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DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN WATER, SEDIMENT AND SOIL USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT. The purpose of this study was to develop a method for the extraction and determination of 2,4-dichlorophenoxyacetic acid (2,4-D) from the soil, sediment, and water, and to investigate its stability in water using high-performance liquid chromatography (HPLC). The recoveries of the spiked compound from water and soil samples was found to be in the range of 80 to 100%. The yield of 2,4-D during acidification of the corresponding amine was 93.84-99.98%. A calibration curve for the method showed good linearity ($R^2 \geq 0.9996$). The LOD was determined to be 0.45 µg/mL while the LOQ was 2 µg/mL. From the analysis of the samples, 2,4-D was not detected in sediment or soil samples from the Wafiko or Kontola sites (Ethiopia), respectively. The 2,4-D concentrations in soil samples from Bochessa, and water samples from Wafiko and Sher sites (Ethiopia) were high, ranging from 68.22 to 167.7 mg/L which exceeded the United States Environmental Protection Agency (US EPA) regulatory agency standards of 70 µg/L. A 45-day experiment on spiked water samples from Lake Koka (Ethiopia) demonstrated that the acidic form of 2,4-D is stable in water with 73.46 ± 2.00% recoveries. The developed method can be used to determine 2,4-D residues in soil, sediment, and water.

KEY WORDS: 2,4-Dichlorophenoxyacetic acid, Lake Koka, Sediment, Soil, Stability, Water

INTRODUCTION

The environment is continually affected by modern agricultural practices such as the extensive use of pesticides. It is well known that a significant portion of pesticides applied in the fields enters the environmental components such as lakes, rivers, and oceans, and they may ultimately accumulate in plants and animals. Pesticides may enter the aquatic system via the surface or subsurface hydrological pathways. This is usually triggered by spray drift, runoff water, and drainage water [1]. Improper operations, such as the filling sprayers, cleaning measuring utilities, disposal of used pesticide containers and cleaning of spraying equipment, may also lead to unnecessary accumulation in the environment. Kumar *et al.* stated that the accumulation of these pesticides brings possible harm to the environment [2]. Therefore, assessing the different types of pesticides that runoff into rivers and reservoirs is essential. In particular, water bodies located in the agricultural catchment face more serious challenges due to drainage systems, runoff, and a rapid increase in the application of pesticides [3]. Regardless of its toxicity, it has been reported that over two million tons of pesticides are applied annually across the globe as farming input [4-6].

Boström and Fogelfors have noted that of the agrochemicals utilized, herbicides are the most frequently used pesticides in many countries [7]. One of the most widely used herbicides in the cultivation today for the broadleaf weed control is 2,4-D either in acidic or in its amine salt form. It is classified as a phenoxyalkane acid [8]. Due to their high toxicity to dicotyledonous plants and low toxicity to monocotyledonous plants, 2,4-D herbicides are often used as selective herbicides

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[9, 10]. Apart from their usage as herbicides, these compounds are often used in high-input farming as seedless fruit germination promoters, defoliating agents, and typically as hormone growth regulators [8]. It has been reported that 2,4-D is usually used due to its limited persistence period, low-cost, and well-understood aquatic dynamics. The 2,4-D has been related to polychlorinated dibenzodioxins; most commonly with 2,3,7,8-tetrachloro-*p*-dibenzodioxins. This substance is a well-documented toxin, teratogen, carcinogen, and persistent. 2,4-D has been frequently detected in surface or groundwater worldwide because of its poor biodegradability and volatility [9, 10].

Due to their occurrence at trace levels and the complexity of environmental samples, the analysis of these pesticides requires selective and efficient sample preparation methods that can extract and pre-concentrate them simultaneously, before their instrumental determination [11]. Different researchers have tackled the problems associated with the analysis of 2,4-D by designing different analysis techniques. Mohammadnia *et al*. analyzed 2,4-D in water and food samples using HPLC via dispersive micro-solid phase extraction method using magnetic graphene oxide tert-butylamine nanocomposite, as an efficient sorbent [12]. The applied method is efficient with regard to analytical parameters. However, the micro extraction and sorbent could require additional costs. Similarly Rouhollah and Rezvan employed salt-assisted liquid-liquid extraction technique that was coupled with HPLC for determination of 2,4-D in water and edible seeds [13].

Given the significant increase in 2,4-D usage in Ethiopia [14, 15], it is necessary to develop rapid, environmentally friendly, low-cost, and sensitive methods for monitoring the presence of 2,4-D in environmental samples. In addition, there is a lack of data on the levels of 2,4-D in Ethiopia, making it impossible to take necessary measures. Thus, the purpose of this study was to develop a rapid, sensitive, and selective method for detecting 2,4-D at trace levels in the water, sediments, and soil samples using HPLC.

EXPERIMENTAL

Study area

Lake Ziway and Lake Koka in the Ethiopian Rift Valley were selected as sampling sites for the study. The Lake Ziway watershed, which is primarily comprised of smallholder agricultural lands, covers an area of 7032 km^2, ranging from latitudes 7° 22' 36"N to 8° 18' 21"N and longitudes 37° 53' 40"E to 39° 28' 9" E. It stretches from the western border of the Gurage Mountains to the eastern border of the Arsi Mountains. It is above 3500 m above sea level in two Ethiopian administrative regions, with 73.6 percent located in Oromia Region and the remainder in Southern Nation Nationalities and Peoples Region (SNNPR) [16]. Lake Koka is located at 98 km from Addis Ababa towards southern Ethiopia, at an elevation of 1590 m above sea level. The lake has a surface area of 220 km^2 and a maximum and mean depth of 14 and 9 m, respectively [14]. The lake water conductivity was measured to be 251 S/cm at 25 °C and had a pH of 7.4 during the study period.

Chemical, reagents, and instruments

2,4-D amine-salt solution (dimethyl amine salt) was purchased from Addis Ababa pesticide vendors. Analytical-grade dichloromethane, HPLC grade acetone, methanol, acetonitrile, hexane, sodium sulfate, methanol, ethylacetate, hydrochloric acid and chloroform were obtained from Sigma Aldrich (Seelze, Germany). Whatman filter paper number 1 of medium porosity was obtained from Schleicher and Schuell (Germany). All other ingredients were of analytical grade, unless stated otherwise. The chromatographic analyses were performed using Agilent Technologies, a high performance liquid chromatograph (HPLC) (Agilent 1260 Infinity, Germany) coupled to a diode array detector (DAD).

Sampling and sample preparation

Sampling points were purposively chosen for the sampling of water, soil, and sediment samples. The US EPA method 1699 sampling procedure for water was followed [17]. Water samples were collected from Koka Dam and Lake Ziway (Wafiko and Sher site) for method validation and 2,4-D monitoring, respectively. For 2,4-D monitoring, samples were collected in May 2021 at the following coordinates: 7° 22' 36"N and 8° 18' 21"N, and longitudes of 37° 53' 40"E and 39° 28' 9" E. Three different points were sampled using a bottom water sampler. Six samples were collected in total, with one taken at the surface and another at a depth of 1 m at each point. All the water samples collected for this study were stored in 2 L amber bottles and transported to the organic chemistry laboratory at Addis Ababa University. The water samples not exposed to 2,4-D, for method validation, were collected from Koka Dam after analysing in our lab prior to use.

The US EPA method 5035 for soil sampling was followed [18]. Triplicates (100 g) of soil samples were collected for method validation (spiking) at three different sampling locations in a garden not exposed to 2,4-D herbicide at Addis Ababa University's College of Natural and Computational Sciences (Ethiopia). Absence of 2,4-D herbicide in the samples collected from the garden was confirmed by HPLC analysis and thin layer chromatography (TLC) co-spotting method. The samples were taken using an auger from the land surface to a depth of 15 cm, after the removal of leaves, debris, and roots from the soil surfaces. They were placed in polyethylene soil sample bags and brought to the laboratory. Soil samples for 2,4-D monitoring were collected from Bochessa and Kontola sites at six different sampling locations in polyethylene bags from suspected 2,4-D-exposed agricultural fields near Lake Ziway. The samples were collected using an auger from the land surface to a depth of 10 and 15 cm at each point. Twelve 300 g soil samples were collected and transported to the laboratory.

Sediment sampling was conducted following US EPA method 5035 [18]. Six sediment samples were collected using a sediment grab sampler from three different sampling points at Wafiko and Sher sites. The samples were placed in polyethylene sample bags and transported to the laboratory.

Water samples were filtered through 0.45 cellulose acetate filter papers (0.45 µm, Micro Science and 110 mm Smith F1/KA4, Germany) for further analysis. After filtering the samples, the pH was immediately determined. Soil samples were air-dried for 24 h, homogenized, sieved (150 mesh), and stored at room temperature until the analysis. Sediment samples were suspended in water and then shaken vigorously for half an hour on a shaker. Suction filtration was used to filter the sample. The filtrate was kept for extraction.

For the extraction of spiked 2,4-D from water, about 100 mL of spiked water collected from Lake Koka was measured, acidified with aqueous HCl to pH 1, transferred to a 1 L separatory funnel, and partitioned with ethyl acetate (50 mL × 3) after it was vigorously shaken for 3 min. The organic phases were separated, mixed and washed with sodium bicarbonate saturated solution. The organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness using a rotary evaporator under vacuum, dissolved with 1 mL methanol, transferred to a 2-mL vial, and kept in a refrigerator until analysis. The same procedure was followed to extract samples from Lake Ziway.

For sediment samples, 100 g of the sediment was weighed in a beaker and transferred to a conical flask. 100 mL of distilled water was added and then shaken vigorously for 30 min on a shaker. Suction filtration was used to filter the sample. After that, it was acidified and set aside for 15 min. The acidified compound was extracted with ethyl acetate (50 mL × 3) and concentrated before being dissolved in 1 mL methanol for HPLC analysis.

Soil samples were extracted using a Soxhlet extractor. The spiked soil of 10 g (different concentrations) was transferred into a thimble; a round-bottom flask was placed in a cork ring, anti-bumping granules, and 90 mL methanol were added, and the flask was clamped and transferred to a heating mantle. The thimble was placed in the Soxhlet and 10 mL of solvent

(methanol) was added from the top. Soxhlet apparatus was attached to the water condenser. The methanol in the round-bottomed flask was heated to boiling. The heating process was kept for various extraction times (2, 4, 5, and 7 h) to evaluate complete extraction of the analyte. Then the heating process was stopped; the methanol extract was filtered and concentrated to dryness. The residue was dissolved in acidified water (15 mL) and transferred to a separatory funnel for liquid-liquid extraction which was carried out using ethyl acetate (30 mL × 3). The organic phases were separated, mixed, dried over sodium sulfate, filtered, and concentrated, dissolved with 2 mL methanol, transferred to the 2-mL vial, and kept in the refrigerator until analysis. Percentage yields were determined for various extraction times. The extraction took place in triplicates of every variable (time of extraction and concentration). The same procedure was followed in monitoring 2,4-D acid from the soil samples obtained from Bochessa and Kontola agricultural fields in Ziway.

Extraction of 2,4-D amine salt (a standard) from the 2,4-D amine solution

Samples were Soxhlet-extracted at 2, 4, 5, and 7 h to determine the optimal extraction time. An appropriate solvent for extracting 2,4-D amine from soil was determined. Different volumes of methanol were investigated to determine the highest recovery rate with a minimum possible volume. The 2,4-D amine salt was obtained from the amine solution (86% w/v) by freeze-drying using a Modylo-D Thermos Avant freeze dryer for 24 h at a temperature of -5 °C. The crude 2,4-D amine salt was subjected to recrystallization using acetone, for further purification. The recrystallized sample was acidified to make 2,4-D acid. The purity of the standard samples was confirmed by TLC, nuclear magnetic resonances (NMR), gas chromatography-mass spectrometry (GC-MS), and HPLC.

Confirmation of purity of the 2,4-D amine crystals

The purity of recrystallized 2,4-D amine was checked using ^{1}H- and ^{13}C-NMR. The rycrystallize 2,4-D amine (10 mg) was dissolved in 0.5 mL D_2O and/or $(CD_3)_2CO$ and analyzed using Bruker 500 MHz NMR machine (Germany).

Confirmation of purity of 2,4-D acid

The purity of acidified 2,4-D was confirmed by using thin lyer chromatographic plate and HPLC. Thin-layer chromatography (TLC) was performed with Merck Kieselgel 60 F254 plates which were visualized under UV light and by spraying with vanillin, H_2SO_4 (5%) in MeOH, and cerium molybdate stain. The acidified material was spotted on the TLC plate along with the starting material to check the complete transformation of the amine form into its acidic form.

Instrumental working procedure for the quantitative determination of 2,4-D using HPLC-DAD

The HPLC-DAD used was Agilent made 1260 infinity (USA) equipped with G1311B quaternary pump, G1329B auto-liquid sampler, G1315C diode array detector (DAD), and G1316A thermal column compartment (TCC). The mobile phase was acetonitrile : water (75:25, 0.2% formic acid) for 8 min in an isocratic mode. The injection was made via the autosampler and the injection volume ranges from 5-10 µL in a standard mode. Reversed phase C-18 column (UAS made by Agilent) with a particle size of 5 µm, an internal diameter of 4 mm, and a length of 250 mm was used for the separation and identification of components. The DAD wavelength was set at 230 and 280 nm for 2,4-D determination.

Recovery percent of the spiked samples

Pesticide-free soil was spiked following the principle described by Tor *et al*. [19]. A sieved soil sample (10 g) was weighed in a beaker and spiked at the required concentration level by adding

different volumes of the standard (0.25 mL and 2 mL of 1 g/mL solution). Water samples were spiked following the principle by Rezaee *et al*. [20]. 15 (100 mL each) water samples were measured in 15 different beakers. Every 100 mL of the water was then spiked with 100 mg of 2,4-D amine salt. The accuracy of the analytical method was evaluated through recovery studies for both the soil and the water samples. The recovery percent of the spiked samples were calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Compound mass after extraction}}{\text{Compound mass spiked}} \times 100\%$$

Calibration curve construction

A 100 mL of 100 mg/L 2,4-D standard solution was prepared and serially diluted to 0.25, 1, 10, 20, 40, 60 and 80 mg/L. Then 1 mL of the sample from each concentration was transferred into HPLC vials and analyzed at 25 °C with an injection volume of 10 μL. Data was recorded at 230 and 280 nm wavelengths. All the analyses were done in triplicate.

Determination of 2,4-D in water, sediment, and soil samples

The analysis was carried out by US EPA method 1699 for the determination of pesticides in soil, water, and sediment samples [17]. A total of 6 water, 6 sediment and 12 soil samples were collected from Lake Ziway. The samples were analyzed in accordance with the aforementioned method on spiked soil and water samples. HPLC was used for quantitation using external standard method.

Determination of the stability of 2,4-D amine in lake water

A stability test was conducted by spiking 2 L of the lake water with 2 g recrystallized 2,4-D amine salt. The samples were extracted on time course bases for over a period of 45 days from zero hour to check the degradation of the compound. For the analysis, the spiked lake water was placed on a shaker for 5 min to maintain homogeneity of the solution and 100 mL was measured and acidified to pH 1 using HCl, extracted with ethyl acetate (EtOAc) three times. The organic phases were combined and washed with sodium bicarbonate, dried over anhydrous sodium sulfate, filtered, and concentrated. This procedure was repeated for all the analysis days (from the 0-45th day). The identity and purity of the compound were checked using TLC co-spotting technique. For the first nine days, spiked water samples were extracted every 24 h. However, after the 10th extraction, the water samples were extracted after every 5 days. The recovery rate of the compound was calculated to monitor the degradation of the compound or the stability in the lake water. The presence of any degradation products was checked by HPLC.

RESULTS AND DISCUSSION

Extraction, recrystallization, acidification, and esterification of the 2,4-D amine salt

A solution of 2,4-D amine turned into brownish crystals after freeze-drying, which was subsequently purified via recrystallization. The recrystallization step was necessary to remove any impurities before preparing working standards. The recrystallized 2,4-D salt turned from brownish to clear crystals. The compound was then acidified to obtain 2,4-D acid for HPLC analysis. The formation of only one spot on a developed TLC plate confirmed the purity of every compound at each step which NMR also confirmed.

Recovery ranges (% yield) of 2,4-D acid

The 2,4-D amine salt was acidified, and extracted in order to verify the yield of the acid. It was found that the yield was in the range of 93.84-99.98%. Based on this and other findings, the stability test and real samples analyses were conducted.

Optimal Soxhlet extraction time

Table 1 summarizes the soil extracts obtained after varying the extraction time. The experimental results revealed that for the different levels of spiked amount of the analyte, 4 h of extraction time was optimum. It produced the exact percentage yield as 5 and 7 h of extraction, where 7 h is the recommended time for a standard Soxhlet extraction [21, 22]. This may be attributed to the very fast mass transfer [23] taking place initially but before the establishment of the equilibrium state, which was achieved later around 4 h. Therefore, an extraction time of 4 h was found to be the optimum time and used throughout this study. Similarly, the amount of solvent used was checked all the way from 80 to 150 mL. It has been determined that amount of the extracts obtained after the extractions were pretty much the same for solvent volume ranges from 90 to 150 mL. Hence, 90 mL methanol was used.

Table 1. Recovery (%) of 2,4-D amine salt in spiked soil sample extracted at different times.

Spiked 2,4-D amine salt solution	Duration of extraction (h)	% Recovery
250 µL*	2	45 ± 0.3%
	4	98 ± 0.2%
	5	100 ± 0.2%
	7	100 ± 0.1%
2 mL*	2	60 ± 0.2%
	4	100 ± 0.4%
	5	100 ± 0.2%
	7	100 ± 0.1%

*Commercial solution without any purification.

Comparison of the proposed method with similar literature reports

The figures of merit of the Soxhlet method developed in the presented study for the determination of 2,4-D amine salt in water, sediment, and soil samples have been compared with other reported techniques [8, 24]. Details of the relevant results of the methods and that of this study are provided in Tables 2 and 3. The proposed method involves minimum labor and requires a short extraction time when compared to standard/tradition method. The performances of the developed technique were compared with that of the previously reported techniques of Kashyap *et al.* [8] and Atalay and Hwang [24] in terms of relative recovery and regression coefficient (R^2). The findings confirmed that the developed technique is found to be comparable in terms of percent recoveries. When it comes to the time of extraction and even the percent recovery, the modified Soxhlet experiment conducted by Kashyap *et al.* [8] was found to be better. Furthermore, it could also be noted that the developed method utilizes simpler and traditional laboratory equipment which could be accessible in most common research laboratories.

In summary, the advantages of the extraction method developed in this study include the use of a small volume of organic solvent (90 mL) in comparison to the "traditional" method, a shorter extraction time (4 h) when compared to the "traditional" method as well, and higher recovery rates (91 ± 2%). Table 2 compares the standard and optimized methods in a variety of ways. The study discussed here demonstrates that the extraction procedures used improved the ease, reproducibility, and throughput of 2,4-D herbicide analysis.

Table 2. Comparison of recoveries (%) of 2,4-D amine salt in the spiked soil sample with literature data.

Soil sample	% recoveries using the standard method [8]	% recoveries using the modified method [8]	* % recoveries using optimized method (methanol)
1	55 ± 5%	95 ± 5%	90 ± 3%
2	60 ± 4%	91 ± 2%	91 ± 2%
3	50 ± 4%	94 ± 5%	94 ± 5%
4	58 ± 6%	96 ± 1%	93 ± 2%
5	49 ± 6%	93 ± 4%	88 ± 2%
6	55 ± 5%	90 ± 2%	91 ± 5%

*2,4-D amine salt (purified).

Table 3 summarizes the comparison of this study and other studies in terms of recovery with percent relative standard deviation (%RSD) in spiked soil samples, the amount of solvent, time for extraction and concentration, and also the linearity of the method. According to the results, the optimized method appears to be an effective tool for sample extraction using unmodified available laboratory apparatus and without additional clean-up. It has a higher extraction efficiency than the widely used standard Soxhlet, particularly when it comes to extracting 2,4-D amine salt from the soil. The findings indicate that this method facilitated sample extraction, identification, and quantification. A one-tailed paired t-test performed in excel on the recovery rates of the optimized method and the standard method with variance unknown gave a p-value of 1.0051×10^{-7}. The obtained p-value was less than 0.05, hence recoveries obtained in this study were significant and did not occur by chance.

Table 3. Comparison of the standard method, modified and the optimized methods from literature for 2,4-D extraction.

Methods	Type of solvent	Amount of solvent (mL)	Extraction time (h)	Average recovery	LOD (µg/L)	R^2	References
Soxhlet-HPLC (UV)	Methanol	80-150	4	91.2 ± 3%	0.45	0.9996	This study
Soxhlet-HPLC-(UV)	Acetonitrile	250-500	4	55 ± 4%	5	0.996268	[8] Standard
Soxhlet-HPLC-(UV)	Acetonitrile	25-50	0.5	95 ± 5%	3	0.996268	[8] Modified
Soxhlet-GC-ECD	Methanol	200	48	97%	-	0.99	[24]

Method validation

Spiking the soil samples and water samples for method validation

To validate the method, 2,4-D amine salt was spiked to soil and water samples. The calibration curve and linear ranges of the detector response were determined using standard solutions of 2,4-D acid with concentrations ranging from 1 to 100 mg/L. Precision was determined using repeatability in recoveries and evaluated using replicates when extracting and analyzing soil samples at various spiking concentrations (0.25 and 2 g/mL). The accuracy of the analytical method was established through recovery studies on soil and water samples (Table 4). The presence of the target analytes in the spiked water and soil samples were established using thin-layer chromatography with the spiked sample extract covering the same distance (R_f) as the standard 2,4-D acid. The target analyte's retention time also confirmed its presence in the spiked samples during HPLC analysis.

The method's overall performance was acceptable in recovery (80–88%) (Table 4) and precision. Seven different concentrations of 2,4-D acid in the range of 1-100 mg/L were used to plot calibration curves. The calibration curve was used to verify linearity. The detector response of 2,4-D acid was linear within the given range, with correlation coefficients (R^2) = 0.9996. The LODs and LOQs were 0.45 µg/mL and 2 µg/mL, respectively, which are generally comparable

to the methods reported in literature [8]. The amount recovered shown in Table 4 is lower than the recoveries reported in Tables 1 and 2. The possible reason could be, in this experiment, the spike sample was 2,4-D amine salt, however, the recovery was calculated based on the amount of acid after a chemical transformation. This shows there was a loss of yield during the process.

Table 4. Recoveries (%) of 2,4-D acid from the spiked soil and water samples.

Experiment No.	Recoveries (%) for soil samples	Recoveries (%) for water samples
1	80.0 ± 3.1	83.0 ± 2.7
2	84.0 ± 1.2	88.0 ± 2.3
3	86.0 ± 3.5	84.0 ± 0.6
4	86.0 ± 1.2	84.0 ± 2.7

Detection and determination of 2,4-D in water, soil, and sediment by using HPLC

The proposed HPLC-DAD method was applied for the selective and quantitative extraction and determination of 2,4-D acid in the water, soil, and sediment samples collected from Lake Ziway. The extracts of the samples were analyzed to determine the concentration of 2,4-D. Peak identification was performed by comparing the retention times of analytes to those of pure standard of 2,4-D acid. Quantitative determination of 2,4-D acid was done after constructing the calibration curve of the standard working solutions of 2,4-D acid.

The analytical method was developed to check the presence of the 2,4-D herbicide residues in the water, and soil samples (Table 4). Following similar procedure, the presence of 2,4-D in the samples was conducted. Tables 5 and 6 show the results obtained when the soil, water, and sediment samples were analyzed with HPLC.

Table 5. 2,4-D detected in the water samples taken from Wafiko and Sher site.

Samples	Sampling site	Type of sample	Concentration level (mg/L)	Mass of 2,4-D per 100 mL of water (mg)
1	Wafiko and Sher site	Water sample	142.6	1.43
2			96.68	0.97
3			167.7	1.68
4			128.2	1.28
5			143.4	1.43
6			94.46	0.94

Table 6. 2,4-D detected in soil samples taken from Bochessa site.

Samples	Sampling site	Type of sample	Concentration level (mg/L)	Mass of 2,4-D per 100 mL of water (mg)
1	Bochessa site	Soil sample	75.60	0.76
2			68.22	0.68
3			131.88	1.32
4			98.44	0.98
5			111.76	1.14
6			95.58	0.96

Typical HPLC chromatograms for the samples are shown (Figures 1 and 2). The peak at the retention time of 2.7 min corresponds to 2,4-D acid. From the chromatograms, other organic compounds which were not identified in this research were also observed. There is a high probability that these are also some pesticides that are used in the area.

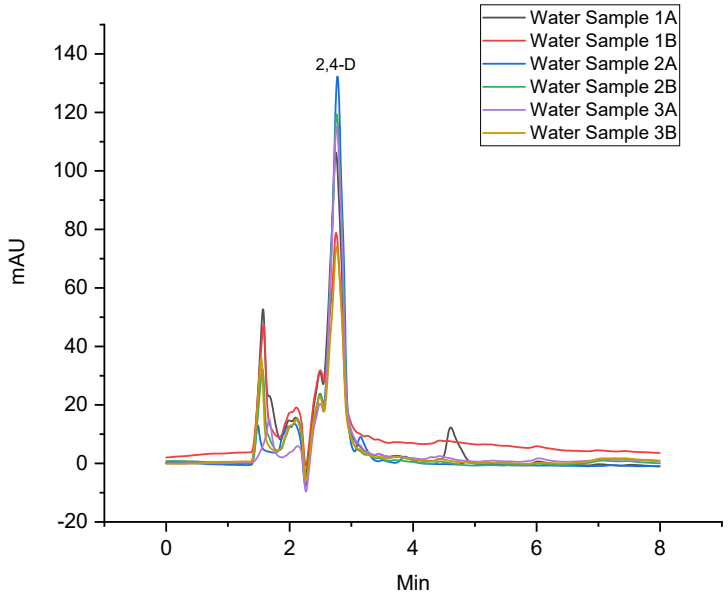

Figure 1. HPLC overlaid chromatograms of the water samples from the Wafiko and Sher site.

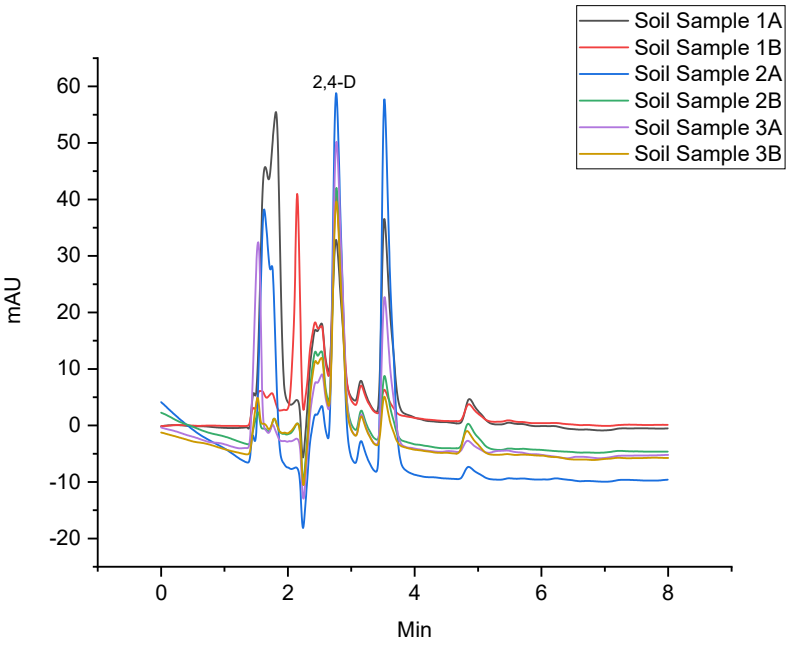

Figure 2. HPLC overlaid chromatograms of soil samples from the Bochessa site.

From the tables, it can be noted that in the soil samples taken from the Bochesa site (Table 6), 2,4-D in its acidic form was detected and the measured concentrations range between 68.22 mg/L to 131.9 mg/L. For the water samples collected from Wafiko and Sher site (Table 5), a significant amount of 2,4-D acid was observed which ranges from 94.46 mg/L to 167.7 mg/L. However, there was no 2,4-D observed in the sediment samples collected from the same site. The findings on sediment can be attributed to the fact that 2,4-D is polar and hence most likely stays dissolved in water. As such, in a lake, it can only accumulate in the sediment if and only if the lake is saturated with the compound which is less likely. Likewise, no 2,4-D was detected in the soil samples obtained from the Kontola site. By the time soil samples were collected, the farmland was already cultivated with cabbage; that was an indication that farmers in the area are well aware of where to apply the herbicide. Cabbage is grouped under broadleaf plants which can easily be affected by 2,4-D. It is effective to control broadleaf weeds as per the information obtained from farmers in the area.

The United States Environmental Protection Agency (US EPA) set the maximum allowable level of 2,4-D at 70 µg/L (0.07 mg/L) [8] in water for human consumption. Similarly, the European Union (EU) set the maximum residue levels, for individual pesticides at 0.1 µg/L and 0.5 µg/L for mixtures of pesticides [25]. However, in Ethiopia, there is no established maximum pesticide residue limit for drinking water except DDT [26].

Based on the findings of this study, 2,4-D in acidic form was detected in the water sample of the Wafiko and Sher sites with a range of 94.46 mg/L to 167.7 mg/L and the soil samples from the Bochesa site 68.22 mg/L and a maximum of 131.9 mg/L which is higher than the limit set by US EPA [8], and the EU [26]. Even if the water from this source is not used for drinking purposes, however, the findings of this study could be used as a warning alarm for the need for continuous monitoring programs to protect the environmental deterioration, and minimize human and animal health risks possibly caused by the future accumulation of the pesticide residues in the study areas.

Table 7. Stability check through percentage recoveries of spiked 2,4-D amine in water.

Days	Time intervals (h)	Amount extracted (mg)
1	0	80.0
2	24	74.3
3	48	72.7
4	72	73.8
5	96	73.9
6	120	73.8
7	144	72.3
8	168	72.1
9	192	75.3
10	216	72.1
15	341	72.1
20	480	72.4
30	720	72.3
40	960	72.5
45	1080	72.3

Stability of 2,4 D acid in water

The results of this study show that 2,4-D is stable in water for more than 45 days. An average recovery of 73.46 ± 2.00% was achieved (Table 7). These findings were inconsistent with Karanth's report on the 10-day stability of 2,4-D in water [27]. However, the results agreed with Toft's report [28], except that in his report, stability for more than 30 days is expected under anaerobic conditions, which was not the case in this study. Although this study was conducted in

an aerobic environment, stability of more than 30 days was obtained. There is a high possibility of protonation of the 2,4-D amine salt in acidic water bodies [29]. The reaction is believed to be very fast, as it requires a single proton transfer. According to the National Pesticide Information Center (USA), 2,4-D amine salts and esters are not persistent under most environmental conditions. Typically, the ester and amine forms of 2,4-D are expected to degrade rapidly to the acid form. Soil half-life values have been estimated at 10 days and 15 days for aquatic environment [29]. This is exactly why this study targeted both 2,4-D amine salt and acid forms at the same time. In every case, the final analysis was conducted after conducting acidification reactions.

CONCLUSION

The proposed method could detect 2,4-D in soil, sediment, and water. Under the optimum conditions, the method was found to be linear over wide concentration ranges with the coefficient of determination of 0.9996; exhibited acceptable precision (%RSD $\leq$ 5%), and satisfactory relative recoveries ranging from 80-100%. Employing the optimized experimental parameters, trace level extraction followed by HPLC-UV determination of the target analytes in the samples collected from the Ethiopian rift valley was successfully achieved. The results indicated that 2,4-D was detected in water from Wafiko and Sher sites and soil samples from the Bochessa site. As the current method was targeted at the extraction of both 2,4-D amine and 2,4-D acid at the same time, the extraction efficiency was found to be satisfactory. Before analyzing standards and real samples, acidification step was found to be crucial so as to convert 2,4-D amine in its acidic form. As a result, all analyses were conducted on 2,4-D acid. The observed 45-day stability of 2,4-D acid suggests the possible hydrolysis of the amine form which can exist in its acidic form. The developed method is simpler, cheap, rapid, and reliable for selective and quantitative extraction of trace level 2,4-D residues.

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