gibe basins. They are an important resource for humans worldwide, especially as food. Commercial and subsistence fishers hunt fish in wild fisheries or farm them in ponds or cages in the ocean (in aquaculture). They are also caught by recreational fishers, kept as pets, raised by fish keepers, and exhibited in public aquaria.

Sixty percent of the Ethiopian population is at risk of malaria, with the highest prevalence reported in Gambella (6%) and Benishangul-Gumuz (3%) regions (Tadesse et al., 2021). Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are instrumental in the significant reduction of malaria morbidity and mortality (WHO, 2017). This can result in the transportation and accumulation of this spray of pesticides to the Baro River by irrigation and other means of water movement mechanisms (Sishu et al., 2022). As a result, these pesticides can enter the fish through gills, skins, and feeding and be stored in the liver and kidney. They can be transferred to living organisms by food chains.

Most farmers in the Gambella region use several highly persistent chemical pesticides (Watiro and Awoke, 2016). These chemicals not only affect the crop, animals, and birds in a specific area but also badly affect the ecosystem balance. If left uncontrolled they can result in mass mortalities or some cases can be served as a source of infection for humans and other vertebrates that consumed fish. Accordingly, fish could be considered one of the significant bio-indicators of these pesticides pollution in freshwater (Daoud et al., 2020).

However, there is no published information available on levels of OCPs in the fishes and other aquatic populations of the Baro River. Therefore, the contamination levels of fish by organochlorine pesticide residues are unknown. The river water is abstracted for domestic and drinking purposes, irrigation, and fishing during its course (Agumassie, 2019). This calls for an extensive study of organochlorine pesticide residue levels of fish in the Baro River. To this effect, the focus of this study was to examine the levels of some persistent OCPs in the fish samples of the Baro River system, Gambella, Southwest Ethiopia.

2. Materials and methods

2.1. Sample collection

The study was conducted in the Baro River, the headwater of the White Nile River. It is located in the province of southwest Ethiopia. It defines part of Ethiopia's border with South Sudan.

Before starting sample collection, preliminary field observation was conducted to determine the specific sampling sites. Sampling sites were purposely identified in a manner that includes an area with limited human interference. Accessibility and stream orders were also considered to determine the locations of specific sampling sites. Three sampling sites were identified depending on different human activities in the use of the Baro River. Those sampling areas were designated as S_1, S_2, and S_3 where S stands for sampling site. The species of fish were identified by a biology staff at Gambella University, Gambella, Ethiopia based on morphometric characteristics. The map of studied area was depicted in Fig. 1 (Muleta, 2021).

The most widely available fish species, *Tilapia Zilli*, Golubtsov and Darkov (2003) and Gebreselassie et al. (2021) were caught using gill nets from Baro River, Gambella, Southwest Ethiopia. They are consumed by a larger part of the community and traded widely in the region. Ten *Tilapia Zilli* fish species were randomly collected from each sampling area and a total of thirty (30) of them were collected for the present study. From each sampling site, 2.6 kg and a total of 7.8 kg of fish samples were collected. For each sample, a 0.25 kg composite sample of target organs (kidneys, livers, edible muscles, and gills) of fish was taken. Samples of fish were transported separately to the laboratory on the same day. The collected fish samples were extracted within 48 h of collection and stored in a refrigerator until time of analysis. It was covered by aluminum foil, stored in the snow-containing fridge, and transported for laboratory analysis to JIJE ATSL, Addis Ababa.

2.2. Chemicals and reagents

Reagents-grade (pesticides-grade) (Aldrin, Heptachlor Epoxide, Dieldrin, Endrin, Chlordane, Endosulfan I, Endosulfan II, *4, 4′*-DDD, *4, 4′*-DDT, DDE, Methoxychlor, β-Lindane, and α-Lindane) were used in all tests. Standard solutions (stock, composite, calibration, spiked, and external) were stored in polytetrafluoroethylene (PTFE)-sealed containers in the dark. Anhydrous sodium sulfate (Na_2SO_4), methylene chloride, hexane, copper powder, and silica gel were obtained from Sigma-Aldrich (Germany).

2.3. Instrument working conditions

The analysis condition of the chromatographic system was Agilent Technologies, 7890B GC with a splitless mode of injection. The detector used was a 5977B MS single quadrupole mass selective detector (Agilent Technologies) with the capillary column DB-5MS, having 30.0 m in length, 0.25 mm in diameter, and 0.25 μm thickness. The GC was run under the following conditions: injector temperature: 250 °C; detector temperature 330 °C; oven temperature: 60 °C for 1 min by 30 °C/min rate to 120 °C and then by 5 °C/min rate to 310 °C. Injected sample volume: 1.5 μL; mode of injection: splitless; carrier gas: Helium. The column flow rate was 1 ml/min and Ithe ionization mode: EMV: Gain Factor: 1 Transfer line temperature: 280 °C; Ion Source temp: 230 °C; Quad temperature: 150 °C with a 77.8 kPa flow rate; runtime: 3 min. Solvent delay: 3 min. Standard peaks were detected by inserting a high concentration of the standard (200 μg/L), and the retention times for OCP were read. The acquisition mode was SIM.

2.4. Solution preparation

The mixture of all concentrated organochlorine pesticide standards was purchased from JIJE Analytical Testing Service Laboratory (JATSL). One thousand milligrams per liter (1000 mg/L) of each organochlorine pesticide was dissolved in hexane in a 5 ml volumetric flask. A lower working concentration of organochlorine pesticides was prepared from a stock solution. It was prepared as follows: First, the volumetric flask was filled with hexane until it is approximately one-third full. Then precisely measured volumes of the concentrated organochlorine pesticide standard mixtures (Aldrin, HCB, Heptachlor Epoxide, Heptachlor, chlordane, Endosulfan I, *4-4′*-DDD, *4-4′*-DDT, DDE Methoxychlor, and α-Lindane) was added to the volumetric flask. The flask was filled with hexane so that the bottom of the solution meniscus lines up with the mark on the neck of the flask and was inverted

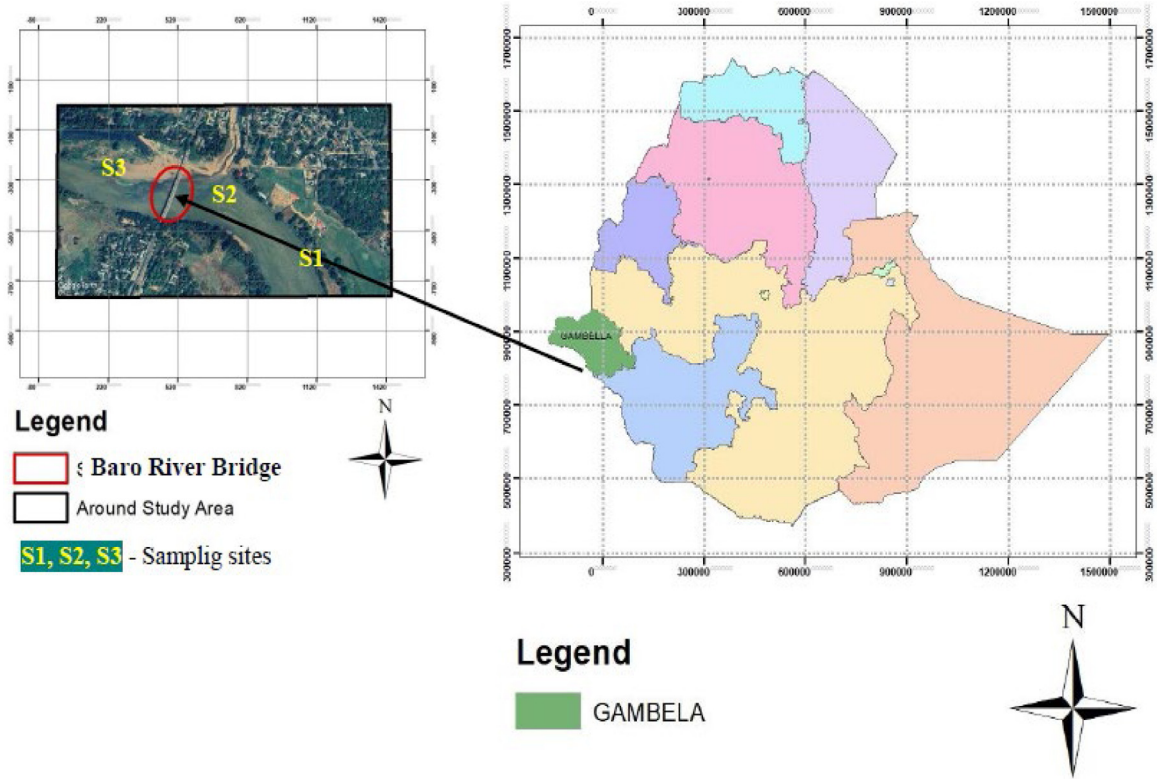

Fig. 1. Map of the sample collection area.

three times to mix well. Finally, the solution was transferred to a clean amber glass bottle. The standard solutions were prepared in acetonitrile containing every thirteen organochlorine pesticides ranging from 5–200 μg/L, and then, the solution was stored in the refrigerator at 4 °C and used for spiking fish samples.

2.5. Sample preparation

2.5.1. Sample extraction

Sample extraction was done using USEPA Method 3540C (Modified USEPA 8082a) Solid fish samples were air-dried. Then the sample was ground so that it passed through a 1-mm sieve. A sufficient sample was introduced into the grinding apparatus to yield at least 10 g after grinding. To calculate sample results on a dry weight basis, a second portion of the sample was weighed at the same time as the portion used for analytical determination. 10 g of solid sample was blended with 10 g of anhydrous sodium sulfate and placed in an extraction thimble. The extraction thimble was drained freely for the duration of the extraction period. Glass wool plugged above and below the sample in the Soxhlet extractor was an acceptable alternative for the thimble.

300 mL of the extraction solvent/hexane-acetone (1:1) was added into a 500 mL round bottom flask containing two clean boiling chips. The flask was attached to the extractor and the sample was extracted for 24 h at 6 cycles/h. Then the extract was allowed to be cooled after the extraction was completed and was concentrated using a rotary evaporator to near dryness. The concentrated sample was bellowed down using nitrogen to fully dry the extract and the concentrate was dissolved with 2 ml hexane.

2.5.2. Sample clean up

Sulfur (S8) interferences were removed using USEPA Method 3660 before silica gel cleanup (You and Lydy, 2004) by using copper. It prevents further degradation of pesticides. The solubility of sulfur in various solvents is very similar to the organochlorine and organophosphorus pesticides. Therefore, the sulfur interference follows along with the pesticides through the normal extraction and cleanup techniques The sample extract was concentrated in a 1.00 mL volume. The sample extract was centrifuged to settle the sulfur crystals and the extract was drawn off with a disposal pipette leaving the excess sulfur in the concentration vessel. Then 1.00 mL of the extract was transferred to a calibrated centrifuge tube. 2 g of cleaned copper powder was added to the centrifuge tube. Vigorously the extract and the copper powder were mixed for 1 min in the mechanical shaker. The phases were allowed to separate. Finally, the extract was separated from the copper by drawing off the extract with a disposable pipette and transferred to a clean vial. The volume of extract remained was still represented 1.00 mL of extracts.

Other interferences were removed using USEPA Method 3630 (silica gel cleanup) (Saranya et al., 2020). The standard column chromatography techniques were packed with a greater amount of silica gel adsorbent and, therefore, have a greater cleanup capacity. The extracts were allowed to reach room temperature since they were in cold storage. 40 mL of hexane and fresh boiling chips were added to the extracts. A 3 g portion of deactivated silica was transferred into a 10 mm ID glass chromatographic column and topped with 3 cm of anhydrous sodium sulfate. Then 10 mL of hexane was added to the top of the column to wet and rinse the sodium sulfate and silica gel. Just prior to exposure of the sodium sulfate layer to the air, the hexane eluate flow was stopped by closing the stopcock on the chromatographic column, and the eluate was discarded. The sample extracts were transferred onto the column. The extract vial was rinsed twice with 2 mL of hexane and each rinse was added to the column. The column was eluted with 80 mL of hexane (Fraction I) at the rate of 5 mL/min followed by the removal of collection flasks and the procedure was repeated with 50 mL of hexane (Fraction II). The third elution was performed with 15 mL of methylene chloride

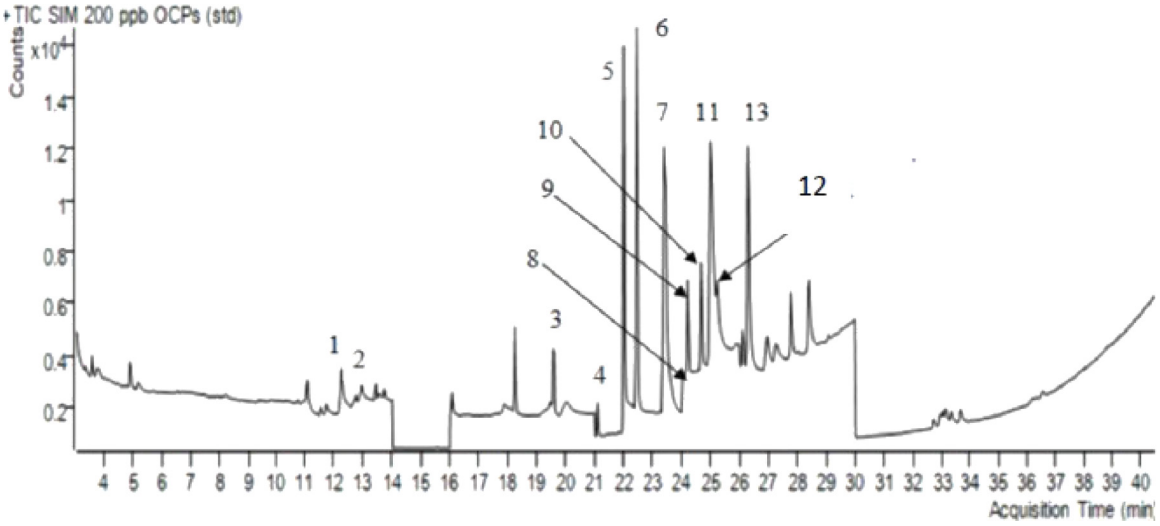

Fig. 2. Chromatogram of organochlorine pesticide standards α-lindane (1), β-lindane (2) aldrin (3), heptachlor epoxide (4), dieldrin (5), chlordane (6), endrin (7), endosulfan I (8), DDE (9), methoxychlor (10), endosulfan II (11), *4, 4′*-DDD (12), and *4, 4′*-DDT (13) at 200 μg/kg.

(Fraction III). Finally, the eluate was collected and ready for GC-MS analysis. The final extract volume was made to be a 10 ml volumetric flask.

2.6. Method validation

The parameters used to validate the methods were linearity, correlation coefficients, recovery, precision, LOD, and LOQ. The linearity of the method was studied with calibration of standard pesticides solution in fish samples at nine concentration levels (5, 10, 20, 40, 60, 80, 100, 150, and 200 μg/kg) for each analyte. The correlation coefficient of each analyte was determined from a calibration curve. Recovery, accuracy, and precision of the method used were determined from the analysis of spiked samples. LOD and LOQ were determined as 3δ and 10δ where δ is the ratio of the standard deviation of calibration lines to respective slopes. The recovery studies were performed by spiking different concentrations (5, 60, and 200 μg/kg) of the analytes to fish in triplicate, and sample preparation and analyte detection followed the same procedure for the unspiked samples.

3. Results and discussion

3.1. Chromatographic analysis of standard solutions

Before analysis, standards were run to check for the column performance, peak height, and resolution. Quantitation and confirmation of pesticides were performed based on the use of transition ions, qualifier ions, and retention times. Identification parameters of organochlorine pesticide standards are summarized in Table 1.

The retention times range is 12.252 ± 0.039–26.332 ± 0.016 min, the minimum RT corresponds to α-Lindane and the maximum was for *4, 4′*-DDT. The SD of retention times range is 0–0.043 min for Methoxychlor and β-Lindane respectively. The retention time acceptance criteria currently recommended by SANTE is on the magnitude of ±0.1 min deviation from the average retention time (Pihlström et al., 2017). Fig. 2 shows a chromatogram of 200 μg/kg of each organochlorine pesticide standard solution.

Table 1
Identification parameters for analytes.

Analytes	Retention time (RT) in minutes ($\bar{X} \pm SD$)	Transition ion (m/z)	Qualifier ions (m/z)
α-Lindane	12.252 ± 0.039	183	109, 219
β-Lindane	12.275 ± 0.043	219	181, 217
Aldrin	19.606 ± 0.011	263	293, 169
Heptachlor Epoxide	21.119 ± 0.011	237	359, 170
Dieldrin	22.499 ± 0.001	373	237, 272
Chlordane	23.471 ± 0.021	246	176, 318
Endrin	23.431 ± 0.013	318	246, 176
Endosulfan I	23.771 ± 0.021	246	318, 176
DDE	24.228 ± 0.012	193	281, 149
Methoxychlor	24.686 ± 0.000	207	193, 235
Endosulfan II	24.699 ± 0.018	207	281, 341
4, 4′-DDD	25.039 ± 0.021	235	237, 165
4, 4′-DDT	26.332 ± 0.016	235	165, 237

Where: $\bar{X}$ is the mean, **SD** = Standard Deviation.

3.2. Method validation

The retention times for standard samples were used for confirmation of the pesticides. Retention time windows were constant for the standard samples and were therefore relied upon for component identification.

3.2.1. Calibration curves for quantification of analytes

Calibration curves were produced with nine different standard concentrations ranging from 5–200 μg/kg. The calibration lines showed excellent linearity in the range of the concentrations of interest and the correlation coefficients (R^2) were found to be 0.999 for all standards and confirmed a linear relationship between the concentrations and peak ratios.

3.2.2. Recovery and precision

Recovery is the proportion of the amount of analyte, present in or added to an analytical portion of the test materials (samples), which is extracted and presented for measurement (Companyó et al., 2009) and it was calculated using Eq. (1). To determine the quality of the methodology, a recovery study was performed

Table 2
Spiked and recovered concentrations of pesticide standards (5, 60, and 200 μg/kg, n = 3).

Analytes	Spiked concentration (μg/kg)	Determined concentration (μg/kg)	Spiked concentration (μg/kg)	Determined concentration (μg/kg)	Spiked concentration (μg/kg)	Determined concentration (μg/kg)
α-Lindane	5	4.65	60	59.7	200	202.8
β-Lindane	5	5.06	60	60.8	200	206.2
Aldrin	5	4.68	60	62.4	200	208.5
Heptachlor Epoxide	5	4.62	60	56.5	200	196
Dieldrin	5	4.9	60	57.1	200	200.3
Chlordane	5	4.22	60	55.4	200	198
Endrin	5	5.15	60	59.4	200	197.9
Endosulfan I	5	4.46	60	58.3	200	207.4
DDE	5	4.71	60	63.2	200	199.7
Methoxychlor	5	4.23	60	57.6	200	210.4
Endosulfan II	5	4.79	60	63.6	200	212.1
4, 4′- DDD	5	4.93	60	62.7	200	200.4
4, 4′- DDT	5	4.37	60	52.4	200	195.8

Table 3
Mean recovery and % RSD (data from Table 2, n = 3).

Analytes	Recovery at 5 μg/kg (%)	Recovery at 60 μg/kg (%)	Recovery at 200 μg/kg (%)	Mean recovery (%)	RSD (%)
α-Lindane	93.0	99.5	101.4	97.97	4.50
β-Lindane	101.6	101.3	103.2	102.03	1.00
Aldrin	93.6	104.2	104.2	100.67	6.08
Heptachlor Epoxide	92.4	94.2	98.0	94.87	3.01
Dieldrin	98.0	95.2	100.1	97.77	2.51
Chlordane	84.4	92.3	99.0	91.90	7.95
Endrin	103.4	99.0	98.9	100.43	2.56
Endosulfan I	89.2	97.2	103.2	96.53	7.28
DDE	94.2	105.3	99.9	99.80	5.56
Methoxychlor	84.6	96.0	100.2	93.60	8.62
Endosulfan II	95.8	106.0	106.1	102.60	5.77
4, 4′- DDD	98.6	104.5	100.2	101.10	3.53
4, 4′-DDT	87.4	87.3	97.9	90.87	6.70

RSD = Relative Standard deviation.

using standard addition methods. The homogenized fish sample was spiked with the mixture of pesticide standards at 5, 60, and 200 μg/kg concentrations with triplicate each (n = 3). The spiked samples were extracted and analyzed as described in the method above. The results revealed that the mean recovery values ranged from 90.87–102.6%.

$$\%\ \text{Recovery} = \frac{(\text{Con.spiked sample} - \text{Con.unspiked sample})}{\text{amount added}} \times 100 \quad (1)$$

Recovery of the method developed was in an acceptable range of 70%–120% (Pihlström et al., 2017). The precision of the method was determined by calculating the relative standard deviation (RSD) of the spiked sample. The percentage relative standard deviation (RSD) values for the precision ranged from 1.00–8.62%. The acceptable level of percent relative standard deviation for precision is ≤20% (Pihlström et al., 2017). Therefore validated RSD values were obtained in the developed GC-MS method. This indicates that the analytical procedures outlined for the OCP determination in this study were reliable, reproducible, and efficient. Table 2 shows the spiked and recovered concentrations of pesticide standards while the mean recoveries and relative standard deviations were shown in Table 3.

3.2.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) are key parameters characterizing the performance of the whole test method at low concentrations. LOD and LOQ were calculated from calibration curves. LOD of an analytical procedure can be described as the lowest concentration of analyte in a sample that can be detected by it, but not necessarily quantified as an exact value (Burns and Valdivia, 2008). LOQ of an analytical procedure can be described as the lowest concentration of the analyte in a sample that can be quantified with suitable accuracy and precision as an exact value (Peters et al., 2007).

As indicated in Table 4, the LOD of each analyte is lower than the minimum concentration of standard used for calibration. Additionally, the lowest calibration level must be equal to, or lower than, the calibration level corresponding to the maximum residue limit(MRL). Thus the MRL of each analyte was not lower than the corresponding LOQ value. The values were between 0.008–0.017 μg/kg for LODs and 0.025–0.052 μg/kg for LOQs.

3.3. Chromatographic analysis of fish samples

The levels of various organochlorine pesticides in three different fish samples from the Baro River are summarized in Table 5. Residues of some of these pesticides were detected in studied fish samples, and accumulation of these contaminants can occur by diffusion from the water across the gills and by transfer from the gut into the body after consumption of contaminated food. When these organic pollutants are taken up by the fish, they bioaccumulate, biomagnify, and remain in the fish until they are eventually consumed by humans (Williams and Ayejuyo, 2015).

The highest concentrations of pesticides were detected in sampling site S_3 and relatively none of the organochlorine pesticides was detected in S_1. Only Endrin was detected in sampling site S_2. The existence of organochlorine pesticide residues in S_2 and S_3 as compared to S_1 may be attributed to the impact of wastewater and chemicals from Gambella City. The S_2 is located in the mid of Gambella city and there are wastewater and chemicals from different parts of the city discharging directly into the river. The S_3 is found below the Gambella city. Different mixed wastewater and chemical effluents from the city join the river just before S_3. Contaminants levels of S_3 fish samples generally followed this pattern: Endosulfan II > Chlordane > Endrin > Endosulfan I > *4,*

Table 4
Calibration parameters, LOD, and LOQ values.

Analytes	Linear range (μg/kg)	Calibration line equations	R^2	LOD (μg/kg)	LOQ (μg/kg)
α-Lindane	5–200	y = 38.18x − 112.5	0.999	0.014	0.046
β-Lindane	5–200	y = 31.22x − 72.97	0.999	0.009	0.030
Aldrin	5–200	y = 28.70x − 34.18	0.999	0.014	0.047
Heptachlor Epoxide	5–200	y = 11.47x + 26.46	0.999	0.011	0.035
Dieldrin	5–200	y = 50.24x + 171.1	0.999	0.009	0.031
Chlordane	5–200	y = 204.70x + 298.3	0.999	0.015	0.048
Endrin	5–200	y = 30.95 x − 39.11	0.999	0.016	0.053
Endosulfan I	5–200	y = 203.6x + 406	0.999	0.02	0.068
DDE	5–200	y = 14.98x + 13.54	0.999	0.008	0.025
Methoxychlor	5–200	y = 10.43x − 24.21	0.999	0.017	0.052
Endosulfan II	5–200	y = 10.23x − 64.38	0.999	0.016	0.054
4, 4′- DDD	5–200	y = 280.82x + 883.59	0.999	0.011	0.037
4, 4′- DDT	5–200	y = 130.40x − 439	0.999	0.009	0.029

Where: R^2 = Correlation coefficient; LOD = Limit of Detection; LOQ = Limit of Quantitation.

Table 5
Organochlorine pesticides detected in Baro River fishes.

Analytes	Sampling sites		
	S_1	S_2	S_3
	Conc. (μg/kg)	Conc. (μg/kg)	Conc. (μg/kg)
α-Lindane	ND	ND	ND
β-Lindane	ND	ND	ND
Aldrin	ND	ND	ND
Heptachlor Epoxide	ND	ND	ND
Dieldrin	ND	ND	ND
Chlordane	ND	ND	17.09
Endrin	ND	14.15	16.55
Endosulfan I	ND	ND	16.34
DDE	ND	ND	ND
Methoxychlor	ND	ND	ND
Endosulfan II	ND	ND	67.08
4, 4′-DDD	ND	ND	3.87
4, 4′-DDT	ND	ND	ND

Where: ND = Not Detected; S_1, S_2 and S_3 represent sampling sites 1, 2 and 3 respectively.

4′-DDD. *4, 4′*-DDD was detected with a minimum concentration of 3.87 μg/kg and Endosulfan II was with a maximum concentration of 67.08 μg/kg.

3.3.1. Chlordane

Organochlorine pesticides tend to accumulate in living organisms, especially in aquatic organisms. The concentration of chlordane in different fish samples is shown in Table 6. The distribution of chlordane typically varied amongst the different fish samples based on their sampling sites. It was not detected in both S_1 and S_2. The same result was obtained in the study conducted by Shinggu et al. (2015) in which no chlordane was detected in *Tilapia Zilli* fish species from Biu Dam. The maximum residue limit of chlordane was found in S_3 with a concentration of 17.09 μg/kg. The study conducted by Ambaye (2016) recorded that the mean concentration of chlordane in fish samples from Tekeze Dam was 0.024 μg/kg which is lower than the result of this study. Additionally, the concentration of chlordane recorded by the study (Bhuvaneshwari and Babu Rajendran, 2012) was found to be 0.37 μg/kg. The presence of chlordane residues in fish products is most likely due to its environmental persistence with a half-life of up to 30 years (Koshlukova and Reed, 2014). Consequently, chlordane still contaminates aquatic environments due to the hydrophobic nature of the compound; it readily adheres to hydrophobic surfaces, such as plastics.

3.3.2. Endrin

Endrin was the sole organochlorine pesticide detected in samples from S_2 and S_3, but not in S_1. Its minimum and maximum residue were found in S_2 and S_3 with concentrations of 14.15 and 16.55 μg/kg respectively. The concentration of Endrin detected in both samples is higher than that detected in the study of Ogunfowokan et al. (2012) which was 0.65 μg/kg. The study conducted by El-Sayed et al. (2021) on *Tilapia Zilli* fish species, in the El-wasta area, detected Endrin with a concentration of 0.217 ± 0.052 μg/kg, which is lower than the present result. The presence of Endrin in fish samples is most likely due to its low water solubility and is absorbed by aquatic populations. If released into water systems it will not hydrolyze or biodegrade but, accumulated in sediments and aquatic organisms, particularly fishes (Benny, 2014).

3.3.3. Endosulfan I

The maximum concentration of Endosulfan I was detected in S_3. But it was not detected in both S_1 and S_2. Its concentration in S_3 was 16.34 μg/kg. The study conducted by Agmas and Adugna (2022) recorded a concentration of Endosulfan I higher than the result of the present study which has a mean concentration of 341.5 ± 32.19 μg/kg. Another comparable study conducted by Olayinka et al. (2015) recorded a higher mean concentration of Endosulfan I than the present study with 102 μg/kg. But the concentration of endosulfan I detected in the fish sample studied by Wenaty et al. (2019) on *Tilapia Zilli* fish species in Lake Victoria was 0.65 ± 0.03 μg/kg, which is lower than the result obtained from the present study.

3.3.4. Endosulfan II

The result of this work indicates that endosulfan II is the most abundant pesticide residue in S_3 fish samples. It was detected with the highest concentration of studied organochlorine pesticides in S_3 of the present study which was 67.08 μg/kg. It was not detected in samples from both sampling sites S_1 and S_2. The study conducted by Olayinka et al. (2015) recorded a concentration of 8.9 μg/kg lower than that of the present study in the same fish species in Lake Victoria. Barakat et al. (2017) recorded a lower concentration of Endosulfan II in *Tilapia Zilli* fish species from Lake Qarun, a protected area of Egypt whose mean concentration was 6.92 μg/kg. A study done by Deribe et al. (2014) in the fish muscle of Lake Hawassa, Ethiopia noted an Endosulfan II maximum concentration of 42.5 μg/kg which is lower than the present result. Its concentration was higher when compared to that of Endosulfan I. This observation is probably because Endosulfan is recalcitrant to degradation with a half-life of 35–67 days of Endosulfan I and 104–265 days of Endosulfan II (Jimenez-Torres et al., 2016). Furthermore, the concentration of Endosulfan II in the present study was higher than the MRLs recommended by various organizations. This may be because Endosulfan contaminates surface waters through spray drift and transport in runoff and may move to targets beyond its use area through atmospheric transport (via volatilization, transport on dust particles, or a combination) (Zamora, 2005).

Table 6
Comparison of concentrations of OCPs (μg/kg) in fish samples from Baro River and that of other studies and maximum residual limits stipulated by some statutory agencies.

Analytes	Wenaty et al. (2019)	EFSA (2007)	Ambaye (2016)	Olayinka et al. (2015)	Present study result	MRLs (Matiur et al., 2021)
α-Lindane	1.1	ND	0.138	–	ND	10
β-Lindane	1.4	ND	–	–	ND	30
Aldrin	–	–	–	7.70	ND	20
Heptachlor Epoxide	–	–	0.041	6.7	ND	20
Dieldrin	2.2	ND	–	12.8	ND	10
Chlordane	2	50	0.024	–	17.09	10
Endrin	–	–	–	12.5	15.35	50
Endosulfan I	–	–	0.816	20.7	16.34	20
4,4'-DDE	–	ND	4.864	–	ND	10
Methoxychlor	–	–	–	–	ND	5
Endosulfan II	0.4	ND	0.816	8.9	67.08	20
4, 4′- DDD	13.2	ND	–	4.52	3.87	30
4, 4′- DDT	–	ND	4.112	–	ND	10

Where: ND = Not Detected.

3.3.5. 4, 4′-DDD

4, 4′-DDD was detected only in sampling site S_3, but not in S_1 and S_2. It is one of the degradation products of *4, 4′*-DDT, indicating a possible transformation process taking place on *4, 4′*-DDT (Wandiga et al., 2002). Its concentration was found to be 3.87 μg/kg. Similar to the present study finding, the study done by Agmas and Adugna (2022) in fish samples of Lake Tana, Ethiopia detects a mean concentration of 5.65 ± 3.12 μg/kg of *4, 4′*-DDD, which is slightly greater than that of this study. A result lower than our finding was found in a study done by Omwenga et al. (2016) that *4, 4′*-DDD in fish samples from Kiambu Kenya was 1.013 μg/kg. Another lower concentration of *4, 4′*-DDD 0.49 μg/kg in fish samples was reported in studies conducted in Gomti River India by Malik and Priyanka (2006). In our finding *4, 4′*-DDT was not detected when compared to its degradation product (*4, 4′*-DDD). This might indicate that the parent compound was currently limited from use around the Baro River. The detected concentration of *4, 4′*-DDD may be due to previous historic events with a long half-life (2–15 years) (Mitiku and Mitiku, 2022).

4. Conclusion

The concentrations of organochlorine pesticide residues in samples of *Tilapia Zilli* fish species collected from three different sites of the Baro River were determined by GC-MS. Analysis was done based on the validated parameters like recovery (%), the LOD, the LOQ, linearity, precision, and RSD (%). The study results show that the river is contaminated with organochlorine pesticides as five of them (Endosulfan II, Endosulfan I, Endrin, *4, 4′*-DDD, and Chlordane) detected positive. Except for the concentrations of chlordane and endosulfan II, all the detected concentrations are below the maximum residual limits (MRLs) set by Codex Alimentarius Commission. The occurrence of these organochlorine pesticide residues in fish samples is sufficient to confirm that these compounds have been used in the areas. Therefore public awareness on pesticide use, health effects, and good agricultural practices that minimize pesticide pollution, and management need to be worked on by concerned bodies. There should also be nationally strict rules and regulations on pesticide use, management, and maximum residue limits. An extensive study with more numbers and varieties of fish samples with the inclusion of other pesticide classes is recommended.

CRediT authorship contribution statement

Ibrahim Umer Keru: Collected the data, Performed the analysis, Contributed analysis tools, Wrote draft paper. **Abiyot Kelecha Geletu:** Conceived and designed the analysis, Wrote the paper. **Kokob Teshome Wondimu:** Contributed and interpreted data.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2023.103126.

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