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Original Research Article

Determination of Selected Pesticide Residues from Gilgel Gibe (I) Hydroelectric Dam Reservoir and Its Tributaries, Jimma Zone, Ethiopia

Meaza Dejene[1], Kassim Kedir[1], Seblework Mekonen[2], Abera Gure[1]*

[1]Department of Chemistry, College of Natural Sciences, Jimma University, P. O. Box 378, Jimma, Ethiopia

[2]Department of Environmental Health Science and Technology, Faculty of Public Health, Jimma University, P. O. Box 378, Jimma, Ethiopia

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ABSTRACT

In this study, the level of selected organophosphorus and organochlorine pesticide residues from water samples of Gilgle Gibe (I) hydroelectric dam reservoir and its potential tributaries, Jimma zone, Ethiopia, was determined by gas chromatography-electron capture detector (GC-ECD). Low density based dispersive liquid-liquid microextraction (LD-DLLME) using toluene (as extractant) and acetonitrile (as disperser) was used for extraction of pesticide residues from the samples. Calibration curves constructed at six concentration points have good linearity with coefficient of determination (r^2) ranging from 0.995 - 0.999. The limits of detection (LOD) and quantification (LOQ) of the method which were determined as 3 and 10 times the signal-to-noise ratio were ranging from 0.0001 - 2.5810 µg/L and 0.0005 - 8.6050 µg/L, respectively. The efficiency of the method was also evaluated using recovery studies by spiking the water samples with known concentrations of the analytes. The obtained recoveries were ranging from 67 - 105% with relative standard deviations of 0.79 - 12.5%. The findings revealed that the studied water samples contain significant amount of the target pesticides, but endrin was not detected in any of the water sample. Methidathion was also detected only in Nada Qalla and Nada Gudda river water samples. The detected residual concentrations of the target pesticides were above the maximum residue limits, except DDT in acute toxic level. The finding indicated that the studied water samples contain considerable amount of the studied residual pesticides that can influence the health of aquatic organisms and other consumers.

Keywords: Pesticide residues, Water samples, Dispersive liquid-liquid microextraction, Gas chromatography-electron capture detector

***Corresponding Author: Tel.: +251-9-12-03-2-08 OR +251-9-88-44-18-60**
E-mail: aberagure@gmail.com, abera.gure@ju.edu.et

Introduction

Pollution of the various compartment of the environment such as water, air and soil is increasing from day to day due to the rise of population, industrialization and urbanization [1]. Pollutions could originate from natural origin and manmade activities such as industrial wastes, use of agricultural chemicals (eg, pesticides) and so on [2, 3]. Pesticides are chemical compounds that are used to kill, control or repel pests such as insects, rodents, fungi and weeds [4]. For instance, organochlorine pesticides (OCP) and organophosphorus pesticides (OPP) are widely used to kill or control insects all over the globe [5].

Extensive uses of pesticides for agricultural and non-agricultural purposes have resulted for the occurrence of their residues in different environmental compartments including water, air and soil [6, 7]. Some pesticides are persistence against biological or chemical degradation. They are also mobile in the environment, strong ability for bioaccumulation in plants and animal tissues, and thus, directly or indirectly affect the health of human beings [8, 9]. Pesticides can enter into the water bodies through leaching, agricultural or urban runoff, drift, etc [8]. Direct or indirect exposure to pesticide residues at even low concentration levels could have an impact on the health of living things [10, 11]. Thus, determination of pesticide residues in surface water and other environmental compartments is crucial.

Determination of pesticide residues from water samples requires sample preparation that involves isolation, cleanup and/or preconcentration steps prior to their instrumental determinations. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are widely used for extraction of pesticide residues from different matrixes including water [12, 13]. However, these methods are time consuming, use large volumes of toxic organic solvents and provide low preconcentration of analytes [14]. Nowadays, several simple and environmentally green alternative sample preparation methods such as solid phase micro-extraction (SPME) [15] and liquid phase microextraction (LPME) [14, 16-20] have been developed for extraction and/or preconcentration of pesticide residues from water and other matrices.

Among LPME techniques, dispersive liquid–liquid microextraction (DLLME) has received abundant attention for extraction and/or preconcentration of various organic and inorganic

pollutants from various matrices [16]. The method was first reported in 2006 [17] and it is characterized by its simplicity of operation, rapidity, low cost, high recoveries, high enrichment factor and environmentally safe [18]. The method uses small volumes of high density [17] or low density organic solvents [18, 19, 21] as well as ionic liquids [14] as extraction solvents. The final extract obtained from DLLME procedure could be analyzed by gas chromatography with various detectors such as flame photometric detector (GC-FPD) [20], electron capture detector (GC-ECD) [21], mass spectrometry (GC-MS) [22] as well as liquid chromatography with mass spectrometry (LC-MS) [23] and so on. For halogen containing pesticides such as OCP, GC-ECD is the most commonly used techniques due to its selectively and sensitivity for such substances [21].

Gilgel Gibe I hydroelectric dam is an artificial Lake formed from Gibe River in Jimma Zone, Oromia Regional State, Ethiopia, to generate hydroelectric power. The dam has also tributaries consisting of Nada Gudda, Nada Qalla, and Nadi Rivers. The dam and its tributaries are surrounded by intensified small scale farmlands which use fertilizers and pesticides for various purposes [24]. Thus, the dam and its tributaries are expected to contain the residues of commonly used pesticides in the area, resulting in serious health effect on aquatic animals and surrounding communities who use the dam water for different purposes such as fish production, irrigation, bathing, drinking and so on [1]. Thus, regular monitoring of the levels of pesticide residues in the dam water and its potential tributaries is crucial.

In Ethiopia, for many years, OCP and OPP have been used for controlling of insecticides on agricultural fields as well as for controlling of malaria at house hold level [11]. Residues of some OCP were also detected in khat [25], teff and red pepper [26] of Jimma Zone, Ethiopia. These evidences clearly indicate the importance of determination of residues OCP and other pesticides in environmental and biological samples to rescue the health of consumers. A study conducted on Gilgel Gibe I hydroelectric dam reservoir and its tributaries Gibe, Nada Gudda, Nada Qalla and Nadi Rivers also indicated the presence of residues of OCP: Aldrin, Dibutylchlorendate, 4,4-DDE, Gamma-chloridane, Edirne, Endosulfan sulfate, Dieldrin, Methoxychlor and Heptachlor epoxide in their water samples [27]. This finding has given an alarm for the possibility of the presence of the residues of the other commonly used pesticides like OPPs in waters of the dam

reservoir and its tributaries. Therefore, in the present study, the levels of selected OPP and OCP residues in the dam reservoir and its tributaries: Gibe, Nada Gudda, Nada Qalla and Nadi River water samples were investigated by using GC-ECD.

Materials and Methods

Study Area

Water samples were collected from Gilgel Gibe I Hydroelectric Dam Reservoir (HDR) and its four potential tributaries: Nada Qalla River (NQR), Nada Gudda River (NGR), Nadi River (NR) and Gibe River (GR). The dam is located at latitude of 7˚49`52.45``N and longitude 37˚19`18.79´´E to the northeast at about 70 km from Jimma town, capital of Jimma zone, Oromia Regional state as well as to southwest at about 260 km from Addis Ababa, capital city of the country, Ethiopia. The dam occupies about 4225 km^2 area and it is largely surrounded by farmlands and villages [28].

Water Sampling

Water samples were collected using grab sampling into 1 L amber glass bottles, which were previously washed with 10% of HNO_3 and thoroughly rinsed with ultrapure water and then dried in an oven at 75 ºC. Before sampling, the bottles were flushed three times with the water to be sampled. The collected water samples were transported to Jimma university analytical chemistry research laboratory in an ice box and kept in refrigerator below 4 ºC until analysis, without any pretreatment. Figure 1 shows map of the study area and the specific sampling sites.

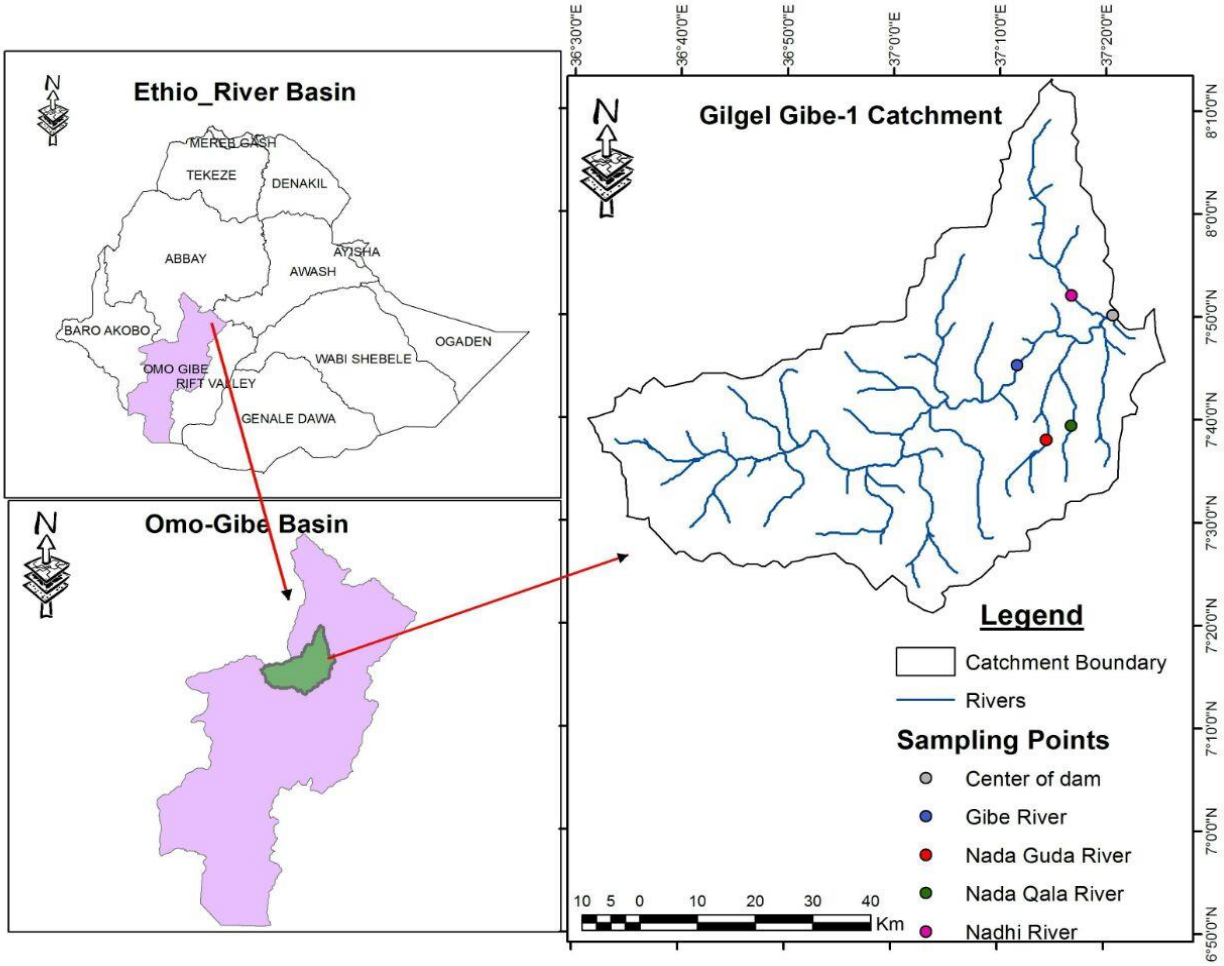

Figure 1. Map of the study area and the specific sampling sites.

Reagents and Materials

All chemicals and reagents used were analytical grade and solvents are HPLC grade. The organic solvents toluene was obtained from Blulux international PLtd (Stockholm, Malmo, Malmohus), n-hexane from Lobachemiepvt. Ltd., (Jehangir villa, Mumbai, India), methanol and acetonitrile were obtained from Carlo Erba reagents S.A.S (Mumbai, India). Sodium chloride was from Tecnopharmchem (Bahadurgarh, India). Whatman filter paper (grade 1 and size 8.5 cm) was used for filtration of the water samples.

Analytical standards of OPPs such as methidiathion, malathion, chloropyrifos, and OCPs including dichloro diphenyl trichloro ethane (DDT), Chlorflurenol methyl, endrin and Dieldrin

were obtained from Sigma Aldrich (St. Louis, MO, USA). Stock standard solutions containing 1000 mg/L of each pesticide were separately prepared in methanol and stored in refrigerator below 4°C. Intermediate working standard solution containing a mixture of 100 mg/L of methidathion, malathion, chlorphyrifos, and Chlorflurenol-methyl as well as 10 mg/L of DDT, endrin and dieldrin analyte was then prepared by diluting appropriate volume of each standard in methanol and then, the solution was stored in the refrigerator at 4 ºC. Working standard solutions were then prepared from the intermediate standard solution by diluting in n-hexane.

Instruments and Equipments

Separation and quantification of the target analytes were performed using Agilent Gas chromatography equipped with an electro capture detector (GC-ECD), auto sampler, pump, column compartment model 7980A (Agilent technologies, Singapore). An HP-5 capillary column (30 m, 0.25 mm inner diameter; 0.25-mm film thickness) coated with 5% phenyl methyl siloxane model 7890A was also obtained from Agilent technologies. A vortex mixer model FB15024 obtained from Fisher scientific (Kunstdal 21, 9900 Eeklo, Belgium) was used for sample preparation.

GC-ECD Operating Conditions

Analyses of the pesticide residues were performed using GC-ECD. The operation conditions of the instrument were adopted from the earlier report [26]. Accordingly, the oven temperature program was initial set at 80 °C which was ramped at 30 °C/min to 180 °C, which was then ramped at 3 °C/min to 205 °C. After 4 min it was again ramped to 290 at 20 °C/min and kept constant for 8 min before ramping to 325 °C at 50 °C/min. Nitrogen (99.99% purity) was used as a carrier gas at a flow rate of 20 mL/min and as a makeup gas at a flow rate of 60 mL/min. An aliquot of 1 µL was injected in split mode at a split ratio of 50:1 and injection temperature of 280 °C. With these conditions the total GC run time was about 28 min. The pesticide residues were detected with µ-ECD operating at a temperature of 300 °C. The gas chromatogram of the target analytes using the above mentioned GC-ECD operating condition is shown in Figure 2.

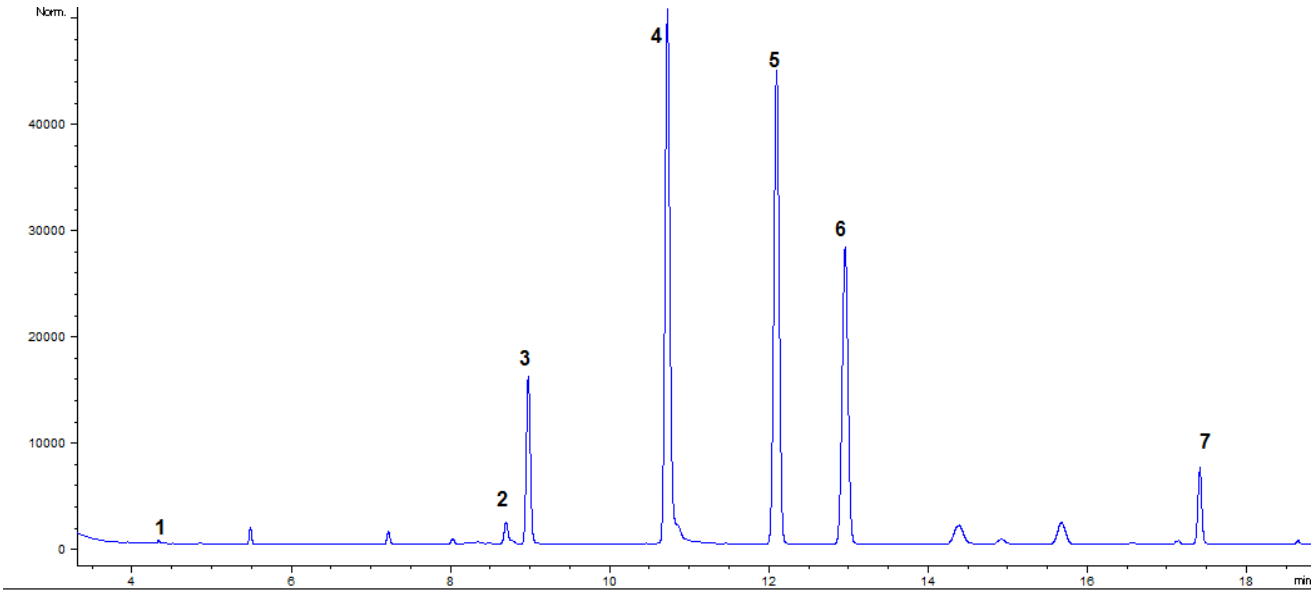

Figure 2. Gas chromatogram of the target OP and OC pesticides. Description of analytes (retention time, t_R, in min); 1) Methidathion (4.328); 2) Malathion (8.708); 3) Chlorphyrifos (8.978); 4) Chlorflurenol-Methyl (10.725); 5) DDT (12.095); 6) Endrin (12.957); and 7) Dieldrin (17.412).

DLLME Procedure

Low density based dispersive liquid-liquid microextraction (LD-DLLME) method which was earlier reported by Shen and coworker [21] was used with minor modification for extraction of the target pesticide residues from water samples. Accordingly, 5 mL water sample was taken into 15 mL centrifuge tube and then, a mixture of 100 µL toluene and 500 µL acetonitrile as extraction and disperser solvents, respectively, was rapidly injected using a 5 mL medical syringe. Subsequently, after the addition of 0.5 g NaCl (i.e., 10%, *m/v*) the content was manual shaken until the salt was completely dissolved. The sample solution was then vortexed for 30 s to enhance homogeneous distribution of cloudy suspension throughout the sample solution, and hence, to accelerate the transfer of analytes from aqueous phase to the extraction phase. The content was then centrifuged for 3 min at 3000 rpm to facilitate phase separation. Finally, 50 µL of the floating organic phase was carefully withdrawn via a micro pipette and transferred into 100 µL insert vial which was housed in 1.5 mL auto sampler vial into inject 1 µL into GC-ECD instrument.

Analytical Method Validation

For quantitative determination of the target analytes, calibration curves were constructed using six concentration points corresponding to 10, 100, 250, 500, 750 and 1000 µg/L for methidathion, malathion, chlorphyrifos and Chlorflurenol-methyl and 0.1, 1, 2.5, 5, 7.5, and 10 µg/L for DDT, endrin and dieldrin. Each concentration level was spiked in distilled water and then extracted using LD-DLLME. All concentration levels were extracted in duplicates and each extract was injected in duplicates. Then, calibration curves were obtained by considering the peak areas as the instrumental response versus the analytes concentrations. The analytical performances of the method was evaluated in terms of linear dynamic range, limits of detection (LOD) and quantification (LOQ), repeatability and relative recovery studies. LOD and LOQ were determined as 3 and 10 times the signal to noise ratio, respectively. Precision (repeatability) of the method was investigated in terms of relative standard deviation (RSD) of replicate determinations. The accuracy of the method was assessed by performing relative recovery studies by spiking pesticide standards at 2.5 and 5 µg/L concentrations levels onto NQR water sample as representative matrix.

Results and Discussion

The calibration curves constructed by considering the peak areas as the instrumental response versus the analyte concentrations were exhibited wide linear ranges and good coefficient of determinations (r^2) ranging from 0.995–0.999. LOD and LOQ of the method were also ranging from 0. 0001-2.5810 µg/L and 0.0005-8.6050 µg/L, respectively. Table 1 shows the analytical performance of the LD-DLLME with GC- ECD for the analysis of OCP and OPP from water samples.

Table 1: Analytical performance characteristics of the LD-DLLME combined with GC-ECD.

Analyte	LDR (µg/L)	r^2	LOD (µg/L)	LOQ(µg/L)
Methidathion	10–1000	0.996	2.5810	8.6050
Malathion	10–1000	0.995	1.3460	4.4850
Chlorphyrifos	10–750	0.996	0.0490	0.1640
Chlorflurenol-methyl	10–800	0.999	0.0060	0.0200
DDT	0.1–8	0.996	0.0001	0.0005
Endrin	0.1–10	0.999	0.0002	0.0010
Dieldrin	0.1–10	0.997	0.0020	0.0070

To study accuracy of the method, relative recovery (%RR) experiments were performed by spiking NQR water sample from a representative sample and distilled water at two concentrations levels (2.5 and 5.0 µg/L). The %RR of the analytes were determined by dividing the peak areas obtained from the differences of the spiked and the unspiked NQR water samples to the peak area obtained for the spiked distilled water times 100, as indicated by the following formula:

$$\%RR = \frac{A_{S(NQR)} - A_{us(NQR)}}{A_{s(DW)}} \times 100$$

Where: $A_{s(NQR)}$, $A_{us(NQR)}$, and $A_{s(DW)}$ are peak areas of spiked NQR, unspiked NQR and spiked distilled water samples.

The obtained average %RR values with RSD in the parenthesis are presented in Table 2.

Table 2: Average %RR and (RSD) of LD- DLLME coupled with GC-ECD (n = 4)

Analyte	**Level-1**	**Level- 2**
Methidathion	98 (7.4)	83 (7.6)
Malathion	76 (4.2)	69 (7.2)
Chlorphyrifos	93 (9.6)	87 (12.5)
Chlorflurenol-methyl	72(8.6)	104 (3.7)
DDT	83 (9.3)	89 (6.3)
Endrin	95 (1.1)	105 (0.8)
Dieldrin	67 (6.9)	82 (11)

The values in bracket are relative standard deviation (RSD %)

With the exception of Dieldrin and Malathion at level 1 and level 2, respectively, the obtained relative recoveries were ranging from 72-105%, demonstrating satisfactory relative recoveries according to IUPAC technical report for the regulatory limits for pesticide residues analysis in water samples [29]. The obtained concentrations of the target pesticide residues (μg/L) in the Dam and the four river water samples are presented in Table 3. Among the studied pesticides chlorphyrifos, Chlorflurenol-methyl, DDT, and dieldrin were detected in all water samples. Methidathion was detected in NQR and NGR water samples. But, endrin was not detected in all studied water samples. One way ANOVA ($p \leq 0.05$) indicated the presence significant differences in the concentrations of the studied pesticide residues in the water samples collected from the Dam and its four potential tributaries.

Table 3: Mean level (µg/L ± SD) of OCPs in water samples (n = 4).

Pesticides	Sample sites							MRL [29]	
	NQR	NGR	NR	GR	HDRR	LSD	CV	Acute	Chronic
Methidathion	142.66 ± 0.79^{a}	110.73 ± 3.24^{b}	ND	ND	ND	14.5	20.5	NA	NA
Malathion	30.82 ± 0.97^{a}	29.92 ± 0.32^{b}	29.67 ± 0.40^{bc}	29.06 ± 0.31^{cd}	28.54 ± 0.29^{d}	0.83	1.82	NA	0.1
Chlorphyrifos	3.17 ± 0.57 ^{c}	10.94 ± 0.29^{a}	1.67 ± 0.10^{d}	3.45 ± 0.42^{c}	5.71 ± 0.59^{b}	0.69	8.97	2.400	0.0043
Chlorflurenol-methyl	8.89 ± 0.05^{b}	8.84 ± 0.04^{b}	9.62 ± 0.47^{ab}	11.19 ± 2.60^{ab}	12.05 ± 1.99^{a}	2.55	16.3	NA	NA
DDT	0.61 ± 0.22^{bc}	0.60 ± 0.20^{c}	0.62 ± 0.01^{abc}	0.70 ± 0.12^{a}	0.67 ± 0.50^{ab}	0.08	8.53	1.100	0.001
Endrin	ND	ND	ND	ND	ND	ND	ND	0.190	0.061
Dieldrin	0.93 ± 0.25 ^{b}	0.92 ± 0.05^{b}	0.94 ± 0.40^{b}	0.97 ± 0.04^{a}	0.95 ± 0.02^{ab}	0.03	2.19	0.360	0.061

ND: not detected, NA: not available, SD: Standerd devation, LSD: least significance difference, CV: coefficient of variance, NQR: Nada Qalla River, NGR: Nada Gudda River, NR: Nadi River, GR: Gibe River, HDR: Hydroelectric Dam Reservoir; and MRL: Maximum residue level

Methidathion was detected at high concentration levels in NQR and NG, 142.66 ± 0.79 µg/L and 110.73 ± 3.24 µg/L, respectively. But, it was not detected in other water samples. This may indicate that the pesticide is excessively used in the study area and thus, can easily mix to the water system via run off, drift or other mechanisms [8]. On the other hand, the compound is low persistent and has short life time, moderate solubility in water and relatively high volatility [30]. These properties may contribute for the absence of the target analytes in other samples. Methidathion is acutely toxic to aquatic organisms [31].

Malathion was detected in all water samples. The lowest and highest malathion concentrations were detected in HDR (28.54 ± 0.29 µg/L) and NQR (30.82 ± 0.97 µg/L), respectively. One-way ANOVA ($p \leq 0.05$) demonstrated the presence of significant difference in the concentrations of the pesticide between NG and NR; NR and GR; as well as between GR & HDR water samples (Table 3). However, NQR, which contains the highest concentration of the pesticide, was significantly different from the other water samples. The lowest concentration of the pesticide in the HDR water sample may be attributed to dilution effect. The concentrations of malathion determined in all water samples were above the MRL of EPA for ambient water quality criteria for aquatic organisms for river water [29]. Thus, the HDR and its tributaries are not suitable for production of aquatic organism such as fish and also other consumption. Malathion is harmful chemical at small concentration level, for aquatic life such as fish and other organisms [31].

Malathion has relatively high water solubility, (i.e., 145 mg/L) and thus, it has high potential to transport in surface water and also ground water. In water it undergoes chemical and microbial degradation and converted to malaoxon and isomalathion, which are more toxic than the parent compound [32]. The rate and extent of its degradation is dependent on the chemical and physical properties of the water system, particularly temperature and the solution pH, in addition to the composition of the microbial population present in the system. Its degradation rate is fast in water at pH > 7.0. Biodegradation also plays a role when pH < 7.0 and its rate of hydrolysis are slower relative to the rate of biodegradation [32].

In the studied water samples, the obtained concentrations of chlorphyrifos were ranging from 1.67 ± 0.1 µg/L to 10.94 ± 0.29µg/L in NR and NGR, respectively. One-way ANOVA ($p \leq 0.05$) indicated that the concentrations of chlorphyrifos in the water samples were significantly

***Corresponding Author: Tel.: +251-9-12-03-2-08 OR +251-9-88-44-18-60**
E-mail: aberagure@gmail.com, abera.gure@ju.edu.et

different, with the exception in NQR and GR samples (Table 3). Chlorphyrifos is one of the highly toxic pesticides to fish and to aquatic invertebrate animals [33]. It is characterized by its low water solubility (1.0 mg/L) or moderate hydrophobicity and volatility [31].

Except NR sample, the studied water samples have very high amount of chlorphyrifos residues, which are higher than the acute and chronic MRL of the pesticide set by EPA for ambient water quality criteria, for aquatic organisms [29]. In NR the observed concentration was below the acute MRL but, it was above the chronic MRL of EPA. This may indicate that the pesticide has been intensively used by the farmers of the study areas. Generally, the water samples were contaminated by chlorphyrifos pesticide at the level that it can cause acute and chronic health effect on aquatic organisms and other consumers of the water.

The concentrations of chlorflurenol-methyl in the studied water samples were ranging from 8.84 ± 0.04 µg/L in NG to12.05 ± 1.99 µg/L in the HDR samples, respectively. One-way ANOVA ($P \leq 0.05$) demonstrated that there was no significance difference in the concentration of the chlorflurenol-methyl among the water samples. But, HDR water exhibited significantly difference in concentration of chlorflurenol-methyl from the others (Table 3).

Chlorflurenol-methyl is an obsolete herbicide and banned for use in the EU or USA [31]. It has actually low mammalian oral toxicity and also not well evaluated for chronic health impacts. It is moderately toxic to fish and aquatic invertebrates [17]. Generally, the studied water samples contain high concentrations of the pesticide, indicating that the surrounding communities are still using and/or had been used the pesticide on their farmlands to control weeds.

DDT was also observed in the water samples, ranging from 0.60 ± 0.20 µg/L in NQR to 0.70 ± 0.12 µg/L in GR, respectively. The observed concentration of DDT in all water samples are below MRL set by EPA, But, in all water samples the detected concentrations were above its chronic toxic effect on aquatic organisms live in fresh water [29]. One way ANOVA ($p \leq 0.05$) indicated the presence of significance differences in concentrations of DDT in NG and GR water samples. The order of the water samples in terms of the concentrations of DDT was NG ≈ NQR ≈ NR < HDR < GR. This indicated that the study areas might be still using DDT, and thus, use of the water from the dam and its tributaries may have long term impact on the health of the

consumers. Literature also indicated the presence of high concentration of DDE, the metabolite of DDT, in the dam and tributaries of the dam [27].

The observed concentrations of dieldrin were ranging from 0.92 ± 0.05 µg/L in NG to 0.97 ± 0.04µg/L in GR, respectively, which are above EPA acute and chronic MRL for fresh water aquatic organisms [19]. Dieldrin is acutely toxic to fish and also persistence in the environment. One way ANOVA ($p \leq 0.05$) indicated that except in GR, there is no significant difference in the concentrations of dieldrin in the water samples. Generally, the observed results showed that the compound may be still intensively used in the study areas. A study conducted on similar area indicated the presence of high concentration of dieldrin in the water samples [27], This may indicate that the pesticide is still used in the area.

Conclusion

In this study, selected OP and OC pesticides including malathion, chlorphyrifos, methidathion, Chlorflurenol-methyl, Endrin, DDT and Dieldrin were determined from Gilgle Gibe I hydroelectric Dam and its potential tributaries water samples using GC-ECD. LD-DLLME was used for extraction and preconcentration of the target pesticides. To perform quantitative determinations, calibration curves were constructed by extracting the spiked distilled samples at six concentration levels. Results of the study demonstrated that all water samples contain the target pesticides, except endrin, which was not detected in all samples. Methidathion was also detected only in NQR and NG water samples. The obtained results indicated that the concentrations of the detected pesticides were above the EPA acute (except DDT) and chronic toxicity MRL set for the ambient water quality criteria for aquatic organisms. Generally, obtained finding indicated that the water samples contain high concentrations of the studied pesticide residues and thus, consumption of the Dam and its tributaries waters may have great effect on the consumers.

Conflict of Interest

The authors declare that there is no conflict of interest regarding publication of this Article

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