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DLLME coupled with HPLC-DAD for enrichment of pesticide residues in environmental sediment and soil samples

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ABSTRACT: Sample pre-treatment during determination of pesticides in sediment and soil is difficult due to matrix effects. For this reason, a low density dispersive liquid-liquid microextraction (LD-DLLME) was developed for the determination of carbaryl, cynazine, atrazine, and propazine. The experimental parameters that could potentially influence performances of the developed analytical technique including the extraction solvent type and volume, disperser solvent type and volume, extraction and centrifugation time, centrifugation speed, salt concentration, and pH were optimized. The optimum experimental values were found to be 50 µL 1-octanol, 0.6 mL acetonitrile, 5 min extraction time, centrifugation at 3500 rpm for 3 min, 10% NaCl and pH 5. At the optimum conditions, the methods offer good linearity (R^2 = 0.998–0.999) for the concentration ranges of 30-800 µg/kg; the detection limit (LOD) ranging from 9-24 µg/kg; precision ≤5.3% RSD, and reproducibility 0.5-5.2% RSD. The accuracy of the method, determined in terms of recovery was found to vary from 74.5-109.7%. Therefore, the developed analytical method could be used for the determination of trace level of pesticides residues in sediment and agricultural soil samples.

Keywords/Phrases: HPLC-DAD, Microextraction, Pesticides residues, Sediment, Soil

INTRODUCTION

Pesticides are used in the agro-industry and health sectors to improve agricultural products and to control vector-borne diseases, respectively. However, excessive use of synthetic pesticides increases the level of pollution in the environment through runoff, volatilization, and bioaccumulation along the food chain (Teshome Tolcha *et al.,* 2021). The pesticide residues in the environment and food are becoming a major concern to the consumers because these substances have harmful effects on other non-targeted organisms than diseases and pests. For example, the uncontrolled and extensive use of carbamate and *s*-triazine pesticides in agriculture raised serious public concern for food safety and the environment (Mnif *et al.,* 2011). Carbamate is a broad-spectrum pesticide used as fungicides, nematocides, acaricides, insecticides, and herbicides. The toxic effect of carbamate pesticides interferes with reproductive systems and fatal development (Niessen, 2010). On the other hand, symmetrical or (*s*)-triazine herbicides are commonly applied to control grassy and broad-leaved weeds. These chemical substances are known to cause possible carcinogenicity and other health effects upon long-term exposure (Teshome Tolcha *et al.,* 2013).

Ethiopia, particularly the Rift Valley area, is greatly exposed to pollution by agrochemical pollutants due to the high investment capacity of the area for commercial farms, including vegetables and floriculture. Because of the rapid intensification of agriculture in Batu areas, located in the southwest zone of the Oromia Region, Ethiopia, there is a general trend of using large amounts of pesticides for pest control (Yosef Alemayehu *et al.,* 2017). The extensive application of these compounds could contaminate ground and surface waters (Teshome Tolcha *et al.,* 2013), sediments and soil (Yared Merdassa *et al.,* 2014). As a result, the environmental or analytical monitoring of these compounds in environmental matrices such as lake water and river sediment and agricultural soils are gaining considerable attention.

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Pesticide residues are found at trace levels. Thus, efficient analytical sample pre-treatment is vital to pre-concentrate and quantitatively extract the contaminants before instrumental analysis. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are usually employed for determination of pesticide residues (Yang *et al.*, 2012). However, LLE has drawbacks, such as the use of large amount of toxic solvents (Negussie Megersa and Samuel Kassahun, 2012) and the formation of tedious emulsions (Chemat *et al.*, 2019). Conversely, the drawbacks of SPE are the use of expensive fibres causing sample carryover effect and thus reduced performance with time, requires the use of organic solvents for column conditioning and elution (Zhang *et al.*, 2012). In modern science, much consideration has been given to advance the analytical sample pre-treatment method which uses less toxic solvents and greatly reduce its volume (Saraji *et al.*, 2014).

Another commonly used miniaturized sample preparation technique is liquid-phase microextraction (LPME), which uses a microliter amounts of the solvent during extraction. A hollow fibre liquid-liquid microextraction (HF-LLME) and single-drop microextraction (SDME) are common LPME methods in use. The drawbacks of HF-LLME are poor reproducibility and air bubbles formation on the hollow fibre surface (Lin *et al.*, 2011). On the other hand, air bubbles formation, unstable droplets, and long equilibrium time are the major drawbacks of SDME (Chen *et al.*, 2010).

To overcome most drawbacks of the SDME and HF-LPME, a dispersive liquid-liquid microextraction (DLLME) was developed by Rezaee and his group (Rezaee *et al.*, 2006) and successfully utilized by quite a number of workers (Chen *et al.*, 2010; Tesfa Bedassa *et al.*, 2015). The use of DLLME has advantages of high extraction recovery, easy to use, high enrichment factors, fast, cost effective, and a significant reduction of volume of organic solvent (Cheng *et al.*, 2011; Ma *et al.*, 2012).

DLLME is nowadays frequently in use in the analysis of various inorganic and organic pollutants in aqueous samples (Farjzadeh *et al.*, 2009; Han *et al.*, 2011; Melwanki and Fuh, 2008; Wang *et al.*, 2010). As solvent utilized in DLLME is not sufficient to extract directly from solid samples, some of the common procedures of DLLME have to be modified. Therefore, the present study aims to develop a low density-dispersive liquid-liquid microextraction, LD-DLLME, technique combined with HPLC-DAD to determine four pesticides (carbaryl, cynazine, atrazine, and propazine) in sediments and soil samples. The application of LD-DLLME has been extended to solid samples by modifying common procedure of DLLME.

MATERIALS AND METHODS

Reagents and materials

HPLC grade solvents, methanol and acetonitrile were obtained from Carlo ErbaReactifs-SDS (Chaussée du vekin, Val de Revil) and Ashland chemical (S. Giuliano MI, Italy), respectively. Sodium chloride (NaCl) and sodium hydroxide (NaOH) were obtained from BDH Laboratory Supplies (Poole, England). Hydrochloric acid (HCl) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was purified with double distiller A8000 Aquatron water still (Bibby Scientific Ltd, Staffordshire, United Kingdom) and deionizer (EASY Pure LF, Dubuque) was used during analysis. Chromic acid was used to clean the glasswares.

Standard solutions preparation

Pesticides standards including carbaryl (97.9%), cynazine (99.9%), propazine (99.9%), and atrazine (99.7%) were obtained from Sigma Aldrich (St. Louis, MO, USA). A stock solution (100 mg/L) of each pesticide standard was prepared in a 25 mL volumetric flask using methanol and stored in a refrigerator. A working solution, 5 mg/L, was prepared once in three days using deionized water. A blank sample was spiked with cynazine (30-800 µg/kg), carbaryl (40-800 µg/kg), atrazine (50-800 µg/kg) and propazine (30-800 µg/kg) and extracted to construct matrix matched calibration curves. Repeatability and reproducibility were performed by extracting the spiked sediment samples at concentration level of 100 µg/kg. Recovery analysis was conducted by extracting spiked samples with all analytes at concentration level of 50 µg/kg.

Instruments

Agilent Technologies 1200 series HPLC, equipped with a quaternary pump, from Agilent Technologies with diode array detector (DAD) (Agilent Technologies, Germany) were used

during analyses. A Phenomenex Luna C18 column (150 x 4.6 mm i.d., 5 µm) was obtained from Phenomenex, Inc. (411 Madrid Avenue Torrance, CA, USA). Agilent Technologies software, LC Chemstation, was employed for data analyses. A filtrating apparatus with a vacuum pump (Quick FIT, England), 25 µL microsyringe (Agilent, Australia), vacuum oven (LABLINE INSTRUMENTS, England), pH meter (Adwa, model 1020, Romania), electronic analytical balance (METTLER AT250, Switzerland), deionizer (EASY Pure LF, Dubuque), vials (2 and 4 mL), centrifuge, Model 800 (China, Beijing), ultrasonic heater (Decon F5100b, England), cellulose acetate filter papers (0.45 µm, Micro Science), and 8 mL centrifuge tube were used resources during the experiments.

Chromatographic conditions

During chromatographic separation, the mobile phase composition was 35% acetonitrile, 43% methanol and 22% water (*v/v*). The isocratic mode mobile phase delivery was set at a flow rate of 0.9 mL/min, and the column temperature was kept at 35˚C. The wavelength for detection was 230 nm with a bandwidth of 4 in reference to wavelength 360 nm having bandwidth 100. The injection volume was 20 µL. The run time (10 min) and post time (1 min) was used. The instrumental response monitored during analysis was the peak area.

Sampling and sample preparation

The soil and sediment samples were collected from agricultural areas around Buchesa River and Batu Lake, Oromia Region, Ethiopia. Geographical locations of the sampling sites is 8˚00'21.94''N latitude and 38˚50'31.78''E longitude with elevation 1637.7 m above sea level. A composite soil sample (10 portions) was taken from farmlands (Ahmed Hussen *et al.,* 2007) Briefly, ten holes of 25 cm depth were made using a spade. Then, 5 cm thick slices, along the vertical wall of the holes, were taken. All collected samples pooled on a methanol rinsed sheet of aluminium foil, each having an area of 3 m^2 and manually mixed.

Soil samples were divided into six portions and a small amount was taken from each portion to make a sub-sample of 1 kg and transported to the laboratory in a chilled insulated box. In the laboratory, the soil samples were air-dried, ground with an electric mill, sieved through a 0.25 mm pore size, wrapped using aluminium foil and stored in a polyethylene plastic bag. The sediment samples were collected following the same procedure for soil sampling and 1.0 g of the sample was subjected to the extraction. The extraction method was developed by using a blank sediment sample which was appropriately washed with acetonitrile and dried to remove possible interferences (Yosef Alemayehu et al., 2017).

Extraction procedure

The sediment and soil samples, 1.0 g each, was spiked at 50 µg/kg in a centrifuge tube and air-dried for 24 h at room temperature to achieve adsorption of the pesticides onto the sample. A 50 µL extraction solvent (1-octanol) and 0.6 mL disperser solvent (acetonitrile) was mixed and rapidly added into the sample with 1 mL syringe. The content was then shaken manually for 1 min. A 5 mL distilled water was then added to the content, after 5 min, and centrifuged at 3500 rpm for 3 min to enhance the phase separation (1-octanol) containing the target analytes. The supernatant was collected in a volumetric flask (5 mL) and 0.6 mL acetonitrile (demulsifier) was injected to the solution. Then, the organic phase was collected using a syringe, dried at room temperature, and reconstituted in 50 µL methanol. Finally, the extract (20 µL) was injected into the HPLC-DAD instrumental system for analyte separation.

RESULTS AND DISCUSSION

Chromatographic working conditions

A series of chromatographic settings were tested to obtain good peak resolution. A tertiary mobile phase composition was studied at different compositions, and the optimum condition was found to be 43% H_2O, 35% ACN, and 22% MeOH. The flow rate of the mobile phase was tested from 0.5–1 mL/min and 0.9 mL/min was selected as optimum flow rate. The injection volume was also investigated over the range of 10–25 µL and injection volume of 20 µL was selected. The effect of column temperature was also studied at 30, 35 and 40^{o}C. Since no significant change was

observed over the temperature range tested, 35°C was selected as optimum temperature.

Optimization of LD-DLLME extraction parameters

The LD-DLLME method usually involves optimization of variables that potentially affect the extraction efficiency, sensitivity and selectivity (Herrera-Herrera *et al.,* 2010). The parameters considered in this particular work are described below.

Selection of the extraction solvent

The selection of appropriate extraction solvent is critical in order to obtain satisfactory extraction efficiency. The extraction solvent should be of low volatility to minimize solvent losses during extraction, low solubility in water, high capability to extract target analytes, and good chromatographic behaviour (Teshome Tolcha *et al.,* 2013). Three extraction solvents, including cyclohexane, *n*-hexane, and 1-octanol, were tested. The experimental results revealed that peak areas were found to be the highest for all the target pesticides when 1-octanol was used as extraction solvent. This observation might be attributed to the high boiling point of 1-octanol (195˚C) relative to cyclohexane (80.75˚C) and n-hexane (69˚C), which results in reducing solvent loss during extraction. Therefore, 1-octanol was used as the solvent of choice for the subsequent experiments.

Selection of disperser solvent

The main role of disperser solvent is to disperse and form fine droplets of extraction solvents. The disperser solvent should be miscible in the organic solvent and water (Yan *et al.,* 2013). In this experiment, the efficiencies of acetonitrile, acetone and methanol, as disperser solvent, were evaluated. Acetonitrile was found to have high extraction efficiency. This efficiency may be because acetonitrile has extraction capability for a wide polarity range of pesticides and the stability of analytes in acetonitrile (Maštovskáand and Lehotay, 2004). Therefore, acetonitrile was selected for the subsequent experiments.

Effect of extraction solvent volume

The extracting solvent volume plays a great role in the extraction efficiency of the LD-DLLME techniques. The optimal extraction solvent volume should offer high extraction efficiency and enough organic phase volume for efficient extraction (Tesfa Bedassa *et al.,* 2015). In this study, the effect of extraction solvent volume was investigated over the range of 30–70 µL, while the other experimental parameters were kept constant.

As indicated in Figure 1, the peak areas of analytes increased with the volume of the extraction solvent up to 50 µL and then lowered with higher volumes. The decrease in the peak areas of the pesticides with further increase in volumes could be due to dilution of the resulting concentrate collected after extraction (Zhou *et al.,* 2009). Therefore, 50 µL of 1-octanol was used as the optimum volume, Figure 1.

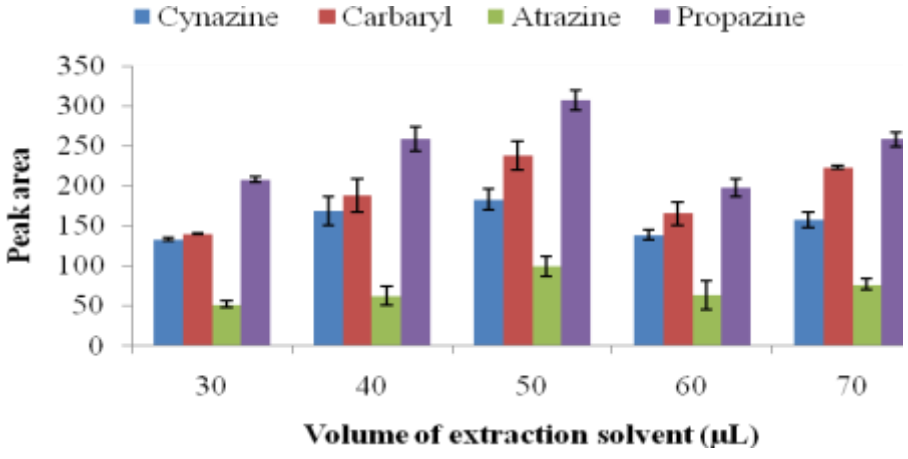


Figure 1. Effect of the extraction solvent volume on the peak areas of carbamate and *s*-triazine pesticides. Experimental parameters: sample size, 1 g; spiked concentration, 100 µg/kg; volume of acetonitrile, 1 mL; extraction time, 5 min; volume of deionized water, 5 mL; centrifugation speed, 3500 rpm for 3 min.; n =3.

Effect of disperser solvent volume

The degree of dispersion of the extraction solvent in the sample depends on the volume of the disperser solvent (Rezaee *et al.,* 2006). To this end, the effect of acetonitrile volume was studied in the range of 0.5–1 mL, Figure 2. The peak areas of all the target pesticides slightly increased with acetonitrile volume up to 0.6 mL and then declined. The most probable reason for decrease in peak area at low volume of acetonitrile could be that the organic extractant fine droplets might not be formed properly. On the other hand, using a higher volume of acetonitrile could enhance the solubility of the pesticides in the aqueous phase due to partitioning of the dispersive solvent in the aqueous sample (Zhang *et al.,* 2010). Based on the experimental results, 0.6 mL acetonitrile was selected.

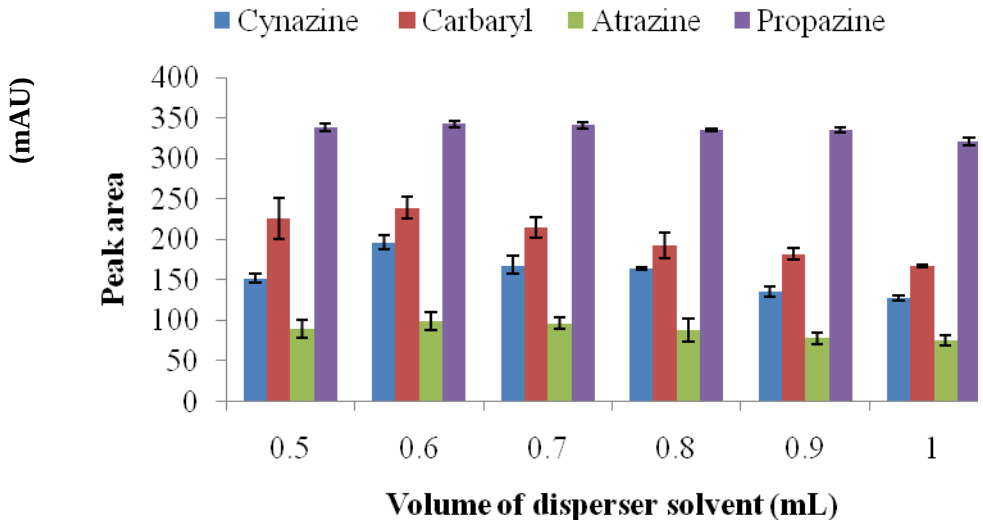


Figure 2. Effect of the disperser solvent volume on the peak areas of carbamate and *s*-triazine pesticides. Experimental parameters: sample size, 1 g; spiked concentration, 100 µg/kg; volume of 1-octanol, 50 µL; extraction time, 5 min; volume of deionized water, 5 mL; centrifugation speed, 3500 rpm for 3 min.; n =3.

Effect of extraction time

In DLLME, extraction time is the time interval between the injection of the disperser solvent containing the extraction solvent and centrifugation (Teshome Tolcha *et al.,* 2013). In the present study, the extraction period was evaluated in the range of 1–9 min. The experimental results revealed that high peak areas for all the target pesticides were achieved at 5 min extraction time. Thus, extraction time of 5 min was adopted in the subsequent extractions.

Effect of centrifugation time and speed

The role of centrifugation is to accelerate collection of the extractant droplets (Tesfa Bedassa et al., 2015). The centrifugation time and speed was studied in the ranges of 1–5 min and 2000–4000 rpm, respectively. The results depicted in Figure 3 showed that the extraction efficiency increased with increasing centrifugation time and remained nearly unchanged after 3 min for most analytes. Similarly, higher peak areas were obtained at the centrifugation speed of 3500 rpm (Figure 4). Therefore, centrifugation time of 3 min and centrifugation speed of 3500 rpm were selected as optimum experimental values for the subsequent experiments.

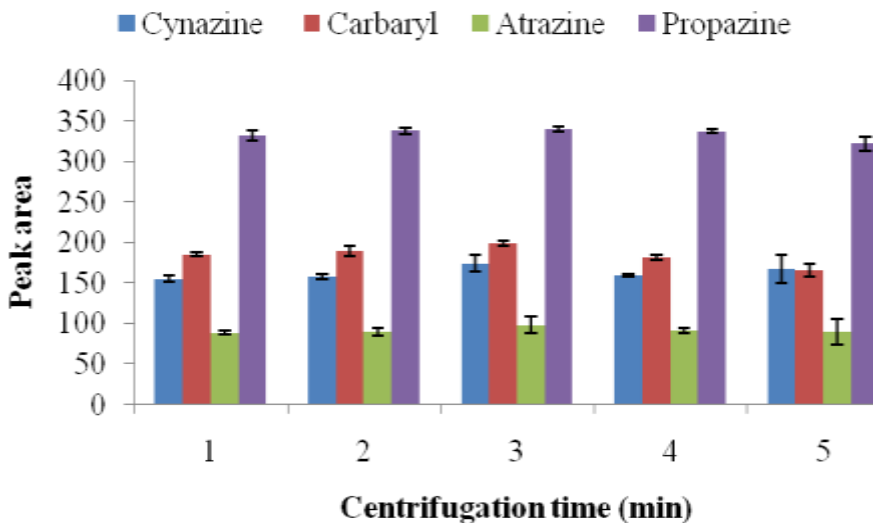


Figure 3. Effect of centrifugation time on the peak areas of carbamate and *s*-triazine pesticides. Experimental parameters: sample size, 1 g; spiked concentration, 100 µg/kg; volume of 1-octanol, 50 µL; volume of acetonitrile, 0.6 mL; volume of de-ionized water, 5 mL; centrifugation speed, 3500 rpm; n = 3

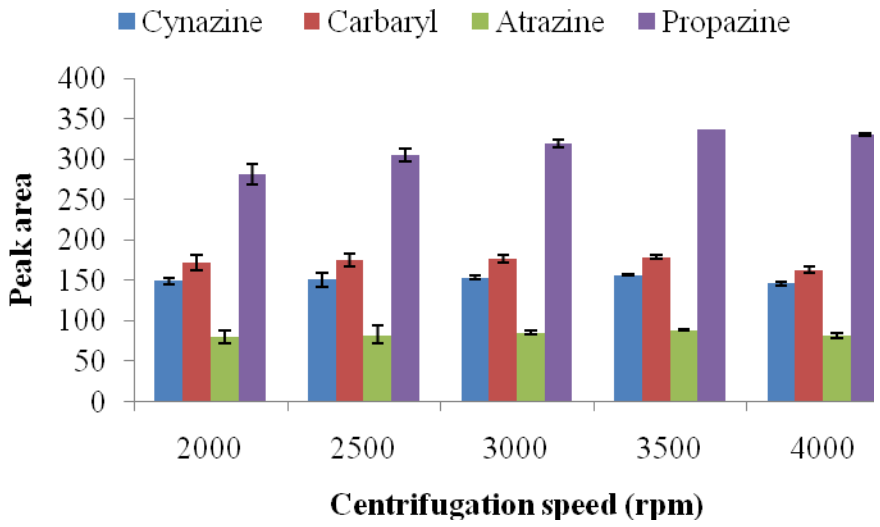


Figure 4. Effect of centrifugation speed on the peak areas of carbamate and *s*-triazine pesticides. Experimental parameters: sample size, 1.0 g; spiked concentration, 100 µg/kg; volume of 1-octanol, 50 µL; volume of acetonitrile, 0.6 mL; volume of de-ionized water, 5 mL; centrifugation time, 3 min; *n* =3.

Effect of salt concentration

In order to assess the influence of ionic strength, a series of experiments were performed by adding varied concentrations of NaCl from 0 to 20% (*m/v*) with interval of 5% in the sample solution, keeping all other parameters constant. The results indicated that the instrumental response (peak areas) of all the target pesticides increased up to 10% and then started to decline on further increase in the added salt concentration. The decrease in peak area after 10% NaCl might be due to the dissolution of sodium chloride in water, which may enhance electrostatic interaction. The electrostatic interaction could most likely cause the extent of analytes transfer to the extraction phase to be reduced (Teshome Tolcha and Negussie Megersa, 2014). Thus, 10% NaCl (*m/v*) was used in the subsequent experiments.

Effect of pH

For efficient extraction of relatively polar compounds, the pH of the sample solution plays a decisive role (Abera Gure *et al.*, 2014). The effect of pH of the sample solution was studied over the

range of 4–8. As shown in Figure 5, peak areas of all the target analytes increased up to pH 5 and then declined on further increase in pH of the sample solution. The results showed the studied analytes to be stable in the weakly acidic environments, whereas possibly degraded in strongly acidic medium (Abera Gure *et al.*, 2014). Hence, pH 5 has been used for further experiments.

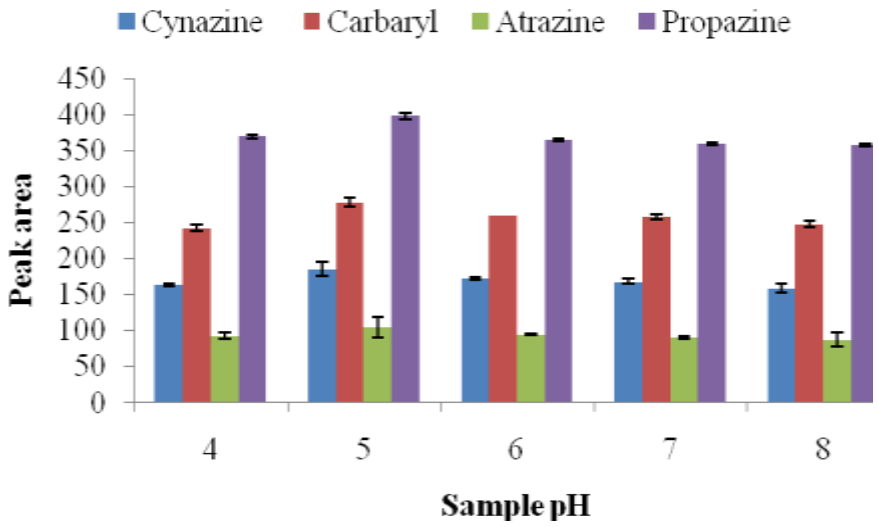


Figure 5. Effect of pH on the peak areas of carbamate and *s*-triazine pesticides. Experimental parameters: sample size, 1 g; spiked concentration, 100 µg/kg; volume of 1-octanol, 50 µL; volume of acetonitrile, 0.6 mL; volume of de-ionized water, 5 mL; centrifugation speed, 3500 rpm; centrifugation time, 3 min; amount of NaCl, 10% (*w/v*); n = 3.

Analytical merits of the method

The proposed LD-DLLME method was evaluated using sediment sample to construct matrix-matched calibration curves. The calibration curves were plotted for five different concentration levels. The calibration curves were obtained using the peak areas as the instrumental response. LOD was calculated as the lowest concentrations yielding S/N ratios of 3 (Yared Merdassa *et al.*, 2014). The Analytical merits of the optimized method are indicated in Table 1.

Table 1. Analytical Analytical merits of the LD-DLLME technique coupled with HPLC-DAD for carbamate and *s*-triazine pesticides under study in blank sediment, n=3.

Analyte	Linear range (µg/kg)	R^2	LOD (µg/kg)	Regression equation	Repeatability (%RSD)	Reproducibility (%RSD)
Cynazine	30–800	0.999	9	Y = 2.38x – 2.17	1.8	2.7
Carbaryl	40–800	0.999	12	Y = 3.04x – 1.04	0.5	2.3
Atrazine	50–800	0.998	24	Y = 1.09x – 1.49	1.5	5.2
Propazine	30–800	0.998	9	Y = 4.89x – 0.76	0.6	0.7

The method offers good correlation ($R^2 \geq 0.998$). The LODs of the method were in the range of 9–24 µg/kg. The precision of the proposed LD-DLLME method was studied in terms of repeatability (intra-day precision) and reproducibility (inter-day precision). Repeatability and reproducibility were investigated by extracting the spiked sediment samples at a concentration level of 100 µg/kg. As indicated in Table 1, the % RSD of the method was in the range of 0.5–1.8% for intra-day precision (repeatability) and 0.7–5.2% for inter-day precision (reproducibility), which ensures the studied precisions to lie within the acceptable ranges (Abera Gure *et al.*, 2014).

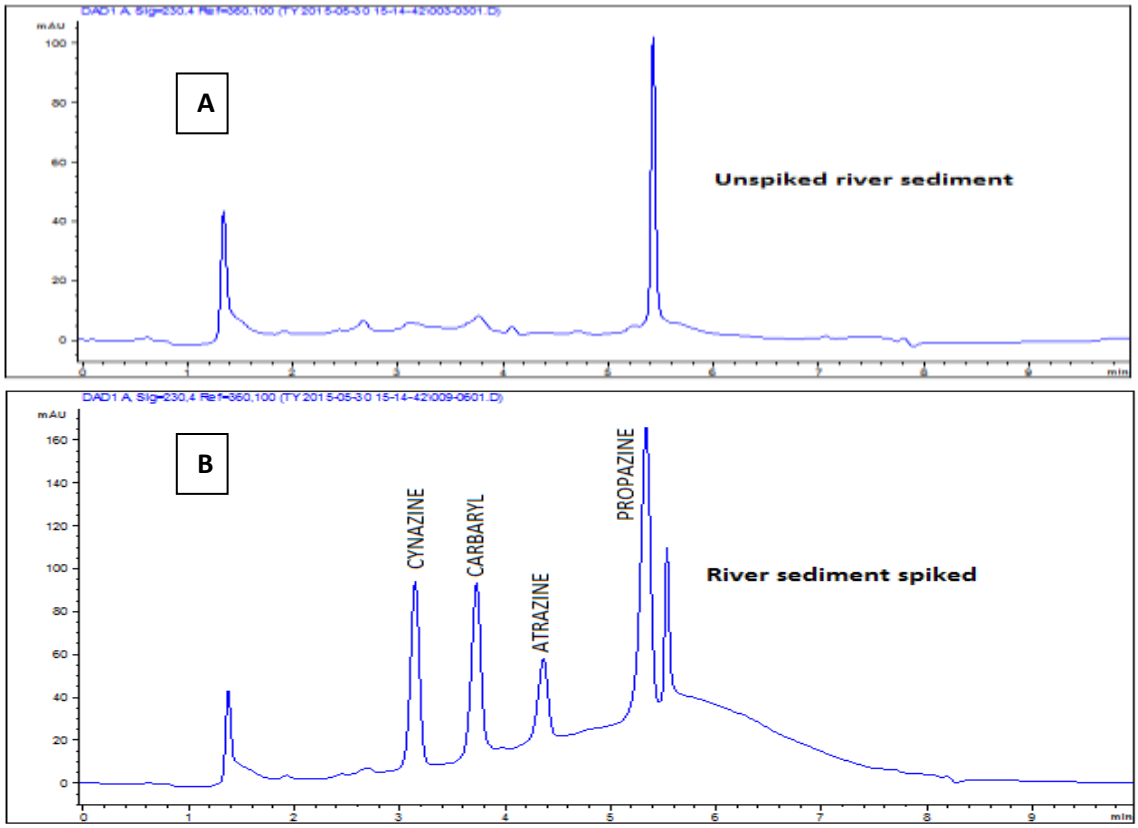
To evaluate the matrix effect of the proposed method, 1g each of the sediment and soil samples were spiked with each *s*-triazine herbicide and carbamate insecticide at concentration level of 50

µg/kg and were extracted using the method. The results are summarized in Table 2. The recoveries ranged from 74.5 to 109.7%, with RSDs varying from 0.8% to 5.3%. These findings demonstrate that the sediment and soil matrix had little or no effect on the LD-DLLME method. Then, the proposed method was applied to the analysis of the target analytes in real sediment and soil samples. As the results in Figure 6 indicate, all the target analytes were not found in the real samples. The sample analyzed may be either free from the residues of target pesticides or containing concentrations below the detection limits. Figure 6 shows the chromatograms of unspiked (A) and spiked river sediments (B), unspiked and spiked Lake sediments, (C) and (D), respectively, and spiked agricultural soil samples (E).

Table 2. %RR and %RSD of the environmental sediment and soil samples (n = 3).

Target analytes	Spiked level (µg/kg)	Lake sediment		River sediment		Agricultural soil	
		%RR	%RSD	%RR	%RSD	%RR	%RSD
Cynazine	unspiked	nd	–	nd	–	nd	–
	50	103.5	2.2	88.5	3.1	95.5	2.3
Carbaryl	unspiked	nd	–	nd	–	nd	–
	50	109.7	0.95	97	2.9	90.9	0.8
Atrazine	unspiked	nd	–	nd	–	nd	–
	50	94.6	4	74.5	3.6	84.5	1.4
Propazine	Unspiked	nd	–	nd	–	nd	–
	50	92.5	3.67	79.9	5.3	85.4	1.7

nd: not detected



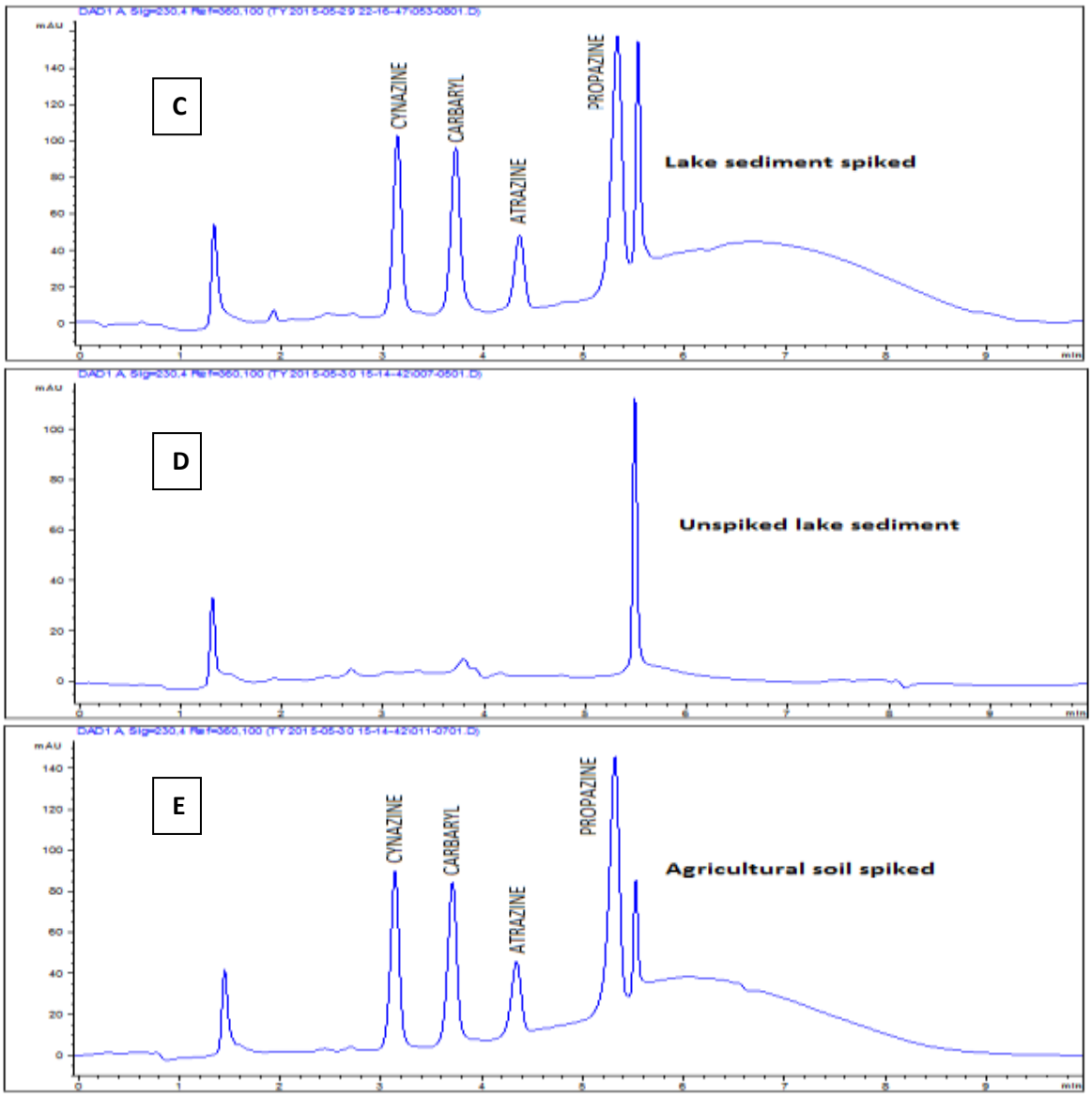


Figure 6. Typical chromatograms for the extracts of unspiked (A) and spiked river sediments (B), Lake sediment unspiked (C) and spiked (D), and spiked agricultural soil samples (E) using the developed LD-DLLME technique.

Comparison of the proposed LD-DLLME with reported literature results

The efficiency of the proposed LD-DLLME method has been compared with other extraction techniques, including ionic liquid-based dispersive liquid-liquid micro-extraction-high-performance liquid chromatography (IL-DLLME-HPLC) (Tong *et al.,* 2012), dispersive liquid-liquid micro-extraction-liquid chromatography (DLLME-LC) (Lingyan *et al.,* 2009), dispersive liquid-liquid micro-extraction-high-performance liquid chromatography (DLLME-HPLC), (Leng *et al.,* 2012; Rajib *et al.,* 2014), and dispersive liquid-liquid micro-extraction gas chromatography (DLLME-GC) (Lana *et al.,* 2013). Details of the comparison are summarized in Table 3. The newly developed extraction method has LOD better than or comparable with most reported extraction techniques. The method also provides a better or similar performance compared with other parameters. The results revealed that the proposed method is sensitive, reproducible, and easy to use in detecting carbamate and *s*-triazine pesticides in environmental sediment and soil samples, and other matrices possessing similar physicochemical properties.

Table 3. Comparison of the proposed LD-DLLME method with reported literature results.

Analytical methods	Sample	Linear range (µg/kg)	LODs (µg/kg)	%RSD	Reference
IL-DLLME-HPLC-UV	Soil and water	2–200	0.94–1.97	3.9–10.1	(Tong *et al.*,2012)
DLLME-LC-FD	Soil	0.1–1000	0.01	4.2	(Lingyan *et al.,* 2009)
DLLME-HPLC-UV-Vis	Soil and water	0.1–100	0.05–0.1	2.1–4.2	(Rajib *et al.,* 2014)
DLLME-HPLC-FLD	Sediment	-	2.3–6.8	< 8.0	(Leng *et al.,* 2012)
DLLME-GC-MS-MS	Sediment	-	0.5–1.8	< 9.2	(Lana *et al.,* 2013)
DLLME-HPLC-DAD	River sediment			2.9–5.3	Current study
	Lake sediment			0.95–4.0	
	Soil	30–800 µg/kg	9.0–24.0	0.8–2.3	

CONCLUSIONS

LD-DLLME analytical method was developed successfully for the rapid and sensitive anlyses of four pesticides (carbaryl, cynazine, atrazine, and propazine) in environmental sediment and soil matrices. Various extraction parameters affecting the extraction efficiency of the method were optimized. Under the optimized conditions, the developed method has offered lower LODs, good repeatability and reproducibility, and relative recoveries. From these experimental observations, the proposed LD-DLLME method could be used to monitor and control carbamate and *s*-triazine pesticides in environmental sediment and soil samples. The method has been applied to real sediment and soil samples. The results indicated that both the sediment and soil samples tested in this work were not contaminated by the target pesticides and containing concentrations below their detection limits.

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REFERENCES

1. Abera Gure, Lara, F. J., Moreno-González, D. Negussie Megersa, Olmo-Iruela, M. and García-Campaña, A.M. (2014). Salting-out assisted liquid-liquid extraction combined with capillary HPLC for the determination of sulfonylurea herbicides in environmental water and banana juice samples. *Talanta* **127:**51–58.
2. Ahmed Husen, Westbom, R., Negussie Megersa, Mathiasson, L., Björklund, E. (2007). Selective pressurized liquid extraction for multi-residue analysis of organochlorine pesticides in soil. *J. Chromatogr. A* **1152**:247–253.
3. Chemat, F., Abert, M., Ravi, V.H.K., Khadhraoui, R.B., Hilali, S., Perinoand, S.A. and Tixier, S.F. (2019). Review of alternative solvents for green extraction of food and natural products: panorama, principles, applications and prospects. *Molecules* **24(16):**3007–337.
4. Chen, H., Chen, R. and Li, S. (2010). Low-density extraction solvent-based solvent terminated dispersive liquid-liquid microextraction combined with gas chromatography-tandom mass spectrometry for the determination of carbamate pesticides in water samples. *J. Chromatogr. A* **1217(8):**1244–1248.
5. Cheng, J., Xiao, J., Zhou, Y., Xia, Y., Guo, F. and Li, J. (2011). Dispersive liquid-liquid microextraction based on solidification of floating organic droplet method for the determination of diethofencarb and pyrimethanil in aqueous samples. *Microchim. Acta*, **172:** 51–55.
6. Farajzadeh, M.A., Bahram, M. and Vardast, M.R. (2009). Optimization of dispersive liquid-liquid microextraction of Co(II) and Fe(III) as their oxinate chelates and analysis by HPLC: application for the simultaneous determination of Co(II) and Fe(III) in water samples. *J. Sep. Sci.* **32(23-24):**4200–4212.
7. Han, D., Yan, H. and Row, K.H. (2011). Ionic liquid-based dispersive liquid-liquid microextraction for sensitive determination of aromatic amines in environmental water. *J. Sep. Sci.* **34(10):**1184–1189.
8. Herrera-Herrera, A., Asensio-Ramos, M., Hernandez-Borges, J. and Rodriguez-Delgado, M. (2010).

Dispersive liquid-liquid microextraction for determination of organic analytes. *Trends Anal. Chem.* **29(7):**728–751.

9. Lana, N.B., Berton, P., Covaci, A., Atencio, A.G., Ciocco, N. F. and Altamiranoo, J. C. (2013). Ultrasound leaching-DLLME based solidification of floating organic droplet for determination of polybrominated diphenyl ethers in sediment samples by gas chromatography-tandem mass spectrometry. *J. Chromatogr. A* **1285:**15–21.
10. Leng, G., Liu, G., Chen, Y., Yin, H. and Dan, D. (2012). Vortex-assisted extraction combined with DLLME for the determination of polycyclic aromatic hydrocarbons in sediment by HPLC. *J. Sep. Sci.* **35(20):**2796–2804.
11. Lin, X., Chen, X., Huo, X., Yu, Z., Bi, K. and Li, Q. (2011). Dispersive liquid-liquid microextraction coupled with high-performance liquid chromatography-diode array detection for the determination of N-methyl carbamate pesticides in vegetables. *J. Sep. Sci.* **34(2):**202–209.
12. Lingyan, F., Xiujuan, L. and Jia, H. (2009). Determination of two pesticides in soil by DLLME combined with LC-FD. *Chromatographia* **70(1)**:1697–1701.
13. Ma, J., Lu, W.and Chen, L. (2012). Recent advances in dispersive liquid-liquid microextraction for organic compounds analysis in environmental water. *Current Anal. Chem.* **8(1):**78–90.
14. Maštovskáand, K. and Lehotay, S.J. (2004). Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. *J. Chromatogr. A* **1040(2)**:259–272.
15. Melwanki, M.B. and Fuh, M.-R. (2008). Partitioned dispersive liquid liquid microextraction: an approach for polar organic compounds extraction from aqueous samples. *J. Chromatogr. A* **1207(1–2):**24–28.
16. Mnif, W., Hassine, A.I.H., Bouaziz, A., Bartegi, A., Thomas, O. and Roig, B. (2011). Effect of endocrine disruptor pesticides: a review. *Int. J. Environ. Res. Public Health* **8(6):**2265–2303.
17. Negussie Megersa and Samuel Kassahun (2012). A new selective liquid membrane extraction method for the determination of basic herbicides in agro-processed fruit juices and Ethiopian honey wine (Tej) samples. *Food Addit. Contam. Part A* **29(5)**: 789–798.
18. Niessen, W.M.A. (2010). Group-specific fragmentation of pesticides and related compounds in liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **1217(28):**4061–4070.
19. Rajib, J., Dhiman, S., Srimanta, M. and Mitali, S. (2014). Preconcentration and recovery of pesticides from soil and water by dispersive liquid-liquid extraction. *Eur. Chem. Bull.* **3(6):**612–616.
20. Rezaee, M., Assadi, Y., M.-R.M. Hosseini, E. Aghaee, F. Ahmadi and S. Berijani (2006). Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J. Chromatogr. A* **1116(1–2):**1–9.
21. Saraji, M. and Boroujeni, M.K. (2014). Recent developments in dispersive liquid-liquid microextraction. *Anal. Bioanal. Chem.* **406:**2027–2066.
22. Tesfa Bedassa, Abera Gure and Negissie Megersa (2015). Low density solvent based dispersive liquid-liquid microextraction and preconcentration of multiresidue pesticides in environmental waters for liquid chromatographic analysis. *J. Anal. Chem.* **70(10)**:1199–1206.
23. Teshome Tolcha and Negussie Megersa (2014). Simultaneous and selective extraction of multiclass pesticide residues in water and sugarcane juice samples. *Am. J. Anal. Chem.* **9(4)**:224–244.
24. Teshome Tolcha, Tura Gemechu, Al-Hamimi, S., Negissie Megersa and Turner, C. (2021). Multivariate optimization of a combined static and dynamic supercritical fluid extraction method for trace analysis of pesticides pollutants in organic honey. *J. Sep. Sci.* **44 (8):**1716–1726.
25. Teshome Tolcha, Yared Merdassa and Negussie Megersa (2013). Low-density extraction solvent based solvent-terminated dispersive liquid-liquid microextraction for quantitative determination of ionizable pesticides in environmental waters. *J. Sep. Sci.* **36(6):**1119–1127.
26. Tong, W., Yu, L., Zhonghua, Y., Haixiang, G. and Zhipiang, Z. (2012). Determination of pyrethroid pesticides in environmental samples using IL-DLLME. *J. Braz. Chem. Soc.* **23(7)**:1327–1333.
27. Wang, Y., You, J., Ren, R., Xiao, Y., Gao, S., Zhang, H. and Yu, A. (2010). Determination of triazines in honey by dispersive liquid-liquid microextraction high-performance liquid chromatography. *J. Chromatogr. A* **1217(26):**4241–4246.
28. Yan, H. and Wang, H. (2013). Recent development and applications of dispersive liquid-liquid microextraction. *J. Chromatogr A* **1295:**1–15.
29. Yang, Z., Liu, Y., Liu, D. and Zhou, Z. (2012). Determination of organophosphorus pesticides

in soil by dispersive liquid-liquid microextraction and gas chromatography. *J. Chromatogr. Sci.* **50(1):**15–20.

30. Yared Merdassa, Liu, J.-f. and Negussie Megersa (2014). Development of a one-step microwave-assisted extraction procedure for highly efficient extraction of multiclass fungicides in soils. *Anal. Methods* **6(9):**3025–3033.
31. Yosef Alemayehu, Teshome Tolcha and Negussie Megersa (2017). Salting-out assisted liquid-liquid extraction combined with HPLC for quantitative extraction of trace multiclass pesticide residues from environmental waters. *Am. J. Anal. Chem* **8(7):**433–448.
32. Zhang, L., Chen, F., Liu, S., Chen, B. and C. Pan (2012). Ionic liquid-based vortex-assisted dispersive liquid-liquid microextraction of organophosphorus pesticides in apple and pear. *J. Sep. Sci.* **35(18):**2514–2519.
33. Zhang, S., Song, C., Li, S., Feng, T., Wang, C. and Wang, Z. (2010). Application of dispersive liquid-liquid microextraction combined with sweeping micellar electrokinetic chromatography for trace analysis of six carbamate pesticides in apples. *Anal. Methods* **2(1):** 54–62.
34. Zhou, Q., Pang, L., Xie, G., Xiao, J. and Bai, H. (2009). Determination of atrazine and simazine in environmental water samples by dispersive liquid-liquid microextraction with high performance liquid chromatography. *Anal. Sci.* **25(1):**73–76.
35. Zhou, Q., Zhang, X. Xie, G. and Xiao, J. (2009). Temperature-controlled ionic liquid-dispersive liquid phase microextraction for preconcentration of chlorotoluron, diethofencarb and chlorobenzuron in water samples. *J. Sep. Sci.* **32(22):**3945–3950.